出國報告（出國類別：開會）

參加 2019 歐盟水產養殖國際研討會

服務機關：台灣中油公司綠能科技研究所
姓名職稱：余宗賢 化學工程師
派赴國家/地區：德國/柏林
出國期間：民國 108 年 10 月 4 日至 10 月 14 日
報告日期：民國 108 年 11 月 12 日

摘　要

本次會議之議題幾乎包括所有水產養殖之議題，共有 56 個會議(sessions)、1039 篇摘要論文、594 篇海報(包含 94 篇之電子海報)、150 個展覽攤位、85 個參與國家、超過 2700 位參與者。

然而，因此國際研討會議題很多，故本人僅能盡可能參與和本組研究方向與未來可能研發方向相關之議題。主要參與之論文發表議題包括 Macroalgae, Climate Change, Sustainable Aquaculture Technologies, Value Addition and Marketing, Mollusc Production and Restoration, Hatchery Technologies and Practices, and Shrimp。本人就將上述之議題摘錄較重要之發表提出探討。

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一、緣起與目的

2019 歐盟水產養殖研討會(Aquaculture Europe 2019)涵蓋的主題有: Climate change (氣候變遷)、Environmental and ecosystem management (環境與生態管理)、Environmental impact of aquaculture - problems coming FROM and TO aquaculture (環境衝擊-水產養殖衍生與遭受之問題)、Aquaculture on offshore structures (離岸水產養殖結構)、Recirculating aquaculture systems and closed containment systems (循環養殖系統與密閉控制系統)、Flow through and raceway systems (溢流池與迴流池系統)、Selective breeding (選擇性養殖)、Processing, co-products and value addition (高附加價值之共產物與程序)等共 38 個議題，結合水產生物科技在工業、環境、食品上的應用。

綠能所環保科技組目前研發方向與此國際研討會之討論主題契合，參加該會議有助於了解目前產業現況，與國際學者、工程師、工業專家進行交流。藉由發表研究成果及聽取與會者的簡報海報，能激發新的創意思維。

二、行程及工作摘要

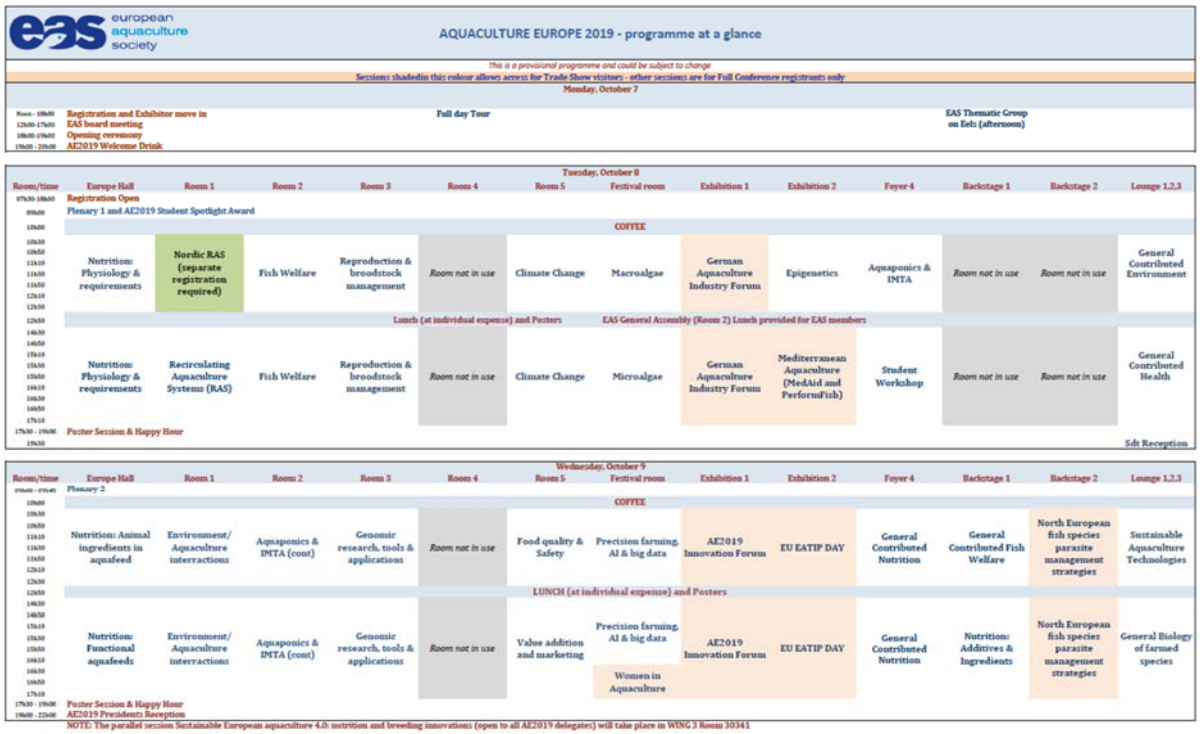
日期	到達地點	詳細工作內容
10/4	台灣→德國	啟程(高雄→德國)
10/5-6	德國	私人行程
10/7-10	德國柏林	參加 2019 Aquaculture Europe 國際研討會
10/11-12	德國柏林	私人行程
10/13-14	德國→台灣	回程(德國→桃園機場→高雄)/飛機上過夜

三、研討會內容摘要

3.1 會議簡介

本次 2019Aquaculture Europe 國際研討會是由歐洲水產養殖學會(European Aquaculture Society)主辦，舉辦時程為 2019 年 10 月 7 日至 10 月 10 日，興辦地點為英國柏林之 ESTREL 飯店會議中心。此會議除邀請多位學者專家進行專題演講外，並由世界各國學者專家共同與會進行論文口頭發表、海報發表及研究經驗交流。本次會議主要議程包括 Climate change (氣候變遷)、Environmental and ecosystem management (環境與生態管理)、Environmental impact of aquaculture - problems coming FROM and TO aquaculture (環境衝擊-水產養殖衍生與遭受之問題)、Aquaculture on offshore structures (離岸水產養殖結構)、Recirculating aquaculture systems and closed containment systems (循環養殖系統與密閉控制系統)、Flow through and raceway systems (溢流池與迴流池系統)、Selective breeding (選擇性養殖)、Processing, co-products and value addition (高附加價值之共產物與程序)等共 38 個議題。

各議題之口頭發表時間和研討室詳如下表所示，然各口頭發表之研發題目與海報發表題目則如附件一與附件二所示。



AQUACULTURE EUROPE 2019 - programme at a glance

This is a provisional programme and could be subject to change

Sessions shadedin this colour allows access for Trade Show visitors - other sessions are for Full Conference registrants only

Monday, October 7

Noon - 18h00	Registration and Exhibitor move in	Full day Tour	EAS Thematic Group on Eels (afternoon)
12h00-17h00	EAS board meeting		
18h00-19h00	Opening ceremony		
19h00 - 20h00	AE2019 Welcome Drink		

Tuesday, October 8

Room/time	Europe Hall	Room 1	Room 2	Room 3	Room 4	Room 5	Festival room	Exhibition 1	Exhibition 2	Foyer 4	Backstage 1	Backstage 2	Lounge 1,2,3
07h30-18h00	Registration Open												
09h00	Plenary 1 and AE2019 Student Spotlight Award												
10h00	COFFEE												
10h30 10h50 11h10 11h30 11h50 12h10 12h30	Nutrition: Physiology & requirements	Nordic RAS (separate registration required)	Fish Welfare	Reproduction & broodstock management	Room not in use	Climate Change	Macroalgae	German Aquaculture Industry Forum	Epigenetics	Aquaponics & IMTA	Room not in use	Room not in use	General Contributed Environment
12h50	Lunch (at individual expense) and Posters						EAS General Assembly (Room 2) Lunch provided for EAS members						
14h30 14h50 15h10 15h30 15h50 16h10 16h30 16h50 17h10	Nutrition: Physiology & requirements	Recirculating Aquaculture Systems (RAS)	Fish Welfare	Reproduction & broodstock management	Room not in use	Climate Change	Microalgae	German Aquaculture Industry Forum	Mediterranean Aquaculture (MedAid and PerformFish)	Student Workshop	Room not in use	Room not in use	General Contributed Health
17h30 - 19h00	Poster Session & Happy Hour												
19h30													5dt Reception

Wednesday, October 9

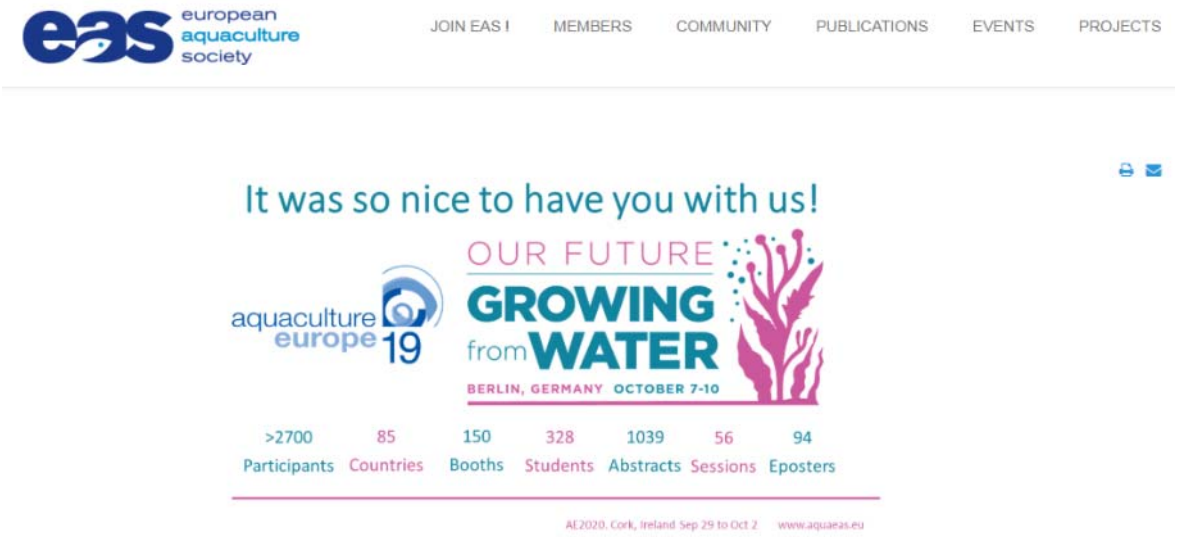
Room/time	Europe Hall	Room 1	Room 2	Room 3	Room 4	Room 5	Festival room	Exhibition 1	Exhibition 2	Foyer 4	Backstage 1	Backstage 2	Lounge 1,2,3
09h00 - 09h45	Plenary 2												
10h00	COFFEE												
10h30 10h50 11h10 11h30 11h50 12h10 12h30	Nutrition: Animal ingredients in aquafeed	Environment/ Aquaculture interractions	Aquaponics & IMTA (cont)	Genomic research, tools & applications	Room not in use	Food quality & Safety	Precision farming, AI & big data	AE2019 Innovation Forum	EU EATIP DAY	General Contributed Nutrition	General Contributed Fish Welfare	North European fish species parasite management strategies	Sustainable Aquaculture Technologies
12h50	LUNCH (at individual expense) and Posters												
14h30 14h50 15h10 15h30 15h50 16h10 16h30 16h50 17h10	Nutrition: Functional aquafeeds	Environment/ Aquaculture interractions	Aquaponics & IMTA (cont)	Genomic research, tools & applications	Room not in use	Value addition and marketing	Precision farming, AI & big data Women in Aquaculture	AE2019 Innovation Forum	EU EATIP DAY	General Contributed Nutrition	Nutrition: Additives & Ingredients	North European fish species parasite management strategies	General Biology of farmed species
17h30 - 19h00	Poster Session & Happy Hour												
19h00 - 22h00	AE2019 Presidents Reception												

NOTE: The parallel session Sustainable European aquaculture 4.0: nutrition and breeding innovations (open to all AE2019 delegates) will take place in WENG 3 Room 30341

Thursday, October 10													
Room/time	Europe Hall	Room 1	Room 2	Room 3	Room 4	Room 5	Festival room	Exhibition 1	Exhibition 2	Foyer 4	Backstage 1	Backstage 2	Lounge 1,2,3
09h00 09h20 09h40 10h00 10h20 10h40 11h00	Nutrition: Additives & Ingredients (cont)	Pathogens, diseases & treatments	Selective Breeding	Molluscs	Governance, policy, regulations & planning	Sustainable systems for large scale production - closed, offshore or both	Percid Fish	Escapes/ Interactions farmed & wild fish	Environmental & Ecosystem Management	General Topics Session	Aquaculture in Central & Eastern Europe (NACEE)	Shrimp Industry Forum	Baltic Aquaculture
11h20 11h45 12h30	Plenary 3 AE2019 Poster Awards												
12h50	LUNCH (at individual expence) and Posters												
14h30 14h50 15h10 15h30 15h50 16h10 16h30	Nutrition: Additives & Ingredients	Pathogens, diseases & treatments	Selective Breeding	Molluscs	Education, knowledge management, transfer & extension networks	Cage systems & offshore structures	Percid Fish	Laboratory aquatic models & ornamentals	Hatchery technologies & practices	General Topics Session	NACEE (cont)	Shrimp	Compounds in Lipid Transport

3.2 與會心得

本次會議之議題幾乎包括所有水產養殖之議題，共有 56 個會議(sessions)、1039 篇摘要論文、594 篇海報(包含 94 篇之電子海報)、150 個展覽攤位、85 個參與國家、超過 2700 位參與者。



然而，因此國際研討會議題很多，故本人僅能盡可能參與和本組研究方向與未來可能研發方向相關之議題。主要參與之論文發表議題包括 Macroalgae, Climate Change, Sustainable Aquaculture Technologies, Value Addition and Marketing, Mollusc Production and Restoration, Hatchery Technologies and Practices, and Shrimp。本人就將上述之議題摘錄較重要之發表提出探討。

一、Macroalgae

(1)

主題：

Brown seaweeds – A status report of offshore cultivation and expected market developments in Europe (褐藻 – 歐洲地區離岸養殖及預期市場發展之現況報告)

講者：

Diogo Raposo, Frank Neumann, Jon Funderud

摘要：

褐藻為全球水產養殖生產比例最高之海藻，主要是因為東南亞大量養殖海帶(kelp)，例如 *Saccharina japonica* (昆布 Kombu)和 *Undaria pinnifatida* (裙帶菜 Wakame)。海藻是亞洲地區非常重要的食品，但在歐洲卻非為主要食品。然而，因為海藻在烹飪和飲食的價值已經越來越受到歐洲廚師和食品產業的注意。

在歐洲養殖褐藻之最初考量是為了生物燃料，但大規模之海藻養殖場需投入大量的資金和自動化技術。而且，直到最近數年，歐洲業者才意識到褐藻可作為食品，且相較於生物燃料，食品之銷售價格又較高。在 2014 年，海藻能源解決方案公司(Seaweed Energy Solution, SES)建立了一個試驗性養殖場，主要為驗證每年收獲 100-150 公噸海藻之技術可行性；故於 2014 年之後，許多歐洲公司開始積極採用類似之養殖技術，特

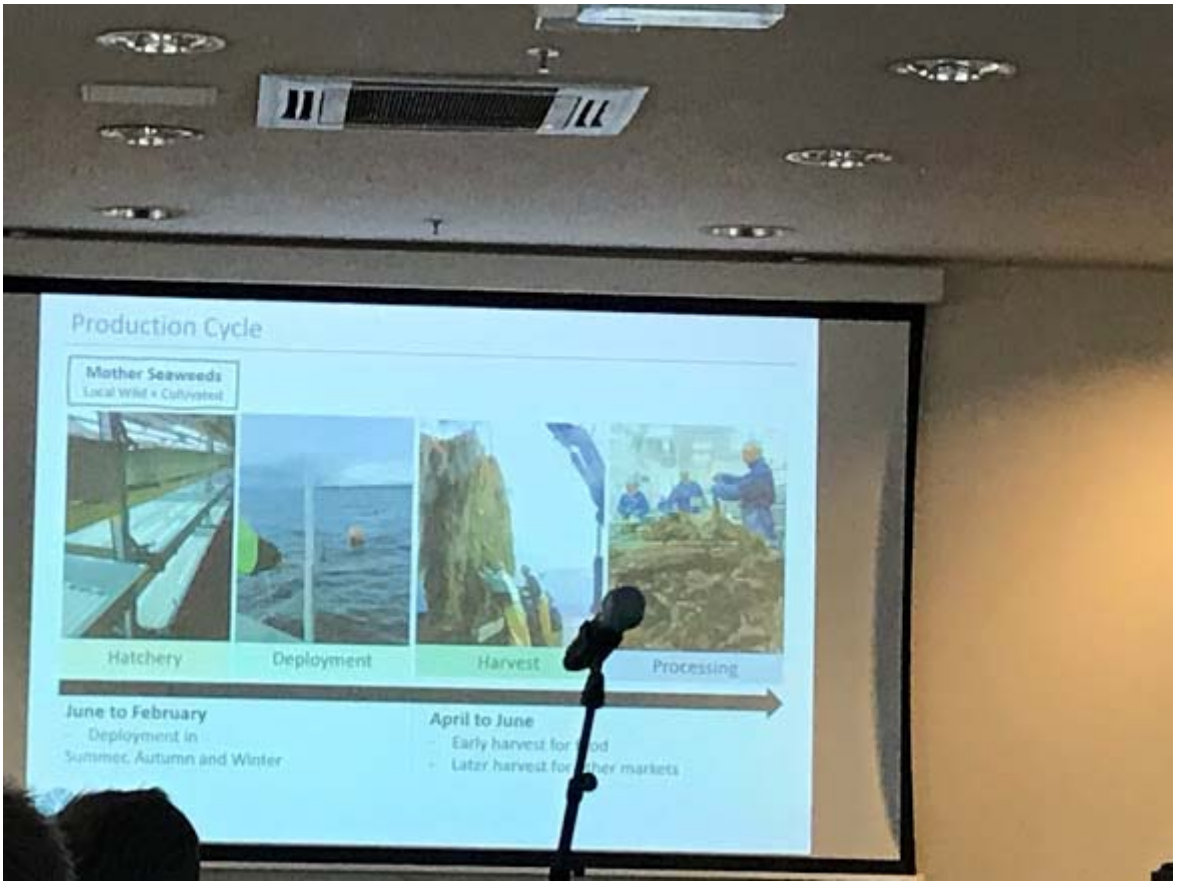
別是在挪威；挪威某一個典型之褐藻養殖場，其盈虧平衡產量為每年 200-500 公噸(取決原料銷售價格)。

褐藻對健康益處和高價化合物已經慢慢得到認可。海藻是碘的天然來源，在歐洲人缺乏碘一直是衛生部門關注的問題。海藻也是 50 種不同的礦物質和微量元素以及 Omega-3 脂肪酸的來源。因褐藻含有較多的谷氨酸(glutamate)，故可提食品的鮮味和增強的口味。

總體而言，海藻是一種高度永續且節能的食物資源，因不需使用淡水，也不需在海洋養殖場中施加化學肥料或化學品，更不會在陸地上留下任何跡象。因此，海藻作為食品成分的使用對環境有著巨大的影響。

海藻生長會吸收大量的營養物質（N，P）和 CO_2，因此可改善水質和增加生物多樣性等效益。海藻森林更是生物多樣性的增強劑，例如增加幼魚、甲殼類動物和其他魚類的庇護所和產期。

但如果持續大規模養殖海藻亦具有一些潛在威脅，例如育種和疾病所造成的基因退化。因此，目前已有許多研究專題正在探討此潛在風險，包括 GENIALG、MACROSEA、KELPPRO 和 SEABEST 等專案，並讓全球瞭解瞭藻種植的實際狀況和未來的前景。



(2)

主題：

Large-scale cultivation of seaweed in west Cork: An Irish success story (科克西部之大規模海藻養殖計畫：愛爾蘭之成功案例)

講者：

Silvia Blanco Gonzalex, Fiona Wanjiku Moejes, Julie Maguire

摘要：

海藻富含生物活性化合物(bioactive compounds)，包括抗氧化劑、可溶性膳食纖維、蛋白質、礦物質、維生素、植物化學物質和多元不飽和脂肪酸，海藻作為食物的潛力超出了日本料理的使用範圍。幾個世紀以來，海藻在愛爾蘭飲食中一直扮演著重要的角色，從果凍狀布丁中使用卡拉膠（*Chondrus crispus*），到在烘焙中使用紫紅藻（*Palmaria palmata*）。

全球有超過 95%的海藻來自海藻養殖場，中國生產大約 47%的海藻生物量。然而，歐洲海藻養殖產業仍處於起步階段，人工養殖之海藻產量<3%。儘管與全球市場相比規模較小，但歐洲擁有大西洋海岸線，不僅營養豐富，且水質清潔，其大西洋海岸線約有 3000 多種海藻，提供高庫存多樣性和高品質的生物量。

海藻可在單一養殖或整合水產養殖系統，其系統亦可在陸地上（槽池或跑道池）和海上（長繩結構）中生產。在歐洲，海帶（如 *Alaria*, *Saccharina*, *Laminaria* 等褐藻）的生

產遵循亞洲國家所使用的技術。生產技術基本原理，a.收集孢子體(sporophyte)、b.在陸地設施生產微型配子體(gametophyte)，並將受精卵(zygote)植種於繩索、c.培養受精卵發芽至特定尺寸後，再將繩索移植至開放水域、d.放置於開放水域之海上生長，直到收穫。

全球海藻生物經濟預估，至 2021 年，將迅速增加到約 176 億美元。愛爾蘭之《海洋變化策略》指出，2020 年海藻市場目標規模達到 3 000 萬歐元。然而，野生海藻便無法滿足需求，故必須轉向為人工養殖海藻。

位於愛爾蘭西南部的班特里海洋研究站有限公司（Bantry Marine Research Station Ltd, BMRS）是歐洲唯一擁有大規模海上海藻養殖場的機構之一。BMRS 以 6 公頃的場地為基礎，目前每年在班特里灣種植超過 10 噸（濕重）的翅藻(*Alaria esculenta*)。翅藻含有各種高價化合物，包括海藻黃質(fucoxanthin)和褐藻多酚(phlorotannins)。

依據 BMRS 之養殖試驗結果，發現最高翅藻收成量是在 2017 年 12 月 09 日放養，在 2018 年 5 月 28 日收成，每 1 米繩索之平均收成量為 12 公斤（濕重）。重量隨著時間的增加而增加，但葉片的長度在 5 月初可達到最大值（2.25 米），之後它們變得更寬，而不是更長。總體而言，第 2 年度的翅藻增長速度比第 1 年來得快，平均葉片更長。

而在海藻黃質之檢測分析結果方面，養殖三週後之翅藻比最後收成之含量還要來得高；此外，利用冷凍乾燥之樣品，其海藻黃質含量亦較烘箱乾燥之樣品來得高。

(3)

主題：

Perspectives for indoor seaweed (*Ulva ohnoi*) cultivation in photobioreactors (以光化反應器於室內培養海藻 *Ulva ohnoi*)

講者：

Erik Malta1, Leen Bastiaens, Kathy Elst, María del Mar Agraso Martínez

摘要：

以 75 公升之光化反應器(PBR)培養海藻(*Ulva ohnoi*)，以 f/2 作為營養源，光源為 LED 燈(150 μmol/m^2/s，16:8 L:D 週期)，初始藻種濃度為 60-70g/PBR，故溫度便為生長主要控制因子。

3 月至 7 月份之氣溫最高，平均約 16-24˚C。此週期之平均增長率為 10% d^{-1}（平均產量為 104g FW）。PBR-1）。以每支 PBR 放養 60~70 克的初始濃度，可獲得最佳生物量產量。生長主要是由室溫決定的，在最冷的月份時，其生長會受到限制。

(4)

主題：

Initial uptake capacity of ammonium in *Saccharina latissima* in an IMTA context (在 IMTA 實場環境下其糖海帶 *Saccharina latissima* 之銨攝取能力)

講者：

Erik Malta1, Leen Bastiaens, Kathy Elst, María del Mar Agraso Martínez

摘要：

挪威是世界領先的大西洋鮭魚生產國（*Saomo salar*），具挪威政府的長期目標，是希望在 2050 年將目前的產量提高四倍。然而，鮭魚生產所產生的廢棄物，已經對環境造成影響，因此需要更加注重生產的環境可持續性，包括改善資源和能源利用率。

鮭魚飼料之總氮，約有 39%會溶解至海水中，其中又以溶解性無機鹽氮（dissolved inorganic nitrogen, DIN）和銨（NH^+）為主。這多研究推估，糖海帶(*Saccharina latissimi*) 可從鮭魚養殖場之排放物中去除 5-40%DIN。

研究結果發現，*S. latissima* 可快速適應不同濃度的 NH^+，且調整並增加其攝取率。如圖 1 所示，在 50 分鐘內觀察到最大攝取率。而且，NH^+的攝取量都會隨著濃度的增加而呈現線性增加。*S. latissima* 的營養史似乎會影響其攝取量，因為氮耗盡的實驗組中，發現 NH^+的攝取速度明顯快於氮飽和控制組。

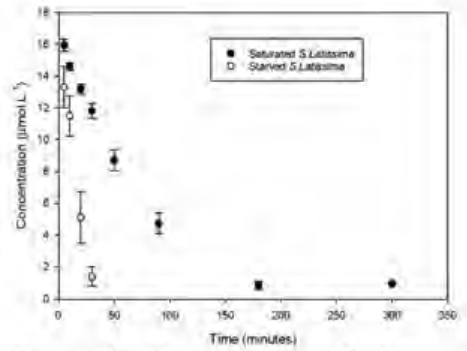


Figure 1: Time course of decrease in ammonium concentration (initially16µmol L^{-1}) in culture medium with juvenile plants of *Saccharina Latissima* for nitrate saturated and depleted plants (n=5±SD)

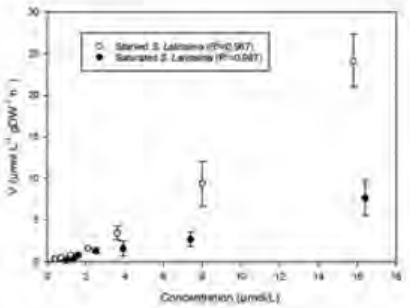


Figure 2: Ammonium uptake rates for *Saccharina Latissima* from 5 to 50 minutes related to available concentration for both nitrate saturated and depleted plants (n=5±SE, linear regression with R^2=0,987 for depleted and saturated).

(5)

主題：

Optimization of antioxidant activity in sea vegetables grown in recirculating aquaculture systems (RAS) (循環養殖系統培養海洋蔬菜之抗氧化能力最佳化探討)

講者：

Sofiia Tretiak, Jakop Schwoerbel1, Ramona Bosse, Frederike Reimold, Ina Enders, Dietmar Hoffmann, Joachim Henjes, Bela Buck, Laurie C. Hofmann

摘要：

此研究探討日長(day length)和光強度對於石蓴(Ulva sp.)生長速率、氧氣生產量和自由基清除活性之影響。此外，同時也探討鹽度、乾燥脫水、日光劑量、和紫外光等因子對於龍鬚菜(*Gracilaria vermiculophylla*)生長速率和自由基清除活性之影響。

研究結果發現，最佳抗氧化活性的條件不一定最適合生長或光合作用。例如高鹽度與脫水程度，會降低龍鬚菜之生長速率和光化系統 II 之量子效率，但抗氧化活性最高（圖 1）。

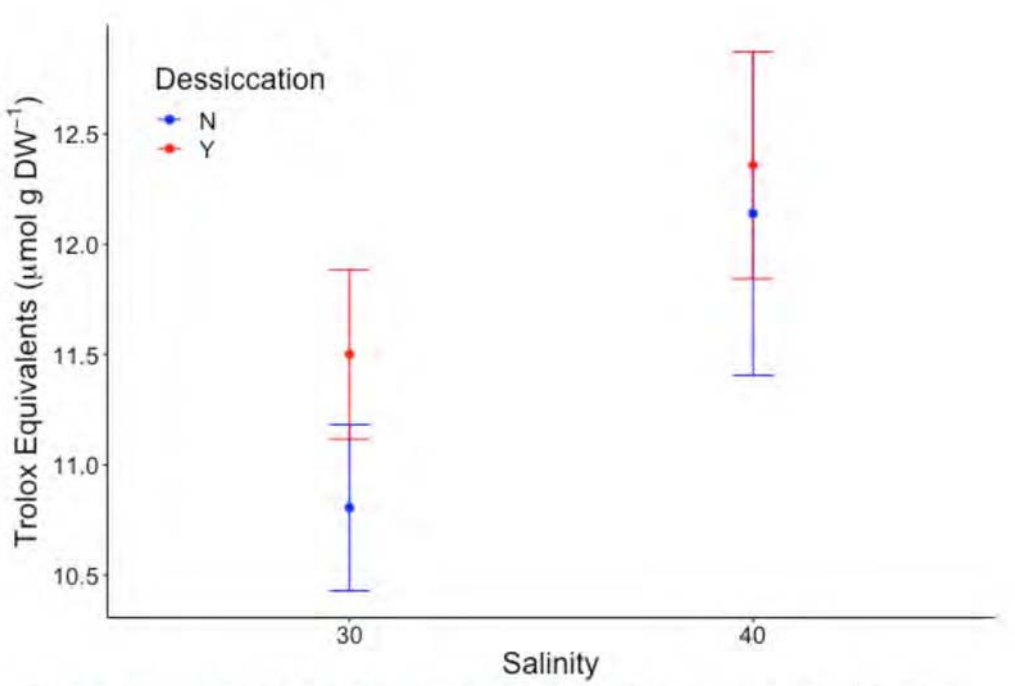


Figure 1. The mean (±SE) radical scavenging activity (as Trolox equivalents) of *Gracilaria vermiculophylla* under different salinity conditions with (Y) and without (N) exposure to desiccation.

(6)

主題：

European seaweed value chains in an international perspective (以國際觀點探討歐盟海藻價值鏈)

講者：

Sander van den Burg, Maggie Skirtun, Miriam Bernard

摘要：

目前，全球海藻產業以亞洲國家為主，中國和印尼是主要原料生產國。近年來，加工設施大多集中在中國，故印尼和智利生產的海藻大部分都以原料型式出口到中國進行進一步加工。歐洲地區，海藻大都是依賴野生捕撈，特別是在法國、愛爾蘭和挪威。杜邦(Dupont)、阿爾蓋亞(Algaia)和 FMC 等公司使用收穫的海藻生物量生產海藻酸鹽(alginates)。

近年來之研究證明，在歐洲各海域進行海藻養殖是可行的，且海藻養殖被認為是未來農業生產的重要來源。初創企業和老牌公司亦開始開發新的生產系統和機械，以便進行大規模生產和收穫，並探索新的消費品、飼料和非食品應用。對海藻的關注也反映在政策中，如荷蘭阿姆斯特丹氣候協議 (Klimaatakkoord)草案和 Noordzee 2030 草案，以及歐洲各國政府通過研究專案方式，提供資金給予研究和擴大規模生產，例如 GENIALG（www.genialgproject.eu）和 ProSeaweed（www.proseaweed.eu）。

從經濟和價值鏈的觀點，一個不可避免的問題即是，剛起步的歐洲海藻養殖部門，如何與全球主要生產者(亞洲國家)相互競爭。歐洲大規模的海藻生產，勢必要進入全球海藻供應鏈，然而其生產成本和高端產品市場銷售亦是需注意的地方。

歐洲部門目前可專注於利基市場，開發新的高價值產品和新市場。開發海藻的新標準，例如永續性和產品品質，可有別於"全球"海藻。
對分別位於荷蘭和中國的 SWOT 分析，突出了中國在海藻養殖方面的長期經驗，以及生產和加工基礎設施的可利用性。歐洲和中國海藻產業似乎相距甚遠，它們面臨著自身未來的挑戰。然而，歐洲海藻產業需克服的這些挑戰，似乎不是那麼困難。歐洲海藻產業還很年輕，且處於早期發展階段，因此擴大規模可採用高品質播種材料、降低成本的機械化和公眾的積極看法。為了實現後者，並滿足消費者和零售商的需求，該部門需要以高度的透明度證明其生產過程對環境具有正面效益。另一方面，近年中國海藻產業幾乎沒有創新作法，仍依賴勞力密集方式生產，且對於永續性和環境影響仍是一大隱憂。
根據初步分析，中國與歐洲海藻部門之間有很大的合作空間，特別是在發展永續育種、生產和捕撈方法方面。共同關注的問題是通過技術進步、改進生產過程，需要更高品質的苗木和價值鏈的透明度。

二、Climate Change

(1)

主題：

Forecasting the impact of future climate scenarios on cultured mussels growth in the Galician Rías (NW SPAIN) (預測未來氣候情境對於西班牙加利西亞下海灣區之養殖貽貝生長影響)

講者：

Isabel Fuentes-Santos, Xosé Antón Álvarez-Salgado1, Uxío Labarta1, M José Fernández-Ririz, Susan Kay, Solfrid Sætre Hjøllo

摘要：

西班牙西北地區主要養殖貝類養殖，因此當地養殖戶對未來氣候情景對生產系統的影響感到關切。ClimeFish 是一個持續進行的 H2020 專案，致力於研究氣候變化對整個歐洲淡海水養殖產業的影響。此篇研究的目的是預測未來氣候情景對加利西亞地區貝類生長的影響。
此篇研究使用 Fuentes-Santos 等人（2019 年）在觀測（2006-2015 年）下提出的動態模型類比了解貝類殼和軟組織生長，並預測了 RCP4.5 和 RCP8.5 排放情景下的氣候條件（2016-2055 年以十進位間隔計算）。
SST 預測因氣候模型而異。SST 與 POLCOMS-ESM 之預測結果得知，其目標年(2046-2055 年)之年平均氣溫幾乎沒有增加；然而，根據 CSIRO 預測結果，其目標年上升幅度約為 1℃。在 RCP8.5 情境下，在目標年逐漸增加到 1.5-2℃。MPI-ESM 預測預計夏季太陽輻照度將下降，春季和秋季的日照率將明顯增加。
圖 1 強調養殖播種時間對貝類生長的重要性。與夏季和秋季播種的貝類相比，2 月份播種的貝類達到目標重量的時間更少，而 6 月份播種的幼苗的培養長度最大。圖 2 顯示，在 POLCOMS 條件下，培養長度應略有增加，但隨著 CSIRO 和 NorESM 預測，培養長度應減小。然而，與播種時程表相比，氣候變化對貝類生長的影響很小。

此篇研究結果亦說明，氣候變化幾乎不會影響未來 30 年加利西亞的貝類生長。然而，對氣候變化對貝類水產養殖的影響進行適當分析需要解決其他因素，如種苗供應和有害藻華現象，這些因素決定了播種和收穫計畫。

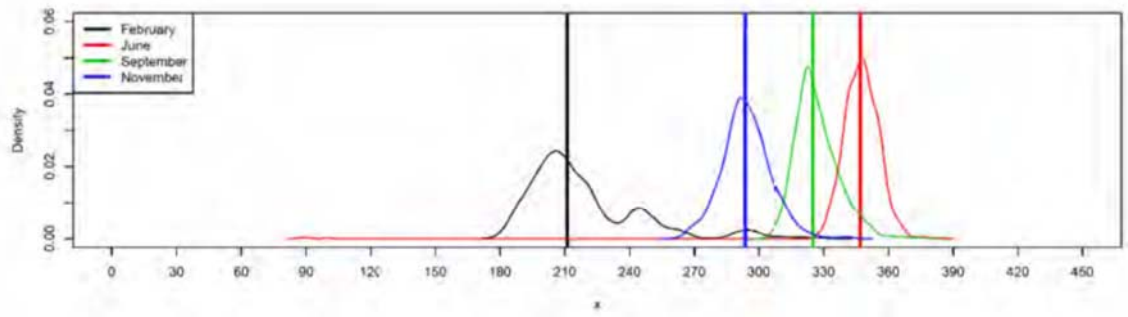


Fig. 1 Time (days) to reach the optimal flesh weight (8g) under observed environmental conditions (2006-2015) in the Ría de Ares-Betanzos. Vertical lines indicate the median.

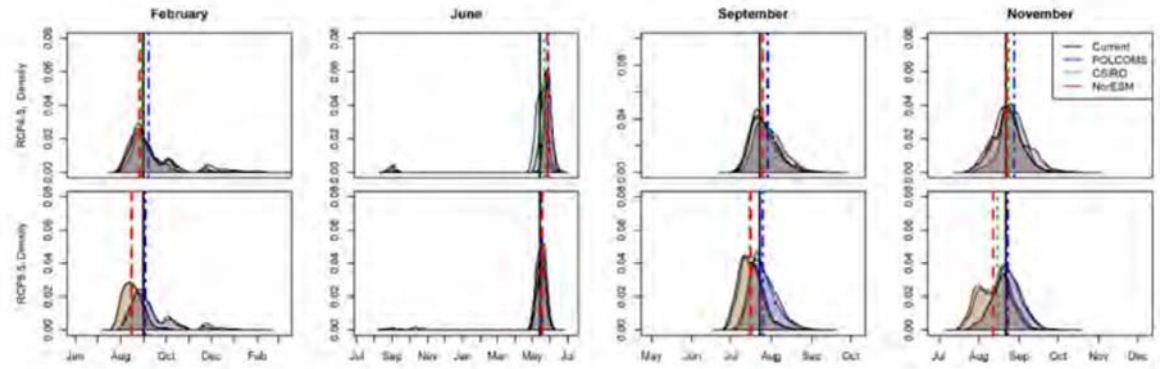


Fig. 2: Density distribution of the harvesting dates for the target size (8g flesh weight) under current (black) and RCP4.5 (top) and RCP8.5 (bottom) conditions for the three climate models in 2046-2055. Vertical lines identify the median of each distribution.

(2)

主題：

Modelling the impacts of climate change on shellfish aquaculture (模擬氣候變遷對於貝類養殖之影響)

講者：

A.M. Cubillo1, J.G. Ferreira, J. Lencart e Silva1, C.M. Kreiss, P. Kamermans, C. Saurel, J. Icely, B. Fragoso, S. Kay

摘要：

此研究建立一種模擬方法，以分析氣候變化對具有商業利益的養殖貝類物種的潛在直接（溫度和糧食供應）和間接（病原體）影響。

針對四種貝類和位置：北海的太平洋牡蠣和藍貝（荷蘭）、北海的藍貝（丹麥）和歐洲大西洋西南部海岸（葡萄牙）的地中海貝類。探討的重點在於貝類生產（生長和死亡率）影響、水質相關的環境外部因素變化、以及後端的社會經濟影響。
支援模擬工作包括：（1）多應力實驗室試驗（溫度 x 食物濃度 x 氧飽和度），主要在參數化氣候變化對雙殼體生長的直接影響;（2）降尺度之物理和生物地球化學模擬，主要利用幾個 IPCC 情境預測未來氣候變遷。
此研究亦使 FARM 模式，探討氣候變遷的直接影響，例如溫度變化對於貝類養殖之影響；另使用 ABC 模式，評估氣候變遷中宿主和病原體相互作用的影響。
根據目前氣候情況，再與 21 世紀中葉（2040-2060 年）和本世紀末（2080-2100 年）的情景進行比較。針對每一種情況，此研究都考慮的 2 種情境：（1）中度情況（IPCC RCP 4.5）;（二）極端情況（IPCC RCP 8.5）。
此研究所介紹的建模工具，主要是期望提高水產養殖部門對氣候變化潛在影響的認識，並希望水產養殖產業能預先做出適當反應，這些調適作法包括開發預警方法、創新養殖模式、基礎設施、位置選擇和商業市場。

(3)

主題：

PAN-European modelling of pacific oyster (*Crassostrea gigas*) growth in the offshore environment: Indicators, regional comparison & climate change (歐洲太平洋牡蠣在近海環境生長之模擬：指標、區域差異和氣候變遷)

講者：

Stephanie Palmer, Laurent Barillé, Pierre Gernez, Susan Kay, Stefano Ciavatta, Yoann Thomas, Philippe Glize

摘要：

海產需求逐漸增加和野生漁業捕撈量逐漸下降，因此海水養殖產業呈現上升趨勢。然而，在傳統上進行養殖的潮間帶地區，擴大貝類養殖範圍是有限的，故擴大在近海生產是一個解決辦法。因此，此研究在於探討太平洋牡蠣（*Crassostrea gigas*）養殖於歐洲和非洲西北部近海環境之生物永續性，以及評估未來氣候變遷對於太平洋牡蠣生長可能之衝擊。
透過牡蠣生長資料，此研究建立幾項產業相關指標，包括養殖至市場需求規格最少之時間、主要市場所需之規格大小和品質指標，基本市場分類為"特殊"、"良好"或"一般"。C.gigas 養殖區域需考是海水鹽度、洋流速度、溫度、和養殖區域之葉綠素範圍，此研究亦考慮兩個深度範圍：<25 公尺深度(適合小規模養殖業者)和<200 公尺深度(可供更大的規模作業投資能力業者)。
再將本世紀初期和末期之氣候預測情景之日增長圖譜轉換為指標圖（如圖 1），因此可在此區域發現了幾個"熱點"，供更詳細地研究。某些區域具有較高生長性，但空間區域較小；而有些區域，在世紀初的參考時期，具有較高生長性，但卻會受到氣候變遷的不良影響。

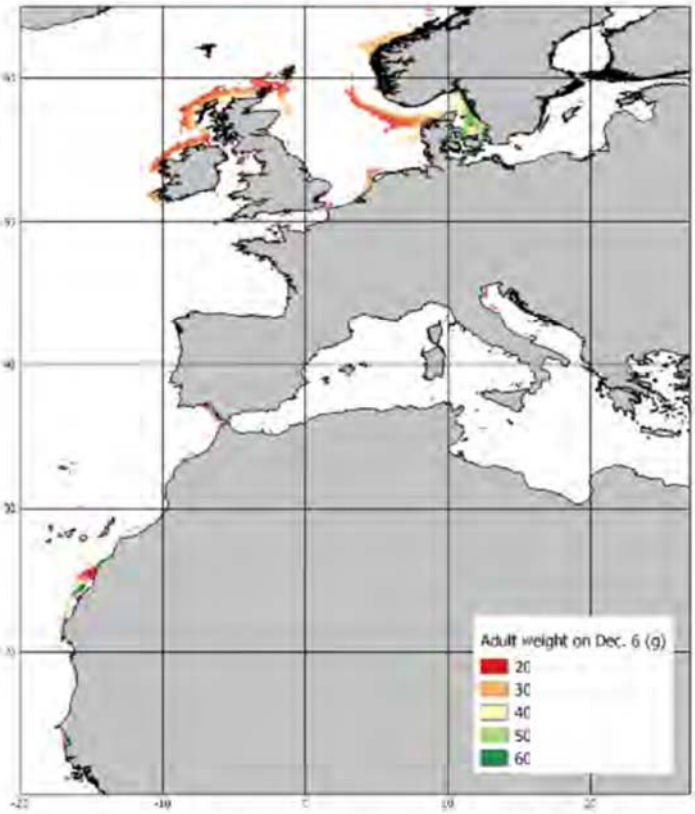


Figure 1. The model domain extent and an example of a mapped Pacific oyster growth indicator, adult weight in time for the December market, for the early-century (2000-04) reference period. Indicator maps and raw modelled growth data are also available for the two future scenarios (2090-99; RCP 4.5 and 8.5).

三、Sustainable aquaculture technologies

(1)

主題：

Tools for new and flexible approaches for aquaculture licensing and regulation (新穎且具彈性之許可和規範管理方法工具)

講者：

P. O'Donohoe*, F. Kane, J. Casserly and S. McLoughlin

摘要：

The H2020 project TAPAS (Tools for Assessment of Planning and Aquaculture Sustainability, http://tapas-h2020.eu/)，主要目的在於協助歐盟會員國能建立一套一致且有效地規範架構，以利歐洲水產養殖部門可以永續成長。因此，此篇研究在於建立一套新的策略和模式，以利水產養殖產業可永續發展。

透過協商和訪談，了解許可證制度存在的關鍵問題和瓶頸。根據收集的資料，並透過提出新的方法和創造新的工具，以改善管理和許可證制度的效率。在與利害關係人和產業界持續協商期間，對靈活的方法和工具進行實地探討，以確保這些方法的可接受性和實用性。

此研究簡要概述利害關係人投入所產出之結果，並就建立新的、靈活的水產養殖許可證和管制政策的方法和工具提出了結論性建議。

(2)

主題：

Farming warmwater fish on land in a cold country – A crazy idea or a new sustainable seafood supply chain? (在寒帶國家養殖溫水魚種：瘋狂想法，還是新的永續海產供應鏈？)

講者：

Kristina Bergman, Patrik Henriksson, Sara Hornborg, Max Troell, Malin Jonell, Friederike Ziegler

摘要：

陸地養殖溫水物種，例如吳郭魚(*Oreochromis niloricus*)、尖齒鬍鯰(*Clarias gariepinus*)、白蝦(*P. vannamei*)，構成一項瑞典的新興食物部門。

幾家小型但發展迅速的公司，自認為比傳統水產養殖更具永續性，這幾家小型公司開始在循環養殖系統（RAS）生產海鮮，其海產作為鮭魚（吳郭魚）、金槍魚（新鮮克鯰魚）、鰻魚（煙燻鯰魚）和進口蝦（白蝦）的替代品，以向有環保意識的瑞典消費者銷售。

此篇研究使用生命週期評估（LCA）方法，分析其中一家公司海鮮生產的環境壓力。LCA 廣泛應用於產品供應鏈之環境衝擊特徵化和確認改善項目，並且用於評估多項水產養殖生產系統。

此研究在瑞典養殖的吳郭魚和尖齒鬍鯰，並評估其產品供應鏈之環境壓力（如溫室氣體排放和優養化）的結果，以確定環境熱點和改進方案。

結果發現，飼料是最重要的因素，另外還有高度密養殖系統之基礎設施和維護工作。包含高密集資源之飼料成分，如動物副產品，也大大增加了環境足跡。

Landbased tilapia and clarias farm in southern Sweden.
Photo: Gårdsfisk

(3)

主題：

Ecointensification of aquaculture: innovative mortality disposal in a circular economy perspective (水產養殖生態強化：循環經濟觀點之創新死亡率處理)

講者：

Hallstein Baarset and Johan Johansen

摘要：

高密度水產養殖的主要影響包括死亡和廢棄魚類。統計資料顯示，箱網養殖之死魚損失約為 15-20%，相當於生物量的 6-9%。挪威水產養殖業者每年需耗費超過 1 億歐元之成本。

GAIN (Green Aquaculture Intensification in Europe 歐洲綠色水產養殖強化)是最近獲獎的 Horizon 2020 專案，並期望能在專案執行期間向市場提供服務和技術，為歐洲水產養殖生產的生態強化做出貢獻。資源效率、環境衝擊、和整體生產鏈的精度與價值等皆是提高海產自我生產熟練程度和區域穩定性的關鍵要素.

目前挪威水產養殖死亡率的處置方法面臨健康、安全和環境方面的挑戰，而且處理費用高昂。因此，Waister 公司評估一種現場乾燥和乾燥消毒產品運輸的死亡處理創新方法。

根據循環經濟，Waister 公司將依據歐盟和挪威的政府法規對此乾燥產品進行必要的評估。但通過乾燥和消毒產品的安全應用驗證，可能會出現進一步的應用方式，但皆需要對現行法規進行修改或修訂。

Waister 公司在挪威大西洋鮭魚生產商建立了 GAIN 示範設施，用於工業生產規模相關基材的評估、記錄和專業培訓。此示範設設為一種魚體的乾燥系統，用於處理幼鮭生產的魚體。

Waister 公司將再進一步比較現行方法和創新方法，並發現於符合政府規定下，其乾燥產品可能增加的價值及效益。

四、Value addition and marketing

(1)

主題：

Novel banana by-products in seabass (*Dicentrarchus labrax*) diets (添加香蕉副產物於海鱸魚飼料之探討)

講者：

S. Ramírez-Bolaños, S. Díaz, N. Díaz-Padilla, A. Ventura-Castellano, R. Quirós-Pozo, L. Robaina

摘要：

加那利群島(Canary Islands)的香蕉是一個非常穩定的產業，每年約可生產 4 億噸香蕉，大約會產生 10%生質殘留物，且多數會直接堆置於香蕉園中，而造成污染的問題。Life BAQUA（LIFE 15/ENV/ES/000157）發現可從香蕉殘留物（banana pseudostem）中提取纖維，此為一項可生物降解材料，可用於包裝材料。

在香蕉纖維提取過程中，會產生一個重要的副產品：紙漿。此外，香蕉作物還產生其他重要的殘留物，如香蕉花。亦有研究表示，香蕉生質殘餘物含有大量的抗氧化劑，亦可開發作人類或動物產品。
將香蕉漿（BT）和香蕉花（BF）等兩種香蕉殘餘物添加於海鱸魚飼料中，並以等蛋白和等脂肪方式添加，以測試不同添加比例對於海鱸魚的生長情。除了生化成分、脂肪酸、消化和肝臟組織學外，亦於試驗結束時，採集生長和性能參數。
試驗結果發現，以 BT4 (表添加 4%之香蕉漿)飼料養殖 2 個月後之魚重，可較控制組之魚重增加約 23.38±1.56 克；而餵養 BF6 (表添加 6%之香蕉花)之最後魚重量約可增加 25.58±0.89 克。

(2)

主題：

Fish quality: a psychological perspective (魚肉品質：心理學的觀點)

講者：

J. Simons, C. Vierboom, M. Ley, and S.-A. Johnigk

摘要：

由已開發國家的魚類和海產購買行為文獻回顧中，了解感官、健康關注、飲食習慣、便利性、自行處理程序、價格和魚類供應等為魚類消費數量之主要決定因素。此外，產品屬性，例如原產國、生產方法、保存方法、產品創新、包裝和生態標籤，亦會影響不同產品的選擇。除購買行為外，還有永續性和消費者願付價格(willing to pay, WTP)等，亦會影響購買意願。
此研究利用形態心理學的理論框架，探討特定產品之影響。此方法是以產品為導向，確定產品滿足心理需求或動機的能力。在定性訪談和小組討論中收集對產品影響的見解，參與者在每天的直播中描述產品的含義。由於定性方法具有解釋性，因此容易產生偏差，因此透過專業心理學家團隊進行研究，以提高結果的有效性。此外，市場資料提供額外的見解，並說明減少偏頗的解釋。
2017 年 11 月和 12 月在德國進行了 10 次深入訪談和 3 次小組討論，每次有 8 至 10 人參加。鑒於產品的心理影響取決於文化環境，成果不能簡單地轉移到其他國家。
在德國，魚和魚產品的具體心理特徵因消費水準低而得到增強，每年約為 14 公斤/人，而肉類和肉製品的消費量約為 90 公斤/人/年。
魚同時具有迷人的和威脅性。此外，魚可以很容易地與假期和冒險的記憶相關聯。魚的"明亮"面與"黑暗"面形成鮮明對比。腐敗且臭掉的魚容易與中毒危險連想在一起，且會破壞魚類迷人的形象和對魚的胃口。而且，魚類可能生長的污染環境，故以魚類作為食物也會引起人類對於食魚的負面想法。魚骨也是另一個問題，可能在準備、料理和進食需要小心。訪談參與者通常會在餐廳吃新鮮的魚，因此不需要靠自己處理魚。然而，新鮮魚類僅占德國魚類總消費量的 10%左右。
魚的新鮮程度同時也是一道屏障，故高價市場額外售販已處理好的魚類產品，提供消費者選擇。

(3)

主題：

Towards improved communication in aquaculture: exploring consumers' perceptions and attitudes (改養水產養殖的溝通：探討消費者的看法與態度)

講者：

Sofia C. Franco, Sharron Kuznesof, Bruna Simões, Beth Clark and Peter Jackson

摘要：

民眾的觀念被認為是水產養殖擴張的制約因素之一，因為此觀念不僅意味著海鮮採購和消費態度，而且意味著民眾對該產業的支持與社會的認可。然而，要建立社會認可，必須先瞭解觀念、態度和基本因素，再用予改善企業、第三部門和監管機構的運作方式和與公眾互動方式。

雖然有一些研究著重於分析消費者的知識和消費習慣、對養殖或野生捕獲海產的偏好、關鍵議題的相關性、歐洲媒體報導的趨勢；然而，目前民眾對水產養殖的看法和相關的態度，例如支持或反對的看法，其研究文獻仍然很少，水產養殖產業的社會認可相關研究也是最近才開始在文獻中出現。因此，在目前有限的證據下，仍較無法去告知民眾，提升接受度或增加消費的有效策略；另外，對於應向公眾或社區傳達哪些內容以及這種參與應如何進行宣傳，存在高度不確定性。在其他角色方面，例如企業社會責任或許可證，在塑造水產養殖形象方面的作用也仍然缺乏探索。

對歐洲水產養殖的看法和態度進行系統文獻回顧，並將文獻回顧結果告知重點小組。重點小組旨在調查海產的採購和消費行為，以及公眾對水產養殖生產和產品的看法和態度；包括不同人群的英國消費者（低頻、高頻）、非消費者（一般、素食）和鄰近經營水產養殖設施的社區（年齡較大、年齡較小的社區）等共進行 6 次重點調查（每次 6-8 人）。

還與英國不同利害關係人（生產商、加工商、零售商、第三部門和監管機構;每組 3-4 人）進行一系列深入訪談，以確定民眾感知問題、溝通挑戰和途徑，以提高參與度。這項研究旨在支援制定最佳傳播作法，改善民眾對水產養殖部門的參與，以及永續的海產生產和消費。

五、Hatchery technologies and practices

(1)

主題：

Improving microdiet technology for first-feeding gilthead seabream (*Sparus aurata*) larvae (改善微粒飼料技術於第一次餵食金頭鯛)

講者：

Wilson Pinto, Sofia Engrola, Bruno Nunes, Rita Colen, André Santos, André Lopes and Luís E.C. Conceição

摘要：

金頭鯛（*Sparus aurata*）幼苗餵食方法通常是在孵化後的頭 30 天內餵食活獵物，並在第二個月完全改為餵食商業微粒飼料。儘管具有挑戰性，但開發一種在第一次餵食時提供的微粒飼料對孵化場極為有利，儘管在這個階段幼苗已經降低獵物和消化能力。

由於微粒飼料往往比活體動物接受程度低、消化性差，特別是在複雜蛋白質方面，因此在製作微粒飼料時必須考慮這些限制。

例如，透過在微粒飼料配方中加入蛋白質水解酸鹽，增強其吸引力並導引幼苗消化道進一步成熟，可以減輕這些因素。首次餵食海帶的微粒需要由小顆粒（約 100 μm）組成，表面與體積比高，導致蛋白質水解到養殖水體，致使溶出過量氮氧化物。微封裝提供了控制這些水溶性營養素溶出的可能性，提高微粒飼料攝食的營養價值。這種控制對於在第一餵食的海帶中成功導入微粒飼料似乎至關重要。

此研究目的在於開發一種微封裝原型，以減少微粒飼料中常用的一種蛋白質水解液的溶出。該原型隨後被添加到商業微粒飼料中，添加比例為 8.5 和 30%，從孵化至孵化後 34 日皆餵食此添加微封裝原型之商業飼料，並測試金頭鯛生長速率和存活率。

在海水中浸泡兩小時後，新型微封裝原型只釋放出 25%的水溶性蛋白質含量，比參考曲線（同期溶出 90%）中獲得的值低 65%。

在孵化後的 12 日，在消化道中用微粒飼料觀察到的幼苗數量比餵食活獵物的多。在這個年齡，在 CAP8.5 和 CAP30 中觀察到的幼苗體重明顯高於對照治療（圖 1）。在孵化後之 34 日，來自 CAP8.5 的幼苗比 CAP30 的幼苗大得多。前處理和對照方法之間沒有顯著差異。幼苗存活率不受微飲食的影響（約為所有實驗之 4-7%）。

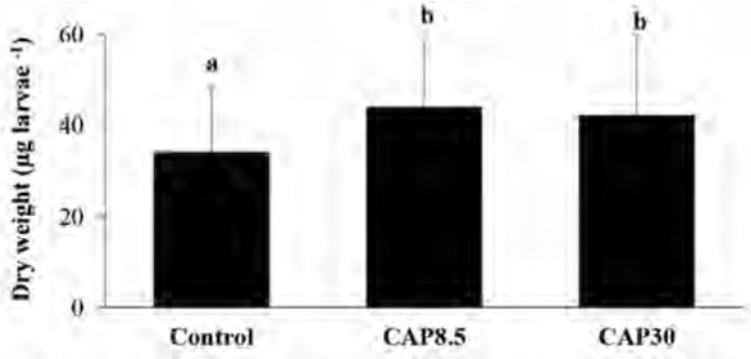


Fig. 1. Dry weight of 12 day-old seabream reared under different dietary treatments. Results are expressed as means ± standard deviation ($n = 60$).

(2)

主題：

Salinity reduction benefits european eel larvae: a review (鹽度降低對歐洲鰻魚幼苗之影響：綜合討論)

講者：

S.N. Politis, E. Syropoulou, E. Benini, S.R. Sørensen and J. Tomkiewicz

摘要：

歐洲鰻魚（*Anguilla anguilla*）是一種廣鹽性的魚種，會為繁殖(產卵)而的遷移易海中，導致歐洲鰻魚幼苗可自然存活於低滲透環境中。在此環境中，血漿滲透率必須低於環境，故歐洲鰻魚幼苗需要通過脫鹽過程以保持滲透平衡，避免滲透水損失。有趣的是，在水產養殖可透過降低鹽度來促進更佳之生長速率和存活率。因此，此研究探討少鹽度對圈養生產歐洲鰻魚幼苗的影響，以評估最佳條件飼養歐洲鰻幼苗。

在所有實驗中，當鹽度保持在 36 psu (practical salinity unit)（對照組）時，幼苗死亡率最高，這與藻海(Sargasso Sea)產卵區的鹽度相似。先前之研究結果顯示，越早和更快的將鹽度降低，其幼苗存活率越高。
降低鹽度試驗中，特別是在 0 dph (days post hatch, 孵化後日數)和 4 psu （最快降低）試驗，可降低死亡率、增加魚體面積、減少油滴利用率，提高生長效率。然而，在 3 dph 才開始減少鹽度，可能導致更高的死亡/畸形比例。因此，當鹽度緩慢降低，使得幼苗和 RAS 生物濾膜可緩慢的適應鹽度降低，故幼苗存活率增加至 50%。此外，當鹽度迅速減少時，幼苗存活率同樣得到改善，此結果與先前實驗結果一致。考量孵化場生產，這是一種更實在、更經濟的高效方法 - 減少幼苗生產對 RAS 的需求。因此，快速減少鹽度的方法可以應用於既有 RAS，且僅需設定鹽度步驟，並分別確保每個系統的水質穩定性和生物濾液的微生物平衡。
此外，劇烈之鹽度變化，其幼苗存活率與對照組實驗相似，因為它們在 36 psu 時也飼養到第 6 天；但令人驚訝的是，幼苗急劇地移動到 50%鹽度，竟能夠快速適應極端的生理變化。因此，在哪個階段或年齡應用這種急劇的變化，以抵消在前 6 dph 期間觀察到的高死亡率是後續應持續探討之問題。
此結果亦證明，可以應用大幅度降低鹽度，應用此種最經濟的鹽度降低方法，便可使幼苗產量增加 50%，而且只需要 2 個 RAS 系統控制鹽度即可。

(3)

主題：

Impact of water purification and conditioning on in vitro produced embryos and larvae of european eel (*Anguilla anguilla*) (水質純化和調理對歐洲鰻魚之外胚胎和幼苗影響)

講者：

S.R. Sørensen, E. Syropoulou, S.N. Politis, Benini, E., Hambly A.C., Tomkiewicz J.

摘要：

2012 年，水產養殖魚類產量首次超過漁業產量，目前是世界上增長最快的糧食生產部門。然而，集約式魚類養殖生產容易爆發疾病，其中以致病菌為主要原因，又以幼苗階段更容易受到集約式水產養殖的感染。
幼苗在集約化水產養殖中暴露於來自外部和內部來源的大量微生物，但其相對重要性取決於所使用的飼養技術。通過優化底層基礎、營養鹽、過濾和水力停留時間等操作條件，這可能有助於支援更有利的微生物群落，從而提高循環水產養殖系統的穩定性和養殖成功率。

研究成果得知，通過微生物處理來調節循環水養殖系統之水質，可有效提升養殖環境的穩定性，增加歐洲鰻魚的胚胎飼養成功率。

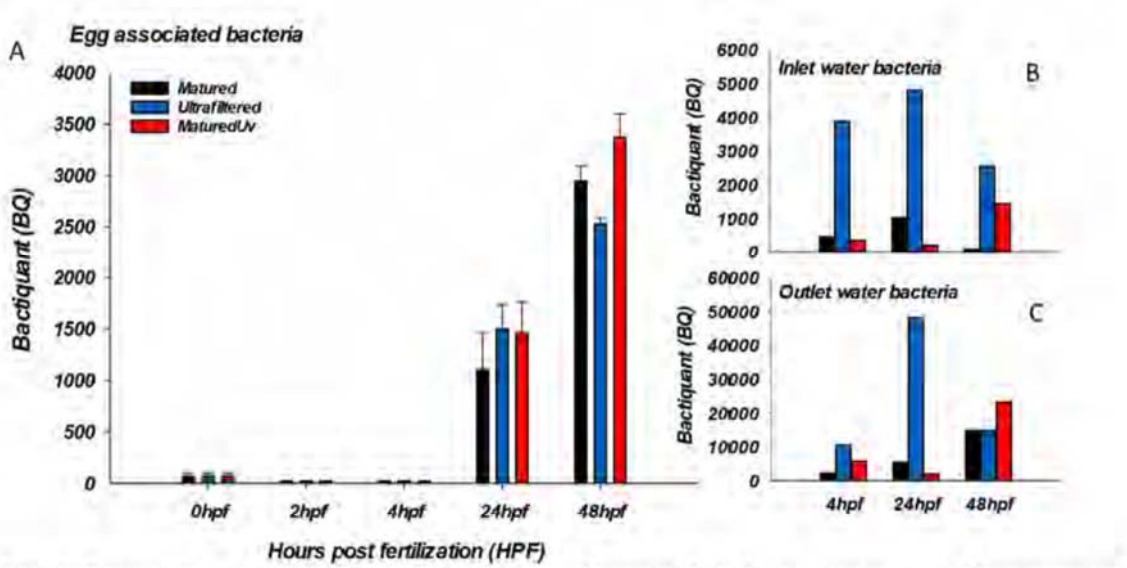


Fig. 1: Bacterial activity (Bactiquant) on incubated eggs and in the rearing water of European eel eggs reared using Matured (MSW), Ultra filtered (UFSW), and Matured UV treated water (UVSW). A: Egg-associated bacteria colonizing the egg of European eel between 0 h post fertilization (HPF) to 48 HPF. B: Bacteria levels of water inlet to the egg incubator. C: Bacteria levels of water and outlet of the egg incubator.

六、Shrimp

(1)

主題：

Improving automatic feeding protocols in semi-intensive pond culture of pacific white shrimp (*Litopenaeus vannamei*) (改善白蝦半集約式養殖之自動化飼料餵食協定)

講者：

João Reis, Melanie Rhodes, D. Allen Davis

摘要：

白蝦養殖的成功取決於改進飼料管理和降低工作力成本。蝦是食用多餐性的食肉動物，因為儲存食物的胃容量有限，故透過餵食多餐方式可提高白蝦養殖成效。先前研究成果發現，由每天兩次餵食轉變為多次餵食系統可提高白蝦生長率和生產量。

以先前研究成果為基礎，此研究目標是為自動餵養系統開發標準餵養協定（standard feeding protocol, SFP），以提高太平洋白蝦(*Litopenaeus vannamei*)半集約式漁塭生產中的增長率。

在第一次試驗中，在 16 個 0.1 公頃的戶外池塘進行 13 周的試驗，以 26 尾蝦/m^2 放養，並餵食 35%的蛋白質大豆優化飼料。四種餵食方式：使用自動計時器提供三種固定餵養處理，分別為 SFP130、SFP145、SFP160 提供 130、145 和 160% 的固定餵養處理，以及第四種餵食方式是使用聲學饋送系統（AQ1）。

試驗結果表示。在不同餵食方法之存活率和 FCR (feed conversion ratio, 飼料轉換率)並無明顯之統計差異。然而，增加飼料餵食量可使產量提高，又以 AQ1 聲學餵養系統之產量最高，因為 AQ1 方法投入更多的飼料量，蝦子可獲得更多的飼料量，故生產之蝦子更大隻，產量也更高。

(2)

主題：

Growth performance and survival of whiteleg shrimp *Penaeus vannamei* under varying stocking densities (不同放養密度之白蝦生長狀況和存活率)

講者：

A. Segelken-Voigt and R. Bochert

摘要：

白蝦(Penaeus vannamei)是全球養殖最多的甲殼類動物之一，不僅可戶外之漁塭養殖，也可在室內使用清水循環水養殖系統（RAS）養殖。然而，水質和放養密度是白蝦養殖最重要管理參數。

此研究探討不同白蝦放養密度，白蝦苗為第 12 天之後期蝦苗（postlarvae, PL 12）和約 2.5g 幼苗重量，評估養殖 2 週和 4 週後之生產成效。以鹽度為 20 ppt 之海水進行個別養殖試驗，每個試驗皆進行三重覆，循環水養殖系統體積為 100 L。 PL 12 放養密度分別為 6、35 和 70 尾/L，幼蝦為 300、600、900 和 1200 尾/m^2。在實驗終止時，對蝦子的長度、重量比和存活率進行比較。

後期蝦苗(PL 12)在 6 和 35 尾/L 的放養密度下，存活率較高，超過 50%，存活率僅為 30%。6 尾/L 的比生長速率（SGR）為 3.5%/天，35 和 70 尾蝦/L 為 2.8%/天。

在幼蝦放養試驗中，兩個低密度組之存活率比兩個高密度組來得高，2 個低密度組之存活率約為 90%，而 2 個高密度組約為 70%。試驗結果亦表示，低放養密度的 SGR 較高，為 3.3%/天（300 和 600 幼蝦/m^2），而 2.5%（900 和 1200 幼蝦/m^2）。

(3)

主題：

Organic and conventional marine shrimp farms in brazil: macrobenthos occurrence inside and in the outlets of the ponds (巴西之有機傳統海洋養蝦場：漁塭內部和出水部出現大型底棲性生物)

講者：

Juliana Schober Goncalves Lima and Ulfert Focken

摘要：

巴西北里奧格蘭德州(Rio Grande do Norte State, Brazil)的 Guaraira 瀉湖(Lagoon)被大量海洋養蝦場包圍，這些養殖場大都為半集約式和集約式養殖。其中一些養殖場是在 1924 年時，與前淡水湖和附近海域建立人工連接後，開始發展為海洋養魚場，而其他養殖場是最近才建立的。

雖然大眾對巴西對蝦業的生態永續性和國際市場價格的下降感到擔憂，但 Guaraira 瀉湖周圍的海洋養蝦業正在穩步增長。

2001 年，位於 Guaraira 瀉湖的一個傳統養蝦場開始發展有機養殖，將天然池塘生物群作為主要食物來源，而傳統養蝦系統則以飼料為主要食物來源。2004 年，該養殖場被認證為巴西第一個甘蔗蝦養殖場。由於放養密度較低，沒有餵食飼料，有機養殖被認為更環保，有機蝦養殖的可變成本大大降低，可以生產更大的蝦，販售更高的價格。

為了比較不同養蝦系統的池塘生態和環境影響，於 2005 年上半年進行有機養殖場和 Guaraira 瀉湖周圍的養殖蝦場，在養殖週期中進行底棲生物的調查，因底棲生物被認為是海水養殖池內外環境變化的良好指標。
底棲生物樣品的採集範圍為 100 mm，篩網尺寸為 1.0mm，且採集之生物體保存於 5% 福馬林中，供日後分析。此外，亦對採集樣本進行分類及豐富度分析。

調查結果顯示，在集約式和半集約式系統的人工飼料養蝦場中，底棲生物密度增加，而且初期之底棲生物豐富度比有機養蝦系統來得高。多毛綱動物(polychaetes)為主要之底棲生物，另亦發現甲殼類動物(crustancean)、貧毛類蠕蟲(oligochaete)和紐蟲(nemertine)。在半集式和集約式系統中，海稚蟲科(Spionidae)的多毛綱動物為主要優勢物種。只有在有機系統中，漁塭內部的生物多樣性才高於外部。
底棲生物的分析結果與土壤有機物、水中營份、氧化還原電位和土壤中的 pH 等因素相關。

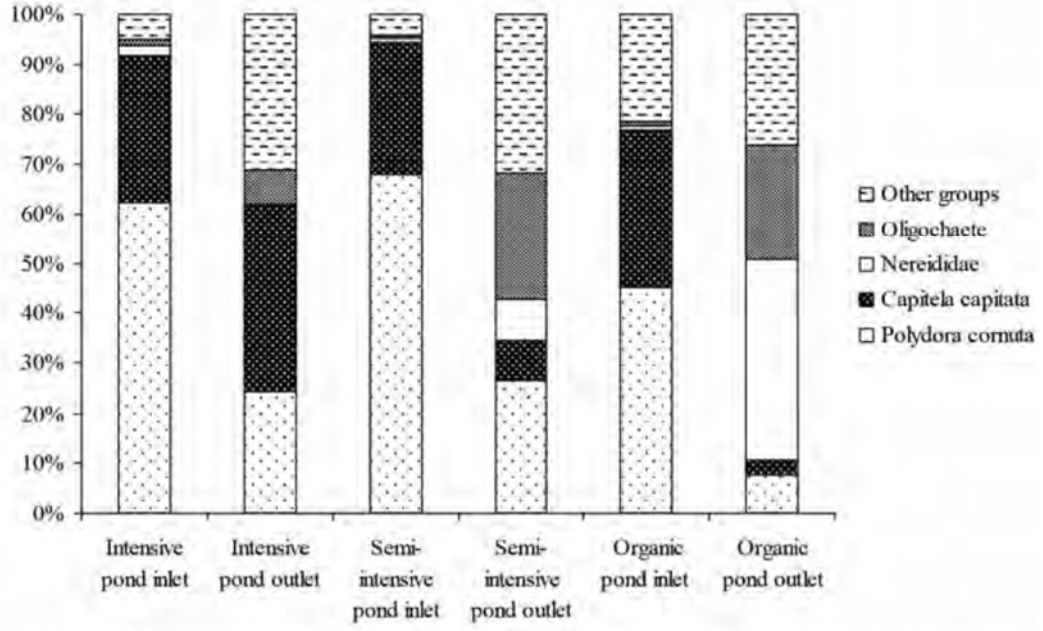
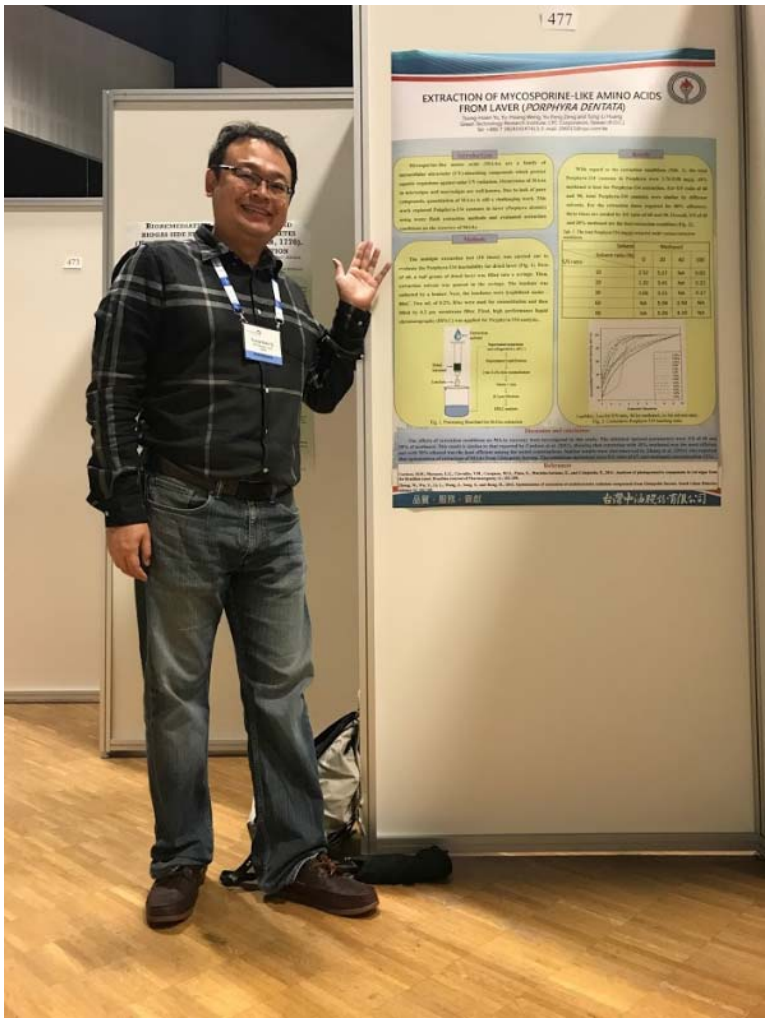


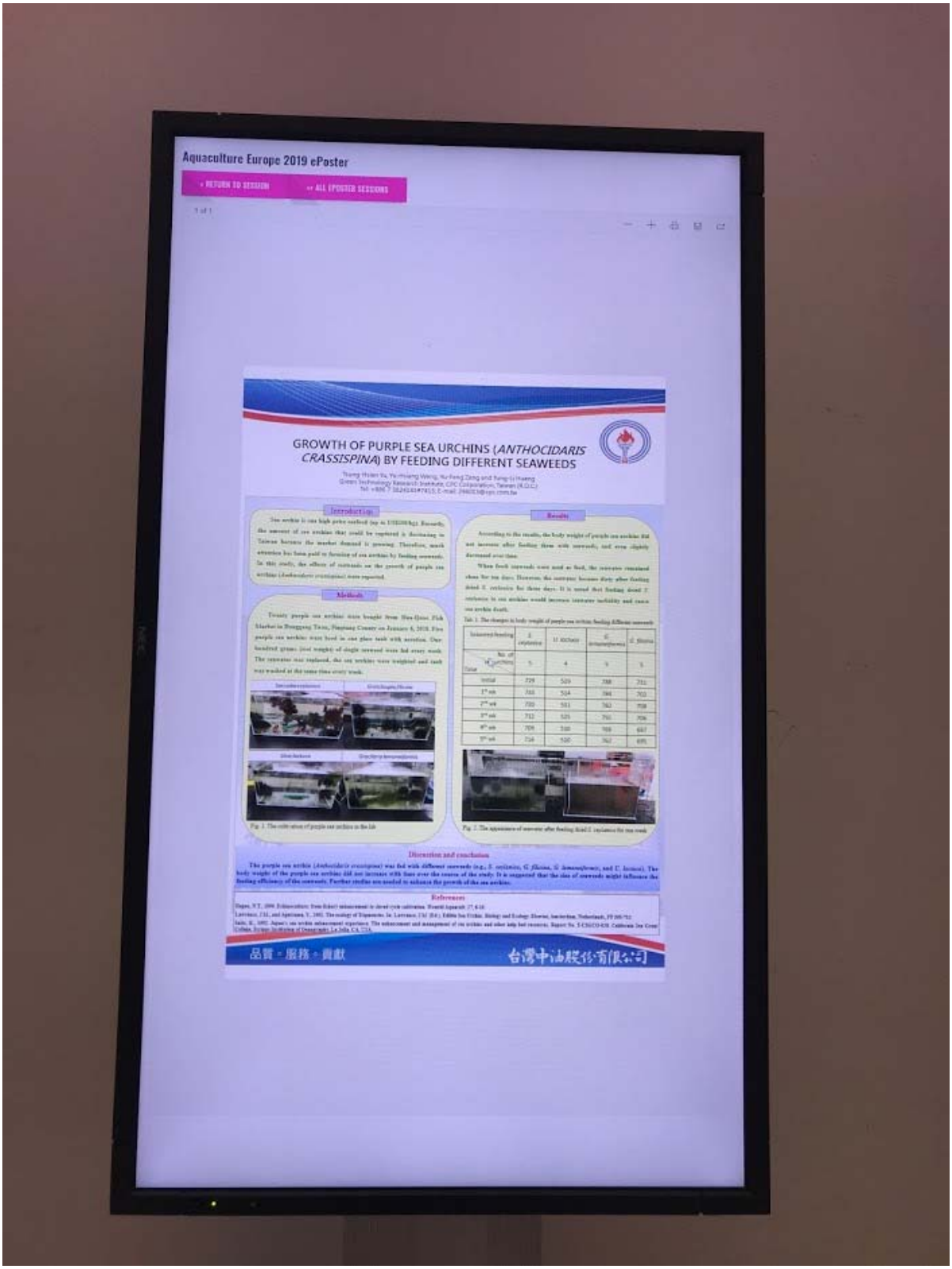
Figure 1. Occurrence (%) of dominant macrobenthos groups inside and at the outlet of the intensive, semi-intensive and organic marine shrimp ponds.

本組參與此國際研討會，共發表 2 篇海報(參與投稿證明如附件三所示)。

第一篇為「EXTRACTION OF MYCOSPORINE-LIKE AMINO ACIDS FROM LAVER *Porphyra dentata*」，此篇海報論文投搞至 Food Quality and Safety 議題類別，Poster ID 為 477。



第二篇海報論文發表為「GROWTH OF PURPLE SEA URCHINS *Anthocidaris crassispina* BY FEEDING DIFFERENT SEAWEEDS」，此篇海報論文投搞至 Seletive Breeding 議題類別，Poster ID 為 556。然因海報張貼看板僅有 500 個，故本海報論文被發表於電子海報(e-Poster)。



四、心得與建議

本次會議之議題幾乎包括所有水產養殖之議題，共有 56 個會議(sessions)、1039 篇摘要論文、594 篇海報(包含 94 篇之電子海報)、150 個展覽攤位、85 個參與國家、超過 2700 位參與者。

然而，因此國際研討會議題很多，故本人僅能盡可能參與和本組研究方向與未來可能研發方向相關之議題。主要參與之論文發表議題包括 Macroalgae, Climate Change, Sustainable Aquaculture Technologies, Value Addition and Marketing, Hatchery Technologies and Practices, and Shrimp。本人就將上述之議題摘錄較重要之發表提出探討。

目前歐洲已逐漸重視海藻，並且開始海藻養殖產業，以及海藻高值化研究。雖然，歐洲海藻產業仍無法與東南亞地區相比較，但歐洲將朝向高品質和高單價之海藻產品進行生產，以和東南亞地區之海藻有所區別。

氣候變遷對於歐洲水產養殖產業之影響有所區別，對於既有養殖物種或原本生存於當地之物種將會造成影響，例如當地養殖之貝類。然而，因氣候變暖，歐洲某些地區水溫亦慢慢變高，因此可養殖一些溫帶和亞熱帶之魚物。

另就養殖技術而言，大多傳統養殖方式皆為半集約式和集約式，高密度養殖易造成水質惡化和致病菌生長，而導致魚蝦生病死亡；因此，目前已推出許多自動化和 AI 水質監控技術，可促使水質較為穩定。

此國際研討會規模很大，許多 sessions 同時進行，故無法同時聆聽到好的發表，僅能有所取捨，挑選較適本組研發方向之 sessions 聆聽。此外，本國際研討會之發表海報數量也很多(高達 594 篇)，但海報參觀時間較短，因此無法全部細讀海報內容和有趣的海報，甚為可惜。

綜合上述，參加本次國際研討會學習到許多有趣的研究方向和內容，另亦攜回研討會摘要論文集，以及紙本海報攝影和電子海報，後續皆可再回顧研讀，或與發表者討論。

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TUESDAY, October 8

PLENARY 1 AND STUDENT SPOTLIGHT AWARD

Tuesday, October 8 09.00 - 10.00 Europe Hall
Chair: Stefan Meyer

09.00 Charles R. Tyler
THE ENVIRONMENT AND FISH HEALTH

Student Spotlight Award

10:00 BREAK

AQUAPONICS AND IMTA

Tuesday, October 8 10.30 - 12.50 Foyer 4
Chairs: Ben Kozen, Hendrik Monsees

10.30 Luca Grosso, Alessandra Fianchini, Stefano Cataudella, Michele Scardi, Arnold Rakaj
INTEGRATED MULTITROPHIC AQUACULTURE WITH THE SEA URCHIN *Paracentrotus lividus* AND THE SEA CUCUMBER *Holothuria tubulosa:* AN INNOVATIVE MODEL OF SUSTAINABLE AQUACULTURE

10.50 Filipe Soares, Ana Nobre, Tomé Silva, Rui Pereira, Helena Abreu, Luísa Valente, Luís Conceição
NUTRIENT-BASED MODELS AS COMPLEMENTARY TOOLS TO ASSESS FINFISH AMMONIA EXCRETION AS THE PRIMARY NUTRIENT SOURCE IN A SEAWEED COMMERCIAL IMTA FARM

11.10 Frank Kane, Joanne Casserly
IMTA AND SMARTER MONITORING FOR GREENER AQUACULTURE

11.30 **M.A. Lorenzo**, M.A. Poli, E.C. Legarda, I. Pinheiro, M.A. Martins, W.Q. Seiffert, F.N. Vieira
INTEGRATED MULTITROPHIC AQUACULTURE APPLIED TO SHRIMP REARING IN A BIOFLOC SYSTEM WITH TILAPIA AND SARCOCORNIA

11.50 **Philipp Sandmann**, Adrian A. Bischoff
UTILIZATION OF AFRICAN CATFISH *Clarias gariepinus* SEDIMENTS FOR CULTIVATING THE POLYCHAETE *Hediste diversicolor* (O.F. MÜLLER, 1776) UNDER DIFFERENT TEMPERATURE REGIMES

12.10 **Gösta Baganz**, Daniela Baganz, Georg Staaks, Hendrik Monsees, Werner Kloas
COMMERCIAL AQUAPONICS: TWO ECONOMIC SCENARIOS FOR A DOUBLE CIRCUIT SYSTEM

12.30 **Vincent Gennotte**, A.A. Forchino, S.M. Takedo Tchuindjo, J. Leroy, P. Maesen, C. Mélard, C. Rougeot, R. Pastres, D. Brigolin
ENVIRONMENTAL AND ECONOMIC ASSESSMENT OF A PILOT AQUAPONIC PRODUCTION: A LIFE CYCLE APPROACH

EPIGENETICS

Tuesday, October 8 10.30 - 12.50 Exhibition 2
Chair: Elena Sarrapoulou

10.30 **Erick Perera**, Serhat Turkmen, Paula Simó-Mirabet, Maria J. Zamorano, Hanlin Xu, Marisol Izquierdo, Jaume Pérez-Sánchez
STEAROYL-COA DESATURASE-1 IS EPIGENETICALLY REGULATED BY BROODSTOCK NUTRITION IN GILTHEAD SEA BREAM *Sparus aurata*

10.50 **Anne-Catrin Adam**, Marit Espe, Takaya Saito, Paul Whatmore, Jorge M.O. Fernandes, Kaja H. Skjaerven
EPIGENETIC EFFECTS OF DIETARY MICRONUTRIENTS IN ATLANTIC SALMON *Salmo salar* MUSCLE

11.10 **Hanlin Xu**, Shajahan Ferosekhan, Serhat Turkmen, Juan Manuel Afonso, Daniel Montero, Marisol Izquierdo
IMPROVED USE OF LOW FM AND LOW FO DIETS IN GILTHEAD SEABREAM *Sparus aurata* JUVENILES OBTAINED BY COMBINED BROODSTOCK SELECTION AND NUTRITIONAL PROGRAMING

11.30 **Suvra Roy**, Parisa Norouzitallab, Peter Bossier, Daisy Vanrompay
EPIGENETIC INHERITANCE AND LONG-LASTING PROTECTION AGAINST VIBRIOS INDUCED BY A PLANT-DERIVED COMPOUND IN BRINE SHRIMP MODEL

11.50 **Delphine Lallias**, E. Quillet, M. Bernard, C. Ciobotaru, N. Dechamp, J.-M. Le Calvez, M. Bideau, L. Goardon, L. Labbé, M. Dupont-Nivet
ANALYSIS OF GENOMEWIDE PATTERNS OF DNA METHYLATION IN RESPONSE TO AN EARLY TEMPERATURE STRESS IN RAINBOW TROUT

12.10 **Serhat Turkmen**, Hanlin Xu, Marisol Izquierdo
NUTRITIONAL PROGRAMMING OF LIPID-METABOLISM IN GILTHEAD SEA BREAM: TRANSGENERATIONAL EFFECTS OF BROODSTOCK SELECTION AND FEEDING

MACROALGAE

Tuesday, October 8 10.30 - 12.50 Festival
Chair: Jorunn Skjermo

10.30 **Diogo Raposo**, Frank Neumann, Jon Funderud
BROWN SEAWEEDS – A STATUS REPORT OF OFFSHORE CULTIVATION AND EXPECTED MARKET DEVELOPMENTS IN EUROPE

10.50 **Silvia Blanco González**, Fiona Wanjiku Moejes, Julie Maguire
LARGE-SCALE CULTIVATION OF SEAWEED IN WEST CORK: AN IRISH SUCCESS STORY

11.10 **Erik Malta**, Leen Bastiaens, Kathy Elst, Maria del Mar Agraso Martinez
PERSPECTIVES FOR INDOOR SEAWEED *Ulva ohnoi* CULTIVATION IN PHOTOBIOREACTORS

11.30 **Siv A. Etter**, S. Forbord, V.R. Dahlen, A. Handa, Y. Olsen, K.I. Reitan
INITIAL UPTAKE CAPACITY OF AMMONIUM IN *Saccharina latissima* IN AN IMTA CONTEXT

11.50 **Sofiia Tretiak**, Jakop Schwoerbel, Ramona Bosse, Frederike Reimold, Ina Enders, Dietmar Hoffmann, Joachim Henjes, Bela Buck, Laurie Hofmann
OPTIMIZATION OF ANTIOXIDANT ACTIVITY IN SEA VEGETABLES GROWN IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

12.10 **Sander van den Burg**, Maggie Skirtun, Mirjam Bernard
EUROPEAN SEAWEED VALUE CHAINS IN AN INTERNATIONAL PERSPECTIVE

NORDIC RAS
(SEPARATE REGISTRATION REQUIRED)

Tuesday, October 8 10.30 - 12.50 Room 1

GENERAL CONTRIBUTED ENVIRONMENTAL

Tuesday, October 8 10.30 - 12.50 Lounge 1-3
Chair: Daniel Taylor

10.30 Festus I. Adeosun
THE FISHES OF AKOMOJE RESERVOIR DRAINAGE BASIN IN LOWER RIVER OGUN, NIGERIA: DIVERSITY AND ABUNDANCE

10.50 Ingrid Askeland Johnsen, Alison Harvey, Anne D. Sandvik, Ola Ugedal, Bjørn Ådlandsvik, Vidar Wennevik, Kevin A. Glover, Ørjan Karlsen
SALMON LICE *Lepeophtheirus salmonis* INDUCED MORTALITY OF ATLANTIC SALMON *Salmo salar* DURING POST-SMOLT MIGRATION IN NORWAY

11.10 Maggie Skirtun, Sander van den Burg, Trond Selnes
MARINE RESOURCES IN CIRCULAR CLIMATE SMART FOOD SYSTEMS: A CASE STUDY OF SEAWEED CULTIVATION IN THE NORTH SEA

11.30 Alda Miftari
ECONOMIC ISSUE AND GOOD ATTRIBUTES OF SUSTAINABLE AGRICULTURE SYSTEM WITH A FOCUS ON CAP REFORM AND WATER FRAMEWORK DIRECTIVE

11.50 Lindsay Brager, Peter Cranford, David Wong, Brent Law
CANCELLED ALTERNATIVE METHODS FOR MONITORING BENTHIC ORGANIC ENRICHMENT EFFECTS AND APPLICABILITY ACROSS A RANGE OF ENVIRONMENTAL AND AQUACULTURE CONDITIONS

12.10 Henry Peterson
WASHINGTON STATE'S POLLUTION IDENTIFICATION AND CORRECTION PROGRAM'S WORK TO PROTECT AND RESTORE SHELLFISH BEDS AND PUBLIC HEALTH

12.30 Rodrigo Roubach, F.C.M. Godoi, F.S. David, N.S. Fialho, M.R. Brande, G.W. Bueno
ENVIRONMENTAL SUSTAINABILITY INDICATORS APPLIED IN SMALL SCALE FAMILY FISH FARM AT THE ATLANTIC FOREST IN SOUTH AMERICA

CLIMATE CHANGE

Tuesday, October 8 10.30 - 17.30 Room 5
Chairs: Myron Peck, Orestis Stavrakidis

10.30 Michelle Barbosa, Caroline Schwaner, Emmanuelle Pales Espinosa, Bassem Allam
OCEAN ACIDIFICATION AND MULTIPLE STRESSORS AFFECT LARVAL BUT NOT JUVENILE STAGES OF THE EASTERN OYSTER *Crassostrea virginica*

10.50 Patrícia Anacleto, Mariana Carmona, Ana Luísa Maulvault, Vera Barbosa, Marta Santos, Pedro Pousão-Ferreira, Luísa M.P. Valente, António Marques, Rui Rosa
FISH ENERGY BUDGET OF THE ZEBRA SEABREAM *Diplodus cervinus* UNDER OCEAN WARMING AND ACIDIFICATION

11.10 I. Martins, A. Azevedo, R. Ramos, M.H. Abreu, R. Pereira, L.M.P. Valente
HOW WILL THE PRODUCTION OF AN ECONOMICALLY RELEVANT ALGA *Gracilaria* sp. VARY WITH TEMPERATURE RISE?

11.30 **António Marques**, A. L. Maulvault, Rui Rosa, Mário S. Diniz
CLIMATE CHANGE AND EMERGING CHEMICAL CONTAMINANTS IN MARINE ORGANISMS: BIOACCUMULATION, ECOTOXICOLOGY AND PUBLIC HEALTH IMPACTS

11.50 **Remigiusz Panicz**, Beata Calka, André Sobral Lopes, João Lencart e Silva, João Gomes Ferreira, Jacek Sadowski, Paulina Hofsoe-Oppermann, Slawomir Keszka, Nick Taylor, Adam Kennerley, James Guilder, Susan Kay
PATHOGEN AND BIOMASS GROWTH RISK ASSESSMENT MODEL FOR INLAND AQUACULTURE IN EASTERN EUROPE UNDER CLIMATE CHANGE

12.10 **Myron Peck**, Pauline Kamermans, Tom Doyle, Nick Taylor, Joao Ferreira
RISKS AND OPPORTUNITIES OF CLIMATE CHANGE TO EUROPEAN AQUACULTURE: ADVANCES MADE IN THE EU CERES PROJECT

12.30 **Lena Schenke**, Tamás Bardócz, Kyra Hoevenaars
CLIMATE CHANGE IMPACTS ON THE EUROPEAN ISLANDS' AQUACULTURE SECTOR

12:50 **LUNCH**

14.30 **Isabel Fuentes-Santos**, Xosé Antón Álvarez-Salgado, Uxío Labarta, Mª José Fernández-Reiriz, Susan Kay, Solfrid Saetre Hjøllo
FORECASTING THE IMPACT OF FUTURE CLIMATE SCENARIOS ON CULTURED MUSSELS GROWTH IN THE GALICIAN RÍAS (NW SPAIN)

14.50 **Alhambra Gadea Martínez Cubillo**, J.G. Ferreira, J. Lencart e Silva, C.M. Kreiss, P. Kamermans, C. Saurel, J. Icely, B. Fragoso
MODELLING THE IMPACTS OF CLIMATE CHANGE ON SHELLFISH AQUACULTURE

15.10 **Stephanie Palmer**, Laurent Barillé, Pierre Gernez, Susan Kay, Stefano Ciavatta, Yoann Thomas, Philippe Glize
PAN-EUROPEAN MODELLING OF PACIFIC OYSTER *Crassostrea gigas* GROWTH IN THE OFFSHORE ENVIRONMENT: INDICATORS, REGIONAL COMPARISON AND CLIMATE CHANGE

15.30 **Sally McGee**
THE SHELLFISH GROWERS CLIMATE COALITION: A BUSINESS PARTNERSHIP OF 100+ COMPANIES TAKING ACTION TO SECURE A LOW CARBON FUTURE FOR THE BENEFIT OF SHELLFISH AND THE ENVIRONMENTS THEY DEPEND ON

15.50 **Gergo Gyalog**, Béla Csukás, László Berzi-Nagy, Mónika Varga
IMPACT OF CLIMATE CHANGE ON CARP YIELDS IN HUNGARIAN POND AQUACULTURE

16.10 **Orestis Stavrakidis-Zachou**, Konstadia Lika, Nikos Papandroulakis
FORECASTING CLIMATE CHANGE IMPACTS ON GREEK AQUACULTURE PRODUCTION: A CLIMEFISH CASE STUDY

16.30 **Øivind Bergh**, Ingrid Johnsen, Lars Asplin, Nina Sandlund, Anne D. Sandvik, Joao G. Ferreira, Nick Taylor
CLIMATE CHANGE AND MITIGATION OF ITS IMPACTS OF NORTH EUROPEAN AQUACULTURE

16.50 **Cornelia Martina Kreiss**, Giulia Micallef, Tom Doyle, Ferit Rad, Virginia Martín, Joao Gomes Ferreira, Alhambra Cubillo, Joao Lencart e Silva, Eleni Papathanasopoulou
FUTURE PROFITABILITY OF TYPICAL FINFISH FARMS IN EUROPE

17.10 **John Icely**, A. Cubillo, M. Elliot, J. Ferreira, B. Fragoso, P. Kamermans, S. Kay, A. Kennerley, C. Kreis, A. Marques, D. Matias, M. Peck, J. Pinnegar, J. Pinnegar, H. Rambo, K. Smyth, V. Stetzenmuller, N. Taylor
POTENTIAL EFFECTS OF CLIMATE CHANGE ON OFFSHORE AQUACULTURE OF MEDITERRANEAN MUSSEL *Mytilus galloprovincialis* ON THE SW COAST OF PORTUGAL

NUTRITION: PHYSIOLOGY AND REQUIREMENTS

Tuesday, October 8 10.30 - 17.30 Europe Hall
Chair: Alessio Bonaldo, Antony Philip, Anne-Catrin Adam

10.30 **Sofia Morais**, S. Puchol, J.M. Cerdá-Reverter, A.R. Angotzi
TASTE SENSING AND GUT FEELING: POSSIBLE INVOLVEMENT OF TASTE RECEPTOR TYPE 1 FAMILY IN SEABREAM *Sparus aurata*

10.50 **David Domínguez**, Mohamed Cherkes, Ramon Fontanillas, Maria Jesus Zamorano, Marisol Izquierdo
EFFECTS OF DIFFERENT LEVELS OF VITAMINS B1, B9 AND B12 IN DIETS LOW IN FISHMEAL ON GILTHEAD SEA BREAM *Sparus aurata* FINGERLINGS

11.10 **Rui Magalhães**, F. Coutinho, I. Guerreiro, A. Couto, C. Serra, H. Peres, A. Oliva-Teles
OXIDATIVE STRESS STATUS AND MICROBIOTA OF GILTHEAD SEA BREAM *Sparus aurata* FED DIFFERENT DIETARY ARA/EPA/DHA RATIOS

11.30 **N. Gilannejad**, F.J. Moyano, G. Martínez-Rodríguez, M. Yúfera
REGULATION OF THE RHYTHMICITY OF PROTEOLYTIC DIGESTIVE FUNCTION IN GILTHEAD SEABREAM UNDER DIFFERENT DAILY FEEDING PROTOCOLS

11.50 **Serena Busti**, Luca Parma, Nicole Francesca Pelusio, Lorenzo Morsiani, Lorenzo Mariani, Marina Silvi, Maurizio Mazzoni, Francesco Dondi, Manuel Yúfera, Neda Gilannejad, Francisco Javier Moyano, Pier Paolo Gatta, Alessio Bonaldo
EFFECTS OF DIFFERENT FEEDING FREQUENCIES ON GROWTH, FEED UTILIZATION, PLASMA BIOCHEMISTRY AND DIGESTIVE CONDITIONS OF GILTHEAD SEA BREAM *Sparus aurata* FED WITH DIFFERENT FISH MEAL AND FISH OIL DIETARY LEVELS

12.10 **Sofia Engrola**, Raquel Carrilho, Rita Colen, Rita Teodósio, Jorge Dias, Cláudia Aragão
IMPROVING GILTHEAD SEABREAM *Sparus aurata* JUVENILES ADAPTABILITY TO ADVERSE CONDITIONS VIA NUTRITION

12.30 **P. Wischhusen**, M. Briens, P.A. Geraert, P. Antony Jesu Prabhu, L. Larroquet, V. Véron, S.J. Kaushik, B. Fauconneau, S. Fontagné-Dicharry
PARENTAL AND DIRECT FEEDING EFFECTS OF DIETARY SELENIUM IN RAINBOW TROUT *Oncorhynchus mykiss* FRY

12:50 **LUNCH**

14.30 **David Huyben**, Teresa Grobler, Douglas Tocher, Bente Ruyter, Brett Glencross
OPTIMISING LEVELS OF DIETARY FATTY ACIDS AND LIPIDS OF ATLANTIC SALMON UNDER NORMOXIC AND HYPOXIC CONDITIONS

14.50 **Marit A.J. Nederlof**, Ep H. Eding, Anne-Jo van Riel, Isabelle Leguen, Stephane Panserat, Patrick Prunet, Johan W. Schrama
EFFECT OF EARLY LIFE HYPOXIA AND DIET COMPOSITION ON GROWTH FEED INTAKE AND OXYGEN CONSUMPTION OF RAINBOW TROUT *Oncorhynchus mykiss* IN LATER LIFE

15.10 **Antony Jesu Prabhu Philip**, Marta Silva, May-Helen Holme, Thea Morken, Sofie Remø, Robin Ørnsrud, Pedro Araujo, Erik-Jan Lock, Rune Waagbø
IMPACT OF DIETARY ZINC LEVEL ON THE ZINC STATUS OF ATLANTIC SALMON PARR, SMOLT AND POST-SMOLT FED LOW FISH MEAL DIETS

15.30 **Claudia Figueiredo-Silva**, C. Boggino, M. Sun, Maria Mastoraki, I. Papadakis, A. Theodoridou, M. Pavlidis, S. Chatzifotis
EFFECTS OF COMPLEXED TRACE MINERALS AT DIFFERENT INCLUSION RATES IN COMMERCIAL SEA BASS *Dicentrarchus labrax* DIETS

15.50 **Nicole Francesca Pelusio**, Luca Parma, Serena Busti, Stefano Porcelli, Lorenzo Mariani, Lorenzo Morsiani, Francesco Dondi, Enric Gisbert, Maria Angeles Esteban, Federica D'Amico, Matteo Soverini, Marco Candela, Pier Paolo Gatta, Alessio Bonaldo
EFFECTS OF REARING DENSITY ON GROWTH, WELFARE INDICATORS AND DIGESTIVE CONDITIONS OF GILTHEAD SEA BREAM *Sparus aurata* FED DIFFERENT FISH MEAL AND FISH OIL DIETARY LEVELS

16.10 **Thomas Staessen**, M.C.J. Verdegem, E.H. Eding, M.A.J. Nederlof, J.W. Schrama
EFFECT OF DIETARY NON-PROTEIN ENERGY SOURCE (STARCH VS. FAT) ON TOTAL BILE ACID POOL SIZE AND COMPOSITION OF RAINBOW TROUT *Oncorhynchus mykiss*

16.30 **Karthik Masagounder**, Paulo Rema, Jorge Dias
EFFECTS OF INGREDIENT INCLUSION LEVEL AND FECES COLLECTION METHOD ON THE APPARENT NUTRIENT AND ENERGY DIGESTIBILITY OF INGREDIENTS IN NILE TILAPIA

16.50 **Caroline Candebat**, Mark Booth, Igor Pirozzi, M. Basseer Codabaccus
SULFUR AMINO ACID REQUIREMENTS AND INTERACTIONS IN JUVENILE YELLOWTAIL KINGFISH *Seriola lalandi*

17.10 **Monica B. Betancor**, Aurelio Ortega, Fernando de la Gándara, Matthew Sprague, Douglas R. Tocher, Gabriel Mourente
DEVELOPMENT OF FEEDS FOR JUVENILE ATLANTIC BLUEFIN TUNA *Thunnus thynnus* L: EFFECT OF LIPID LEVEL AND SOURCE

REPRODUCTION AND BROODSTOCK MANAGEMENT

Tuesday, October 8 10.30 - 17.30 Room 3
Chair: Constantinos Mylonas

10:30 **Yonathan Zohar**
FROM FERTILITY TO STERILITY OF FARMED FISH – HORMONES AND GENES

10.50 **Jurgen Adriaen**, Joachim Claeyé, Thomas Abeel, Wouter Meeus, Heidi Arnouts
EFFECT OF TEMPERATURE AND DIFFERENT SPAWNING AGENTS ON REPRODUCTION SUCCESS OF BURBOT IN CAPTIVITY

11.10 **Andrea Miccoli**, Ioannis Fakriadis, Irini Sigelaki, Anastasios Raftopoulos, Constantinos C. Mylonas
SPAWNING KINETICS OF GREATER AMBERJACK *Seriola dumerili* IN RESPONSE TO DIFFERENT DOSES OF GnRHa IMPLANTS

11.30 **Carlos Marrero Alemán**, Wendy González López, Sandra Ramos Júdez, Isabel Navarro, N. Duncan
ARTIFICIAL FERTILISATION IN SENEGALESE SOLE *Solea senegalensis:* INDUCTION WITH GNRHa AND DETERMINATION OF EGG QUALITY

11.50 **Michelle Grace Pinto Jørgensen**, Einar Eg Nielsen, Elin Kjørsvik, Jonna Tomkiewicz
DOSE DEPENDENT EFFECTS OF CARP PITUITARY EXTRACT ON INDUCTION OF OVARIAN DEVELOPMENT IN EUROPEAN EEL *Anguilla anguilla*

12.10 **Johanna Kottmann**, Ian A.E. Butts, Michelle G.P. Jørgensen, Peter Ravn, Jonna Tomkiewicz
EFFECTS OF EXOGENOUS GONADOTROPIN TYPE (CARP VS. SALMON PITUITARY EXTRACT) ON OFFSPRING QUALITY IN EUROPEAN EEL

12.30 **Arjan P. Palstra**, Ignacio Giménez Nebot, Pauline Jéhannet, P. Mark Lokman, William Swinkels, Leon T.N. Heinsbroek
TESTING RECOMBINANT GONADOTROPINS FOR THE PROPAGATION OF EUROPEAN EEL *Anguilla anguilla,* PRETREATED BY FEMINIZATION, SIMULATED MIGRATION AND STEROID IMPLANTS

12:50 **LUNCH**

14.30 **Javed Khan**, Alvin Setiawan, M.J. Wylie, Steve Pether, J.E. Symonds
PROGRESS IN HAPUKU *Polyprion oxygeneios* PRODUCTION FROM F1 BROODSTOCK

14.50 **Dario Vallainc**, Francesca Leggieri, Barbara Loi, Gianni Brundu, Gemma Gimenez Papiol
CANCELLED HATCHERY PRODUCTION OF FLATHEAD GREY MULLET *Mugil cephalus* JUVENILES

15.10 **Paul Engler**, Jean-Michel Le Calvez, David Guilet, Laurent Labbé, Pierre Chicoteau
IMPACTS OF A DIETARY SUPPLEMENTATION OF RAINBOW TROUT *Oncorhynchus mykiss* BROODSTOCK WITH A LOW DOSE OF DRY GRAPE EXTRACT ON THE METABOLOMIC PROFILE OF THEIR EGGS AND THE GROWTH PERFORMANCES OF THEIR OFFSPRING

15.30 **Adrien Marc**, Jarrod Guppy, Dean Jerry, Peter Mulvey, Paige Bauer, Damien Paris
EVALUATING BARRAMUNDI *Lates calcarifer* SPERM QUALITY USING HIGH THROUGHPUT ADVANCED REPRODUCTIVE TOOLS

15.50 **Francesca Bertolini**, Michelle Grace Pinto Jørgensen, Christiaan Henkel, Jonna Tomkiewicz
UNRAVELLING THE CHANGES DURING INDUCED SEXUAL MATURATION OF FEMALE EUROPEAN EEL *Anguilla anguilla* THROUGH RNA-SEQ: WHAT HAPPENS TO THE LIVER?

16.10 **Ferosekhan Shajahan**, Cathaysa Pérez-García, Serhat Turkmen, Hanlin Xu, Juan Manuel Afonso, Ramon Fontanillas, Sadasivam Kaushik, Marisol Izquierdo
INFLUENCE OF GENETIC SELECTION FOR GROWTH AND DIETARY n-3 LC-PUFA LEVELS ON REPRODUCTIVE PERFORMANCE IN GILTHEAD SEA BREAM *Sparus aurata*

16.30 **Ingun Næve**, Maren Mommens, Augustine Arukwe, Jonni J. Virtanen, Md. Enamul Hoque, Elin Kjørsvik
ESTABLISHING A NON-INVASIVE METHOD FOR SEXUAL MATURATION MONITORING IN ATLANTIC SALMON *Salmo salar* MALES AND FEMALES USING ULTRASOUND TECHNOLOGY

16.50 **Ana Pombo**, Eliana Venâncio, Pedro M. Félix, Ana C. Brito, João Sousa, Francisco Azevedo e Silva, Tomás Simões
DOES THE DIET OF A *Holothuria tubulosa* BROODSTOCK INFLUENCE VIABILITY AND LARVAL DEVELOPMENT?

17.10 **Leila Basti**, Jyoji Go, Kiyohito Nagai
EFFECTS OF MONO AND MIXED BLOOMS OF CHARMFUL ALGAE *Alexandrium, Karenia* AND *Chattonella* ON THE REPRODUCTION OF JAPANESE PEARL OYSTER *Pinctada fucata martensii*

FISH WELFARE

Tuesday, October 8 10.30 - 17.30 Room 2
Chair: MIchalis Pavlides

10.30 **Albin Gräns**, Jennifer Bowman, Per Hjelmstedt, Nicole van Nuland
CARBON DIOXIDE STUNNING OF RAINBOW TROUT *Oncorhynchus mykiss:* THE EFFECTS OF DIFFERENT SEASONAL TEMPERATURES AND THE RELIABILITY OF VISUAL INDICATORS OF CONSCIOUSNESS

10.50 **Jaume Pérez-Sánchez**, Juan Antonio Martos-Sitcha, Javier Sosa, Dailos Ramos-Valido, Francisco Javier Bravo, Cristina Carmona-Duarte, Henrique Leonel Gomes, Josep Àlvar Calduch-Giner, Enric Cabruja, Aurelio Vega, Miguel Ángel Ferrer, Manuel Lozano, Juan Antonio Montiel-Nelson, Juan Manuel Afonso
AEFishBIT: SMART DEVICE FOR TRACKING FISH BEHAVIOUR AND ACTIVITY

11.10 **Carolina Gutierrez Rabadan**, Charlotte Spreadbury, Sonia Consuegra del Olmo, Carlos Garcia de Leaniz
DEVELOPMENT AND VALIDATION OF OPERATIONAL WELFARE INDICATORS (OWI) FOR FARMED LUMPFISH *Cyclopterus lumpus* L.

11.30 **Xavier Gutierrez**, Felipe A. Briceno, Rafael Leon, Patricio Feest, Rafael Leon, Patricio Feest
LINKING FRESH WATER QUALITY AND FISH WELFARE WITH SEA WATER FEEDING PERFORMANCE IN CHILEAN SALMON FARMING: A MODELLING TOOL BASED ON SMOLT PHYSIOLOGICAL STATUS

11.50 **Mark Schumann**, Cornelius Becke, Andreas Müller-Belecke, Roland Rösch
RAINBOW TROUT WELFARE INDEX MODEL 1.0

12.10 **Jian Zhao**, Shengyu Hang, Yanci Wen, Songming Zhu, Zhangying Ye
DEEP LEARNING-BASED MONITORING OF THE LOCAL UNUSUAL BEHAVIORS FOR FISH SCHOOL IN INTENSIVE AQUACULTURE

12.30 **Jeroen Brijs**, E. Sandblom, M. Axelsson, K. Sundell, H. Sundh, A. Kiessling, C. Berg, A. Gräns
REMOTE MONITORING OF CARDIOVASCULAR PERFORMANCE TO GAUGE STRESS RESPONSES OF RAINBOW TROUT *Oncorhynchus mykiss* IN AQUACULTURE

12:50 **LUNCH**

14.30 **Alexander Jaramillo-Torres**, Torunn Forberg, Elvis Chikwati, Armin Kousha, Abdelaziz Adam Mohammed, Yanxian Li, Åshild Krogdahl, Trond M. Kortner
MICROBIOTA-RELATED INDICATORS OF GUT HEALTH IN ATLANTIC SALMON *Salmo salar*

14.50 **Jessica Petereit**, C. Hörterer, R. Pastres, L. Conceição, J. Johansen, J. Aerts, B.H. Buck
THE EFFECT OF ALTERNATIVE FEED INGREDIENTS ON GROWTH AND STRESS RESPONSE IN JUVENILE TURBOT *Scopthalmus maximus* KEPT IN RECIRCULATING AQUACULTURE SYSTEMS

15.10 **Francisco Guardiola**, Martha Reyes-Becerril, Verónica Sánchez, Carlos Angulo
YEASTS β-GLUCANS IMPACT ON IMMUNE RESPONSE IN PLASMA AND SKIN MUCUS OF PACIFIC RED SNAPPER *Lutjanus peru* CHALLENGED WITH *Aeromonas hydrophila*

15.30 **Lluis Tort**, Annais Carbajal, Felipe E. Reyes-Lopez, Oriol Tallo-Parra, Manel López-Béjar
CORTISOL IN PLASMA, SKIN MUCUS AND SCALES. A COMPARATIVE VIEW OF THE HYPOTHALAMIC-PITUITARY-INTERRENAL AXIS ACTIVITY IN DIFFERENT FISH MATRICES

15.50 **Linda Tschirren**, Nicola Rhyner, Andreas Seitz, Constanze Pietsch
GENE EXPRESSION LEVELS IN DISTINCT BRAIN AREAS OF CARPS AFTER POSITIVE AND NEGATIVE ACUTE STRESSORS

16.10 **Raminta Kazlauskaite**, Bachar Cheaib, Joshka Kaufmann, Chloe Heys, Alex Kitts, Martin Llewellyn
SALMOSIM: BUILDING ARTIFICIAL ATLANTIC SALMON *Salmo salar* GUT SYSTEM

16.30 **Luis André Barbas**, Jhenneff Feitosa, Marcelo Torres, Brenda Costa, Moisés Hamoy
EUGENOL INDUCES A SEIZURE-LIKE STATE DURING SHORT-TERM EXPOSURE IN FISH

16.50 **Maureen Ellis**, L. Dunn, M. Haskell, S. Jarvis, S. Rey, J.F. Turnbull
NOVEL USE OF QUALITATIVE BEHAVIOUR ASSESSMENT TO MONITOR WELFARE IN FARMED ATLANTIC SALMON *Salmon salar*

17.10 **Lina Weirup**, Johan Aerts, Carsten Schulz, Henrike Seibel
ASSESMENT OF CHRONIC STRESS AND WELFARE STATUS IN RAINBOW TROUT *Oncorhynchus mykiss* AT DIFFERENT STOCKING DENSITIES IN COMMERCIAL FLOW-THROUGH SYSTEMS IN GERMANY

AE2019 INDUSTRY FORUM – GERMAN FARMERS' DAY

Tuesday, October 8 10.30 - 17.30 Exhibition 1
Chair: Stefan Meyer

10.30 Bernhard Feneis
WELCOME FROM VDBA

Birgit Schmidt-Puckhaber
WELCOME FROM DLG

10.50 Birgit Schmidt-Puckhaber
PRODUCTION SYSTEMS IN EUROPE - HOW DO WE PRODUCE TOMORROW?

10.50 Bernhard Feneis,
POLITICAL AND SOCIETAL FRAMEWORK CONDITIONS - EUROPEAN AQUACULTURE PRODUCERS' PERSPECTIVE

11.20 Daniel Żarski,
TOWARD INNOVATION IN POND-BASED PRODUCTION TECHNOLOGY

11.35 Gert Füllner
CARP POND CULTURE - OUTDATED OR FUTURE TECHNOLOGY?

11.50 Martin Oberle,
COMPOSITION AND MINERALIZATION OF CARP POND SOIL

12.05 Jan Masilko
HORSEBEAN AS FEED STUFF FOR COMMON CARP - INFLUENCE ON GROWTH AND FLESH QUALITY

12.20 Andreas Müller-Belecke
INTENSIVE CARP FRY PRODUCTION - IMPROVING PERFORMANCE AND EFFICIENCY WITH STATE-OF-THE-ART FEED AND TECHNOLOGY

12.35 Szymon Łakomiak,
FROM SALMONIDS TO STURGEONS - SECOND LIFE OF OUTDATED TROUT PRODUCTION SYSTEMS

12.50 LUNCH

14.10 Werner Kloas
NEW SOLUTIONS FOR "OLD" PROBLEMS

14.10 Alexander Tautenhahn
HEALTHY FISH - STRATEGIES TO REDUCE FISH LOSSES

14.40 Thomas Wachinger
SHARING INSIGHTS FROM A BAVARIAN INDOOR-RAS FARM FOR TROPICAL SHRIMP PRODUCTION

14.55 Paul Sindilariu
RAS - FUTURE FOR SWISS AQUACULTURE?

15.10 Dominik Ewald
USE OF DIGITAL SYSTEMS IN AQUACULTURE - ARE WE READY FOR "BIG DATA"?

15.25 BREAK

15.50 Stefan Meyer
ANIMAL WELFARE AND CONSUMER ACCEPTANCE

15.50 Helmut Wedekind
SCIENCE-BASED INSIGHTS INTO ANIMAL WELFARE IN AQUACULTURE

16.10 Johannes Simons
ENJOY FISH! A GERMAN TROUT CASE STUDY ON CONSUMER ACCEPTANCE FOR FISH PRODUCTS FROM AQUACULTURE

16.30 Stefan Johnigk, Bernhard Feneis, Stefan Hofer
"FUTURE PERSPECTIVES…" - ROUND TABLE DISCUSSION

RECIRCULATING AQUACULTURE SYSTEMS (RAS)

Tuesday, October 8 14.30 - 17.30 Room 1
Chairs: Astrid Buran Holan, Carlos Octavio Letelier Gordo

14.30 Paula Rojas-Tirado, Åse Åtland, Carlos Letelier-Gordo
THE EFFECT OF DIFFERENT WATER SOURCES ON THE POTENTIAL H2S-FORMATION WITHIN RAS

14.50 Ragnhild Fossmark, Kari Attramadal, Trond Rosten, Deni Koseto, Jenny Nesje, Ingrid Bakke, Niels Jørgensen, Gema Raspati, Stein Østerhus, Olav Vadstein
MICROBIAL COMMUNITY DYNAMICS IN RECIRCULATING AQUACULTURE SYSTEMS REARING ATLANTIC SALMON PARR *Salmo salar* WITH REDUCED ORGANIC LOADING THROUGH MEMBRANE FILTRATION

15.10 Jeremiah Minich, Khattapan Jantawongsri, John Bowman, Abigail Elizur, Stewart Fielder, Rob Knight, Eric Allen
HATCHERY SYSTEM (RAS VS FT) EFFECTS ON FISH MICROBIOTA FOR ATLANTIC SALMON *Salmo salar* AND YELLOWTAIL KINGFISH *Seriola lalandi*

15.30 Felipe do Nascimento Vieira, Frederike Schmachtl, Mirko Boegner, Ana Clara C. da Silva, Vitor F. Silva, Walter Q. Seiffert, Cláudia Machado, Daniele dos Santos, Gabriela S. Ferreira
STRATEGIES FOR WATER PREPARATION IN A BIOFLOC SYSTEM: EVALUATION OF A CHEMOAUTOTROPHIC, HETEROTROPHIC AND MATURE SYSTEM FOR PACIFIC WHITE SHRIMP REARING

15.50 Desislava Bögner, Mirko Bögner, Fredericke Schmachtl, Nicolas Bill, Jörn Halfer, Kai Lorkoswki, Matthew J. Slater
DEMYSTIFYING CRITICS ABOUT HYDROGEN PEROXIDE UTILIZATION IN RECIRCULATING AQUACULTURE SYSTEMS

16.10 **Wanhe Qi**, Songming Zhu
LOW CONCENTRATION PMS/UVA-LED COMBINATION AS A POTENTIAL ALTERNATIVE DISINFECTION METHOD FOR RECIRCULATING AQUACULTURE SYSTEMS

16.30 **Angela Boley**, Wolf-Rüdiger Müller
EVALUATION OF A MEMBRANE-DENITRIFICATION REACTOR (MDR) IN A RECIRCULATION AQUACULTURE SYSTEM UNDER REAL CONDITIONS

16.50 **Jaime Orellana**, Silvia Gómez, Felipe Hurtado
BIOREMEDIATION OF ORGANIC SLUDGE FROM A MARINE RECIRCULATING AQUACULTURE SYSTEM USING THE POLYCHAETE *Abarenicola pusilla*

17.10 **Alberto Monteleone**, Shane Hunter, Simeon Deguara
AQUABIOTECH GROUP – ABT INNOVIA: EXCELLENCE THROUGH QUALITY AND INNOVATION

MICROALGAE

Tuesday, October 8 14.30 - 17.30 Festival
Chair: Kjell Inge Reitan

14.30 **Dorinde M.M. Kleinegris**, Jeroen H. de Vree, Pia Streinrücken, Hanna Böpple, Hans T. Kleivdal
THE NATIONAL ALGAEPILOT MONGSTAD: PRODUCTION OF MICROALGAE FOR AQUACULTURE

14.50 **Mariluz Bagnoud**, Alexandre Bagnoud, Cyril Mahmed, Roger Röthlisberger, Saloua Sadok
INTEGRATION OF A NEW PHOTOSYNTHETIC BIOFILM FILTER IN RECIRCULATING AQUACULTURE SYSTEMS AND DIRECT BIOMASS RECOVERY

15.10 **Robert Röllig**, Harry W. Palm, Adrian Bischoff-Lang, Hilary Jane Lewis Karlson, Malene Fog Lihme Olsen, Lars Jørgensen
MICROALGAE CULTIVATION FOR BIOREMEDIATION OF NUTRIENTS UNDER AQUAPONIC CONDITIONS

15.30 **A. Roulston**, S. Glover, J. Long, R. Roulston
COMPACT PHOTOBIOREACTORS FOR LIVE MICROALGAE PRODUCTION IN AQUACULTURE HATCHERIES AND NURSERIES

15.50 **Christos Latsos**, Pieter Oostlander, Gabrielle Verbeeke, Maria Barbosa, Rene Wijffels, Jasper van Houcke
Rhodomonas salina PRODUCTION: GOING FROM LAB TO SEMI-COMMERCIAL SCALE

16.10 **Hideaki Matsui**, Satoshi Kono, Kazuki Okita, Tomonari Kotani
THE MICROALGA *Isochrysis* AS AN ENRICHMENT DIET FOR ROTIFERS: EFFECTS OF HARVEST TIMING ON ROTIFER VITALITY AND FATTY ACID PROFILES

16.30 **Ana Viskovic**, Francisco Guardiola, Inês Alexandra Sa, Marina Machado, Filipa Fontinha, Rita Azeredo, Benjamin Costas, Ana Couto
EVALUATION OF HEALTH STATUS IN EUROPEAN SEA BASS *Dicentrarchus labrax* L. JUVENILES FED DIETS WITH PARTIAL REPLACEMENT OF FISH MEAL BY MICROALGAE MEAL

16.50 **Ifakat Tülay Çagatay**, Mehmet Özbas, Noha Sati, Hasan Emre Yilmaz
ANTIMICROBIAL ACTIVITY OF *Nannochloropsis* spp. AGAINST FISH PATHOGENS

17.10 **Joseph Penhaul Smith**, C. Beveridge, V.A. Laudicello, L. McEvoy, A.D. Hughes, J.G. Day
MICROALGAL MIXOTROPHY AS A SOURCE OF FEED FOR *Mytilus edulis* LARVAE

PERFORMFISH / MEDAID INDUSTRY FORUM

Tuesday, October 8 14.30 - 17.30 Exhibition 2
Chairs: Katerina Moutou, Bernardo Basurco

14.00 **Katerina Moutou,** Alicia Estévez
PerformFISH and **MedAID**: TWO YEARS LATER

14.20 **Leonidas Papaharisis**, Kantham Papanna, Dimitrios Chatziplis, Valia Economou, Tereza Manousaki, Costas S. Tsigenopoulos
INCREASING DISEASE RESISTANCE OF FISH FOR PARASITES THROUGH BREEDING: THE CASE OF THE EUROPEAN SEA BASS AGAINST THE MONOGENEAN *Diplectanum aequans* AND THE COPEPOD *Lernanthropus kroyeri*

Costas Tsigenopoulos
HERITABILITY ESTIMATES FOR DISEASE RESISTANCE IN EUROPEAN SEA BASS: OPEN SEA CHALLENGES TO MONOGENEAN PARASITES *Diplectanum aequansis* AND *Lernanthropus kroyeri*

14.50 **Kostas Tzokas, George Koumoundouros**
DO ALL DEFORMITIES MATTER?

15.10 **Javier Villa, Nikos Papandroulakis**
NOVEL TECHNOLOGY TO IMPROVE FEEDING MANAGEMENT

15.30 **Giovanna Marino, Dane Desnica**
KEY PERFORMANCE INDICATORS FOR EFFICIENT BENCHMARKING IN MEDITERRANEAN AQUACULTURE

15.50 **José Pérez**
SOCIAL ACCEPTABILITY AND GOVERNANCE. STAKES, CHALLENGES AND RECENT EXPERIENCES

16.10 **José L. Fernández Sánchez**
ECONOMIC FINDINGS IN PRODUCTION AND MARKETS OF MEDITERRANEAN AQUACULTURE

16.30 **María Soledad Izquierdo López**
OPEN DISCUSSION AND CONCLUSIONS

STUDENT WORKSHOP
EAS Avengers – AquaIndustry Challenges

Tuesday, October 8 14.30 - 17.30 Foyer 4
Chair: Kathrin Steinberg

14.30 Kathrin Steinberg
Welcome

14.40 Ana Nobre – SPAROS
FEEDNETICS as a tool for students and young professionals

15.00 EAS SG Travel Grant Winners - Industry and Research Experiences
Ana Costa
Kerrie Ni Dufaigh
Rodrigo Mendes
Sandra Langi
Sofiia Tretiak

15.20 Kunststoff Spranger

15.40 EAS SG National Coordinators
Status of Aquaculture, Irene Brandts (Spain)
Ivo Monteiro (Portugal)
Pauline Wischhusen (France)
Guido Bonello (Italy)
Elisavet Syropoulou (Greece)

16.05 Lourdes Reig and Rosa Flos (UPC)
How students perceive aquaculture: should we be worried? How could it be approached from the curricula?

16.25 Andrew Richardson (NC)
Issues and Problems within the Industry

16.40 Ideas and Solutions for the Industry
Group Discussions and Networking

17.20 Aideen Kearney (NC), Silvia Blanco (NC)
Outlook to Ireland for AE2020

GENERAL CONTRIBUTED HEALTH

Tuesday, October 8 14.30 - 17.30 Lounge 1-3

Chair: TBA

14.30 **Sebastian Rakers**, Lisa Jordan, Mikolaj Adamek, Dirk Scheel, Anna Becker, Marina Gebert
CELL MODELS FOR THE DEVELOPMENT OF VACCINES FOR AQUACULTURE FISH

14.50 **Kerrie Ní Dhufaigh**, Eugene Dillon, Eugene MacCarthy, Orla Slattery
BIOCHEMICAL AND PROTEOMIC CHARACTERISATION OF EXTRACELLULAR PROTEINS FROM THE PROTOZOAN PARASITE *Paramoeba perurans* REVEALED BY AN *IN VITRO* MODEL

15.10 **Dongdong Wang**, Gilbert Van Stappen, Nancy Nevejan
FOLLOWING THE INFECTION PROCESS OF VIBRIOSIS IN PACIFIC OYSTER *Crassostrea gigas* AND BLUE MUSSEL *Mytilus edulis* LARVAE USING FLUORESCENCE LABELING AND HISTOPATHOLOGY METHODS

15.30 **David Straus**, T. Meinelt, D. Liu, L.-F. Pedersen, C. Good, J. Davidson
A RETROSPECTIVE OF OUR INTERNATIONAL RESEARCH COLLABORATION ON THE USE OF PERACETIC ACID IN AQUACULTURE

15.50 **Luisa M. Vera**, Eric Leclercq, Karina Gajardo, Mathieu Castex, Hervé Migaud
DEVELOPMENT OF A PRACTICAL REPETITIVE STRESS CHALLENGE MODEL FOR SEAWATER ATLANTIC SALMON *Salmo salar*

16.10 **Uun Yanuhar,** Nico Rahman Caesar, Feri Setiawan, Muhammad Musa
IDENTIFICATION OF Chlorella sp. USING THE INTERNAL TRANSCRIBED SPACER SEQUENCE AND ITS ROLE AS INHIBITOR STRESS CAUSED BY RNA VIRAL NERVOUS NECROSIS INFECTION ON THE HUMPBACK GROUPER

WEDNESDAY, October 9

PLENARY 2

Wednesday, October 9 09.00 - 10.00 Europe Hall
Chair: Stefan Meyer

09.00 Alexander Wever
PRODUCER, TRADE AND CONSUMER – A PRESENTATION/PANEL SESSION

10:00 BREAK

NUTRITION: ANIMAL INGREDIENTS IN AQUAFEED

Wednesday, October 9 10.30 - 12.50 Europe Hall
Chair: Luisa Valente
Sponsored by

AQUASOJA

10.30 Ana Basto, Margarida R.G. Maia, Jaume Perez-Sanchez, Josep Calduch, Elisabete Matos, Luísa M.P. Valente
DEFATTED YELLOW MEALWORM *Tenebrio molitor* LARVAE MEAL: A PROMISING FISHMEAL SUBSTITUTE FOR EUROPEAN SEABASS

10.50 Constant Motte, Morgane Henry
REPLACING FISH MEAL WITH DEFATTED INSECT MEAL (YELLOW MEALWORM *Tenebrio molitor*) IMPROVES THE GROWTH AND IMMUNITY OF EUROPEAN SEABASS *Dicentrarchus labrax* L.

11.10 Mónica Costa, Benjamin Costas, Marina Machado, Carla Teixeira, Sergio Fernández-Boo, Tiago Sá, Sónia Batista, Alexandra Marques, Fernando Miranda, Luísa M.P. Valente
ANCHOVY AND GIANT SQUID HYDROLYSATES CAN ENHANCE GROWTH AND THE IMMUNE RESPONSE OF EUROPEAN SEABASS *Dicentharcus labrax* FED VEGETABLE BASED-DIETS

11.30 Luisa Valente, Inês Campos, Elisabete Matos
LOCALLY PROCESSED LAND ANIMAL BY-PRODUCTS AS POTENTIAL CANDIDATES TO REPLACE FISHMEAL AND FISH OIL IN PRACTICAL DIETS FOR EUROPEAN SEABASS

11.50 Elisabete Matos, Inês Campos, Luísa M.P. Valente, Pedro Marques, Fausto Freire
LIFE-CYCLE ASSESSMENT OF ANIMAL FEED INGREDIENTS: POULTRY FAT, POULTRY BY-PRODUCT MEAL AND HYDROLYZED FEATHER MEAL

12.10 Jessica Jaxion-Harm
EFFECTS OF DIETARY PHOSPHOLIPIDS ON EARLY STAGE ATLANTIC SALMON *Salmo salar* PERFORMANCE: A COMPARISON AMONGST PHOSPHOLIPID SOURCES

12.30 Gabriella Do Vale Pereira, Felipe Soares, Ana Margarida Fernandes, Tome Silva, Bruna Silva, Jorge Dias, Bela Buck, Johan Johansen, Luis Eugenio Castanheira Conceicao
ASSESSMENT OF SUSTAINABLE AQUAFEED FORMULATIONS USING TWO APPROACHES: GROWTH TRIAL AND A DYNAMIC NUTRIENT-BASED MATHEMATICAL MODEL

FOOD QUALITY AND SAFETY

Wednesday, October 9 10.30 - 12.50 Room 5
Chair: Johannes Pucher

10.30 Valeska Weymann
EFFICIENT GUIDE TO PRODUCERS FOR GOOD AQUACULTURE PRACTICES AT ALL STAGES OF PRODUCTION

10.50 Miguel Angel Pardo, Andrea Gustinelli, Monica Caffara, Maria Letizia Fioravanti, Kurt Buchmann, Santiago Pascual, George Rigos, Csaba Székely, Diana Sandor, Gabor Cech
A EUROPEAN SURVEY ON ZOONOTIC HELMINTHS REVEALS A NEGLIGIBLE RISK OF INFECTION FROM FARMED FISH TO HUMANS

11.10 Andrew D. Younger, Robson V. de Sousa, Mickael Teixeira Alves, Carlos Campos
AN INVESTIGATION OF HOW THE CLASSIFICATION STATUS OF SHELLFISH PRODUCTION AREAS MAY BE AFFECTED BY THE NUMBER OF *Escherichia coli* RESULTS ASSESSED

11.30 Christian Schlechtriem
INVESTIGATIONS ON NATURE AND LEVEL OF RESIDUES IN FARMED FISH AS PART OF THE EUROPEAN PESTICIDE REGULATION

11.50 Johannes Pucher, K. EL Arbani, E. Strauch
MICROBIAL COMMUNITIES IN BIOFLOC SYSTEMS FOR WHITELEG SHRIMP *Litopenaeus vannamei*

12.10 Y.D. Ahongo, T. Kerneis, L. Goardon, L. Labbé, J. Bugeon, P-Y. Rescan, F. Lefèvre
FLESH QUALITY RECOVERY IN FEMALE RAINBOW TROUT *Oncorhynchus mykiss* AFTER EGG PRODUCTION

12.30 Olajumoke Omosowone, Sunday Olatoye
BIOACTIVITY OF GARLIC *Allium sativum* AGAINST *Dermestes maculatus* ON SMOKE-DRIED AFRICAN CATFISH *Clarias gariepinus*

SUSTAINABLE AQUACULTURE TECHNOLOGIES

Wednesday, October 9 10.30 - 12.50 Lounge 1-3
Chair: Alexander Brinker

10.30 Pauline O'Donohoe, Frank Kane, Joanne Casserly, Sean McLoughlin
TOOLS FOR NEW AND FLEXIBLE APPROACHES FOR AQUACULTURE LICENSING AND REGULATION

10.50 Kristina Bergman, Patrik Henriksson, Sara Hornborg, Max Troell, Malin Jonell, Friederike Ziegler
FARMING WARMWATER FISH ON LAND IN A COLD COUNTRY – A CRAZY IDEA OR A NEW SUSTAINABLE SEAFOOD SUPPLY CHAIN?

11.10 Hallstein Baarset, Johan Johansen
ECOINTENSIFICATION OF AQUACULTURE: INNOVATIVE MORTALITY DISPOSAL IN A CIRCULAR ECONOMY PERSPECTIVE

11.30 **Rui Rocha**, Sílvia Pires, Diogo Martins, Andreia Rodrigues, Pearl Ofoegbu, Vitória Pereira, Ana Costa, Amadeu Soares
COCKLES HARVESTING, TRANSPORT AND PACKAGING: HOW TO IMPROVE THE QUALITY OF PRODUCT IN CONSUMERS TABLE

11.50 **Anneliese Ernst**, Patrick Maurer, Uwe Waller
SOURCES AND SINKS OF BACTERIA IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

12.10 **Thomas Adams**, Dmitry Aleynik, Andrew Dale, Max Holloway, Keith Davidson
CHOOSING THE BEST LOCATIONS FOR SALMON AQUACULTURE SITES: INSIGHTS FROM BIOPHYSICAL MODELS AND THE MOVE TO MORE EXPOSED SITES

12.30 **Langley Gace**
THE CRITICAL SUCCESS FACTORS OF AN OPEN OCEAN FARMING OPERATION

GENERAL CONTRIBUTED FISH WELFARE

Wednesday, October 9 10.30 - 12.50 Backstage 1
Chair: Lina Weirup

10.30 **Joan Martorell-Ribera**, Mareen Nipkow, Torsten Viergutz, Christian Plinski, Tom Goldammer, Ulrike Gimsa, Alexander Rebl
STRESS HORMONES EVOKE CHARACTERISTIC EXPRESSION PROFILES IN SALMONID HEAD KIDNEY CELLS UNDER IMMUNE STIMULATION

10.50 **Constanze Pietsch**, Andreas Seitz, Nicola Rhyner, Marcel Arter, Linda Tschirren
NEW INSIGHTS INTO THE APPETITE GENE REGULATION IN COMMON CARP *Cyprinus carpio*

11.10 **Asa Johannesen**, Pascal Klebert, Øystein Patursson
THE EFFECT OF WAVES ON VERTICAL DISTRIBUTION OF SALMON IN AN EXPOSED SALMON CAGE

11.30 **Susana M.F. Ferreira**, Ana F. Ruas, Adriana G. Gaveta, Sílvia C. Gonçalves
ASSESSMENT OF FEED DOSES TO BE ADMINISTERED TO *Garra rufa* (HECKEL, 1843) UNDER AN ICHTHYOTHERAPY REGIMEN

11.50 **M. Carla Piazzon**, Fernando Naya-Català, Josep A. Calduch-Giner, Paula Simó-Mirabet, Amparo Picard-Sánchez, Ariadna Sitjà-Bobadilla, Jaume Pérez-Sánchez
SEX, AGE AND BACTERIA: HOW THE INTESTINAL MICROBIOTA IS MODULATED IN A PROTANDROUS HERMAPHRODITE FARMED FISH

12.10 **Josip Barisic**, Brian Quinn, S. Cannon
BLOOD BIOCHEMISTRY DURING SEAWATER TRANSFER: INVESTIGATION INTO THE CAUSES OF FAILED FISH SYNDROME IN RAINBOW TROUT *Oncorhynchus mykiss*

12.30 **Erik Sandblom**, Henrik Seth, Brankica Djordjevic, Lars Niklasson, Henrik Sundh, Bo Algers, Lotta Berg, Torbjörn Lundh, Michael Axelsson, Jeffrey Lines, Snuttan Sundell, Albin Gräns, Anders Kiessling
STUNNING OF ARCTIC CHAR *Salvelinus alpinus* WITH CARBON DIOXIDE AND ELECTRIC FIELD EXPOSURE: PHYSIOLOGICAL MECHANISMS OF ACTION AND WELFARE IMPLICATIONS

PRECISION FARMING, AI AND BIG DATA

Wednesday, October 9 10.30 - 16.30 Festival
Chair: Martin Fore

10.30 **Mohammadmehdi Saberioon**, Petr Cisar, Laurent Labbé, Pavel Soucek, Pablo Pelissier
APPLICATION OF HYPERSPECTRAL IMAGERY TO DISCRIMINATE DIFFERENT DIETS OF LIVE RAINBOW TROUT *Oncorhynchus mykiss*

10.50 **Dinara Bekkozhayeva**, Mohammadmehdi Saberioon, Petr Cisar
INDIVIDUAL PHOTO-IDENTIFICATION OF FISH WITH VISIBLE SKIN PATTERNS (SUMATRA BARB *Puntigrus tetrazona)* USING COMPUTER VISION

11.10 **Petr Cisar**, Mohammadmehdi Saberioon, Dinara Bekkozhayeva, Rudolf Schraml
COMPUTER VISION BASED FISH INDIVIDUAL IDENTIFICATION AS AN ALTERNATIVE TO FISH TAGGING

11.30 **Christian Schellewald**, A. Stahl, O. Markovic, M. Markovic, I. Hammerset, E. Moen, H. Trengereid, L.M. Sunde
TOWARDS AN INDIVIDUAL CHARACTERISATION OF FARMED SALMON

11.50 **Joao G. Ferreira**, Nick Taylor, Alhambra Cubillo, Joao Lencart e Silva, Roberto Pastres, Øivind Bergh, Nicola McPherson, James Guilder
THE ABC MODEL – A PARADIGM SHIFT IN INTEGRATED CARRYING CAPACITY MODELLING

12.10 **Stepan Papacek**, Petr Cisar, Karel Petera
CFD SIMULATION OF FLUID FLOW AND RTD ANALYSIS FOR AQUACULTURE SYSTEMS: WITH AND WITHOUT FISH

12.30 **Fearghal O'Donncha**, Scott C. James
A MACHINE-LEARNING-BASED APPROACH TO FORECAST HARMFUL ALGAL BLOOMS

12:50 **LUNCH**

14.30 **René Alvestad**, Kristian H. Liland, Ingrid Måge, Chris Noble, Bjørn-Steinar Sæther
CHARACTERISTICS AND QUALITY OF PRODUCTION DATA IN AN ATLANTIC SALMON *Salmo salar* L. PRODUCER'S LEGACY DATABASE

14.50 **Petter Olsen**
SHOULD THE AQUACULTURE INDUSTRY USE BLOCKCHAIN TECHNOLOGY FOR DATA RECORDING?

15.10 **Jon Grant**, R. Filgueira, M. Burke, C. Stockwell, E. Milos, L. Torgo, F. Donncha
FISH FARM SENSORS AND THE INTERNET OF THINGS

15.30 **Vagelis Chalkiadakis**, George Livanos, Michalis Zervakis, Konstantia Moirogiorgou, Nikos Papandroulakis
AN AUTONOMOUS UNDERWATER VEHICLE FOR AUTOMATED INSPECTION OF AQUACULTURE NET PEN CAGES

15.50 **Eleni Kelasidi**, Biao Su, Martin Føre, Kevin Frank, Walter Caharija, Christian Schellewald, Leif Magne Sunde
AUTONOMOUS UNDERWATER ROBOTS FOR SAFER AND MORE EFFICIENT OPERATIONS IN FISH FARMS

16.10 **Maxime Lafont**, Ambre Vallauri, Samuel Dupont, Charlotte Dupont
DIGITIZATION OF AQUACULTURE: REAL-TIME MONITORING AND PREDICTION FOR A SUSTAINABLE FUTURE

ENVIRONMENT / AQUACULTURE INTERACTIONS – FROM AND TO AQUACULTURE

Wednesday, October 9 10.30 - 17.30 Room 1
Chair: Shawn Robinson

10.30 **Christopher Naas**, Werner Kloas
META-ANALYSIS OF GROWTH PERFORMANCE OF FRESHWATER FISHES UNDER SALINE ENVIRONMENTS IN AQUACULTURE

10.50 **Renée K. Bechmann**, Maj Arnberg, Emily Lyng, Stig Westerlund, Shaw Bamber, Alessio Gomiero, Thorleifur Agustsson, Alfhild Kringstad, Les Burridge
SENSITIVITY OF NORTHERN SHRIMP *Pandalus borealis* TO COMMERCIAL FORMULATIONS USED AS ANTI-PARASITIC DRUGS

11.10 **Vidar Wennevik**, Kevin Glover, Ola Diserud, Øystein Skaala, Peder Fiske, Monica Solberg, Kjetil Hindar, Terje Svåsand, Sten Karlsson, Lasse Andersen, Ellen Grefsrud
ENVIRONMENTAL IMPACTS OF AQUACUTLURE: ASSESSING THE RISK OF FURTHER GENETIC INTROGERSSION OF DOMESTICATED SALMON IN WILD POPULATIONS IN NORWAY

11.30 **Erika M.D. Porporato**, Mattia Andreoletti, Giuseppe Arcangeli, Daniele Brigolin, Roberto Pastres
USING SATELLITE DATA FOR ASSESSING THE RISK OF FAECAL BACTERIA CONTAMINATION IN MUSSEL FARMS

11.50 **Jurica Jug-Dujakovic**, Damir Kapetanivic, Snježana Kazazic, Anamarija Kolda, Darija Vukic Lušic, Ana Gavrilovic
SEASONAL VARIATIONS IN THE ABUNDANCE OF *Vibrio* sp. IN THE MARINE CAGE FARM AREA OF MALI STON BAY, EASTERN ADRIATIC

12:50 **LUNCH**

14.30 **Shawn Robinson**, Benjamin Forward, Terralynn Lander, Jonathan Day
TRACKING THE MICROBIOME IN INVERTEBRATES AS A TOOL FOR OPTIMIZING CULTURE CONDITIONS

14.50 **Johannes Müller**, Natacha Nogueira, Carlos Andrade
THE REASONS BEHIND THE ESCAPES OF *Sparus aurata* FROM MADEIRA ISLAND (PORTUGAL) FISH FARMS – RISK ASSESSMENT THROUGH THE ANALYSIS OF ARCHIVE DATA

15.10 **Arnaldo Atucha**, Nuria García Bueno
RELATIONSHIP BETWEEN MODELS OF DISPERSION OF ORGANIC WASTE FROM FISH FARM AND THE VARIATION OF THE ISOTOPE NICHE AND BIOACCUMULATION OF TRACE ELEMENTS IN BIOFILM

15.30 **Tale Skrove**, Yngvar Olsen
EFFECTS OF NUTRIENT EMISSIONS FROM AN EXPOSED FISH FARM ON THE UPPER WATER COLUMN

15.50 **Peter Cranford**, Lindsay Brager, Deanva Elvines
TOWARDS A REVISED CLASSIFICTION SCHEME DESCRIBING BENTHIC IMPACTS OF MARINE AQUACULTURE BASED ON SEDIMENT GEOCHEMISTRY

16.10 **Brent A. Law**, Paul S. Hill, Adam Drozdowski, Fred Page
PROOF-OF-CONCEPT APPLICATION OF THE BENTHIC BOUNDARY LAYER TRANSPORT (BBLT) MODEL TO SALMON AQUACULTURE WASTE DISPERSAL

16.30 **Alessio Gomiero**, Kjell Birger Øysæd, Marte Haave, Tanja Kogel, Mona Gjessing, Trygve Berg Lea, Catarina Martens, Trude Olafsen
TRACKING OF PLASTIC EMISSIONS FROM AQUACULTURE INDUSTRY

16.50 **Marcelo Hidalgo**, Michiel Fransen
MINIMIZING THE IMPACT OF MARINE LITTER AND AQUACULTURE GEAR IN THE AQUACULTURE INDUSTRY GLOBALLY

17.10 **Maitri Thakur**, Wenhao Chen, Shraddha Mehta, Nicholas Holden, Gudrun Olafsdottir
IMPACT HOTSPOT ANALYSIS OF THE NORWEGIAN FARMED SALMON VALUE CHAIN

AQUAPONICS AND IMTA - CONTINUED

Wednesday, October 9 10.30 - 17.30 Room 2
Chairs: Ben Kozen, Hendrik Monsees

10.30 **Benz Kotzen**
'WHEREFORE ART THOU AQUAPONICS – THE HERD OF ELEPHANTS IN THE ROOM': FUTURE AQUAPONICS IN EUROPE TOWARDS SURVIVAL GROWTH AND SUCCESS

10.50 **H.W. Palm**, U. Knaus, S. Appelbaum, G. Burnell, B. Kotzen
THE NEW DEFINITION OF AQUAPONICS: FUTURE PERSPECTIVES AND CONSTRAINTS

11.10 **Hendrik Monsees**, Johanna Suhl, Maurice Paul, Werner Kloas, Dennis Dannehl, Sven Wuertz
LETTUCE *(Lactuca sativa*, VARIETY SALANOVA) PRODUCTION IN DECOUPLED AQUAPONIC SYSTEMS

11.30 **Mathilde Eck**, Iris Szekely, Sébastien Massart, M. Haïssam Jijakli
STUDY OF THE EVOLUTION OF MICROORGANISMS COMMUNITIES IN AN AQUAPONIC SYSTEM OVER THE COURSE OF A FULL LETTUCE GROWTH CYCLE

11.50 **Thomas Tomson**, M. Haïssam Jijakli
SAPRISTI, AN INNOVATIVE MODULAR TOOL IN THE IMPLEMENTATION OF A METHODOLOGY FOR THE RECOVERY OF FISH FARMING EFFLUENT BY AQUAPONICS

12.10 **Nlkos Vlahos**, Ioannis Mitsopoulos, Kostas Babouklis, Emmanouil Kapetanios, Stavros Frangou, Anastasios Manolios, Kalliopi Giannoulatou, Eirini Mardoglou
DESIGN, AQUASCAPE AND CONDITIONING OF A BRACKISH AQUAPONICS SYSTEM

12.30 **Anton Rossbach**
FRESH-WATER-PRODUCING SEAWATER-AQUAPONICS AS A SOLUTION FOR THE DRAUGHT IN THE NORTHEAST OF BRAZIL

12.50 **LUNCH**

14.30 **Benoît Stalport**, Pierre Raulier, Nicolas De Cock, Frédéric Lebeau, M. Haissam Jijakli
DEVELOPMENT OF AN INDIVIDUAL-BASED GENERIC AQUAPONIC MODEL USING OBJECT ORIENTED PROGRAMMING

14.50 **Raulier Pierre**, Benoît Stalport, Frederic Lebeau, Doriane Stagnol, Caroline Bini, Bart Leenknegt, Thomas Abeel, Sara Crappé, Nick Pannecouque, Germain Desmet, Christophe Hermans, Noémie Lardinois, Charlotte Boeckaert, Herinaina Andriandroso, Jad Nassard, Bertrand Vandoorne, Vincent Lefevere, Haissam Jijakli
SMART AQUAPONICS: DEVELOPMENT OF A TOOL FOR EDUCATION, DECISION SUPPORT AND MONITORING FOR AQUAPONICS

15.10 **Alexander Boedijn**, Esteban Baeza, Rob Van den Ven, Carlos Espinal, Ragnheidur Thorarinsdottir, Maja Turnšek
MODELLING AND DIMENSIONING AQUAPONICS AS THERMAL TREATMENT NETWORKS THAT OPTIMIZE GEOTHERMAL ENERGY USE

15.30 **Simon Goddek**, Alyssa Joyce, Oliver Koerner, Karel Keesman
A FULLY INTEGRATED SIMULATION MODEL OF MULTI-LOOP AQUAPONICS: PUTTING THEORY INTO PRACTICE

15.50 **Edson Panana**, B. Delaide, P. Bleyaert, S. Teerlinck
ORGANIC REDUCTION AND NUTRIENT RECOVERY PERFORMANCES OF PIKEPERCH *Sander lucioperca* SLUDGE AEROBIC DIGESTION AND LETTUCE GROWTH PERFORMANCE IN ITS EFFLUENTS

16.10 **U. Knaus**, H.-D. D. Hübner, H.W. Palm
AQUAPONICS (S.L.) PRODUCTION OF SPEARMINT *Mentha spicata* AND BASIL *Ocimum basilicum* WITH BIOHUMIN AND AFRICAN CATFISH *Clarias gariepinus*

16.30 **Gilles Stouvenakers**, Sébastien Massart, M. Haïssam Jijakli
AQUAPONIC WATER SUPPRESSIVENESS ON *Pyhtium aphanidermatum* ROOT ROT IN LETTUCE AND ITS ORIGIN

16.50 **Sarah Milliken**, Benz Kotzen, Ranka Junge, Morris Villarroel, Tjasa Griessler Bulc
AQU@TEACH: INNOVATIVE EDUCATIONAL TOOLS TO PROMOTE LEARNING AMONG EUROPEAN STUDENTS USING AQUAPONICS

17.10 **Luigi Petrocchi Jasinski**, G. Galliano, L. Rossi
TOWARDS SUSTAINABLE AWARENESS: IMPLEMENTATION OF AN AQUAPONIC CODE OF PRACTICE

AE2019 INNOVATION FORUM

Wednesday, October 9 10.30 - 17.30 Exhibition 1
Chair: Stefan Meyer

Aquaculture development requires upscaling production systems and developing new solutions for producing more to supply a growing market with healthy products. EAS wants to promote and support start-ups and emerging business models in the aquaculture sector, by matching ideas to those that can push the development of new companies and new products. With all the new knowledge presented at our events, we have a wealth of possibility for innovation and value creation to help develop the sector.

This is the EAS Aquaculture Europe Innovation Forum and its inaugural event will take place during our AE21019 event in Berlin on Wednesday, October 9th. In partnership with Hatch Blue and the German Startups Association, the day will be made up of introductory speakers and then pitches from 12 new companies that wish to seek further investment to develop. Speakers include Olaf Birkner on "raising awareness for your start-up"; "What an investor looks for in a start-up" from Viggo Halseth, Chief Innovation Officer, Nutreco and the "European Innovation Council: a one-stop-shop to high-potential innovators" from Sigi GRUBER, Head of the Healthy Oceans & Seas Unit, in the Directorate General for Research and Innovation at the European Commission. A teaser on 'lessons learned' will be provided by Rob van de Ven, Landing Aquaculture, The Netherlands and former EAS Student Group chair.

10.30 Short introduction and welcome messages from our partners:
"Dynamic AgriTech Eco-System - Focus: Marine and Aquaculture",
Dominik Ewald, German Startups Association
Wayne Murphy, COO and co-founder HATCH Aquaculture Accelerator
Session I TECHNOLOGY Chair Stefan Meyer
10.40 "How to get yourself noticed - Raising awareness for your start-up"
Olaf Birkner - Tech founder and advisor digital marketing and innovations
11.00 Introduction of panellists that will judge the pitches of this session
Dominik Ewald – Monitorfish, Angela Schultz-Zehden – Sustainable Projects
Lucille Bonnet – High-Tech Gründerfonds and Margriet Drouillon – Gent University

11.10 Microbia Environnement – Early warning for Harmful Algal Blooms (HABs)
11.20 Networking Break
11.30 Umitron – Data services (IoT, remote sensing, AI…)
11.40 Tellspec – Fish quality control and fraud
11.50 onCyt – Real time monitoring of micro-organisms
12.10 Teaser: Rob van de Ven - Landing Aquaculture
12.20 Watergenics – Sensors for dissolved oxygen and nutrients
12.30 Inalve – Concentrated microalgal production
12.40 Wrap-up of session. Lunch break (on your own). One-to-one meetings.
Session II: PRODUCTION Chair Bjorn Myrseth
14.10 "What an investor looks for in a start-up"
Viggo Halseth, Chief Innovation Officer Nutreco
14.30 Session II Introduction of panellists that will judge the pitches of this session
Viggo Halseth – Nutreco, Robin Shields – Scottish Aquaculture Innovation
Harald Sveier - Lerøy Seafood Group and Johan Verreth – EAS Past President
14.40 SEAentia – Land based production of meagre
14.50 SEADUCER – Production equipment for oysters
15.10 Arbiom – Feed protein source from wood
15.20 Genetirate – selective breeding services (HATCH)

15.30 Univiv (HATCH)
15.40 Networking Break
15.50 Dynaspace – algae detection by state-of-the-art satellite remote sensing (HATCH)
16.00 NSS - Nitrogen Sensing Solutions – multiparametric sensor for water analysis (HATCH)
16.20 Aquaculture Health Lab – Rapid diagnostics
16.30 Tunatech – Blue biofouling bioresources
16.40 Panel deliberations
16.40 "European Innovation Council: a one-stop-shop to high-potential innovators"
Sieglinde Gruber, European Commission DG R&I
16.55 Session chairs wrap up and announce the winners of the AE2019 Innovation Forum
17.30 AE2019 Happy Hour in Exhibition Area

EU EATIP DAY – LOW IMPACT – HIGH OUTPUT
Promoting food security and new value chains in aquaculture

Wednesday, October 9 10.30 - 17.30 Exhibition 2
Chair: Sigi Gruber, Alexandra Neyts

Aquaculture in Europe provides safe food of the highest quality and nutritional value, across a wide range of products adapted to consumer preferences and lifestyles. European policies and strategies recognise its potential as a full-fledged sector of the blue bioeconomy and as an essential approach to acquiring global food and nutrition security. Future-proofing our food systems in terms of sustainability, resilience and competitiveness, calls for strengthening circularity and resource efficiency.

According to the report "Food from the Oceans"[1], the biggest potential for increasing seafood production is through mariculture, especially at lower levels in the ocean food chain. In addition, the production of shellfish, algae and other low-trophic organisms contributes to carbon sequestration and eutrophication mitigation. It is therefore important to consider their suitability to produce food and feed, as well as other high value compounds for use in cosmetics, personal care, pigments, etc. Other ways of supporting the circular bio-economy are the use of waste from one aquatic species as feed for another, and the introduction of alternative feed ingredients from insects, unicellular organisms, fungi, wood and other terrestrial plants.

As a joint effort, the Healthy Oceans & Seas Unit of the European Commission, Directorate General Research and Innovation (DG RTD), and the European Aquaculture Technology and Innovation Platform (EATiP) are setting focus on the impact of European research for the benefit of aquaculture development within Europe as well as worldwide. Through three consecutive sessions, they present some major outcomes of technology and innovation efforts that contribute to tackle the bottlenecks and opportunities in aquaculture towards the overall goal of ensuring food and nutrition security. Pitching the latest results from EU funded projects will be followed up by industry-driven panel discussions.

AQUAEXCEL[2020] is a key aquaculture research project funded by the EU, which integrates leading European aquaculture research facilities that work towards bringing aquaculture research in Europe to a new level. The latest industry-relevant outputs will be presented and discussed at an interactive brokerage session, creating a forum for engagement and exchange between aquaculture researchers and potential industry beneficiaries of these innovative aquaculture outcomes.

10:30 SESSION 1: Setting the scene – How can European research and innovation stimulate sustainability and competitiveness of the aquaculture sector?
Policy context and stakeholders' engagement driving food security and new value chains in aquaculture. Incl. introductory talks by the European Commission and EATiP.

11:25 SESSION 2 – Low trophic production technologies and new feed resources
New findings, future bottlenecks and opportunities on sustainable production of novel food, feed and added value products from alternative low-trophic resources.

14:10 SESSION 3 – Efficient management systems to optimize sustainability and competitiveness
Sustainable, resilient, climate friendly and competitive practices through eco-intensification, intelligent management and new systems for the production of low-trophic species.

NORTH EUROPEAN FISH SPECIES PARASITE MANAGEMENT STRATEGIES

Wednesday, October 9 10.30 - 17.30 Backstage 2
Chair: Emma Bello Gomez

Disease prevention and management are essential for the sustainability of the European aquaculture industry. The diversity of species and farming practices throughout Europe involves a significant number of threats related to a large variety of pathogens that hamper production and require specific preventive and curative practices and tools ensuring a high level of biosecurity of aquaculture production and related seafood products. Among other disease-related threats, parasites and related infections can cause significant damages to farmed fish species and can result in poor growth performance, impaired welfare, and high mortality rates with significant consequences in terms of production and economic performance.

Over the last four years, the EU-funded project **ParaFishControl** (634429) has been working to develop innovative solutions and tools for the prevention, control and mitigation of the most harmful parasitic species affecting the main European farmed fish species, namely: Atlantic salmon, rainbow trout, turbot, gilthead seabream, European sea bass and common carp.

The **"North European Fish Parasite Management Strategies in Aquaculture Farms" workshop** will focus on the parasites which have been most damaging for fresh water and marine farmed species in North Europe (salmon, carp and trout) - *Lepeophtheirus salmonis*, *Tetracapsuloides bryosalmonae*, *Sphaerospora molnari*, *Ichthyophthirius multifiliis*, *Paramoeba perurans* and *Saprolegnia parasitica* as well as zoonotic helminths. During the workshop, industry representatives will provide an overview of the most prevalent issues related to parasitic diseases in North European aquaculture farms. **ParaFishControl** partners will then present the **new tools and techniques** developed within the project to **diagnose, prevent and treat** these diseases. The workshop will provide attendees with new knowledge to better manage their farms, and greatly reduce population loss in a cost-effective way.

More information about the event and full programme of the ParaFishControl workshop will be available online at: https://bit.ly/2IwQw3J

AE2019 INNOVATION FORUM - (1 TO 1 MEETINGS)

Wednesday, October 9 10.30 - 17.30 Backstage 3
Chair: Stefan Meyer

GENERAL CONTRIBUTED NUTRITION

Wednesday, October 9 10.30 - 17.30 Foyer 4
Chair: Andrew Richardson

10.30 **Barbara Rossi**, Maria Angéles Esteban, José María García-Beltrán, Alberto Cuesta, Andrea Piva, Ester Grilli
ANTIOXIDANT EFFECT OF THYMOL AND SORBIC ACID IN FISH HEPATOCYTES*IN VITRO*

10.50 **Florence Perera**, Bjørn Grønevik, Mette Sørensen, Ørjan Hagen
THE EFFECT OF TOTAL REPLACEMENT OF FISH OIL WITH RAPESEED OIL ON GROWTH, BODY COMPOSITION AND GUT HEALTH OF JUVEILE LUMPFISH *Cyclopterus lumpus*

11.10 **Kamil Mert Eryalçin**, Marisol Izquierdo
VITAMIN AND MINERAL COMPOSITIONS OF THE ROTIFER *Brachionus plicatilis* (MÜLLER, 1786) CULTURED WITH DIFFERENT FEEDS

11.30 **Erik Höglund**
ANTI-INFLAMMATORY FEEDS AND TRYPTOPHAN METABOLIC PATHWAYS TO STRESS RESILIENCE IN FISH

11.50 **Susana M.F. Ferreira**, Gonçalo Branco, Pedro M. Santos, Andreia Raposo, Ana Pombo
EFFECTS OF VEGETABLE DIETS ON THE GROWTH, GAMETOGENIC AND OFFSPRING DEVELOPMENT OF THE SEA URCHIN *Paracentrotus lividus* (LAMARCK, 1816)

12.10 **Monica B. Betancor**, Alexa MacEwan, Matthew Sprague, Daniel Montero, Fernando Norambuena, Olga Sayanova, Lihua Han, Johnathan Napier, Marisol Izquierdo, Douglas R. Tocher
FEASIBILITY OF AN OIL DERIVED FROM A GM-OILSEED CROP AS A SUBSTITUTE FOR FISH OIL IN FEEDS FOR EUROPEAN SEA BASS *Dicentrarchus labrax*

12.30 **Zainal Abdin Muchlisin**, Abdullah A. Muhammadar, Nur Fadli, Kartini Eriani, Irma Dewiyanti, Mahfud Mahfud
APPLICATION OF PROBIOTIC AND PAPAIN ENZYME IN THE DIET OF TROPICAL SHORTFIN EEL *Anguilla bicolor*

12.50 **LUNCH**

14.30 **Xiaoting Zheng**, Adam F. Feyaerts, Patrick Van Dijck, Peter Bossier
EVALUATION OF THE ANTI-VIBRIO ACTIVITY OF ESSENTIAL OILS AND THEIR COMPONENTS IN GNOTOBIOTIC ARTEMIA TEST SYSTEM

14.50 **Sjo Zwart**, Frank Ruyseveldt
EVALUATING THE EFFECT OF DIFFERENT FEED PHOSPHATES ON BLOOD PARAMETERS, CORPSE ANALYSIS, INTESTINAL HISTOLOGY AND GROWTH RATE OF KOI CARP *Cyprinus carpio*

15.10 **Amal Biswas**, Fumiaki Takakuwa, Shinichi Yamada, Akihisa Matsuda, Hiroki Kihara, Jonas Miller, Josh Silverman, Renee Saville, Allan LeBlanc, Hideki Tanaka
METHANOTROPH *Methylococcus capsulatus* MEAL AS AN ALTERNATIVE PROTEIN SOURCE FOR YELLOWTAIL *Seriola quinqueradiata*

GENOMIC RESEARCH, TOOLS AND APPLICATIONS

Wednesday, October 9 10.30 - 17.30 Room 3
Chairs: Klaus Kohlmann, Ross Houston

10.30 **Yang Jin**, Rolf Erik Olsen, Thomas Nelson Harvey, Mari-Ann Østensen, Keshuai Li, Nina Santi, Olav Vadstein, Jon Olav Vik, Simen Rød Sandve, Yngvar Olsen
DOMESTICATION-ASSOCIATED LIPID METABOLISM REGULATION IN ATLANTIC SALMON

10.50 **Manu K. Gundappa**, A.C. Bertolotti, R.M. Layer, M.D. Gallagher, C.M. Hollenbeck, T. Nome, S.R. Sandve, D. Robledo, R.D. Houston, I.A. Johnston, S. Lien, D.J. Macqueen
THE STRUCTURAL VARIATION LANDSCAPE IN ATLANTIC SALMON AND IT'S POTENTIAL CONTRIBUTION TO DISEASE RESISTANCE

11.10 **Nicholas Robinson**, Luqman Aslam, Diego Robledo, Ross Houston, Hooman Moghadam, Borghild Hillestad, Matthew Kent, Matthew Baranski, Aleksei Krasnov, Solomon Boison
QTL AND EQTL FOR SALMONID ALPHAVIRUS VIRAL LOAD IN ATLANTIC SALMON – IMPLICATIONS FOR PANCREAS DISEASE RESISTANCE AND TOLERANCE

11.30 **Muhammad Luqman Aslam**, S.A. Boison, C. Jacq, O.J. Hansen, V. Puvanendran, A. Mortensen, P. Sae-Lim
GENETICS OF LICE EATING ABILITY IN LUMPFISH *Cyclopterus lumpus* USING ddRAD SEQUENCING

11.50 **Jan Pawlowski**, Kristina Cermakova, Tristan Cordier
MONITOR THE ENVIRONMENTAL IMPACTS THROUGH ARTIFICIAL INTELLIGENCE APPLIED TO METAGENOMICS

12.10 **Emilie Cardona**, Jérôme Montfort, Hugues Parinello, Laurent Journot, Julien Bobe, Sandrine Skiba-Cassy
CIRCULATING MICRORNA IN PLASMA AND OVARIAN FLUID AS POTENTIAL NON INVASIVE BIOMARKERS OF NUTRITION AND REPRODUCTION OF FEMALE RAINBOW TROUT *Oncorhynchus mykiss*

12.30 **Fabio Sarais**, Henrike Rebl, Marieke Verleih, Bernd Köllner, Alexander Rebl, Tom Goldammer
MOLECULAR CHARACTERIZATION OF THE NKIRAS PROTEIN FAMILY IN RAINBOW TROUT *Oncorhynchus mykiss*

12.50 **LUNCH**

14.30 **Mikhail Ozerov**, Anti Vasemägi, Riho Gross
DECIPHERING THE GENOME OF THE RIVER MONSTER – THE EUROPEAN CATFISH *Silurus glanis*

14.50 **Daniel Zarski**, Aurelie Le Cam, Joanna Nynca, Christophe Klopp, Slawomir Ciesielski, Jerome Montfort, Pascal Fontaine, Andrzej Ciereszko, Julien Bobe
TRANSCRIPTOMIC PROFILING OF EGGS OF PIKEPERCH *Sander lucioperca* REVEALS NOVEL EGG-QUALITY-ASSOCIATED TRANSCRIPTS

15.10 **Carolina Peñaloza**, Tereza Manousaki, Rafaella Franch, Alexandros Tsakogiannis, Anna Sonesson, Luca Bargelloni, Costas Tsigenopoulos, Ross Houston
DEVELOPMENT OF A COMBINED SPECIES SNP ARRAY FOR THE EUROPEAN SEA BASS AND THE GILTHEAD SEA BREAM

15.30 **Anna K. Sonesson,** Siri Storteig Horn, Theo Meuwissen, Aleksei Krasnov, Borghild Hillestad, Hooman Moghadam, Bente Ruyter
GENOMICS OF OMEGA-3 FATTY ACID CONTENTS IN ATLANTIC SALMON

15.50 **Luca Bargelloni**, Marianna Pauletto, Serena Ferraresso, Massimiliano Babbucci, Giuseppe Radaelli, Lisa Maccatrozzo, Daniela Bertotto
MUSCLE TRANSCRIPTOME RESPONSE TO FASTING-REFEDING IN FAST-GROWING COMPARED TO AGE- OR SIZE-MATCHED SLOW GROWING INDIVIDUALS IN THE GILDHEAD SEA BREAM

16.10 **Tom L. Jenkins**, Charlie D. Ellis, Carly L. Daniels, Eduarda M. Santos, Jamie R. Stevens
DETERMINING THE HERITABILITY OF GROWTH TRAITS IN CULTURED EUROPEAN LOBSTERS USING GENOMICS

16.30 **Inés González-Castellano**, Chiara Manfrin, Alberto Pallavicini, Ana M. González-Tizón, Andrés Martínez-Lage
NOVEL INSIGHTS INTO SEX-RELATED GENES IN THE COMMON LITTORAL SHRIMP *Palaemon serratus* BY GONADAL TRANSCRIPTOMIC ANALYSIS

16.50 **Jeremiah Minich**, Eric Allen, Rob Knight
CONSIDERATIONS FOR MICROBIOME RESEARCH IN AQUACULTURE: OVERCOMING CHALLENGES WITH LOW BIOMASS ENVIRONMENTS, REDUCING COSTS, ADDRESSSING TECHNICAL NOISE, AND META-ANLAYSES

17.10 **Julien Nguinkal**, Lidia de los Ríos-Pérez, Nadine Schäfer, Frieder Hadlich, Marcus Stüecken, Olaf Wolkenhauer, Ronald M. Brunner, Alexander Rebl, Marieke Verleih, Dörte Wittenburg, Tom Goldammer
THE DRAFT GENOME OF PIKEPERCH *Sander lucioperca*

VALUE ADDITION AND MARKETING

Wednesday, October 9 14.30 - 17.30 Room 5
Chairs: Marija Banovic, Johannes Simons

14.30 **Johan Johansen**, Lars Svenningsson, Hallstein Baarset
ECOINTENSIFICATION OF AQUACULTURE: CAPTURE AND VALORISATION OF SLUDGE FROM AQUACULTURE WASTEWATER

14.50 **Andreas Müller-Belecke**, Nicolas Borchert, Rafael Valbuena, Adam Erdös
FISHMEAL FROM ENSILAGED FRESHWATER FISH PROCESSING BY-PRODUCTS IN A RAINBOW TROUT DIET: GROWTH PERFORMANCE, PRODUCT QUALITY AND ENVIRONMENTAL IMPACT

15.10 **Sara Ramírez-Bolaños**, S. Díaz, N. Díaz-Padilla, A. Ventura-Castellano, R. Quirós-Pozo, L. Robaina
NOVEL BANANA BY-PRODUCTS IN SEABASS *Dicentrarchus labrax* DIETS

15.30 **Jose Luis Fernández Sánchez**, Jose Manuel Fernández Polanco, Ignacio Llorente García, Elisa Baraibar Diez, Maria Dolores Odriozola Zamanillo, Manuel Luna García, Ladislao Luna Sotorrio
THE EFFECT OF PRICE AND PRODUCTION CHANGES ON FIRMS' PROFITABILITY AND RISK: A SIMULATION WITH EUROPEAN PRODUCERS OF CULTURED SEA BASS AND SEA BREAM

15.50 **Guilherme Wolff Bueno**, Maicon R. Brande, Naor Silveira Fialho, Elisa M. Godoy, Pablo Gallardo Ojeda, Rodrigo Roubach
FINANCIAL RISK EVALUATION OF COMMERCIAL PRODUCTION OF NILE TILAPIA *Oreochromis niloticus* IN NET-CAGES, SÃO PAULO, BRAZIL

16.10 **Marija Banovic**, Machiel Reinders, Anna Claret, Luis Guerrero, Athanasios Krystallis
WHAT SEPARATES WINNERS FROM LOSERS? EVIDENCE FROM A CROSS-COUNTRY INVESTIGATION ON CONSUMER ACCEPTANCE OF NEW AQUACULTURE PRODUCTS

16.30 **Johannes Simons**, Carl Vierboom, Michael Ley, Stefan Johnigk
FISH QUALITY: A PSYCHOLOGICAL PERSPECTIVE

16.50 **Sofia C. Franco**, Sharron Kuznesof, Bruna Simões, Beth Clark, Peter Jackson
TOWARDS IMPROVED COMMUNICATION IN AQUACULTURE: EXPLORING CONSUMERS' PERCEPTIONS AND ATTITUDES

17.10 **Johannes Simons**, Carl Vierboom, Michael Ley, Stefan Johnigk
CHALLENGES OF CONSUMER ORIENTED COMMUNICATION ABOUT SCIENTIFICALLY BASED SUSTAINABILITY STANDARDS FOR AQUACULTURE

FUNCTIONAL AQUAFEEDS

Wednesday, October 9 14.30 - 17.30 Europe Hall

Chair: Pedro Gomez Requeni

Sponsored by Biomar

14.30 **Jie Wang**, Peng Lei, Amr A.A. Gamil, Leidy Lagos, Yang Yue, Kristin Schirmer, Liv Torunn Mydland, Margareth Øverland, Åshild Krogdahl, Trond M. Kortner
RAINBOW TROUT *Oncorhynchus mykiss* INTESTINAL EPITHELIAL CELLS AS A MODEL FOR STUDING GUT IMMUNE FUNCTION AND EFFECTS OF FEED ADDITIVES

14.50 **Carmen Tatiana Kalinowski**, L.E. Robaina, L. Larroquet, V. Veron, S. Kaushik, S. Fontagné-Dicharry
INFLUENCE OF DIETARY ASTAXANTHIN ON THE OXIDATIVE STRESS RESPONSE CAUSED BY EPISODIC HYPEROXIA IN RAINBOW TROUT

15.10 **Paul J. Midtlyng**
ANTI-INFLAMMATORY EFFECTS OF BETA-1,3/1,6 GLUCAN SUPPLEMENTED FEED IN ATLANTIC SALMON *Salmo salar*

15.30 **Nicola Tallarico**, Christophe Bodenreider, Geoff Horst
POSITIVE EFFECTS OF SUPPLEMENTING ALGAL 1,3-BETA GLUCAN ON THE SURVIVAL OF ATLANTIC SALMON *Salmo salar* IN CHALLENGE CONDITIONS

15.50 **Yanxian Li**, Leonardo Bruni, Alexander Jaramillo-Torres, Trond Kortner, Elvis Chikwati, Ikram Belghit, Erik-Jan Lock, Åshild Krogdahl
GUT HEALTH AND MICROBIOTA IN POST-SMOLT ATLANTIC SALMON *Salmo salar* FED LARVAE MEAL FROM BLACK SOLDIER FLY *Hermetia illucens*

16.10 **Nafsika Karakatsouli**, Kostas Domalis, Socratis Panopoulos, Antonio Coli, Constantina Anastasiadou, Maria Rati, Spyros Bantounas, Alkisti Batzina
INVESTIGATION OF EARLY INTRODUCTION OF DRY FEED ON GILTHEAD SEABREAM *Sparus aurata* LARVAL DEVELOPMENT AND GROWTH PERFORMANCE AFTER METAMORPHOSIS

16.30 **Delano Dias Schleder**, Priscila Costa Rezende, Felipe Nascimento Vieira
COMBINATIONS OF BROWN SEAWEEDS AS FEED ADDITIVES IMPROVED THERMAL SHOCK RESISTANCE OF PACIFIC WHITE SHRIMP POST-LARVAE REARED IN BIOFLOC SYSTEM

16.50 **Ricardo Pereira**, Luísa Valente, Sónia Batista
ANTIOXIDANT AND IMMUNE-MODULATORY POTENTIAL OF MARINE ALGAE IN DIETS FOR EUROPEAN SEABASS

17.10 **Leong-Seng Lim**, Rossita Shapawi, Gunzo Kawamura
AMINO ACIDS AS FEEDING STIMULANT IN THE DEVELOPMENT OF SOYBEAN-BASED DIET FOR JUVENILE GROUPER *Epinephelus fuscoguttatus*

GENERAL BIOLOGY OF FARMED SPECIES

Wednesday, October 9 14.30 - 17.30 Lounge 1-3

Chair: Ivo Monteiro

14.30 **Orestis Stavrakidis-Zachou**, Konstadia Lika, Jorge Alarcon, Abdulaziz M. Al-Suwailem, Nikos Papandroulakis
BIOLOGICAL PERFORMANCE OF MEAGRE *Argyrosomus regius* UNDER HIGH TEMPERATURE

14.50 **Dieter Steinhagen**, M. Adamek, M. Dietrich, F. Teitge, M. Ganter, I. Baumann, V. Jung-Schroers, V. Piackova, G. Gela, M. Kocour, A. Ciereszko, D. Steinhagen
THE MULTIFUNCTIONAL GILL – THE ACHILLES' HEEL OF FISH HEALTH?

15.10 **Smaragda Tsairidou**, A. Hamilton, D. Robledo, J. Bron, R.D. Houston
OPTIMISING GENOMIC SELECTION WITH LOW DENSITY MARKERS IN FARMED ATLANTIC SALMON

15.30 **Benan Gulzari**, Kasper Janssen, Hans Komen
IDENTIFICATION OF FACTORS EXPLAINING THE GROW-OUT PERFORMANCE OF EUROPEAN SEABASS *Dicentrarchus labrax* AND GILTHEAD SEABREAM *Sparus aurata* IN THE MEDITERRANEAN

15.50 **Sergio Vela-Avitúa**, Ingunn Thorland, Vasileios Bakopoulos, Kantham Papanna, Arkadios Dimitroglou, Leonidas Papaharisis, Bruno Guinand
ADVANCED SELECTIVE BREEDING FOR DISEASE RESISTANCE IN EUROPEAN SEA BASS *Dicentrarchus labrax*

16.10 **Laureana Rebordinos**, Aglaya Garcia-Angulo, Emilio García, Silvia Portela-Bens, María Esther Rodríguez, Belen Molina, Manuel Alejandro Merlo, Marco Anaya, Alberto Arias, Ismael Cross
CONSTRUCTION ON AN INTEGRATED GENETIC MAP OF BENEFIT TO THE *Solea senegalensis* AQUACULTURE

16.30 **Carlos Andrade**, Natacha Nogueira, Mariana Martins
MONITORING IMPACTS OF MACROPLASTICS IN MADEIRA ISLAND'S OFFSHORE FISH FARM OF *Sparus aurata*

16.50 **Maria Mastoraki**, Lydia Katsika, Efthimia Antonopoulou, Laura Gasco, Stavros Chatzifotis
THE EFFECT OF FISHMEAL SUBSTITUTION WITH THREE DIFFERENT INSECT MEALS ON GROWTH PERFORMANCE, NUTRIENT UTILIZATION AND DIGESTIBILITY OF GILTHEAD SEA BREAM *Sparus aurata*

NUTRITION: ADDITIVES AND INGREDIENTS - CONTINUED

Wednesday, October 9 14.30 - 17.30 Backstage 1
Chair: Katerina Kosoulaki, Kiron Viswanath

BD # 356 **Luca Grosso**, Alessandra Fian*chini*, Stefano Cataudella, Michele Scardi, Arnold Rakaj
EFFECTS OF DIFFERENT DIETS ON THREE SIZE CLASSES OF SEA URCHIN *Paracentrotus lividus*

14.30 **Marialena Kokkali,** Francisco J. Barba, Francisco J. Marti-Quija, Dorinde Kleinegris, Katerina Kousoulaki
RELEASE OF PHENOLICS AND OTHER ANTIOXIDANTS FROM MICROALGAE *Phaeodactylum tricornutum* AND *Tetraselmis chuii* FOLLOWING BEAD MILLING

14.50 **Alessandra Roncarati**, Marina C.T. Meligrana, G. Enrico Magi, Francesca Mariotti
RAINBOW TROUT *Oncorhynchus mykiss* GROWING TRIAL USING DIETS WITH DIFFERENT DOSAGES OF THE SAME ADDITIVE

15.10 **Maria Angéles Esteban**, Barbara Rossi, José María García-Beltrán, Alberto Cuesta, Andrea Piva, Ester Grilli
DOSE-EFFECT RESPONSE OF A BLEND OF ORGANIC ACID AND NATURE-IDENTICAL COMPOUNDS ON GILTHEAD SEABREAM *Sparus aurata* L.

15.30 **Roel Maas**, Marc Verdegem, Yueming Dersjiant-Li, Johan Schrama
IMPACT OF NSP LEVEL AND ENZYMES (PHYTASE AND XYLANSE) ON NUTRIENT UTILIZATION, GROWTH PERFORMANCE IN NILE TILAPIA *Oreochromis niloticus*

15.50 **Frederik Kaiser**, Carsten Schulz
EFFECTS OF RAPESEED PROTEIN PRODUCTS SUPPLEMENTED WITH GLUCOSINOLATES AND PHYTIC ACID ON THE GROWTH PERFORMANCE OF RAINBOW TROUTS *Oncorhynchus mykiss*

16.10 **Heba Abdel-Ghany**, Mohamed Salem, Samia Abu-Elkhair
USE OF AN EXOGENOUS ENZYMES AND PROBIOTICS COCKTAIL TO ENHANCE THE GROWTH PERFORMANCE OF RED TILAPIA *Oreochromis niloticus* × *O. mossambicus* AND ELIMINATING THE BACTERIAL COUNT IN ITS EFFLUENTS

16.30 **Trine Ytrestøyl**, Bente Ruyter, Bjarne Hatlen, Elena Shumilina, Allessandra Ciampa, Marta Bou, Aleksei Krasnov, Tone-Kari Østby, Alexander Dikiy
HOW DIET COMPOSITION AND ENVIRONMENTAL FACTORS AFFECT THE UPTAKE AND METABOLISM OF ASTAXANTHIN IN ATLANTIC SALMON *Salmo salar*

SUSTAINABLE EUROPEAN AQUACULTURE 4.0: NUTRITION AND BREEDING INNOVATIONS

Wednesday, October 9 14.30 - 17.50 Room 30341, Wing 3, Estrel Hotel
Chair: Antti Kuse

Session: Sustainable European aquaculture 4.0: nutrition and breeding innovations

In recent years, advancements in enabling technologies have led to completely novel innovations that are now being implemented within the aquaculture industry. This session presents the latest developments in the interacting fields of digital information technology, genomics and circular economy applied in fish feeding, nutrition and selective breeding. They together produce more sustainable aquaculture practices and more robust, healthy, nutritious and resource-efficient farmed fish. The results originate from three Horizon 2020 EU-projects, AquaIMPACT, FutureEUAqua and iFishIENCi, funded under the topic 'Sustainable European Aquaculture 4.0: Nutrition and Breeding Innovations'. In these complementary projects, researchers and companies together develop more sustainable aquaculture corresponding to the UN's Sustainable Development Goals.

14.30 Antti Kause
AquaIMPACT – GENOMIC AND NUTRITIONAL INNOVATIONS FOR GENETICALLY SUPERIOR FARMED FISH

14.50 Lars Ebbesson, Dominique Durand, Tamás Bardócz
iFishIENCi: INTELLIGENT FISH FEEDING THROUGH INTEGRATION OF ENABLING TECHNOLOGIES AND CIRCULAR PRINCIPLES

15.10 Åsa Maria Espmark
FutureEUAqua – FUTURE GROWTH IN SUSTAINABLE, RESILIENT AND CLIMATE FRIENDLY ORGANIC AND CONVENTIONAL EUROPEAN AQUACULTURE

15.30 Giuseppe Lembo, Pierluigi Carbonara, Sebastien Alfonso, Walter Zupa, Maria Teresa Spedicato
THE ROLE OF INTERNET OF THINGS FOR HEALTHY FISH AND ENVIRONMENT IN THE EUROPEAN AQUACULTURE

15.50 Franck Le Gall, Ahmed Abid, Philippe Cousin, Elisa Ravagan
MODELLING DATA IN THE CONTEXT OF AQUACULTURE

16.10 Steven Prescott, Tamas Bardocz
iFishIENCi: WILL INCREASED EFFICIENCY THROUGH THE USE OF BREAKTHROUGH INNOVATIONS AND PRINCIPLES OF CIRCULAR ECONOMY TRANSLATE INTO IMPROVED SUSTSAINABILITY FOR EUROPEAN AQUACULTURE?

16.30 Elena Mente, K. Kousoulaki, N. Vlahos, A. Vasilaki, E. Antonopoulou, A. Jokumsen, P. Lembo, I. Nengas
FEED AND NUTRITION IN ORGANIC AQUACULTURE

16.50 Daniel Montero, S. Torrecillas, D.R. Tocher, M. Vandeputte, R. Fontanillas, G. Rosenlund, P. Haffray, B. Ruyter, A. Sonesson, J. Bastiaansen, A. Kause
DEVELOPMENT OF FISH FEEDS AND FEEDING STRATEGIES FOR GENETICALLY SUPERIOR FISH FROM BREEDING PROGRAMMES

17.10 John Bastiaansen
THE IMPACT OF GENOMIC SELECTION IN COMMERCIAL BREEDING PROGRAMMES FOR FOUR IMPORTANT SPECIES IN EUROPEAN AQUACULTURE

17.30 Anne Kettunen, Marie Lillehammer
POWER CALCULATIONS FOR OPTIMISATION OF THE EXPERIMENTAL DESIGN TO DETECT G X E: SALMON EXPERIMENTS IN FutureEUAqua

WOMEN IN AQUACULTURE

Wednesday, October 9 16.30 - 17.30 Festival
Chair: Alistair Lane

Jointly organised by EAS and The Fish Site, the Women in Aquaculture session will offer firsthand insights into how women can overcome perceived gender-related obstacles and build thriving careers right across the aquaculture sector. Panellists will discuss key issues related to the benefits of diversity in the workforce and ways to ensure that aquaculture organisations pursue recruitment policies that allow talented people, regardless of gender, to succeed.

SESSION HOSTS
Synnøve Hellund, NOFIMA AND EATIP
Rob Fletcher, The Fish Site

PANEL MEMBERS
Lara Barazi, Kephalonian Fisheries
Ole Christiansen, BIOMAR
Ben Hadfield, MOWI Scotland
Matthijs Metselaar, Benchmark Animal Health
Birgit Schmidt-Puckhaber, German Agriculture Society
Selina Stead, University of Stirling

ENVIRONMENTAL AND ECOSYSTEM MANAGEMENT

Thursday, October 10 09.00 - 11.20 Exhibition 2
Chair: Oyvind Berg

09.00 **Pierre Gernez**, Laura Zoffoli, Thomas Lacour, Virginie Raimbault, Véronique Séchet, Victor Martinez Vicente, Tristan Harmel
HIGH RESOLUTION SATELLITE REMOTE SENSING OF RED TIDES IN SHELLFISH FARMING COASTAL WATERS

09.20 **Roberto Pastres**, Suzanne B. Bricker, Bela H. Buck, Luis Conceição, Joao Gomes Ferreira, Jon Grant, Johan Johansen, David C. Little, Carmen Sotelo, Chang Bo Zhu
TOWARDS THE ECOLOGICAL INTENSIFICATION OF EUROPEAN AQUACULTURE: THE GAIN PROJECT

09.40 **Maren Lyngsgaard**, Per Dolmer, Daniel Taylor, Bent Vismann
BLUE BIOMASS – A WAY FORWARD FOR MITIGATION MUSSELS

10.00 **Ellen Sofie Grefsrud**, Terje Svåsand, Lasse Berg Andersen
RISK ASSESSMENT OF NORWEGIAN SALMON FARMING – A NEW APPROACH

10.20 **Marcos Carvajalino Fernández**, N.B. Keeley, I. Fer, B.A. Law, R.J. Bannister
IMPACT OF BOTTOM SUBSTRATE TYPE ON THE RESUSPENSION OF ATLANTIC SALMON *Salmo salar* FAECAL MATERIAL

10.40 **Thorsten Stoeck**, Thomas A. Wilding, Tristan Cordier, Jan Pawlowski, Verena Dully, Larissa Frühe, Marc Coulson
ESTABLISHING BENTHIC ECOLOGICAL STATUS AROUND SALMON AQUACULTURE CAGES USING DNA-BASED ENVIRONMENTAL MONITORING

11.00 **Øivind Bergh**, Alexander Christian Beck, Guldborg Søvik, Erik Olsen, Trude H. Thangstad, Genoveva Gonzalez-Mirelis, Fabio Grati, Luca Bolognini
SPATIAL CONFLICTS OF COASTAL FISHERIES WITH LARGE SCALE SALMONID AQUACULTURE IN A NORWEGIAN FJORD ENVIRONMENT ANALYZED BY GIS AND STAKEHOLDER SURVEYS

SUSTAINABLE SYSTEMS FOR LARGE SCALE PRODUCTION - CLOSED, OFFSHORE OR BOTH

Thursday, October 10 09.00 - 11.20 Room 5
Chairs: Asa Espmark, Hans Bjelland

09.00 **David Kristiansen**, Biao Su, Stefan Vilsen, Per C. Endresen, Carina Norvik, Zsolt Volent
DESIGN CONSIDERATIONS AND RESEARCH CHALLENGES FOR CLOSED FISH CAGES IN CURRENTS AND WAVES

09.20 **Sharada Navada**, Frédéric Gaumet, Olav Vadstein, Claudia Spanu, Øyvind Mikkelsen, Jelena Kolarevic
IMPROVING SALINITY ADAPTATION IN NITRIFYING BIOREACTORS BY SEAWATER PRIMING

09.40 **Enrique Pino Martinez**, Pablo Balseiro Vigo, Markus Braanaas, Valentina Tronci, Cindy Pedrosa, Naouel Gharbi, Sigurd Handeland, Tom Nilsen
INFLUENCE OF WATER TEMPERATURE AND FEEDING REGIME ON THE INCIDENCE OF EARLY SEXUAL MATURATION IN ATLANTIC SALMON *Salmo salar* L. POSTSMOLTS DURING THE FRESHWATER STAGE

10.00 **Khurram Shahzad**, Harald Takle, Karl F. Ottem, Magnus Stendal, Kevin T. Stiller, Yuriy Marchenko, Jelena Kolarevic
CFD MODELING OF SEMI-CLOSED CONTAINMENT FLOATING SYSTEM WITH FLEXIBLE WALLS EFFECT OF INLET ORIENTATION ANGLE ON THE FLOW FIELD HYDRODYNAMICS

10.20 **Christopher Good**, Travis May, Curtis Crouse, John Davidson, Natalie Redman, Megan Murray, Anna DiCocco, Brian Vinci, Steven Summerfelt, Lars Ebbesson, Sigurd Handeland, Tom Ole Nilsen, Sigurd Stefansson, Bendik Fyhn Terjesen, Frode Mathisen, Åsa Maria Espmark
DETERMINING THE IMPACT OF PHOTOPERIOD, FEEDING REGIME, AND PLOIDY ON ATLANTIC SALMON *Salmo salar* POST-SMOLT HEALTH, GROWTH PERFORMANCE, AND MATURATION IN FRESHWATER RECIRCULATION AQUACULTURE SYSTEMS

10.40 **John Davidson**, Steven Summerfelt, Brian Vinci, Kevin Schrader, Christopher Good
EVALUATING THE FEASIBILITY OF INCOPORATING MEMBRANE BIOLOGICAL REACTORS WITHIN RAS: EFECTS ON WATER USE, WATER QUALITY, AND RAINBOW TROUT *Oncorhynchus mykiss* PERFORMANCE

11.00 **Uwe Waller**, Klaus Kimmerle, Sabine Boehmer, Uwe Köhler
SUSTAINABLE AQUACULTURE OF ATLANTIC SALMON *Salmo salar* ON BOARD A LARGE SAIL SHIP

BALTIC AQUACULTURE

Thursday, October 10 09.00 - 11.20 Lounge 1-3
Chair: Mathis von Ahnen

09.00 **Monika Normant-Saremba**, Hanna Ladkowska, Halina Kendzierska, Barbara Dmochowska, Katarzyna Smolarz
FROM LARVAE TO PLATE – AN EXPERIMENTAL LAND-BASED RAS CULTURE OF *Litopeanaeus vannamei* IN POLAND

09.20 **Xiaoyu Huang**, Carlos Letelier-Gordo
NITROGEN REMOVAL FROM A SALT-WATER RECIRCULATING AQUACULTURE SYSTEM (RAS) USING A STEP FED BATCH REACTOR (SFBR) OPERATED UNDER EXTERNAL AND INTERNAL CARBON SOURCES

09.40 **Jouni Vielma**, Gunno Renman, Agnieszka Renman, Jani Pulkkinen
PHOSPHORUS REMOVAL FROM RECIRCULATING AQUACULTURE SYSTEMS (RAS) EFFLUENT BY REACTIVE COLUMN FILTERS

10.00 **Sanni Aalto**, Jani T. Pulkkinen, Suvi Suurnäkki, Jouni Vielma, Marja Tiirola
AUTO- AND HETEROTROPHIC NITROGEN REMOVAL IN WOODCHIP REACTORS TREATING RECIRCULATING AQUACULTURE SYSTEM EFFLUENTS

10.20 **Suvi Suurnäkki**, Jani Pulkkinen, Marja Tiirola, Sanni Aalto
GEOSMIN PRODUCERS AND MICROBIAL COMMUNITY COMPOSITION IN COMMERCIAL RECIRCULATING AQUACULTURE FARMS

10.40 **Anniina Runtuvuori**, Heidi Kunttu, Gabriel Almeida, Elina Laanto, Kati Mäkelä, Mathias Middelboe, Lotta-Riina Sundberg
ISOLATION AND CHARACTERIZATION OF NEW *Flavobacterium columnare* AND ITS BACTERIOPHAGES FROM FISH FARMS

11.00 **Miriam von Thenen**, M. Maar, H.S. Hansen, K.S. Schiele
A GIS SUITABILITY ANALYSIS FOR SELECTING MUSSEL FARM SITES IN THE SOUTH-WESTERN BALTIC SEA

GOVERNANCE, POLICY, REGULATIONS AND STRATEGIC PLANNING

Thursday, October 10 09.00 - 11.20 Room 4
Chair: John Bostock

09.00 **Qingyin Wang**
CANCELLED SOME CONSIDERATIONS ON THE SUSTAINABLE DEVELOPMENT OF AQUACULTURE – CHALLENGES, CONSTRAINTS AND PROSPECTS

09.20 **Keith Jeffery**, Stephen Mangi, Heather Conejo-Watt, Angela Muench, Kieran Hyder
INTEGRATION OF FISHERIES AND AQUACULTURE INTO A MORE HOLISTIC SEAFOOD PRODUCTION SYSTEM

09.40 **Øivind Bergh**
CONSTRAINTS AGAINST GLOBAL DEVELOPMENT OF AQUACULTURE: COMPETITION FOR SPACE, RESOURCES AND SOCIETAL COMMITMENT

10.00 **Jenny Weitzman**, Ramon Filgueira, Jon Grant
TOWARDS AN ECOSYSTEM APPROACH TO AQUACULTURE: DEVELOPING A HOLISTIC FRAMEWORK FOR CARRYING CAPACITY OF SALMON AQUACULTURE IN CANADA

10.20 **Trevor Telfer**, Hanne Kaas, Anne Lise Middelboe, Lynne Falconer, Pauline O'Donohoe
AN 'AQUACULTURE SUSTAINABLITY TOOLBOX' FOR FLEXIBLE LICENSING AND REGULATION OF AQUACULTURE IN EUROPE

10.40 **Gudrun Olafsdottir**, David Cook, Shraddha Mehta, Ingunn Yr Gudbrandsdottir, Maitri Thakur, Sigurdur G. Bogason
GOVERNANCE AND PERCEIVED POWER IN THE SALMON VALUE CHAIN

11.00 **Tim Knöpfel**, Bernhard Brümmer, Stephan Wessels
EFFICIENCY OF RAINBOW TROUT PRODUCTION IN NORTH-WEST GERMANY

ESCAPES / INTERACTION OF FARMED AND WILD FISH

Thursday, October 10 09.00 - 11.20 Exhibition 1
Chair: Kjetil Hindar

09.00 **Sten Karlsson**, Tonje Aronsen, Geir Bolstad, Ola H. Diserud, Ingerid J. Hagen, Kjetil Hindar
ESTIMATING GENETIC INTROGRESSION OF FARMED SALMON – P(WILD); METHODOLOGY, ACHIEVEMENTS AND PROSPECTS

09.20 **Ola H. Diserud**, Kjetil Hindar, Sten Karlsson, Kevin A. Glover, Øystein Skaala
ESCAPED FARM SALMON AFFECT THE GENETICS OF WILD SALMON *Salmo salar* POPULATIONS ALL ALONG THE NORWEGIAN COAST

09.40 **Tony Farrell**, Yangfan Zhang, Mark Polinski, Phillip Morrison, Colin Brauner, Kyle Garver
THE IMPACT OF A PISCINE ORTHOREOVIRUS (PRV) INFECTION ON THE RESPIRATORY PERFORMANCE AND CAPACITY OF ATLANTIC SALMON AND SOCKEYE SALMON IN BRITISH COLUMBIA, CANADA

10.00 **Monica F. Solberg**, Grethe Robertsen, Line E. Sundt-Hansen, Kjetil Hindar, Kevin A. Glover
DOMESTICATION LEADS TO INCREASED PREDATION SUSCEPTIBILITY

10.20 **Marie Lillehammer**
FITNESS EFFECTS OF INTROGRESSION OF ESCAPED FARMED SALMON INTO WILD POPULATIONS

10.40 **Kevin A. Glover**, Monica Solberg
CRYPTIC INTROGRESSION: WILL PLASTICITY AND NATURAL SELECTION MASK THE EFFECTS OF GENE-FLOW FROM DOMESTICATED ESCAPEES ON NATURAL POPULATIONS?

11.00 **Øystein Skaala**, Francois Besnier, Reidar Bordstrøm, Bjorn Barlaup, Anne Sørvik, Eirik Normann, Britt Østebø, Michael Hansen, Kevin Glover
A LONG-TERM STUDY OF DOMESTICATED AND WILD ATLANTIC SALMON IN THE RIVER GUDDALSELVA

EUROSHRIMP INDUSTRY FORUM

Thursday, October 10 09.00 - 11.20 Exhibition 2

Chairs: Gregor Jaehne, Stefan Meyer

INFORMAL MEETING FOR ALL SHRIMP ENTHUSIASTS

Meet and greet. We offer the opportunity to meet each other without any program constraints and discuss the topics that interest you the most.

WORKSHOP OPENING

Dr. Matthew Slater, Head of Aquaculture Research Group at the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research (AWI)

STAKEHOLDER SURVEY "RECIRCULATION AQUACULTURE SYSTEMS – POSITIONS OF THE ORGANIC FOOD SECTOR

Dr. Stefan Bergleitner, Naturland e.V.

DIVERSIFICATION OF GLOBAL SHRIMP FARMING THROUGH TRANSPARENCY – THE NEW ASC METRICS METHODOLOGY

Kathrin Steinberg, Aquaculture Stewardship Council (ASC)

THE DEVELOPMENT OF INEXPENSIVE SALT MIXTURES FOR INLAND INTENSIVE SHRIMP *Litopenaeus vannamei* FARMING

Dr. Andrew Ray, Kentucky State University, College of Agriculture, Communities and the Environment, School of Aquaculture and Aquatic Sciences

FIGHTING AND AVOIDING VIBRIO IN INTENSIVE CULTURE OF *Litopenaeus vannamei*: OVERVIEW OF RESEARCH DONE AT CREVETEC FARM

Eric De Muylder, CreveTec

PRODUCTION OF POSTLARVAE *Litopenaeus vannamei* IN EUROPE

Nicola Scalise, EcoShrimp

EXPERIENCES FROM A NEW SHRIMP RESEARCH FACILITY IN LITHUANIA

Nerijus Nika, Kleipeda Science and Technology Park

NATIONAL PILOT PLATFORMS CONECTING THE SHRIMP SECTOR IN VIETNAM, THAILAND AND BANGLASDESH

David Basset, European-Asian Technology and Innovation Platform (EURASTIP)

APPLICATION OF THAILAND TO GET POSITIVE LISTED AS A COUNTRY TO EXPORT LIVE SHRIMP INTO THE EU

Dr. Patrick Sorgeloos, Ghent University, Department of Animal Production

SUPPORT LETTER AND WORKSHOP CLOSING

Dr. Matthew Slater, Head of Aquaculture Research Group at the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research (AWI)

NUTRITION: ADDITIVES AND INGREDIENTS - CONTINUED

Thursday, October 10 09.00 - 16.50 Europe Hall
Chair: Katerina Kosoulaki, Kiron Viswanath

09.00 **Antigoni Vasilaki**, Katerina Kousoulaki, Tor Andreas Samuelsen, Giorgos Pyrenis, Dimitra Kogiannou, Kriton Grigorakis, Eleni Fountoulaki, Elena Mente, Ioannis Nengas
EFFECTS OF NOVEL INGREDIENTS ON GROWTH PERFORMANCE IN EUROPEAN SEA BASS *Dicentrarchus labrax*

09.20 **Sandrine Skiba**, Christine Burel, Thierry Kerneis, Laurent Labbé, Frédéric Terrier, Geneviève Corraze, Nadège Richard, Yann Marchand, Mathilde Dupont-Nivet
SUPLEMENTING AQUAFEEDS WITH NEW ALTERNATIVE INGREDIENTS: A PROMISING SOLUTION TO IMPROVE PLANT-BASED DIET EFFICIENCY IN RAINBOW TROUT

09.40 **Pabodha Weththasinghe**, Leidy Lagos, Jon Øvrum Hansen, Liv Torunn Mydland, Margareth Øverland
THE EFFECT OF DIETARY BLACK SOLDIER FLY LARVAE *Hermetia illucens* MEAL AND PASTE ON GROWTH PERFORMANCES AND IMMUNE PARAMETERS IN ATLANTIC SALMON *Salmo salar*

10.00 **Mette Sørensen**, Katerina Kousoulaki, Dorinde Kleinegris, Chris Andre Johnsen, Anjana M. Palihawadana, Marialena Kokkali, Viswanath Kiron
WHOLE OR BROKEN CELLS OF *Phaeodactylum tricornutum* AND *Tetraselmis chuii* FED TO ATLANTIC SALMON *Salmo salar*

10.20 **Ricardo Pereira**, Luísa Valente, Mónica Costa, Luís Baião, Sónia Batista, Sergio Fernández-Boo, Benjamín Costas, Luís Cunha, Rui Lima
EXPLORING THE BENEFITS OF NATURAL ANTIOXIDANTS IN DIETS FOR EUROPEAN SEABASS UNDER A CIRCULAR ECONOMY CONTEXT

10.40 **Tiziana Bongiorno**, Andrea Di Biase, Mauro Vasconi, Vittorio M. Moretti, A. Lopez, Aldo Tava, Domenico Carminati, Giuliana D'Imporzano, Marina Montedoro, Fabrizio Adani, Gabriel F. Acién Fernández, Javier F. Alarcón, Katia Parati
GROWTH PERFORMANCE AND QUALITY TRAITS OF SIBERIAN STURGEON *A. baerii* JUVENILES FED DIETS INCLUDING *Nannochloropsis gaditana* AND *Scenedesmus almeriensis* MICROALGAE MEAL

11:20 **BREAK**

11:45 **PLENARY 3 – POSTER AWARDS – LUNCH**

14.30 **M. Ferreira**, P. Campelos, M.L. Nunes, V. Barbosa, M. Barata, A. Marques, L. Ribeiro, P. Pousão-Ferreira, J. Dias, S. Sousa, V.F. Domingues, L.M.P. Valente
BIO-FORTIFICATION OF GILTHEAD SEABREAM *Sparus aurata* WITH DIETS CONTAINING ALGAE AND SELENISED YEAST

14.50 **Marta Carvalho**, Ramón Fontanillas, Grethe Rosenlund, Daniel Montero, Marisol Izquierdo
THE COMBINATION OF POULTRY AND ALGAE OILS AS REPLACERS OF FISH OIL IN DIETS FOR GILTHEAD SEA BREAM *Sparus aurata* FINGERLINGS

15.10 **Ana Catarina Matias**, Marisa Barata, Ravi Luna Araujo, Jorge Dias, Pedro Pousão-Ferreira
TAURINE MODULATES PROTEIN TURNOVER IN SEVERAL TISSUES OF MEAGRE JUVENILES FED WITH PLANT-BASED DIETS

15.30 **David San Martin**, Bruno Iñarra, Mikel Orive, Alicia Estevez, Joan Nazzaro, Ricard Fenollosa, José Miguel Martínez, Anna-Maria De Smet, Jaime Zufía
BREWERS' SPENT YEAST AND GRAIN AS ALTERNATIVE INGREDIENTS FOR AQUACULTURE FEED

15.50 **Ivan Viegas**, Mariana Palma, Francisca Silva-Brito, Emanuel Silva, João Rito, Paulo Rema, Elisabeth Plagnes-Juan, Stéphane Panserat, Leonardo Magnoni
METABOLIC UTILIZATION OF DIETARY GLYCEROL BY RAINBOW TROUT *Oncorhynchus mykiss:* A MULTI-TRIAL EVALUATION

16.10 **Jon Øvrum Hansen**, Leidy Lagos, Peng Lei, Felipe Eduardo Reveco-Urzua, Ricardo Tavares Benicio, Line Degn Hansen, Liv Torunn Mydland, Margareth Øverland
DO DOWN-STREAM PROCESSING OF BAKERS YEAST *Saccharomyces cerevisiae* AFFECT DIGESTIBILITY AND IMMUNE RESPONSE IN ATLANTIC SALMON *Salmo salar?*

16.30 **Luis Conceicao**, Filipe Soares, Ana Nobre, Tomé Silva
PREDICTING LONG-TERM EFFECTS OF SMALL CHANGES IN AQUAFEEDS PERFORMANCE USING A DYNAMIC METABOLIC MODEL

SELECTIVE BREEDING

Thursday, October 10 09.00 - 16.50 Room 2
Chairs: Christos Palaikostas, Marc Vandeputte

09.00 **Hugues de Verdal**, Marc Vandeputte, François Allal, Mathieu Besson, Wagdy Mekkawy, Dirk-Jan de Koning, John A.H. Benzie
ARE GENETICS AND GENOMICS HELPFUL TO IMPROVE FEED EFFICIENCY IN NILE TILAPIA *Oreochromis niloticus?*

09.20 **Hanne Dvergedal**, Jørgen Ødegård, Margareth Øverland, Liv Torunn Mydland, Gunnar Klemetsdal
NOVEL INDICATOR TRAITS FOR FEED EFFICIENCY IN ATLANTIC SALMON *Salmo salar*

09.40 **Marc Vandeputte**, Clémence Fraslin, Pierrick Haffray, Anastasia Bestin, François Allal, Martin Prchal, Martin Kocour, Mathilde Dupont-Nivet
HOW TO GENETICALLY INCREASE FILLET YIELD IN FISH: RELEVANT GENETIC PARAMETERS AND METHODS FOR THE ESTIMATION OF GENETIC GAIN

10.00 **Martin Prchal**, Martin Kocour, Marc Vandeputte, Antti Kause, Alain Vergnet, Jinfeng Zhao, David Gela, Lucie Genestout, Anastasia Bestin, Pierrick Haffray, Jérôme Bugeon
MORPHOLOGICAL PREDICTORS OF SLAUGHTER YIELDS USING 3D DIGITIZER AND THEIR UTILIZATION IN COMMON CARP BREEDING PROGRAM

10.20 **Anne Kettunen**, Hooi Ling Khaw, Dennis Brandborg Nielsen, Borghild Hillestad, Hooman Moghadam
SLAUGHTER QUALITY OF ATLANTIC SALMON *Salmo salar* USING COMPUTERIZED TOMOGRAPHY

10.40 **Samuel Bekele Mengistu**, Han A. Mulder, John A.H. Benzie, Hans Komen
GENETIC PARAMETERS FOR RESILIENCE BASED ON FLUCTUATIONS IN BODY WEIGHT IN NILE TILAPIA GROWN IN AERATED AND NON-AERATED PONDS

11.00 **Clemence Fraslin**, A. Bestin, N. Dechamp, J. D'Ambrosio, F. Krieg, E. Belmonte, C. Poncet, P. Hocde, P. Haffray, Y. Guiguen, F. Phocas, E. Quillet
GWAS REVEALS SEVERAL GENOMIC REGIONS GOVERNING SPONTANEOUS XX-MALENESS IN RAINBOW TROUT

11:20 **BREAK**

11:45 **PLENARY 3 – POSTER AWARDS – LUNCH**

14.30 **Luca Bargelloni**, Oronzo Tassiello, Ludovica Montanucci, Massimiliano Babbucci, Serena Ferraresso, Paolo Carnier
EXPLORING MACHINE LEARNING METHODS FOR GENOMIC PREDICTION OF DISEASE RESISTANCE AND BODY LENGTH IN THE GILTHEAD SEA BREAM

14.50 **Christos Palaiokostas**, Henrik Jeuthe, Dirk-Jan Koning
IMPLEMENTATION OF LOW COVERAGE GENOTYPE BY SEQUENCING IN THE SELECTIVE BREEDING PROGRAM OF ARCTIC CHARR *Salvelinus alpinus* IN SWEDEN

15.10 **Binyam Dagnachew**, Solomon Antwi Boison, Matt Baranski
IMPUTATION AND GENOMIC PREDICTION ACCURACIES FOR SEA LICE RESISTANCE IN ATLANTIC SALMON

15.30 **Rama Bangera**, Sergio Vela-Avitua, Marie Lillehammer, Marcela Salazar, Morten Rye, Edna Constanza Erazo, Andres Suarez, James Cock, Nicholas Robinson
GENOMIC SELECTION TO IMPROVE WHITE SPOT SYNDROME VIRUS (WSSV) RESISTANCE IN A *Litopenaeus vannamei* BREEDING PROGRAM

15.50 **Alejandro Gutierrez**, Jane Symonds, Nick King, Konstanze Steiner, Tim Bean, Ross Houston
POTENTIAL OF GENOMIC SELECTION FOR THE IMPROVEMENT OF PACIFIC OYSTER RESISTANCE TO OSTREID HERPESVIRUS (OSHV-1)

16.10 **Sara Faggion**, Daniela Bertotto, Massimiliano Babbucci, Giulia Dalla Rovere, Rafaella Franch, Mauro Bovolenta, Stanislas Laureau, Francesco Pascoli, Anna Toffan, Luca Bargelloni, Paolo Carnier
GENETIC AND GENOMIC PARAMETERS FOR VNN RESISTANCE, BODY WEIGHT AND CORTISOL CONCENTRATION IN EUROPEAN SEA BASS *Dicentrarchus labrax* L.

MOLLUSC PRODUCTION AND RESTORATION

Thursday, October 10 09.00 - 16.50 Room 3
Chair: Camille Saurel

09.00 **Pernille Nielsen,** Camille Saurel, Pedro S. Freitas, Pascal Barreau, Lene Friis Moller, Jens Kjerulf Petersen
IS THERE A NEED FOR FLAT OYSTER Ostrea edulis RESTORATION IN DENMARK?

09.20 **Linda Tonk**, Pauline Kamermans, Remment ter Hofstede, Tony van der Hiele, Tjeerd Bouma, Tom van der Have, Karin Didderen
FLAT OYSTER *Ostrea edulis* RESTORATION IN OFFSHORE WIND PARKS: AN OPPORTUNITY FOR INNOVATION OF SCOUR PROTECTION

09.40 **Susanne Vogeler**, Xiaoxu Li, Garry H. Wikfors, Stefano Carboni, Alyssa Joyce
N-METHYL-D-ASPARTATE RECEPTOR AND NITRIC OXIDE PATHWAYS AS POTENTIAL REGULATORS OF LARVAL METAMORPHOSIS IN BIVALVE SPECIES: INVESTIGATION OF NEW PATHWAYS FOR COMMERCIAL HATCHERY APPLICATIONS

10.00 **Kristina Arranz**, Iñaki Urrutxurtu, Irrintzi Ibarrola, Miren Bego Urrutia, Daniel Prieto, Enrique Navarro
SEED CONDITION AND DIETARY N CONTENT AFFECTS C/N HOMEOSTASIS IN TISSUES OF JUVENILE CLAMS *Ruditapes philippinarum*

10.20 **Eva Hartog**, Tony van der Hiele, Jouke Heringa, Niels Wagenaar, Jacob Capelle
COPING WITH THE INVASIVE JAPANESE OYSTER DRILL *Ocinebrellus inornatus* ON OYSTER CULTURE PLOTS

10.40 **Kati Michalek**, David Vendrami, Michaël Bekaert, Joanna Wilson, David Green, Thomas Wilding, Kim Last, Joseph Hoffman
SPECIES COMPOSITION, INTROGRESSION AND SHELL PLASTICITY IN FARMED *Mytilus* spp. ON THE WEST COAST OF SCOTLAND

11:20 **BREAK**

11:45 **PLENARY 3 – POSTER AWARDS – LUNCH**

14.30 **Ramón Filgueira**, Leah Strople, Tore Strohmeier, Samuel Rastrick, Øivind Strand
MUSSELS OR TUNICATES: THAT IS THE QUESTION. EVALUATING EFFICIENT AND SUSTAINABLE RESOURCE USE BY LOW-TROPHIC SPECIES IN AQUACULTURE SETTINGS

14.50 **Anna-Lucia Buer**, Lukas Ritzenhofena, Marie Maarb, Gerald Schernewskia
PRODUCTION POTENTIAL OF BLUE MUSSEL *Mytilus* spp. FARMING ALONG THE SALINITY GRADIENT OF THE GERMAN BALTIC SEA

15.10 **M. Maar**, A. Holbach, K. Timmermann, D. Taylor
SPATIAL MODELLING OF BLUE MUSSEL FARM PRODUCTION POTENTIAL IN THE WESTERN BALTIC SEA

15.30 **Daniel Taylor**, Camille Saurel, Pernille Nielsen, Finn Bak, Niels-Peter Nielsen, Jens Kjerulf Petersen
OPTIMIZING PRODUCTION OF MITIGATION MUSSELS

15.50 **Gerald Schernewski**, Rene Friedland
ZEBRA-MUSSEL (DREISSENA) CULTIVATION IN BALTIC COASTAL WATERS – AN OPTION FOR BLUE GROWTH AND WATER POLICY IMPLEMENTATION?

16.10 **Lars Kjerulf Petersen**, Nardine Stybel
LOCAL ACCEPTANCE OF MUSSEL CULTIVATION IN THE BALTIC SEA

16.30 **Andrew van der Schatte Olivier**, S.K. Malham, L. Le Vay, M. Christie, J. Wilson, S. Allender, S. Schmidlin, J. Brewin, L. Jones
POTENTIAL FOR USING MUSSEL FARMING *Mytilus edulis* IN NUTRIENT REMEDIATION IN ESTUARIES OF THE UNITED KINGDOM

PERCID FISH

Thursday, October 10 09.00 - 16.50 Festival
Chair: Daniel Zarski

09.00 Carole Rougeot
REVIEW ON SEX DETERMINATION PROCESS, ALL-FEMALE PRODUCTION AND GROWTH PERFORMANCES IN EURASIAN PERCH *Perca fluviatilis*

09.20 Marcus Stüeken, Tobias Rapp, Fabian Schäfer, Daniel Zarski, Ralf Bochert, Werner Kloas
THE EFFECT OF LIGHT SPECTRUM ON GONADAL DEVELOPMENT IN PIKEPERCH *Sander lucioperca* (L., 1758)

09.40 Oleksandr Malinovskyi, Lukáš Veselý, Miroslav Blecha, Jiří Kříšřan, Tomáš Policar
THE SUBSTRATE SELECTION AND SPAWNING BEHAVIOR OF PIKEPERCH *Sander lucioperca* L. BROODSTOCK UNDER POND CONDITIONS

10.00 Leila El Mohajer, Sirine Selmi, Pascal Fontaine, Sylvain Milla
THE SEARCH FOR THE MATURATIONS INDUCING STEROID (MIS) IN EURASIAN PERCH *Perca fluviatilis*

10.20 Uros Ljubobratovic, Ferenc Demeny, Geza Peter, Oleksandr Malinovskyi, Maciej Kwiatkowski, Andras Ronyai, Akos Horvath
THE EFFECT OF DIFFERENT HORMONAL INDUCTION STRATEGIES ON PIKEPERCH *Sander lucioperca* L. GAMETE QUALITY OBTAINED AFTER OUT-OF-SEASON ARTIFICIAL REPRODUCTION

10.40 Daniel Zarski, Gergely Bernath, Imen Ben Ammar, Sebastien Baeckelandt, Zoltan Bokor, Pascal Fontaine, Akos Horvath, Patrick Kestemont, Syaghalirwa N.M. Mandiki
REPEATED HORMONAL INDUCTION OF SPERMIATION AFFECTS STRESS BUT NOT IMMUNE RESPONSE IN PIKEPERCH *Sander lucioperca*

11.00 Gergely Bernáth, Levente Várkonyi, Ferenc Fodor, Tamás Koltai, Ádám Bodnár, József Molnár, Levente Zete Láng, Tibor Izsák, Árpád Ferincz, Ádám Staszny, Béla Urbányi, Zsolt Szári, Zoltán Bokor
THE METHODICAL IMPROVEMENT OF SPERM CRYOPRESERVATION IN VOLGA PIKEPERCH S*ander volgensis*

11:20 BREAK

11:45 PLENARY 3 – POSTER AWARDS – LUNCH

14.30 Edson Panana, S. Teerlinck
EFFECT OF PHOTOPERIOD ON PIKEPERCH *Sander lucioperca* LARVAE PERFORMANCE IN RECIRCULATING AQUACULTURE SYSTEMS

14.50 Aiman Imentai, Christoph Steinbach, Carlos Yanes-Roca, Tomas Policar
EFFECT OF FEEDING STRATEGY WITH ROTIFERS *Brachionus plicatilis* ON PIKEPERCH *Sander lucioperca* LARVAL PERFOMANCE

15.10 Katarzyna Palinska-Zarska, Piotr Niewiadomski, Jaroslaw Król, Daniel Zarski
NUTRITIONALLY CHALLENGED EURASIAN PERCH LARVAE *Perca fluviatilis* SHEDS LIGHT ON ADAPTABILITY OF FISH TO COMPOUND DIET

15.30 **Sandra Langi**, Edson Panana, Ceder Alloo, Gilbert Van Stappen, Wouter Meeus
REPLACEMENT OF FISH MEAL USING ALTERNATIVE PROTEIN SOURCE IN FEEDS FOR PIKEPERCH *Sander lucioperca* DURING GROW OUT PHASE

15.50 **Stefan Heidemann**, Marcus Stüeken, Ralf Bochert, Tobias Rapp
THE EFFECT OF LIGHT COLOUR ON PERFORMANCE PARAMETER OF JUVENILE PIKEPERCH *Sander lucioperca*

16.10 **Tobias Rapp**, Moritz Tielmann, Ralf Bochert, Marcus Stüeken
FEEDING MANAGEMENT OF JUVENILE PIKEPERCH *Sander lucioperca* UNDER PRODUCTION CONDITIONS

16.30 **Alain Pasquet**, Marielle Thomas, Yannick Ledoré, Lucas Schaeffer, Sarah Nahon, Joël Aubin, Christophe Jaeger, Aurélie Wilfart, Alain Benard, Thomas Lecocq
ICTHYODIVERSITY AS A DRIVING FORCE TO PROMOTE NEW AGRO-ECOLOGICAL APPROACH FOR *Sander lucioperca* REARING

AQUACULTURE IN CENTRAL AND EASTERN EUROPE

Thursday, October 10 09.00 - 16.50 Backstage 1
Chair: Laszlo Varadi

09.00 **Gennady G. Matishov**, Elena N. Ponomareva, Marina N. Sorokina
PRESENT BIOTECHNOLOGY OF AQUACULTURE OF SOUTH RUSSIA

09.20 **Koushik Roy**, Mario Precanica, Petr Dvorak, Jaroslav Vrba, Sadasivam J. Kaushik, Jan Mraz
IS THERE A EUTROPHICATION CONCERN IN SUPPLEMENTARY FEED BASED COMMON CARP FARMING?

09.40 **Eva Kerepeczki**, Péter Palásti, G. Gyalog
ECOSYSTEM SERVICES PROVIDED BY FRESHWATER FISHPONDS

10.00 **Szilvia Keszte**, Kata Tóth-Ihász, Réka Balogh, Árpád Ferincz, Ádám Stasznyi, Vilmos Józsa, Béla Urbányi, Balázs Kovács
MOLECULAR GENETIC DIVERSITY OF HUNGARIAN SILVER PRUSSIAN CARP *Carassius auratus gibelio* POPULATIONS BASED ON MITOCHONDRIAL D-LOOP SEQUENCES

10.20 **Béla Urbányi**, Balázs Kriszt, Sándor Szoboszlay, Judit Háhn, László Friedrich, Gábor Jónás, Gergely Bernáth, Zsolt Csenki-Bakos, Zsolt Czimmerer, Péter Palotás, Katalin Rákóczi, Zsófia Tarnai-Király, Brigitta Nyiro-Fekete, Gábor Bordós, László Zanathy, Adrienn Micsinai
INTRODUCTION OF THE ENVIRONMENT AND FOOD SAFETY RISKS OF POND FISH PRODUCTION – CAN CARP CONSUMERS BE SATISFIED?

10.40 **Alexandre Tahar**, Alan Kennedy, Ronan Cooney, Sarah Naughton, Emer O'Neill, Siobhan Kavanagh, Andy Fogarty, Neil Rowan, Eoghan Clifford
SUPPORTING THE SUSTAINABLE DEVELOPMENT OF THE IRISH FRESHWATER AQUACULTURE INDUSTRY

11.00 **Edouard Royer**, Daniele Brigolin, Roberto Pastres
IMPLEMENTING PRECISION FISH FARMING ON A RAINBOW TROUT FARM: A DATA ASSIMILATION APPROACH

PATHOGENS, DISEASES AND TREATMENT

Thursday, October 10 09.00 - 16.50 Room 1
Chairs: Giorgos Rigos, Egdar Brun

09.00 **Gabriel Arriagada**
THE ROLE OF EPIDEMIOLOGY IN THE SURVEILLANCE AND CONTROL OF PREVALENT FISH DISEASES – THE CASE OF SEA LICE IN FARMED SALMONIDS IN CHILE

09.20 **Tülay Akayli**, Ozgur Canak, Remziye Eda Yardimci, Cigdem Urku, Dilek Okmen
ENTEROBACTER INFECTIONS OF CULTURED RAINBOW TROUT *Oncorhynchus mykiss*

09.40 **Trina Galloway**, Jacob Seilø Torgersen, Jørgen Ødegård, Tim Martin Knudsen
ROBUST SALMON SKIN – IS THERE A GENETIC COMPONENT?

10.00 **Carolina Barroso**, J.V. Neves, P.N.S. Rodrigues
THE MULTIPLE FUNCTIONS OF PISCIDINS IN THE EUROPEAN SEA BASS *Dicentrarchus labrax*

10.20 **Mikolaj Adamek**, Marek Matras, Andy Dowson, Verena Jung-Schroers, Veronika Piackova, David Gela, Martin Kocour, Jerzy Adamek, Rafal Kaminski, Michal Reichert, Dieter Steinhagen
COMPREHENSIVE APPROACH TO DEVELOPMENT OF COMMON CARP STRAINS RESISTANT TO DISEASES CAUSED BY INFECTIONS WITH CYHV-3, CEV AND SVCV

10.40 **Jose Gallardo**, Carolina Figueroa, Pamela Veloso, Lenin Espin, Debora Torrealba, Juan Manuel Afonso, Carlos Soto, Drian Dixon, Pablo Conejeros
FAMILIAR VARIATION EXPLAINS REDUCED PROTECTION OF COMMERCIAL VACCINES AGAINST BACTERIAL PATHOGENS IN ATLANTIC SALMON

11.00 **Lluis Tort**, Felipe E. Reyes-López, Xiaohong Liu, Ali R. Khansari, Mariana Teles, Gonzalo Martínez, Juan M. Mancera
VACCINES INDUCE A SIGNIFICANT IMMUNE RESPONSE BUT NOT A ROBUST NEUROENDOCRINE REACTION IN BRAIN AND PITUITARY TISSUES OF SEABREAM *Sparus aurata*

11:20 **BREAK**

11:45 **PLENARY 3 – POSTER AWARDS – LUNCH**

14.30 **Paul J. Midtlyng**
NUCLEIC ACID VACCINES ARE EFFECTIVE FOR CONTROL OF VIRAL INFECTIONS AND DISEASE IN SALMONID AQUACULTURE

14.50 **Hans Petter Kleppen**, Eirik Baardsen, Juan J.J. Martinez, Ingrid S. Larsen, Cyril Frantzen
USE OF BACTERIOPHAGES TO CONTROL *Yersinia ruckeri* IN SALMON FARMING

15.10 **Hector Abelardo Ocampo**, Andres Vasavilbazo-Saucedo, Norma Alamaraz-Abarca
ANTIVIRAL EFFECTS IN *L. vannamei* OF FEED ADDITIVE OF MICROENCAPSULATED PHENOLIC EXTRACTS OF *M. umbellata*

15.30 **Parini Manuela**, Matteo Calligaris, Alessio Paoli
1-MONOGLYCERIDES OF SHORT- AND MEDIUM-CHAIN FATTY ACIDS PROVED TO BE EFFECTIVE IN DECREASING THE MORTALITY CAUSED BY *Flavobacterium psychrophilum* AND IN REDUCING THE ANTIBIOTIC TREATMENTS IN RAINBOW TROUT *Oncorhynchus mykiss,* WITH FAVORABLE ECONOMIC RETURN FOR THE FISH PRODUCERS

15.50 **Dibo Liu**, Thomas Meinelt, David L. Straus, Petra Bartschat
TOXICITY OF PERACETIC ACID (PAA) PRODUCTS: IMPACT OF WATER QUALITY, PRODUCT COMPOSITION AND FISH SPECIES

16.10 **Dimitra Kogiannou**, Chrysanthi Nikoloudaki, Antigoni Vasilaki, Giorgos Pyrenis, Ioannis Nengas, George Rigos
PHARMACOKINETIC COMPARISON OF DIFFERENT DOSING STRATEGIES OF IN-FEED ADMINISTERED PRAZIQUANTEL IN GREATER AMBERJACK *Seriola dumerili*

16.30 **Claudia Tschesche**, Michaël Bekaert, James E. Bron, Armin Sturm
DELTAMETHRIN RESISTANCE IN SALMON LICE: MITOCHONDRIAL AND NUCLEAR SINGLE NUCLEOTIDE MARKERS

GENERAL TOPICS SESSION

Thursday, October 10 09.00 - 16.50 Foyer 4
Chair: TBA

09.00 **Diogo Thomaz**
THE POTENTIAL OF ARTIFICIAL INTELLIGENCE TO SUPPORT AQUACULTURE FARM MANAGERS: PRELIMINARY RESULTS OF THE APPLICATION OF MACHINE LEARNING ALGORITHMS FOR THE EARLY PREDICTION OF BATCH PERFORMANCE

09.20 **Mohamad Fadjar**, Sri Andayani, Chung-Hun Liu, Jefri Anjaini
EFFECTS OF SQUID *Loligo* sp. INK EXTRACT TO WHITE SHRIMP *Litopeneus vannamei* CypA GENE POLYMORPHISM IN WHITE FECES SYNDROME (WFS) CASE

09.40 **Michael Besmer**, Frederik Hammes, Konstanze Schiessi
MONITORING SHORT-TERM MICROBIOLOGICAL DYNAMICS WITH FULLY AUTOMATED ONLINE FLOW CYTOMETRY IN REALTIME

10.00 **Rayaprolu Srinivas**
EFFECT OF ORGANIC ACIDS IN GROWTH, SURVIVAL AND CONTROLLING CONTINUOUS MORTALITIES OF *Litopenaeus vannamei* CULTURE AT KATTUKALAVA, WEST GODAVARI DISTRICT, ANDHRA PRADESH, INDIA

10.20 **Majid Mohammad Nejad**, Hajimohammad Shirmohammadli
CHANGES IN PROTEIN, FAT, ASH AND DRY MATTER IN DIFFERENT WEIGHTS OF COMMON CARP

10.40 **Inmaculada González Cabrera**, Elena Proietti, Ricardo Haroun Tabraue
THE DEVELOPMENT OF AQUACULTURE IN THE CANARY ISLANDS: NEW PERSPECTIVES AFTER THE PUBLICATION OF THE “PROAC” AND THE “NEW CANARY AUTONOMY STATUTE”

11.00 **Nyiko C. Mabasa,** Clifford L.W. Jones, Mark Laing
SOLVING THE PROBLEM OF SALT ACCUMULATION IN RECYCLED BREWERY EFFLUENT THROUGH THE INTEGRATION OF WATER TREATMENT, AGRICULTURE AND AQUACULTURE

11:30 PLENARY 3 – POSTER AWARDS – LUNCH

14.30 Mohammad Sayyadbourani, Mohammad Pourkazemi, Alireza Valipour, Seied Reza Seied Mortezaei
MONOCULTURE OF PIKEPERCH *Sander lucioperca* IN INTENSIVE SYSTEM OF IRAN

14.50 Olarinke Victoria Adeniyi, Faith Omowumi Dosunmu
GROWTH RESPONSE AND BLOOD INDICES OF AFRICAN SHARPTOOTH CATFISH *Clarias gariepinus* TO DIETS FORTIFIED WITH WILD LETTUCE *Latuca virosa* LEAF EXTRACT

15.10 Javad Daghigh Roohi, Abdolhossein Dalimi, Mohammad Pourkazemi, Mohaddes Ghasemi, Shokoofeh Shamsi
MOLECULAR AND PHYLOGENETIC ANALYSIS OF *Dactylogyrus spp.* PARASITES IN CULTIVATED SILVER CARP *Hypophthalmichthys molitrix* AND BIG HEAD CARP *Hypophthalmichthys nobilis* IN IRAN

15.30 Yenitze Fimbres Acedo, Mauricio Emerenciano, Masato Endo, Kevin Fitzsimmons, Rodolfo Garza, Rosalia Servin, Francisco Magallon
TILAPIA PRODUCTION WITH BIOFLOC TECHNOLOGY IN THREE DIFFERENT TROPHIC CONDITIONS

15.50 Yenitze Fimbres Acedo, Mauricio Emerenciano, Kevin Fitzsimmons, Masato Endo, Rodolfo Garza, Rosalia Servin, Francisco Magallon
HYDROPONIC CULTURE IN NFT USING EFFLUENTS FROM BIOFLOC TECHNOLOGY IN THREE DIFFERENT TROPHIC CONDITIONS

PLENARY 3 AND POSTER AWARDS

Thursday, October 10 11.45 - 12.50 Europe Hall
Chair: Stefan Meyer

11.45 Dina Dziuba
MANAGING RISKS IN A CHANGING ENVIRONMENT

CAGE SYSTEMS AND OFFSHORE STRUCTURES

Thursday, October 10 14.30 - 16.50 Room 5
Chairs: Bela Buck, Hans Bjelland

14.30 Heidi M. Føre, P.C. Endresen, Grim Eidnes, Morten Bondø, Carina Norvik, Andrei Tsarau, David Kristiansen, David Kristiansen, Hans V. Bjelland
HYDRODYNAMIC LOADS ON EXPOSED FISH FARMS

14.50 Kevin Heasman, Malcolm Smeaton, Nicholas Scott, Bela H. Buck
THE INFLUENCE OF STRUCTURE ORIENTATION TO WATER CURRENTS AND WAVES AND THE EFFECT OF WATER DEPTH ON STRUCTURAL STRESS AND SHELLFISH RETENTION IN EXPOSED OCEAN ENVIRONMENTS

15.10 Ajie B.K. Pribadi, Luca Donatini, Evert Lataire
MUSSEL CULTIVATION IN THE BELGIAN NORTH SEA

15.30 **Maximilian Felix Schupp**, Gesche Krause, Vincent Onyango, Bela H. Buck
MULTI-USE POTENTIAL OF MARINE AQUACULTURE WITHIN OFFSHORE WIND FARMS IN THE GERMAN BIGHT: A SWOT ANALYSIS

15.50 **Laurent Barillé**, Stéphanie Palmer, Pierre Gernez, Yoann Thomas, Stefan Simis, Philippe Glize
SATELLITE-MAPPED BIOLOGICAL POTENTIAL FOR MOVING PACIFIC OYSTER AQUACULTURE OFFSHORE

16.10 **Mark Capron**, Michael Chambers, Jim Stewart, Kelly Lucas, Zach Moscicki, Corey Sullivan, Stacy Krueger-Hadfield
RESTORATIVE AQUACULTURE WITH OFFSHORE STRUCTURES

16.30 **Nina Bloecher**, Kevin Frank, Lionel Eisenhauer, Morten Bondø, Oliver Floerl
THE IMPACTS OF BIOFOULING CONTROL MEASURES ON FISH HEALTH AND INFRASTRUCTURE, AND HOW TO MITIGATE THEM

HATCHERY TECHNOLOGIES AND PRACTICES

Thursday, October 10 14.30 - 16.50 Exhibition 2
Chair: Ignacio Fernandez Monzon

14.30 **Barbara Loi**, Francesca Leggieri, Gemma Giménez Papiol, Dario Vallainc
GROWTH RATE AND SURVIVAL IN JUVENILES OF GREY MULLET *Mugil cephalus* REARED UNDER THREE SALINITY LEVELS

14.50 **Vincenzo Alessandro Laudicella**, Christine Beveridge, Stefano Carboni, John Day, Mary K. Doherty, Elaine Mitchel, Michele Stanley, Phil D. Whitfield, Adam D. Hughes
LIPIDOMICS: SPOT ON LIPID METABOLISM DURING NURSERY PRODUCTION OF JUVENILE BLUE MUSSELS *Mytilus edulis* L. REARED UNDER DIFFERENT DIET REGIMES

15.10 **Wilson Pinto**, Sofia Engrola, Bruno Nunes, Rita Colen, André Santos, André Lopes, Luís E.C. Conceição
IMPROVING MICRODIET TECHNOLOGY FOR FIRST-FEEDING GILTHEAD SEABREAM *Sparus aurata* LARVAE

15.30 **Jan Giebichenstein**, Julia Giebichenstein, Christopher Heiduk, Björn Ronge, Casten Schulz
BALLAN WRASSE *Labrus bergylta* LARVAL REARING, A COMPARATIVE STUDY OF DIFFERENT FEEDING REGIMES AND MICRO DIETS BASED ON THE COPEPOD *Acartia tonsa* AS START FEED

15.50 **Sebastian Nikitas Politis**, E. Syropoulou, E. Benini, S.R. Sørensen, I.A.E. Butts, J.J. Miest, D. Mazurais, A. Servili, J.-L. Zambonino-Infante, J. Tomkiewicz
SALINITY REDUCTION BENEFITS EUROPEAN EEL LARVAE: AN OVERVIEW

16.10 **Sune Riis Sørensen**, E. Syropoulou, S.N. Politis, E. Benini, A.C. Hambly, J. Tomkiewicz
IMPACT OF WATER PURIFICATION AND CONDITIONING ON *IN VITRO* PRODUCED EMBRYOS AND LARVAE OF EUROPEAN EEL *Anguilla anguilla*

16.30 **Stefan Spreitzenbarth**, Andrew Jeffs
ARTIFICAL WORLD – NEW INSIGHTS ON OCTOPUS EGG REARING AND LARVAL FEEDING BEHAVIOUR

LABORATORY AQUATIC MODELS AND ORNAMENTALS

Thursday, October 10 14.30 - 16.50 Exhibition 1
Chair: Julien Bobe

14.30 **Julien Bobe**, Caroline Cheung, Thaovi Nguyen, Stephanie Gay, Amelie Patinote, Emilie Cardona, Violette Thermes
WHAT MAKES A FISH EGG ABLE TO BE FERTILIZED AND SUBSEQUENTLY DEVELOP INTO AN EMBRYO: NEW INSIGHTS FROM CRISPR/CAS9 GENOME EDITING IN MODEL SPECIES

14.50 **Laia Ribas**, J. Moraleda-Prados, M. Caballero-Huertas, S. Joly, N. Roher
ZEBRAFISH AND LPS, MODEL TOOLS FOR DECIPHERING EPIGENETIC CHANGES DURING SEX DIFFERENTIATION

15.10 **J. Min**, Rob Knight, Barbara Nowak, Todd Michael, Eric Allen
DEVELOPMENT OF A *Scrombidae* MODEL MARINE FISH FOR DISEASE ECOLOGY, PACIFIC CHUB MACKEREL

15.30 **Ana P.L. Costa**, Igor S. Bernardo, Davide A.M. Silva, Andreia C.M. Rodrigues, Catarina R. Marques, Amadeu M.V.M. Soares, Rui J.M. Rocha
TESTING DIFFERENT PROTOTYPE DIETS FOR CORAL AQUACULTURE

15.50 **Guro Løkka**, Alexander Jaramillo-Torres, Karina Gajardo, Jorge Arturo Vargas-Abúndez, Elvis Chikwati, Elin Valen, Trond M. Kortner, Åshild Krogdahl
ADULT ZEBRAFISH AS MODEL ANIMAL FOR THE EVALUATION OF POSSIBLE HEALTH EFFECTS OF SEAWEEDS IN THE DIET

16.10 **Boris Gomelsky**, Jeffrey Warner, Noel Novelo, Alexander Kramer, Brandylyn Thomas
INHERITANCE AND EXPRESSION OF RED-EYE KOI MUTATION IN KOI *Cyprinus carpio* × GOLDFISH *Carassius auratus* HYBRIDS

16.30 **Antonia Theodoridi**, Michail Pavlidis, Luisa Dalla Valle, Aleka Tsalafouta
BEHAVIORAL DIFFERENCES IN COPING STYLES BETWEEN WILD-TYPE AND GR-KNOCKOUT ZEBRAFISH GENERATED WITH THE CRISPR/CAS9 TECHNOLOGY

SHRIMP

Thursday, October 10 14.30 - 16.50 Exhibition 2
Chair: Matt Slater

14.30 **Joao Reis**, Melanie Rhodes, D. Allen Davis
IMPROVING AUTOMATIC FEEDING PROTOCOLS IN SEMI-INTENSIVE POND CULTURE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

14.50 **Alexandra Segelken-Voigt**, Ralf Bochert
GROWTH PERFORMANCE AND SURVIVAL OF WHITELEG SHRIMP *Penaeus vannamei* UNDER VARYING STOCKING DENSITIES

15.10 **Gerardo Rodríguez-Quiroz**, Arturo Polanco-Torres, Píndaro Álvarez-Rúiz
EARTHWORM VERMICOMPOST TO ENHANCE SHRIMP WHITE SHRIMP *Litopenaeus vannamei* GROWTH AND INHIBIT AHPND DISEASE IN A EXPERIMENTAL CULTURE

15.30 **Eran Hadas**, Asaf Elkayam, David Hazut
MOLTING CAN BE A MAJOR CAUSE OF MORTALITY IN INTENSIVE SYSTEMS (RAS) FOR SHRIMP *Litopenaeus vannamei* CULTURE

15.50 **Yustian Rovi Alfiansah**, Sonja Peters, Jens Harder, Christiane Hassenrück, Astrid Gärdes
STRUCTURE AND CO-OCCURRENCE PATTERNS OF BACTERIAL COMMUNITIES ASSOCIATED WITH WHITE FAECES DISEASE OUTBREAKS IN THE PACIFIC SHRIMP *Litopenaeus vannamei*

16.10 **Vikash Kumar**, Peter Bossier, Kartik Baruah
PIRABVP TOXIN MEDIATES *IN VIVO* PATHOGENICITY OF *Vibrio parahaeolyticus* AHPND STRAIN: ROLE OF NOVEL PLANT-BASED HSP70 INDUCING COMPOUND IN PROTECTION AGAINST AHPND STRAIN IN SHRIMP SPECIES

16.30 **Ulfert Focken**, Juliana Schober Goncalves Lima
ORGANIC AND CONVENTIONAL MARINE SHRIMP FARMS IN BRAZIL: MACROBENTHOS OCCURRENCE INSIDE AND IN THE OUTLETS OF THE PONDS

NACEE PANEL – AQUACULTURE IN CENTRAL AND EASTERN EUROPE

Thursday, October 10 14.30 - 16.50 Backstage 1
Chair: Laszlo Varadi

Concept
The Central and Eastern European (CEE) region of Europe has certain specific features that make this region a special aquaculture region in Europe, which is sometimes called “pond region” or “carp region”. There are however other specificities as well why we may consider this region a special aquaculture region of Europe. For example, the fish consumption of several countries in the region is among the lowest in Europe. The CEE region includes both EU and non-EU countries and this “interface” character also creates challenges and opportunities in aquaculture development. In spite of the gradual social and economic development in the CEE region, aquaculture development is often constrained by limited resources, not only in terms of money, but, for example, in terms of language knowledge and international collaboration. This is well indicated by the low number of participants in international events from CEE countries and relatively low number of publications in international journals. Only few CEE institutions and organisations are members of project consortia. Multi-stakeholder cooperation is also rather weak in the CEE region where strong producers’ associations exist only in few countries. One of the main NACEE objectives is to bridge the gap that still exists between Eastern and Western Europe in the field of aquaculture. The proposed session could contribute to alleviate the situation and facilitate the improvement of collaboration between East and West.

Moderator: Peter Lengyel, General Secretary of NACEE

14:30 **Laszlo Varadi, Zsigmond Jeney**
CEE IN THE EUROPEAN AQUACULTURE SCENE

14:50 **Vitaliy Bekh, Nikolay Barulin, Peter Lengyel**
AQUACULTURE IN CENTRAL AND EASTERN EUROPE: CONSTRAINTS AND OPPORTUNITIES

15:10 **OPEN DISCUSSION**

15:50 **CONCLUDING REMARKS**

EDUCATION, KNOWLEDGE MANAGEMENT, TRANSFER AND EXTENSION NETWORKS

Thursday, October 10 14.30 - 16.50 Room 4
Chair: Georgia Bayliss-Brown

14.30 **Kay Lwin Tun**, Kevin Fitzsimmons, Hillary Egna, Hendrik Stolz, Uwe Scholz, Peter Buri
DEVELOPMENT OF BSC AND MSC FISHERIES AND AQUACULTURE DEGREE PROGRAMS IN MYANMAR

14.50 **Lourdes Reig**, Rosa Flos, Eva Vallejos, Felipe Reyes
PERCEPTION ABOUT AQUACULTURE AMONG UNDERGRADUATE STUDENTS; INFLUENCE OF PERSONAL PROFILE AND PSYCHOGRAPHIC MODULATORS

15.10 **Natacha Nogueira**, Lydia Png, Carlos Andrade
PUBLIC PERCEPTIONS OF AQUACULTURE IN ATLANTIC ISLANDS: IS THERE ROOM FOR MSP?

15.30 **Yik Sung Yeong**, Happy Nursyam, Quoc Phu Truong, Min Pau Tan, Najiah Musa, Siti Azizah Mohd Nor, Mieke Eggermont, David Bassette, Marieke Reuver, Patrick Sorgeloos
THE ASEAN FISHERIES EDUCATION NETWORK; ESTABLISHMENT, EXPANSION AND EXTENSION

15.50 **Marieke Reuver**, Jane Maher, John Bostock, Yeong Yik Sung
ENHANCING EUROPE-ASIA COOPERATION IN AQUACULTURE EDUCATION – ACHIEVEMENTS OF THE EU HORIZON2020 PROJECT - EURASTIP

16.10 **Joseph Molnar**, Isaac Omiat, Moureen Matuha, John Walakira, Gertrude Atukunda, Theodora Hyuha, James Bukenya
SUSTAINING A MOBILE APPLICATION FOR FISH FARMERS IN UGANDA: POLICY CONTRADICTIONS, TECHNICAL CAPABILITIES, AND USER NEEDS

16.30 **John Bostock**, Martyn Haines, John Birger Stav, Marieke Reuver
PROFESSIONAL AQUACULTURE – SUPPORTING SKILLS AND CAREER DEVELOPMENT IN EUROPEAN AQUACULTURE

COMPOUNDS IN LIPID TRANSPORT

Thursday, October 10 14.30 - 16.50 Lounge 1-3
Chair: Ashild Krogdahl

14.30 **Åshild Krogdahl**, Anne Kristine Grostøl Hansen, Elvis Chikwati, Michael H. Penn, Aleksei Krasnov, Ingemar Björkhem, Trond M. Kortner
NUTRIENTS INVOLVED IN DIGESTION AND TRANSPORT OF LIPID ACROSS THE INTESTINAL MUCOSA OF ATLANTIC SALMON *Salmo salar* L.– PART 1: AN OVERVIEW

14.50 **Elvis Chikwati**, Paul Midtlyng, Trond M. Kortner, Jie Wang, Yanxian Li, Weiwen Zhou, Guro Løkka, Alexander Jaramillo-Torres, Åshild Krogdahl
NUTRIENTS INVOLVED IN DIGESTION AND TRANSPORT OF LIPID ACROSS THE INTESTINAL MUCOSA OF ATLANTIC SALMON – PART 2: LIPID MALABSORPTION IN SIX, NORWEGIAN ATLANTIC SALMON *Salmo salar* L FARMS

15.10 **Trond M. Kortner**, Anne Kristine G Hansen, Elvis Chikwati, Aleksei Krasnov, Ingemar Björkhem, Åshild Krogdahl
NUTRIENTS INVOLVED IN DIGESTION AND TRANSPORT OF LIPID ACROSS THE INTESTINAL MUCOSA OF ATLANTIC SALMON *Salmo salar* L. – PART 3: EFFECTS OF DIETARY CHOLESTEROL, PHOSPHOLIPIDS AND BILE SALTS ON INTESTINAL LIPID METABOLISM

15.30 **Anne Kristine G. Hansen**, Trond M. Kortner, Elvis Chikwati, Hans J. Grav, Ingemar Björkhem, Vegard Denstadli, Åshild Krogdahl
NUTRIENTS INVOLVED IN DIGESTION AND TRANSPORT OF LIPID ACROSS THE INTESTINAL MUCOSA OF ATLANTIC SALMON *Salmo salar* L.– PART 4: EFFECTS OF DIETARY PHOSPHATIDYLCHOLINE AND CHOLINE ON INTESTINAL LIPID METABOLISM

NOTES

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CLIMATE CHANGE POSTERS

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0 **John Icely,** A Cubillo, M Elliot, J Ferreira, B Fragoso, P Kamermans, S Kay, A Kennerley, C Kreis, A Marques, D Matias, M Peck, J Pinnegar, H Rambo, K Smyth, V Stetzenm?Iler, N Taylor, N Taylor, X Álvarez-Salgado, N Norte, I Fuentes-Santos, D Brigolin, M Ballesteros, R Chapela, M Fernández-Reiriz, U Labarta, F Pranovi, J Santiago
COMPARISON BETWEEN THE RESULTS FROM THE EU H2020 CERES AND CLIMEFISH PROJECTS ON THE POTENTIAL EFFECTS OF CLIMATE CHANGE FOR AQUACULTURE OF MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*)

1 **M. Virginia Martín**, B.C. Felipe, A. Misol, S. Jerez, F.J. Santamaría, E. Almansa
COMBINED EFFECT OF INCREASING TEMPERATURE AND FEED RESTRICTION IN GILTHEAD SEABREAM *Sparus aurata* JUVENILES

2 **Ingrid A. Johnsen**
FJORD BASIN WATER EXCHANGE IN SILL NORWEGIAN FJORDS

3 **Elisabeth Ytteborg**, Bruce McAdam, Trevor Telfer, Solfrid Sætre Hjøllo, Øystein Hermansen, Lynne Falconer
POTENTIAL IMPACT OF WARMING SEA WATER TEMPERATURES ON ATLANTIC SALMON *Salmo salar* FARMING IN NORWAY

4 **Natacha Nogueira**, João Canning-Clode, Mafalda Freitas, Sonia Gueroun, Jamileh Javidpour
TRIALS WITH JELLYFISH SPECIES: IMPROVEMENTS ON THE BIOLOGY OF THE SPECIES AND REARING TECHNOLOGY

5 **Elke Burgstaller**, Janneke Aelen, Douglas Tenison-Collins, Eddie A.M. Bokkers
GREENHOUSE GAS ASSESSMENT OF ATLANTIC SALMON *Salmo salar* AQUACULTURE ON CERTIFIED (ASC) FARMS

6 **Thuy Pham**, Charlotte Weber, Ragnhildur Friðriksdóttir, Jónas Viðarsson, Sigurður Örn Ragnarsson, Petter Olsen, Tarub Bahri, Elisabeth Ytteborg, Alan Baudron, Michaela Aschan
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7 **Richard Heal**, Joseph Nagoli, M. Mahfujul Haque, Arifuzzaman Syed, Charles Tyler, David Bass
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8 **Balázs Kovács**, Dóra Kánainé Sipos, Réka Balogh, Csaba Guti, Kata Ihász, Szilvia Keszte, Béla Urbányi
MICROSATELLITE SET FOR PARENTAGE ANALYSES OF AFRICAN CATFISH, *Clarias gariepinus* (BURCHELL, 1822)

9 **Rui J. M. Rocha,** D. B. Freitas, J. G. Fonseca, I. B. Oliveira, C. M. Barroso, S. Galante-Oliveira
HIGH PERFORMANCE OF *Venerupis corrugata* LARVAL CULTURE UNDER PROJECTED ACIDIFICATION AND WARMING SCENARIOS UNVEILS THE SPECIES RESILIENCE TO FUTURE CLIMATE CHANGE

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10 **Andreas Müller-Belecke**, Cornelius Becke, Mark Schumann, Roland Rösch
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11 **Tae Young Ahn**
ACUTE TOXICITY AND BIOACCUMULATION IN EELS *Anguilla japonica* BY EXPOSURE OF WATERBORNE CADMIUM

12 **Nuria García Bueno**, Arnaldo Marin
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13 **Nuria García Bueno**, Arnaldo Marin
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14 **Øivind Bergh**, Henning Wehde, Vidar S. Lien
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15 **Lydia Png-Gonzalez**, Patrício Ramalhosa, Soledad Álvarez, Ignacio Gestoso, João Canning-Clode, Natacha Nogueira
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16 **Nicola Rhyner**, Constanze Pietsch
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17 **Fatema Al-Fatle Ali Abdulhur**, Erika Edvine Meleg, Katalin Ihasz, Tamas Molnar, Balázs Kovács, Istvan Lehoczky
CONSERVATION OF NATIVE CYPRINID SPECIES IN HUNGARY – PRELIMINARY RESULTS ON THE GENETIC VARIABILITY OF CRUCIAN CARP *Carassius carassius* (L., 1758) AND TENCH *Tinca tinca* (L., 1758) POPULATIONS AND STOCKS

18 **Lynne Falconer**, Akpojotor Ekpeki, Reed Ozretich, Trevor C. Telfer
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19 **Olaf Kattenpoel Oude Heerink**, Paul J. Van den Brink, Douglas Tenison-Colli
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20 **Elisa Ravagnan**, Anna Nora Tassetti, Gianna Fabi
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24 **Sang-Eun Nam**, Jae-Sung Rhee
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Our Future - GROWING from WATER

All life on earth emerged from water and the aquaculture sector, by definition, relies upon its existence for all or part of its many and varied production cycles. The future of healthy and sustainably produced food lies in the diversity of aquaculture, from single-cell microalgae in bioreactors to fish in sea pens or freshwater ponds. This is reflected in the German aquaculture sector which is unique in its diversity, ranging from extensive cultivation systems in ponds and coastlines to intense indoor farming.

Truly unlocking this potential requires innovative ways to support the aquaculture economy, generation of new business models and support of start-ups. We need to proactively work towards the demands of existing and new markets for all our products, seeking new alliances in international trade and consumer support. Meanwhile, the challenges of climate change and scarcity of natural resources are urgent and require our full attention in respect and appreciation of the ecosystems we work with. As a result there has been huge interest in our sessions focussing on the environmental interactions with aquaculture systems as well as those for nutrient recycling and nutrient supply to aquaculture organisms.

The organisation of such a conference is a huge team effort. The Steering and Local Organising Committees, chaired by Stefan Meyer and Birgit Schmidt-Puckhaber respectively have done a great job over the last two years to structure the event and ensure that all is in place. Overall the degree of response for AE2019 in Berlin has been overwhelming. More than 900 abstracts have been submitted and as a result we have been able to offer an impressive program characterised by highly diverse sessions, thematic workshops and innovative fora. These include new inter- and transdisciplinary platforms to support exchange within the aquaculture community and between various stakeholders to generate new ideas and concepts with economic potential. Our Programme Co-chairs Carsten Schulz and Tomáš Policar have worked incredibly hard to try to accommodate this record number of abstracts. Some difficult decisions have had to be taken by session chairs and some people will of course be disappointed. But we hope that the diversity of the programme will entice you to go out of your 'normal' sessions and listen to others. I dropped in on an Aquaponics session in Las Vegas at a World Aquaculture Conference several years ago and it changed the whole direction of my research!

As you will have seen, EAS has partnered this year with NordicRAS to provide our events back to back and hence allow participants to attend both in one place. We very much hope to pursue this partnership in the years to come.

With all the new knowledge being presented at our events, there is a wealth of possibility for innovation and value creation to help develop the sector. We have therefore developed the EAS Aquaculture Europe Innovation Forum and its inaugural meeting will take place on Wednesday. The event, in partnership with Hatch Blue and the German Startups Association, will consist of plenary speakers and "dragons' den" style pitches from 12 new companies that wish to seek further investment to develop.

The trade exhibition is bigger than ever this year with over 148 booths representing 130 companies from 23 countries and we have a series of special sessions to encourage attendance from exhibitors and trade show visitors. One of these is "Women in Aquaculture," a panel discussion looking at ways to ensure greater gender diversity at all levels of the aquaculture sector. There are also meetings of European associations, the EU EATIP Day and workshops of EU projects.

A special forum has been arranged for students attending AE2019 to enable networking and exchange of ideas. The forum will have a dedicated programme and includes a special student reception. In addition, the opening plenary session will feature the inaugural Aquaculture Europe Student Spotlight Award. This is based on the prize that is presented at the EAS/WAS AQUA events and it allows three finalists the chance to give a short pitch presentation of their work at one of the keynote sessions. The winner is then selected by the audience.

The Estrell Centre is a huge complex so we suggest that you download our EAS Meetings app from the app store or google play which will keep you in touch with all the up to date information on the inevitable last-minute program changes

Finally, I would like to highlight and thank our loyal AE2019 Gold Sponsors Biomar, our session sponsor Sorgal and the Rentenbank in Germany for their support of this event. We would equally like to thank our supporting partner organisations and our media partners.

Berlin is an eccentric city with a superbly integrated transport system and a wealth of cultural and heritage sites. Make sure you take the time to explore its historic streets and buildings.

Let there be life – and a future growing from water!

Gavin Burnell, EAS President 2018-2020

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aquaculture europe 19

ABSTRACTS

AUTO- AND HETEROTROPHIC NITROGEN REMOVAL IN WOODCHIP REACTORS TREATING RECIRCULATING AQUACULTURE SYSTEM EFFLUENTS

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Introduction

Woodchip denitrification reactors offer cost-efficient nitrate removal for treating nitrate-rich effluents of recirculating aquaculture systems (RAS). Currently, woodchip reactors are successfully applied in freshwater RAS (e.g. von Ahnen et al. 2018), where the nitrate removal is considered to be based on active heterotrophic microbial community growing on woodchips, that conducts denitrification i.e. reduces water nitrate into N_2 gas using carbon leaching from the woodchips as an electron donor. However, the production of marine fish species in RAS is increasing, bringing more challenges to the effluent treatment. Recently, nitrate removal was found to be significantly lower in woodchip reactors treating saline than freshwater RAS effluents (von Ahnen et al. 2019), which was suggested to be related to the dominance of autotrophic denitrification i.e. nitrate reduction using other electron donors than organic carbon (e.g. H_2S, HCO_3^-) under saline conditions. In this study, our objective was to examine if HCO_3^- addition affected the dynamics between auto- and heterotrophic denitrifiers, seen as the differences in the nitrate removal, microbial community composition and origin between woodchip reactors receiving freshwater and low salinity (7ppt) RAS effluents.

Materials and methods

The experimental set-up comprised of twelve laboratory-scale woodchip reactors, which received four different RAS effluent types: a) freshwater (F) b) freshwater with $NaHCO_3$ addition (targeted alkalinity of 1.6 g l^{-1}; FS), c) salt water (S), and d) salt water with $NaHCO_3$ addition (targeted alkalinity of 1.6 g l^{-1}; SS), n = 3 per treatment. The experiment was run for 58 days. Inorganic N concentrations were measured weekly and other water chemistry parameters three times during the experiment. For microbial community analysis, water samples from the inlet, outlet, and in the middle of the reactors,

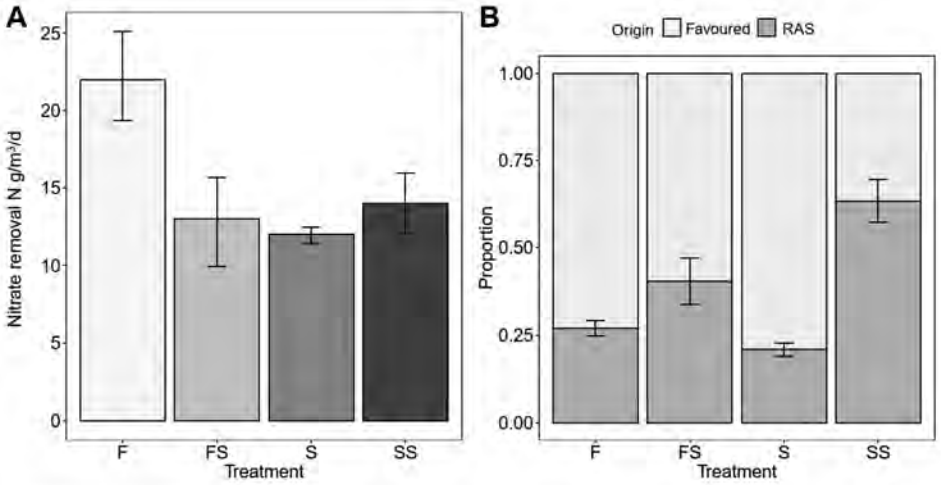

Figure 1. A) Nitrate removal rates (average ± SE), and B) the proportions (average ± SE) of microbes favoured by reactor conditions or originating from woodchip (Favoured) and microbes originating from RAS to woodchip biofilm community predicted with SourceTracker in woodchip reactors receiving four types of effluents (F = freshwater, FS = freshwater + $NaHCO_3$, S = saline water, SS = saline water + $NaHCO_3$).

(Continued on next page)

and three replicates of woodchip biofilm were collected in the end of the experiment. Microbial community composition was analysed using next generation sequencing with IonTorrent PGM targeted on 16S rRNA gene V4 region. Nitrate removal rates were calculated as in von Ahnen et al. (2019). The origin of woodchip biofilm microbes was predicted using SourceTracker method (Knights et al. 2011).

Results and discussion

Freshwater reactors (F) had the highest nitrate removal rates (Figure 1A). The addition of HCO_3^- improved slightly the nitrate removal in saltwater reactors, but suppressed the nitrate removal in freshwater reactors. Furthermore, HCO_3^- addition changed the microbial community composition in freshwater reactors, decreasing the diversity and the abundance of the key denitrifying microbial groups, whereas in two saltwater treatments, the communities were rather similar, but HCO_3^- addition increased the diversity and denitrifier abundance. Based on the SourceTracker (Figure 1B), HCO_3^- addition increased the proportion of microbes originating from RAS in woodchip biofilms in both freshwater and saltwater reactors. Altogether, these results indicate that in freshwater woodchip reactors, heterotrophic denitrification is the most beneficial process, and HCO_3^- addition promoted the abundance of non-denitrifying microbes originating from fish tanks. However, it seems that mixed autotrophic-heterotrophic denitrification system is beneficial under saline conditions, where woodchip carbon-based heterotrophic denitrification is typically suppressed. In addition, the results suggest that a substantial proportion of the autotrophic denitrifiers originates from saltwater RAS. In conclusion, efficient nitrate removal in saltwater effluents requires favourable conditions for autotrophic denitrifiers, e.g. through addition of inorganic electron donors or using H_2S produced in sulfate-rich saltwater RAS.

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Bacillus pumilus ISOLATED FROM HEALTHY HONEY BEES AND ITS EFFECTS ON SOME BIOLOGICAL ASPECTS IN MICE AS POTENTIAL AQUACULTURE PROBIOTIC

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Introduction

A *Bacillus pumilus* is a Gram-positive, aerobic, spore-forming *bacillus* belonging to *Bacillus subtilis* group. *B. pumilus* is widely used in industrial microbiology, such as the production of many of fermented foods, based on its efficiency to degrade a lot of the pollutants, it used in the treatment of wastewater and the degradation of environmental pollutants. *B. pumilus* have the ability to produce several biological compounds such as vanillin, keratinase, xylanase, alkaline serine protease and several other bioactive substances. Numerous *B. pumilus* strains isolated from several environmental sources were applied as microbial probiotics for animals. However, numerous *B. pumilus* are harmful to human, animals, and plants. The present study was aimed to isolate a novel *B. pumilus* strain to evaluate its ability as bacterial probiotics in animals.

Materials and methods

Isolation of microorganisms: *B. pumilus* associated with the digestive tract of healthy honey bees was isolated using 100 honey bees. The digestive tracts of honey bees were extracted. a sterilization of outer surface of the extracted digestive tracts was done using ethanol solutions (70%) and sodium hypochlorite solution (7%).

Procedures and experiments: 60 adult healthy male mice *Mus musculus domesticus* were selected to obtain body weight 25±1 gm then used in the present biological experiment. All mice were grouped into three groups (20 mice for each group), the first group (control group) was dieted a standard mice diet, the second group was dieted with standard mice diet and treated with 0.20 ml of sterile normal saline solution (Orally, the solutions was given daily), the third group was treated as in the second with the replacement of the sterile normal saline solution with the bacterial suspension (The bacterial suspension was prepared from pure culture of *Bacillus pumilus* in a sterile normal saline solution (106 cell/ml)).

Statistical analysis: A completed random design (CRD) was applied in this study and statistical analyses of data were done using IBM SPSS Statistics 23.

Results and discussion

Isolation and identi ication

The aim of this study was to evaluate the biological effects of *B. pumilus* strain isolated from healthy honey bees on several biological parameters in mice. The microscopic features results showed that some the bacteria isolated from the digestive tract of healthy honey bees were rod-shaped, Gram-positive, aerobic, spore-forming bacteria. All the isolates that have previous microscopic features were selected as *B. pumilus* then the biochemical tests using HiBacillusTM Identification Kit (Himedia, india) were done to distinguish between *Bacillus* species. The data in table 1 reported that there are some bacterial isolates may be *B. pumilus* strains where each Voges Proskauer's, citrate, ONPG, catalase, sucrose, mannitol, glucose, arabinose and trehalose positive; and malonate, nitrate reduction and arginine negative bacterial isolates were considered *B. pumilus* strains.

Animal experimentation

A figure 2 shows the biological effects of the *B. pumilus* on body weight (gm) and mortality (%) of the mice. The data confirmed that *B. pumilus* has a positive impact on the body weight (gm) and mortality (%) of the mice. The *B. pumilus* led to a significant ($P<0.05$) increase in the body weight and a significant ($P<0.05$) decrease in the mortality. Differences in the mean concentration of the biological parameters were not significant in all groups. The level of the blood constituents and compounds of mice, which were investigated in this work were within normal levels comparing to the levels mentioned by Mitruka and Rawnsley (1981). The data obtained from this study reported that *B. pumilus* has no unfavorable influence on total protein, glucose, insulin, total cholesterol, WBCs, RBCs, HGB, HCT, PLT, MCV, MCH, and MCHC.

(Continued on next page)

There are several *Bacillus* species such as *B. cereus*, *B. clausii*, *B. pumilus* have been carried in numerous commercial probiotic products for aquaculture and human, and have been characterized for potential attributes including colonization, immunostimulation, and antimicrobial activity that accounted for their probiotic features.

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Table 1: The biochemical tests used to identify *Bacillus pumilus* using HiBacillus™ Identification Kit (Himedia, india).

Biochemical test	Malonate	Voges Proskauer's	Citrate	ONPG	Nitrate reduction	Catalase	Arginine	Sucrose	Mannitol	Glucose	Arabinose	Trehalose
Results	-	+	+	+	-	+	-	+	+	+	+	+

ONPG = o-Nitrophenyl-β-D-Galactopyranoside, + = positive and - = negative.

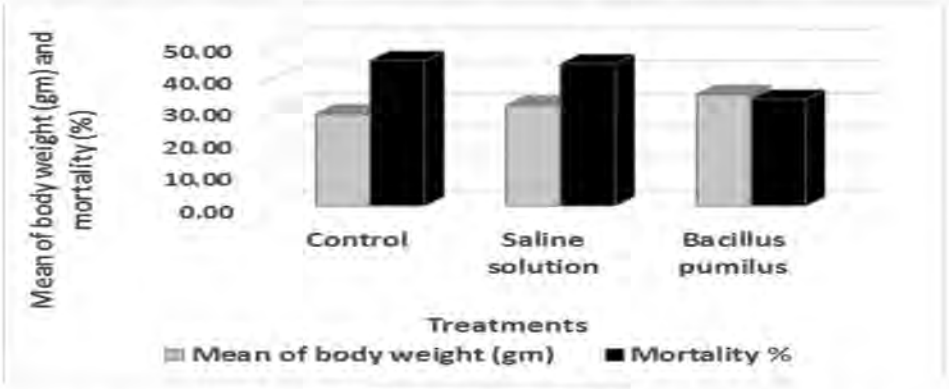

Figure 2: The biological effects of *Bacillus pumilus* on body weight (gm) and mortality of mice.

A STUDY OF DIETARY PATTERN AMONG MALE STUDENTS AT THE UNIVERSITIES OF THAMAR AND AL SAEEDA, YEMEN

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Introduction

Different dietary patterns can have an effect on overall health since pleasure apparently acts as health promoters and worries can adversely affect health. The male population in Yemen has increased rapidly, the number of females, in relation to the total Yemenis population, grew from 51 % in 2015, (central Statistical Organization For the period 2005-2025). World average fish per capita is 20 kg while, Yemen average fish per capita is 4 kg (FAO, 2019). The analysis of dietary patterns becomes the best alternative to evaluating healthy behaviors. In this study, they decreased their weekly consumption of oily fish, sea foods, fresh fruits, cooked and the raw vegetables, pulses and olive oil while they increased their sugar, and fast food intake. The Food Frequency Questionnaire (FFQ) is commonly used to assess dietary patterns associated with clusters of frequently consumed food items.

Methodology

Sampling technique: The study included 300 male students aged 19-29 years were randomly selected three colleges (Medicine, Engendering, and Information technology) of the University of Thamar and Al saeeda.

Statistical analysis: All the data thus collected were entered into the SPSS 17 software.

For Meat/Snacks Score:

If your score is:
22-24 you are generally eating a typical American diet, which could be lower in fat.
18-21 you are making better low-fat food choices.
17 or less you are making the best low-fat food choices. Keep up the great work
If you scored 17 or less, you are doing well, this is the desirable score on this screener.

Results and discussion

Consumption frequency of different protein foods Items by male Students at Thamar and Al Saeeda University

Frequency of eating different food items for male students are mentioned below is presented in table (5). The present sample demonstrated high consumption of proteins food (eggs 72.7%%, bean71.3%, chicken 70%, meat 56.7%)**;** While were (eggs 76.7%, chicken 70.7%, bean 66.7%, meat 64%) among male Students Thamar and Al saeeda Universities respectively. While low consumption of protein food (fish 23.6%, and nuts 17.3%)**;** (fish 25.2%, and nuts 16) among male students of Thamar and Al saeeda Universities respectively. The decrease of fish consumption may be due to Length of distance from the coast, and hence the high cost of fish, as well as fish damage, due to lack of good conservation; and lack of awareness about the importance of fish consumption on overall health promotion. The negative association between nuts and consumption it might be related to the limited financial powe .

Eating habits

Meat/Snacks and fruit/vegetable/fiber intake among male Students of Thamar and Al Saeeda University

Average score for meat/snacks intake shows in fig. (1). Meat/Snacks and intake values were significantly making the best low food choices keep up the great work, where values were less than 17 (15.92) for male medical students, and high than 17, were around (21.22, and 22.52) For male students (engineering and computer faculties) of Thamar university respectively. Decreasing consumption between male medical students may be due to the lack of time among medical students that the meat and fish products need time to get them as well as to prepare them. While for male students of Al saedda university (medical, engineering and computer faculties) were (19, 20.94 and 21.32) respectively. Our results showed that moderate intake of meat/snacks pattern may be protective factors to general (BMI) and central obesity (WHR), respectively.

(Continued on next page)

Table 1. Consumption Frequency of Different Protein Foods Items by male Students at Thamar and Al saeeda university

Items	University	
	Thamar (n = 150) %	Al saeeda (n =150) %
Meat	56.7	64
Poultry	70	70.7
Eggs	72.7	76.7
Fish	23.6	25.2
Milk	51.3	53.3
Cheese	44.7	52.7
Yoghurt	48.7	51.3
Nuts	17.3	16
Peas	37.3	39.3
Beans	71.3	66.7

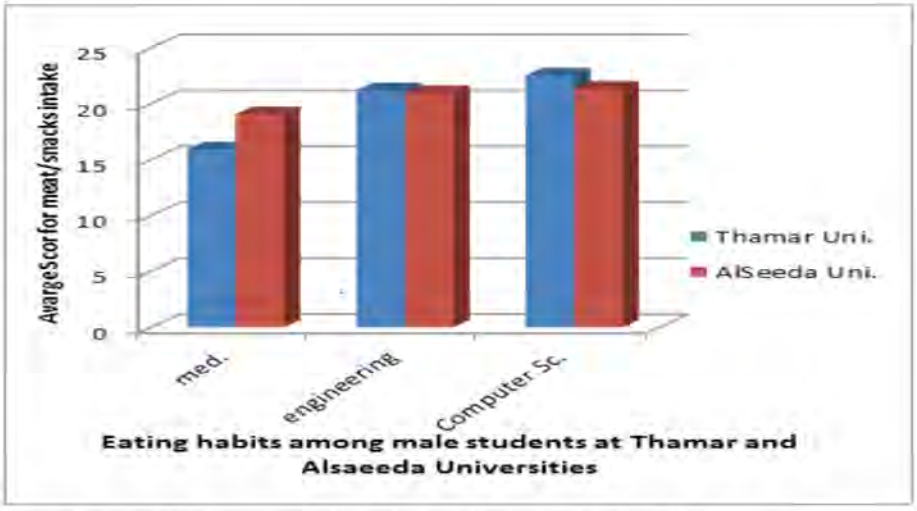

Figure 1. Eating habits among male Students at Thamar and Al Saeeda University.

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USE OF AN EXOGENOUS ENZYMES AND PROBIOTICS COCKTAIL TO ENHANCE THE GROWTH PERFORMANCE OF RED TILAPIA (*Oreochromis niltoticus* × *O. mossambicus*) AND ELIMINATING THE BACTERIAL COUNT IN ITS EFFLUENTS

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Introduction

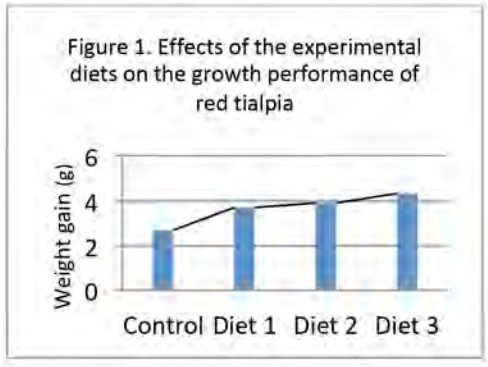
Tilapia (*Oreochromis* sp.) has become one of the most important aquaculture species globally. Artificial diets are the essential feeding source used in tilapia aquaculture. Plant-derived proteins, such as soybean meal are widely used as ingredients in tilapia diets. However, these plant-derived proteins show a low nutrient utilization of by fish which results in low growth performance as well as the pollution of the fish pond environment (Leenhouwer et al., 2007). This limitation in nutrient availability in fish diets is mostly caused by non-starch polysaccharide (NSPs) compounds and/or other anti-nutritional factors in plant-derived proteins (Sinha et al., 2011; Cheng et al., 2013). Therefore, Microbial enzymes are widely used in fish diets to minimize the negative impacts of anti-nutritional factors, thereby improving the nutritive value (Forster *et al.,* 1999), the nutrient utilization (Cheng *et al.,* 2013) and fish growth (Cheng *et al.,* 2013; Saputra *et al.,* 2016). It has been found that the probiotic and multi-enzymes inhibited the pathogenic bacteria (Jiang et al., 2015; Acosta et al., 2016). Therefore, the objective of this study was to evaluate the possible effects of supplementation of an exogenous enzymes and probiotic cocktail on growth performance, feed utilization and the intestinal microbial count of red tilapia (*Oreochromis niloticus* × *O. mossambicus*) and the bacterial count in its effluent

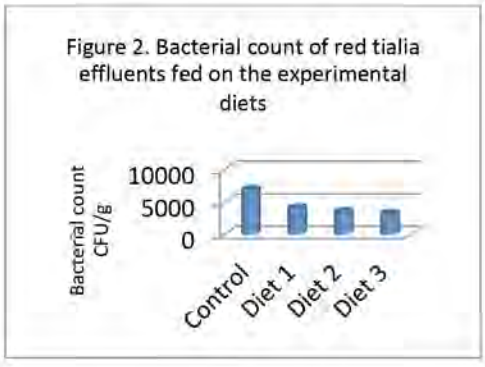
Material and methods

Red Tilapia fry (initial weight 0.35 g) were randomly assigned to four groups. The treatments were performed in triplicate. Four experimental diets were offered to the fish for 60 days. Out of the four diets, three contained the exogenous enzymes and probiotic mixture at various levels of inclusion (1.5 (Diet 2), 2 (Diet 3) or 2.5 (Diet 4) g kg^{-1} diet) and the fourth a control diet was left without any inclusion (Diet 1). The exogenous enzymes and probiotic mixture (Activity/g) is consisted of Cellulase: 1,50,000 CMCU, Arabinase: 2120 U, Beta Galactosidase: 25,000 U, Beta Mannanase: 2,00,000 U, Xylanase: 2,80,000 U, Protease: 7,00800 U, Alpha Amylase: 70,000 U, Lipase: 18,000 U, Phytase: 2500 FTU and Probiotics (CFU/g) (*Lactobacillus acidophilus* 6×109 and *Saccharomyces baulardii* 6×109).

Results

The results demonstrated that red tilapia fed on the exogenous enzymes and probiotic mixture resulted in better growth performance in terms of final body weight (FBW), weight gain (WG) and feed conversion ratio (FCR) compared to the control group ($p < 0.05$) (Figure 1). Fish fed the diets containing the exogenous enzymes and probiotic mixture showed significantly higher whole body protein and lower lipid contents ($p < 0.05$) than the control gtroup. The study demonstrates that test diets supplementation modulated the intestinal bacterial communities in intestines of red tilapia ($p < 0.05$). The inclusion of the exogenous enzymes and probiotic mixture into red tilapia diets minimized the bacterial content of fish tanks effluents $p < 0.05$) (Figure 2).





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Discussion

In the present study, the beneficial effects of multi-enzyme and the probiotic mixture on the performance of red tilapia have been indicated. This is explained by the ability of exogenous enzymes including protease, amylase, cellulose and mannase to solubilize various anti-nutrition factors and improve digestibility and growth performance (Cowieson, 2005). In support, it has been found that a multi-enzyme complex improved growth performance such as FBW, FCR and SGR of snakehead (*Channa argus*) (Dai et al., 2019). In Nile tilapia, fish fed a diet containing the xylanase-expressing probiotic, Bacillus amyloliquefaciens, had better growth performance and health status compared to fish fed a control diet (Saputra et al., 2016). The result of intestinal bacterial count showed that the probiotic and multi-enzymes improved the health by inhibiting pathogenic bacteria which is in agreement with Jiang et al. (2015) and Acosta et al. (2016). The causes for such effects may be that mixture of exogenous enzymes changed the environment of the intestine through an alteration of the pH or intestinal substrates caused by enzyme decomposition (Jiang et al., 2015).

In conclusion, inclusion of the exogenous enzymes and probiotic mixture into red tilapia diets has improved the growth performance, feed utilization, intestinal microbial communities and decreased the bacterial count of fish tanks effluents. However, future studies are needed to determine the conditions under which probiotics interact with multi-enzyme mixtures.

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INFLUENCE OF β-CAROTENE ON THE GROWTH AND FATTY ACID STATUS OF THE YOUNG STELLATE STURGEON *Acipenser stellatus* PALL

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Carotenoid pigments cannot be synthesized by animals *de novo* and have exclusively dietary origin. Each species (or genus) adapts to the accumulation of the pigment that is more accessible. At the same time, in the fish organism there are certain biochemical mechanisms that are capable to modify carotenoids obtained from the diet into species-specific ones

Species-specific carotenoids of sturgeons are zeaxanthin, lutein and β-carotene. Under natural conditions, sturgeon fishes consume a significant amount of carotenoids contained in living food organisms. When artificially reared and in the absence of natural feed, the young fish are almost completely deprived of these pigments

Three variants of feeds were studied, in which 2.4, 4.8 and 7.2 mg of β-carotene were injected per kg of feed. As a result of cultivation, it was determined that the growth, survival rate and the level of ω3 and ω6 fatty acids and their ratio depended on the amount of β-carotene. With its greater concentration in the diet, the growth rate of the fry, in contrast to the survival and fatty acid status, increased by 20, 25 and 10% as compared with the control (Fig. 1).

Thus, at the level of β-carotene equal to 2.4 mg/kg of feed, the survival rate increased by 25%, ω3 / ω6 ratio in total lipids and phospholipids increased by 24.4 and 19.4%, respectively, mainly due to 18:3 and 22:6 fatty acids. At the level of β-carotene being 4.8 mg/kg, the differences of these parameters from the control were less significant, namely, 1.0, 1.9 and 6.7%. At the maximum level of β-carotene, the survival rate decreased by 54%, and that of ω3 / ω6 ratio in lipids by 2.8 times.

Having assessed the effect of different concentrations of β-carotene in the diet of young stellate sturgeon, we should say that 2.4 mg per 1 kg of feed is the optimal amount.

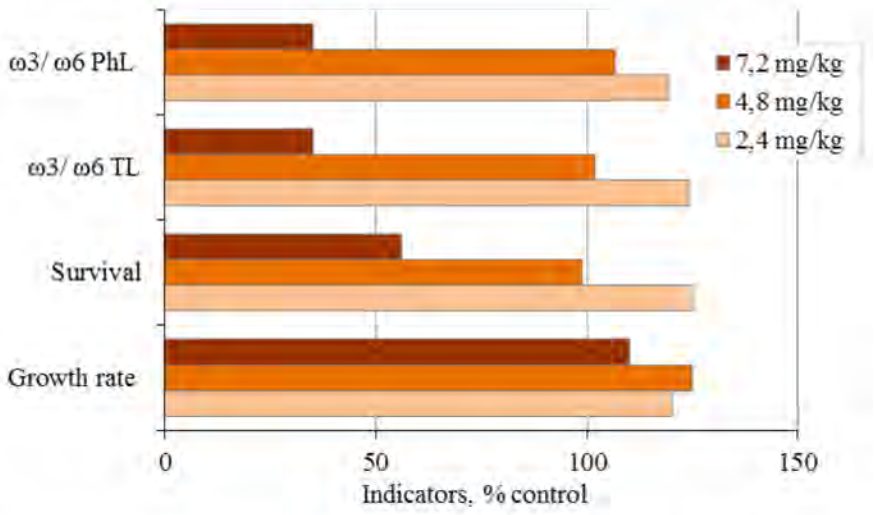

Figure 1: The results of growing young sturgeon consuming feeds with β-carotene:
ω3 / ω6 TL,
ω3 / ω6 PHL,
TL - total lipids,
PhL - phospholipids

SCREENING OF BIOSORPTION CAPACITY OF MACROPOROUS FUNGAL BIOMASS OF *Trichoderma viride* FOR LEAD REMOVAL: A PROPOSED BIOREMEDIATION IN AQUACULTURE

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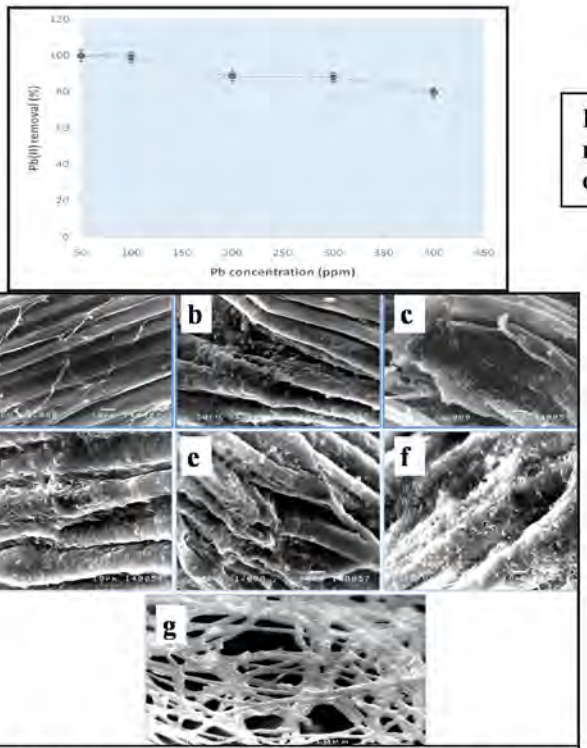
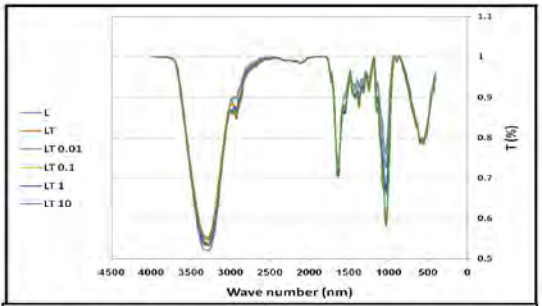
Environment pollution by heavy metals is a global disaster, lead is considered one of the persisting heavy metal pollutants throughout the world due to its continuous anthropogenic generation and non-biodegradability. Its mobilization towards aquaculture escalates its threat due to its introduction to the food chain via aquatic fish and consequently to human beings consuming fish. Removal of heavy metals by conventional methods are not economically and environmental friendly as it produce massive quantity of toxic chemical compounds. Bioremediation and biological treatments especially with filamento s fungi is an alternative methods which have gained an increasing attention for heavy metal removal and recovery due to their upright performances, low cost and huge quantities. This study is a preliminary research for a proposed bioremediation in aquaculture was carried out using 3D macroporous fungal biomat formed of *Trichoderma viride* immobilized on luffah was used for lead removal. The biomate was able to remove up to 79.44% of 400 ppm lead within 24 h and was increased to 89.05% after optimizing temperature and pH. Gamma radiation and NaOH Pre-treatment of the biomat was performed, lead removal increased to 95% within 1 hour of incubation at 30°C and pH 6. FT-IR and SEM spectroscopy indicated some changes in functionality and texture of the immobilized *T. viride* biomat. The re-use of the biomat was efficient for three consecutive cycles and was also used in fixed bed column and showed 89% removal. The biomat is very suitable for use in fixed bed reactors. and may could be used as a biofilter and contribute to water conservation.



SEM images of luffah (a), immobilized luffah biomat (b), immobilized biomat irradiated with 0.01 KGy (c), 0.1 KGy (d), 1 KGy (e), 10 KGy (f), NaOH (g). Magnifications at 1000x.

Pb removal using *T. viride* immobilized macroporous biomats incubated with different initial Pb concentrations



FT-IR spectra of gamma irradiated *T.viride* immobilized macroporous

2)

EFFECTS OF DIETARY FISH OIL SUBSTITUTION BY *Echium* OIL ON ENTEROCYTE AND HEPATOCYTE LIPID METABOLISM OF EUROPEAN SEA BASS *Dicentrarchus labrax* (*Linnaeus,* 1758)

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Introduction

The major carnivorous fish species farmed in Europe have traditionally been fed diets based on fish meal and fish oil (FO). Therefore, the replacement of FO with vegetable oils (VO) in aquafeeds is a viable alternative for the sustainability of the aquaculture industry. The poor ability of marine fish to convert C_{18} polyunsaturated fatty acids (PUFA) to long chain PUFA (LC-PUFA) such as 20:4n-6 (ARA), 20:5n-3 (EPA) and 22:6n-3 (DHA), together with the fatty acid profile of VOs, lead to important changes in the fatty acid composition of flesh of fish fed VOs, and therefore in its nutritional value, which include decreased n-3 LC-PUFA and increased total fat and linoleic acid (18:2n-6, LA) contents. For these reasons, replacement of dietary FO must be approached with caution. In this sense, the *Echium* genus (*Boraginaceae*) seed oils, *Echium* oil, has an interesting fatty acid profile with a good n-3/n-6 PUFA balance and a high content of unusual fatty acids, (stearidonic acid, SDA and γ-linolenic acid, GLA) that have competitive and inhibitory effects in the production of proinflammatory eicosanoids derived from ARA. Enterocytes and hepatocytes play critical roles en lipid metabolism including uptake, oxidation, conversion of fatty acids and the supply of LC-PUFA to the other tissues. Therefore, the aim of this study is to evaluate the effects of 50% substitution of dietary FO by *Echium* oil.

Materials and methods

Sea bass juveniles with an initial body weight of around 150 g were distributed into 9 tanks. Three of them were fed a commercial diet (CD), containing FO, during the whole experiment, 7 months. The other six ones were fed an experimental diet (ED) with 50% FO and 50% VO, during 4 months, that was the time in which VO composition was reflected in the muscle. Thereafter, three of these tanks continued receiving the experimental diet (7 months in total) while the other three turned back to the commercial diet (experimental-commercial diet, ECD) to the end of the experimental period. Fish were fed *ad libitum*, reared under constantly flowing seawater, and natural photoperiod at 19–19.5 °C. At the end of the experiment, the individuals were slaughtered by a sharp blow to the head, individually measured and weighed. Samples of blood, muscle and several tissues were taken for biochemical analysis. In addition, samples of intestine and liver were collected to isolate enterocytes and hepatocytes respectively. Part of both cellular suspensions were incubated with ^{14}C radiolabelled fatty acids to perform fatty acid metabolic studies, and the other were used to carry out the control lipid profile studies. The entire experiment was conducted in accordance with the Spanish law in BOtE 8th April 2013 for protection of experimental animals.

Results

The metabolic studies using radiolabelled fatty acids indicated that although desaturase delta 6 activity is present it is not directly implicated in EPA conversion to DHA, but in the conversion of 18:2n-6 to 18:3n-6 and of 18:3n-3 to 18:4n-3. Elongation activity was evident for several substrates and no delta 5 activity was detected.

After 7 months of experiment the control fatty acid profile of enterocytes and hepatocytes were analyzed and no significant differences in DHA, n-3 LC-PUFA levels and n-3/n-6 index were found between the 3 treatments. The lipid classes profile of control cells was also analized and there were not significant differences in total polar lipids, although there was an increase in triglycerides in ED fish group

Discussion and conclusions

Although the DHA level in the experimental diet was half that of the commercial diet, and there is no DHA production from EPA, it seems that the *Echium* diet has induced a selective retention of DHA. Our present results suggest that the balanced n-6/n-3 composition of *Echium* oil provides a compensatory effect on the fatty acid metabolism and tissue deposition similarly to that reported for *Sparus aurata* by our group (Díaz-López et al., 2009; 2010).

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The balanced composition of *Echium plantagineum* oil not only did not affect the DHA levels in sea bass but enhanced some parameters of interest like polar lipids and LC-PUFA after returning to the control marine based diet. *Echium* oil might be considered as a more sustainable alternative to other VO in animal diets, providing a health prometing profile for human consumption.

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Acknowledgment

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EPIGENETIC EFFECTS OF DIETARY MICRONUTRIENTS IN ATLANTIC SALMON (*Salmo salar*) MUSCLE

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Introduction

Increased dietary levels of plant proteins and oils have been shown to alter the metabolism of amino acids and micronutrients such as folate, vitamin B12 and B6, all of which are important one-carbon (1C) metabolism nutrients. Negative effects of such dietary changes were observed on growth performance and flesh quality in farmed Atlantic salmon (Morkore and Rorvik, 2001; Larsson et al., 2014; Hemre et al., 2016). This study explores how dietary micronutrients affect nutrient-responsive mechanisms in fast muscle of Atlantic salmon using a multi-omics approach. A better understanding of the underlying nutrient-responsive mechanisms, which are of molecular and epigenetic nature, can help to explain phenotypic changes to muscle. DNA methylation regulates mRNA expression, and thereby controls metabolism, which is one underlying mechanistic explanation for nutritional programming. Previous results showed that gene expression patterns and epigenetic regulation in fish are highly sensitive to nutrient levels (Skjaerven et al., 2018). Epigenetic changes in early life stages, or as early impact in pre-smolts, can program life-long consequences on physiology, robustness and growth. The results from this study will provide documentation that has potential to customize feed composition to improve healthy growth and maintain good filet quality through nutrition

Material and methods

Two experimental diets provided by Skretting ARC (Stavanger, Norway) were fed to Atlantic salmon 6 weeks prior to smoltification until 3 months after saltwater transfer in triplicate tanks. Diet A and diet B contained different levels of methionine (6.7 and 9.5 g/kg diet), folate (2.6 and 4.8 mg/kg diet), vitamin B12 (0.15 and 0.18 mg/kg diet) and vitamin B6 (6.75 and 9.31 mg/kg diet), respectively. Diet A includes 1C-nutrients at the requirement level (NRC, 2011) and diet B a surplus of 1C-nutrients to support maximal performance, as determined by others (Espe et al., 2014; Hemre et al., 2016). The main protein sources in the diets were soy protein (24%) and pea protein (15%) concentrates, and the diets included smaller amounts of fishmeal (12%) and krill meal (2%). Lipid source was a mixture of rapeseed (8.1%) and fish oil (12.6%). Muscle from pre- and post-smolts (five individuals per tank, n=3) were analyzed for free amino acids, folate, vitamin B12 and B6, and were sent to global metabolic profiling (n=3). RNA and DNA extracted from the same muscle samples (n=9) was used for RNA-sequencing (RNA-seq) and Reduced Representation Bisulfite Sequencing (RRBS) analysis, respectively.

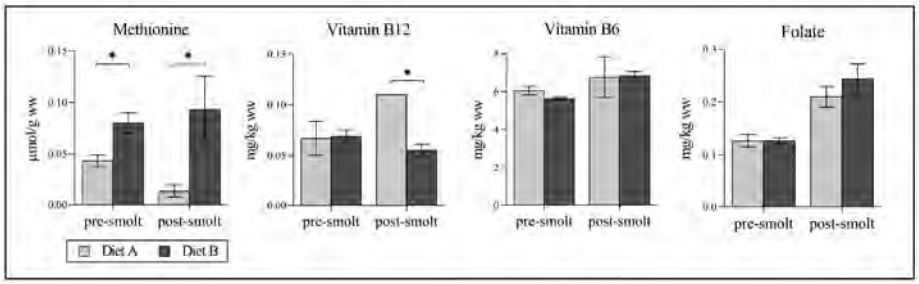

Fig. 1. Methionine, vitamin B12, vitamin B6 and folate levels (mean±SD) in fast muscle of pre- and post-smolt Atlantic salmon fed either diet A (n=3) or diet B (n=3). Asterisk indicates statistical significance using Welch's t-test for significance testing ($p<0.05$, unpublished data).

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Results

Preliminary results showed that salmon fed diet A and diet B grew from a mean body weight (±SEM) of 31.6±0.8 g and 32.2±0.5 g during the freshwater period to a final body weight of 462.5±19.2 g and 539.2±8.5 g after saltwater transfer, respectively. There were no differences in growth performance during the freshwater period, but fish fed diet B had better growth at the end of the saltwater period than fish fed diet A ($p=0.01$). Hepatosomatic index was higher in fish fed diet A (1.64±0.08) compared to fish fed diet B (1.32±0.04) during the saltwater period, but not different in the freshwater period. Feed conversion ratio and protein utilization were not different during either the freshwater or the saltwater period. Unpublished results show that higher methionine levels given with diet B increased methionine in both pre-smolt ($p=0.01$) and post-smolt muscle ($p=0.04$) when comparing with diet A (figur 1). No differences were found in folate and vitamin B6 levels in muscle during the freshwater and saltwater period. Vitamin B12 levels were the same in pre-smolt muscles, but lower in muscle of post-smolts fed diet B compared to diet A ($p=0.003$). Comparing metabolites from a global metabolic profiling of the muscle samples, most of the differences were between pre- and post-smolt than between dietary groups. Most differences between muscles from fish fed either diet A or diet B were found in the amino acid profiles. Differences between pre- and post-smolts relate to amino acids, ether lipids, unsaturated fatty acids, mono- and polyunsaturated fatty acids. Key results from metabolic, gene expression (RNA-seq, in progress) and DNA methylation profiling (RRBS, in progress) will be presented.

Discussion and conclusion

There is limited research on requirement levels for 1C-nutrients for farmed Atlantic salmon especially during periods of extensive growth and development. Diets containing 1C-nutrient levels as for diet B are recommended as they resulted in best performance during smoltification and on-growing saltwater period. Following a multi-omics approach, correlation of metabolic, gene expression and DNA methylation profiles will reveal how nutrient-responsive epigenetic mechanisms can explain how diet affects muscle growth. The knowledge obtained through this study contributes to further development of innovative methods for nutritional programming of better fish health

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COMPREHENSIVE APPROACH TO DEVELOPMENT OF COMMON CARP STRAINS RESISTANT TO DISEASES CAUSED BY INFECTIONS WITH CYHV-3, CEV AND SVCV

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Introduction

Common carp aquaculture is affected by several viral disease caused by infections with cyprinid herpesvirus 3 (CyHV-3), carp edema virus (CEV) and spring viremia of carp virus (SVCV). Especially the koi herpesvirus disease (KHVD) caused by CyHV-3 and koi sleepy disease (KSD) caused by CEV can lead to extremely high losses of common carp in all age categories. However, common carp strains with a different genetic background present diverse susceptibility to some pathogens and this fact could be utilise for development strain with broad resistance to virus infections. Here we present results from first comprehensive evaluation of viral disease resistance of common carp strains and first steps for development of strains with resistance to multiple viral diseases.

Materials and Methods

Common carp strains Amur wild carp (AS), Ropsha scale carp (Rop) Prerov scale carp (PS) and koi were tested for susceptibility to CyHV-3, CEV and SVCV using bath or cohabitation infection models. Furthermore, the virus load, speed of spreading and magnitude of type I interferon responses as a first line of cellular defences against viral infection were measured in skin, gills, head and trunk kidney using RT-qPCR. New crosses were developed based on the results from infection experiments and the survival of F2 generation was measured in experimental ponds.

Results

An infection experiment confirmed significant differences in mortality and virus load during CyHV-3 infection where Rop and AS (mortality of 22% and 47%) were performing better than PS and koi (mortality of 65% and 90%). When the susceptibility to a CEV infection and the development clinical of KSD were investigated, Amur wild carp were more resistant to the infection and did not develop clinical signs for KSD while in koi mortality reached 100%. An infection with SVCV induced low (22%) mortality in PS, while Rop (with 0% mortality) was the most resistant. The evaluation of the viral loads and replication showed significant differences which correlated with mortality. The magnitude of the type I IFN response was not a good marker of resistance because it was positively correlated with virus load.

Discussion and Conclusion

Based on the results resistance results 3 crosses were developed using Rop, AS and highly productive Zator strain. The three new Rop_Z, AS_Z and Rop_AS_Z crosses should provide the resistance to CyHV-3 and SVCV, CEV and all three respectively. The crosses showed higher survival in experimental ponds in the first year of productions, showing that the challenges caused by viral diseases could be addressed by selective breeding. Further infection experiments will follow to measure the disease resistance of the novel crosses.

WIDE DISTRIBUTION OF CARP EDEMA VIRUS IN EUROPE IS CONFIRMED BY RECENT DETECTIONS IN HUNGARY, CROATIA, SERBIA AND LITHUANIA

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Introduction
Koi sleepy disease (KSD) caused by carp edema virus (CEV) infection is considered an emerging disease in certain regions of the world. Especially in Europe the virus was detected only in recent years. However, after its initial detection in UK it came more into focus of diagnostic laboratories, which was followed by multiple detections. Based on this we hypothesise that both CEV and KSD have existed in the European common carp aquaculture since long time. However, due to a particular set of circumstances characteristic for CEV/KSD like: Less dramatic clinical presentation, the season of occurrence, similarity of clinical signs to koi herpesvirus disease (KHVD) or intoxication with ammonia and no ability to detect the virus by cell culturing, it was not detected.

Materials and Methods
A result which could support this hypothesis would be a wide geographical distribution of the virus in main carp producing countries and in countries with a limited carp production. Therefore, samples were collected in Hungary, Serbia, Croatia and Lithuania in 2015-2018 and screened for the presence of CEV DNA with quantitative PCR. DNA sequences of the ORF encoding for P4a core protein of the virus were used for phylogenetic analyses.

Results
Prevalence of CEV in Hungary, one of the largest European common carp producers, was the highest with 76% (13 CEV positive out of 17 locations screened). In contrast, the prevalence in countries with smaller crap production was lower: 14% prevalence was recoded in Croatia where 6 out of 44 locations were CEV positive, 30% prevalence was recorded in Lithuania (6 CEV positive locations out of 20 checked). In Serbia fish with clear signs of clinical KSD from only two farms were sampled and both locations were confirmed to be CEV positive. Phylogenetic analyses indicated the majority of the viral sequences belonged to the genogroup I which is the most common in European aquaculture; however, several Hungarian and Lithuanian sequences were clustering within genogroup IIb. Furthermore, in some cases the detected virus isolates are distributed in geographically related locations. For instance, the same virus sequence was found in neighbouring regions of Hungary and Serbia.

Discussion and Conclusion
Our findings indicate that CEV (especially from genogroup I) is widely spread in the European carp aquaculture. This could indicate that rather than being an emerging pathogen this virus was previously overlooked or misdiagnosed. Therefore, KSD should be considered in whole Europe when investigating disease outbreaks in common carp at temperatures below the optimum for KHVD.

USE OF ZEBRAFISH MODEL IN STUDIES OF VIRAL INFECTIONS AFFECTING CULTURED FISH SPECIES

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Introduction
Zebrafish (*Danio rerio*) is becoming an interesting model for studying immune responses to viral pathogens thanks to significant biotechnological progress in genetics, plethora of available molecular tools, and mutants. The approach using zebrafish model could bring a significant progress in studying the diseases in farmed fish. Here *in vitro* and *in vivo* studies were performed in the search for new viral models which would help explore antiviral responses and immune functions of novel C-type lectin receptors (CTLR) in zebrafish

Materials and Methods
Initially zebrafish derived ZF4 and SJD.1 cells were tested for susceptibility to several fish pathogenic viruses including *cyprinid herpesvirus 1* and *3* (CyHV-1 and CyHV-3), carp edema virus (CEV), *chum salmon reovirus* (CSV), common carp paramyxovirus (CCPV), common carp orthomyxovirus (CCOV), and common carp birnavirus (CCBV). As positive control *spring viremia of carp virus* (SVCV) was used. Antiviral responses based on *vig-1* and *mxa* gene expression were measured by RT-qPCR. Based on the *in vitro* results, CyHV-3, CSV and SVCV were selected for testing *in vivo*. The infections were performed by intraperitoneal injection of the virus into adult zebrafish and by immersion of larvae in virus suspensions. Furthermore, several putative CTLR genes were located in the zebrafish genome, molecularly cloned and analysed for structural transmembrane classification.

Results
In vitro studies demonstrated that SVCV, CSV, CyHV-1 and CyHV-3 but not CEV, CCPV, CCOV and CCBV were able to replicate in the zebrafish cell lines ZF4 and SJD.1. *In vivo* studies showed that both CSV and CyHV-3 induce an up-regulation of *vig-1* and *mxa* expression in kidney and spleen of adult zebrafish after i.p. injection but not in larvae after infection by immersion. SVCV infection was the strongest inducer of an antiviral response. When mRNA expression of the two putative CTLR encoding genes *up463* and *up690* (likely homologues of mammalian MINCLE and SIGNR3) was measured, a strong down-regulation was noticed in adult fish under infection

Discussion and Conclusion
The presented results give a good basis for further functional studies of host pathogen interactions during devastation diseases (like koi herpesvirus disease caused by CyHV-3) affecting finfis aquaculture. Further studies on CTLR will include *in vitro* studies on the binding of bacterial and viral pathogens to recombinant proteins and further *in vivo* studies in which up463 and up690 CRISPR-Cas knockout zebrafish embryos will be infected with various viral or bacterial pathogens.

BYSTANDERS RATHER THAN KILLERS? – WHAT CAN BE LEARNED FROM THE CASES OF PRV-1 IN ATLANTIC SALMON AND PRV-3 IN RAINBOW AND BROWN TROUT IN GERMANY?

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Introduction
Piscine orthoreoviruses (PRVs) are emerging viral pathogens responsible for circulatory disorders in salmonids including heart and skeletal muscle inflammation (HSMI). German aquaculture, with a substantial production of rainbow trout and conservation programs for Atlantic salmon and brown trout in several river systems, is potentially vulnerable to the impact caused by these viruses. Furthermore, German brown trout populations are declining due to the proliferative darkening syndrome (PDS), a lethal disease of unknown etiology affecting this fish in the rhithral region of alpine Bavarian limestone rivers.

Materials and Methods
Heart, kidney and spleen samples were collected from fish from two German farms breeding Atlantic salmon or rainbow trout, experiencing in 2017 some health problems leading to accumulated mortalities of 10% and 20%. Furthermore, archival samples from exposure studies performed in 2008 and 2009, in which brown trout developed PDS, were used. The samples were tested for the presence of PRV RNA with PCR based methods, PDS samples from 2009 were additionally screened in a next-generation RNA sequencing pipeline for pathogen detection.

Results
PCR examination indicated a PRV-1 infection in the Atlantic salmon and a PRV-3 infection in rainbow trout. Further analyses indicated also the presence of *Aeromonas salmonicida* in internal tissues of both species. While PRV-1 was likely the causative agent of the disease in Atlantic salmon, most of the rainbow trout suffered from a systemic infection with *A. salmonicida* and not from PRV-3. Interestingly, PRV-3 RNA was also detected in several organs of the PDS-affected brown trout in 2008 but not in 2009. However, similar virus loads were measured in control fish from 2008, which were not exposed to river water presumably holding the PDS-inducing pathogen and which did not show any signs of the disease.

Discussion and Conclusion
The results from Germany confirm a wide geographical distribution of both PRV-1 and PRV-3 in Atlantic salmon and rainbow trout also in continental Europe. However, a clear association with disease was hampered by the presence of an *A. salmonicida* co-infection. Furthermore, PRV-3 is present in German brown trout populations but is not the causative agent of PDS. Nevertheless, potential diseases induced by PRVs should be considered when investigating mortalities in salmonids.

DEVELOPMENT OF IMPROVED *IN VITRO* SYSTEM FOR TILAPIA LAKE VIRUS REPLICATION

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Introduction

Infections of Nile tilapia with tilapia lake virus (TiLV) cause an emerging disease threatening the production of this fish and food security in several large developing countries. The virus is already present on three continents (Asia, African and South America), thus coordinated effort has to be taken to limit its impact and spreading. We identified several challenges in working with TiLV: 1) the lack of widely available tilapia cell lines, 2) scarcity of molecular tools for currently used E-11 cells for routine TiLV work, originating from snakehead. Therefore, in a first line of studies we explored possibilities of repurposing some cyprinid or salmonid cell lines, and the development of a tilapia based *in vitro* culture system for TiLV studies.

Materials and Methods

Precision cut slices cultures (PCSC) from tilapia brain, gills and liver were used *in vitro*. Furthermore, attempts to raise the cell line form tilapia scales or fins were made. The susceptibility of several cell lines to TiLV (Thai isolate) was measured using: RTG-2, RTgill-W1 (rainbow trout), KFC (common carp) and ZF4 (zebrafish) and compared to the E-11 reference. Susceptibility was measured by observation of CPE, estimation of viral particles produced by the cells and presence of viral RNA in the cells with $TCID_{50}$ and RT-qPCR.

Results

Over eight days culturing periods gill and brain PCSCs were replicating the virus better that liver cultures, they produced more viral particles released to the culture medium, had also higher virus RNA level in the cells. Interestingly, both rainbow trout cell lines were replicating the virus at similar levels as E-11 cells despite their cultivation at suboptimal temperature of 25°C. Also, zebrafish cells were able to replicate the virus although at much lower level, while common carp cells were nonpermissive for TiLV.

Discussion and Conclusion

The use of PCSC will allow monitoring type I IFN responses in tissues susceptible to TiLV, while the use of rainbow trout cells will allow to measure the vulnerability of the virus to type I IFN responses by using recombinant IFN and poly I:C stimulation. Furthermore, lipid raft dependence of viral entry and its blockage by 25-hydroxycholesterol will be studied.

CHOOSING THE BEST LOCATIONS FOR SALMON AQUACULTURE SITES: INSIGHTS FROM BIOPHYSICAL MODELS AND THE MOVE TO MORE EXPOSED SITES

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Introduction

Industry targets for global aquaculture demand an increase in output over the next decade. Salmon farm operators seek to meet this need either by establishment of new sites, or by the expansion of existing sites. However, finding space for new sites can be challenging. The OFF-AQUA project (https://www.sams.ac.uk/science/projects/off-aqua/) is investigating a range of issues associated with moving salmon production sites to more exposed locations in Scottish waters, including physical, ecological, economic and fish welfare issues. A particular environmental challenge in salmon aquaculture is posed by parasitic sea lice. Enhanced availability of host fish at farms can allow lice to reach much greater numbers than they would naturally. It is hypothesised that more exposed locations will experience lower lice abundances. However, this must be weighed against increased physical exposure. We used biophysical models to make a comparison between sites covering a range of exposure levels. We show how outputs can be used by industry, regulators and other stakeholders to help to guide management of new (and indeed existing) sites.

Methods

A meteorological-hydrodynamic model covering the west coast of Scotland was developed (Aleynik et al. 2016, 2018), based on directly coupled unstructured mesh ocean (FVCOM; Chen et al. 2013) and atmospheric (WRF; Skamarock et al. 2008) models. This has been run operationally since 2013. In 2019, the domain was expanded to incorporate more exposed environments. Development also began on local fine-scale models of study sites. A biological particle tracking model (Adams et al. 2016) was used to simulate the spread of "sea lice" larvae from three focal fish farm sites, covering sheltered fjordic through to exposed open environments. Simulations covered a representative range of tide and weather conditions, which dominate local flow patterns (Edwards 2016).

Results

A range of metrics including dispersal patterns, kernels and between-site connectivity allowed characterisation of the proposed sites in the context of their surroundings (Figure 2). Sheltered sites are typically more connected to other sites and have higher "self-infection" rates. While such sites provide the most amenable conditions for operating, some more exposed sites also provided suitable conditions for farming fish

Discussion and conclusion

A range of factors must be taken into account when selecting the most sustainable approach to industry expansion, and choosing sites upon which to focus. Physical conditions at more exposed sites generally lie within the range suitable for fish, but can pose operational difficulties for site managers. More exposed sites offer an opportunity to reduce environmental impacts in terms of sea lice connectivity, with an associated reduction in outbreak frequency and risk to wild fish. They may also offer increased dispersion of excess organic material.

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AN INNOVATIVE PHOTOBIOREACTOR HEATED UP BY GEOTHERMAL WATER FROM COAL MINE FOR THE EXPERIMENTAL CULTIVATION OF *Spirulina*

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Introduction

Arthrospira platensis (*Spirulina sp.*) is a multicellular filamen ous planktonic cyano- bacterium with a long history of human use. Recently, it has gained a lot of attention as a nutritional supplement and food additive due to its high protein content of around 60% and several other reported health benefits (Pan-utai and Iamtham, 2019). Moreover, it can also be used as a complementary ingredient in aquaculture feeds (Usharani et al., 2012). *Spirulina* is commercially cultivated in large outdoor raceway ponds. However, the outdoor ponds are subjected to variable, daily fluctuations in water temperature and sunlight intensity, which in turn are affected by geographic location, season, and pond management strategy (Hidasi and Belay, 2018). Constant temperature, high alkaline conditions and light intensity are the main determinants of biomass production of *Spirulina* (Torzillo et al., 1991). For these reasons, a wide variety of photobioreactors (PBR) has been designed and developed (Huang et al. 2017). The main advantages of this solution instead of open ponds were a higher photosynthetic efficienc , higher concentrations and areal productivities, low contamination, the prevention of water loss caused by evaporation, and a precisely controlled environment. Although the variety of this novel solution, in the case of *Spirulina* remain the problem to maintain the strain at the optimal temperature (35°C), which represents a consistent operating cost.

We report here on the development and testing of a new photobioreactor which used geothermal water from a coal mine to heat the medium for *Arthrospira platensis*. The specific objectives were to design and build the photobioreactor, to evaluate its hydrodynamics and evaluate the growth and productivity of one strain of *Spirulina*.

Materials and methods

The photobioreactor was designed and constructed based on the shape proposed by Bahadur et al. (2013). It is composed by a tubular circuit in Boro-silicate glass. The current design (Patent n. IT 102019000001449) had a volume of 55 L. The temperature is maintained by a continuous heat exchange guaranteed through a coaxial Teflon (R) corrugated tube where geothermal waters circulate. Geothermal waters (40-42 °C) were pumped for underground safety reason, from the Seruci-Nuraxi Figus Coal Mine (Sardinia, Italy), which was recently dismissed. The circulation of the microalgae culture takes place with "air lift" technology. The system is monitored and controlled by a Programmable Logic Controller (PLC) for the temperature and pH control. The pH is regulated through CO_2 insufflation with an electronic valve controlled by the PLC. Actual gas induced liquid circulation velocity was determined using tracer analysis by inserting a colored piece of cotton fiber cloth in the photobioreactor and measuring the time needed to complete a predefined distance (Bahadur et al., 2013). *A. platensis* (M2M strain) was obtained from the National Research Council of Italy (CNR, Florence, Italy) and was grown

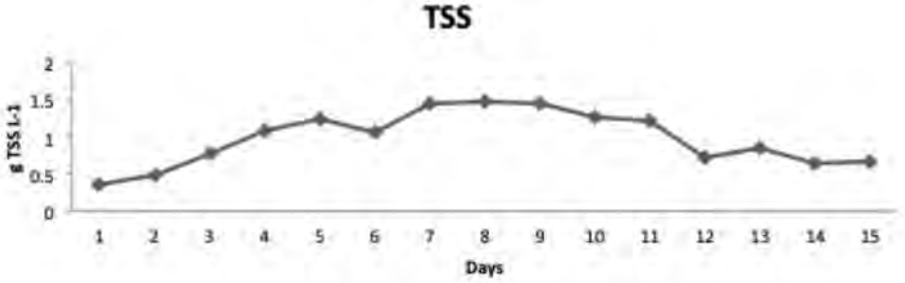

Fig. 1. Biomass algal concentration measured as g L^{-1} for *A. platensis* during 15 days of experiment.

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in Zarrouk medium. The growth was sustained in the semi-batch mode with daily monitoring of temperature, pH, spiral structure under microscope and biomass concentration. The biomass concentration was measured as dry weight biomass in terms of Total Suspended Solids (TSS) (Clasceri et al., 1999). The experiment started in March 2019 and lasted 15 days.

Results

The PBR was designed between September and November 2018 and completely built in December 2018. The liquid circulation velocity test was carried out in January 2019 resulting in a max speed of 0.5 m/s. Preliminary results of TSS showed an average value (± standard deviation) of 1.0 ± 0.3 g L^{-1} during the 15 days of experiment (figure 1). Average biomass production in the photobioreactor was 14.2 g m^{-2}/day. Changes in the spiral structure of *A. platensis* have not been observed during the experiment. The maximum temperature reached by the culture was 34 °C and the minimum was 25 °C. The average pH value (± standard deviation) was 10 ± 1.8.

Discussion and conclusion

The preliminary results obtained indicate that this photobioreactor was suitable for the cultivation of *A. platensis* maintaining good liquid velocity as well as favorable pH and a temperature higher than 25 °C. The value of biomass production was promising considering that the outdoor temperatures during the trial ranged from 5 to15 °C. Further research with different strains and modified culture medium will be conducted to assess the performance of the PBR in the di ferent seasons.

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GROWTH RESPONSE AND BLOOD INDICES OF AFRICAN SHARPTOOTH CATFISH (*Clarias gariepinus*) TO DIETS FORTIFIED WITH WILD LETTUCE (*Latuca virosa*) LEAF EXTRACT

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Introduction
The use of antibiotics in aquaculture is under serious scrutiny and regulation to avoid deleterious consequences of development of antibiotic-resistant pathogen and residues in fish meant for human consumption (FAO, 2011; Gent et al., 2012). Utilization of herbal products as natural alternatives to synthetic antibiotics has shown great potentials. There is paucity of information on the use of *Latuca virosa* in fish production. Therefore this study was conducted to investigate the effects *L. virosa* leaf ethanol extract on the growth performance of *Clarias gariepinus*.

Materials and methods
Fresh leaves of *Latuca virosa* were air-dried at room temperature, ground into meal and the ethanol crude extract was obtained at 1:10w/v and concentrated. Preliminary analysis on the phytochemical components of the extract was investigated qualitatively (Sofowora, 1993). Feeding trial was conducted with 300 *C. gariepinus* fingerlings in triplicates in an indoor static renewal system. The fish were fed diets fortified with 0, 2.5, 5.0, 7.5 and 10ml/kg diets at 3% body weight daily for 84 days. Mortality number and fish body weight were recorded at intervals. Weight Gain (WG), Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) and survival were calculated. Blood samples were obtained from the fish through the caudal vein and analyzed following standard procedures. Data were analyzed using one way analysis of variance at $P = 0.05$.

Results
The phytochemical analysis showed that *L. virosa* contained flavonoids, saponins and terpenoids. The *C. gariepinus* fed diets fortified with 2.5 - 10ml extract/kg diets had significantly higher ($P<0.05$) WG and PER than the fish fed with the control diet (Table I). Fish fed diets containing 2.5 - 10mL extract showed significantly lower FCR and survival than fish fed other diets.

The differences in the red blood cells and globulin were not significant. However, the heterophil values differed significantly ($P<0.05$) with the highest value obtained at 7.5ml/kg diet.

Discussion and conclusion
Fortification of *C. gariepinus* diets with 5 - 10ml extract of *Latuca virosa* enhanced growth performance and nutrient utilization. The improved growth performance and nutrient utilization observed in this study could be attributed to presence of phytochemical (flavonoid, saponin, terpenoids) present in the wild lettuce leaf. The phytochemicals have proven to promote secretion of digestive enzymes, feed digestibility and nutrient utilization leading to increased growth performance of fish (El-Dakar et al., 2015; Adeniyi et al., 2018) as observed in this study.

Blood parameters are significant for evaluation of physiological status of fish in response to diets or other stressor (Fagbenro et al., 2013). The insignificant differences observed on red blood cell counts in the present study is similar to Dada and Olaoye (2015) while contrary observations were reported on heterophil (Pakravan et al., 2011) and globulin (Bahrami et al., 2015) of fish fed diets fortified with herbal extracts. Utilization of *Latuca virosa* extracts at 5.0ml/kg diet is therefore recommended for production of *Clarias gariepinus*.

(Continued on next page)

Table I: Growth performance and blood indices of *Clarias gariepinus* fed diets fortified with *Latuca virosa* leaf extract*

Parameters	Dietary levels of inclusion (ml/kg diet)					
	0.0	2.5	5.0	7.5	10.0	P-Value
Final weight (g)	32.10^{b}	32.24^{b}	41.84^{a}	37.54^{b}	43.79^{a}	0.025
Weight gain (%)	549.05^{c}	557.79^{c}	755.23^{a}	665.60^{b}	779.18^{a}	0.014
Feed Conversion Ratio	1.40^{a}	1.44^{a}	1.30^{b}	1.31^{b}	1.26^{b}	0.019
Protein Efficiency Ratio	1.67^{b}	1.62^{b}	1.80^{a}	1.78^{a}	1.85^{a}	0.007
Survival (%)	95.00^{a}	93.33^{ab}	86.67^{bc}	81.67^{c}	83.33^{bc}	0.011
RBC (10^{6}/µL)	2.56	3.55	3.08	2.85	2.99	0.357
Heterophil (%)	24.00^{c}	30.75^{ab}	25.00^{c}	32.50^{a}	27.00^{bc}	0.007
Globulin (g/dL)	3.53	3.80	3.45	3.65	3.85	0.165

*Means with a different superscripts in a row are significantly different at P<0.05
RBC = Red blood cells

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THE FISHES OF AKOMOJE RESERVOIR DRAINAGE BASIN IN LOWER RIVER OGUN, NIGERIA: DIVERSITY AND ABUNDANCE

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Introduction

The freshwater ecosystem, though highly populated and with rich plant and animal diversity, may be the world ecosystem in greatest danger of extinction of its fish populations. Major causes of threats to global freshwater plant and animal life are overexploitation, water pollution, flow modification, destruction or degradation of habitat, and invasion by exotic species (Dudgeon *et al*., 2006).

Materials and methods

The fishes of Akomoje reservoir drainage basin in Lower River Ogun, Nigeria were studied from June to November, 2017. Fish species composition, abundance, and diversity were investigated bi-monthly. Water quality parameters were also collected and measured *in-situ* and *ex-situ* using standard methods and kit.

Table ii: Abundance and weight of fish species in Akomoje reservoir drainage system

Fish species	TOTAL		Relative Percentage (%)	
	Abundance	Wt(kg)	Abundance	Weight
Clarias gariepinus	56	14.3	5.53	21.25
Chrysichtys nigrodigitatus	610	26.5	60.28	39.38
Tilapia zilli	113	5.8	11.17	8.62
Synodontis budgetti	11	2.5	1.09	3.72
Heterotis niloticus	8	5.2	0.79	7.73
Chrysichtys auratus	51	5.4	5.04	8.02
Synodontis schall	8	0.4	0.79	0.59
Synodontis batensoda	11	0.4	1.09	0.59
Schilbe mystus	6	0.5	0.59	0.74
Oreochromis niloticus	62	2.5	6.12	3.72
Malapterurus electricus	32	0.7	3.16	1.04
Mormyrus rume	11	1.0	1.09	1.49
Parachanna obscura	15	1.2	1.48	1.78
Tilapia mariae	18	0.9	1.78	1.34
Total	**1012**	**67.3**	**100**	**100**

Table iv: Diversity indices of fish species in Akomoje Reservoir drainage system, Nigeria

Diversity index	Values
Number of species (S)	14
Total number of individual species (N)	1012
Simpson's index (D) = Σn (n-1) / N(N-1)	0.39
Simpson index of diversity (1-D)	0.61
Simpson's reciprocal index = (1/D)	2.56
Shannon Diversity index (H) = Σpi ln pi	-1.5749
Shannon's Equitability or Evenness (E_H) = H/1nS	-0.5968

(Continued on next page)

Results

1012 fish species comprising of 14 fish species from 9 families were identified. Bagrids were the most abundant fish family in the reservoir basin and *Chrysichtys nigrodigitatus* was the most dominant species contributing 60.28 % and *Schilbe mystus* was the least abundant species by number (0.59%) and weight (0.74%) (Table ii). In terms of occurrence, common species are *C. nigroditatus*, *T. zilli, O. niloticus, C. gariepinus, C. auratus* and *Malapterurus electricus* representing 91.3% while occasional and rare species represented 6.53% and 2.17% of the fish population. Diversity indices estimates were Simpson's Index (D) = 0.39, Simpson's Index of diversity (1-D) = 0.61, Simpson's Reciprocal Index (1/D) = 2.56, Shannon-Diversity Index (H) = -1.5749, Shannon's equitability (EH) or Evenness (E) of = -0.5968 (Table iv). Results of physical and chemical parameters measured were air temperature (29.23±1.27), water temperature (26.9±0.37^{0}C), dissolved oxygen (6.12±0.70mg/l), and pH (7.6±0.39). There was no significant difference ($p > 0.05$) in temperature, BOD and phosphorus all though the study period. Negative correlation was determined between water quality parameters and between water quality parameters and fish abundance.

Discussion and Conclusion

The fish families and species identified from this water body during the study revealed a decline in the fish assemblage of this water body as there was differences when compared to the findings of Adeosun *et al*. (2012) who recorded thirty-two species from thirteen families from this same water body. Negative correlation obtained between some water quality parameters indicated that increase in one parameter, will result to corresponding decrease in another. Of importance, the high negative correlation between water temperature and pH of the study area indicated that any increase in the water temperature or pH will cause a decrease in either one. The study concluded that the water quality parameters of the study location measured were still within tolerable range for fish survival, however, with the negative correlation obtained between fish abundance and water quality parameters, human activities around this river should be monitored to prevent pollution of the water body. Finally, the fish population of this river basin has been well depleted and thus more attention should be paid to the resource to prevent extinction of important fish species

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DIETARY SUPPLEMENTATION OF AUTOLYSED BREWER'S YEAST ENHANCE GROWTH, LIVER FUNCTIONALITY AND INTESTINAL MORPHOLOGY IN AFRICAN CATFISH, *Clarias gariepinus*

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Introduction

Yeast (*Saccharomyces cerevisiae*) is a unicellular organism used as bioactive and functional feed ingredient with potential to enhance growth performance, health and immunity of aquaculture species (Shurson *et al*, 2018). Dietary supplementation of yeast and yeast products (autolysed yeast inclusive) could enable aquaculture species cope with stress that accompany intensification of aquaculture operations due to suboptimal environmental conditions resulting from overcrowding and overfeeding. To this end, this study was carried out to evaluate the effect of dietary supplementation of autolysed brewer's yeast (Leiber CeFi® Pro) on African catfish *Clarias gariepinus*) growth, health and intestinal morphology.

Materials and Methods

Four iso-nitrogenous and iso-lipidic diets were formulated as Control (containing 0% Leiber CeFi® Pro), 0.3% AY (containing 0.3% Leiber CeFi® Pro), 0.6% AY (containing 0.6% Leiber CeFi® Pro) and 1% AY (containing 1% Leiber CeFi® Pro) diets. The feed ingredients were thoroughly mixed, moistened (200 mL kg^{-1}) and then cold-press extruded to produce 2mm sinking pellets. The diets were oven dried (at 60 ^{o}C) and the proximate composition analysed using AOAC protocols. Three hundred African catfish (*C. gariepinus*) of 22.5±1.15 g fis $^{-1}$ were randomly distributed (20 fish per tank, n = 3 per treatment) into a freshwater flow-through aquaculture system containing 12 tanks (of 33 L capacity each) after two weeks of acclimatization with the control diet (containing 0% Leiber CeFi® Pro). The photoperiod and water temperature (29±0.29 ^{o}C) were maintained at ambient condition and other important water quality parameters were monitored (pH, 6.85±0.34; dissolved oxygen, >5 mg L^{-1} and ammonia, 0.34±0.1 mg L^{-1}).

After seven weeks of feeding and weekly batched-weighing, the growth performance, feed efficiency and somatic indices of the African catfish (*C. gariepinus*) were determined as described by Adeoye et al. (2016)"ISSN":"00448486","abstra ct":"A study was carried out to investigate the combined effect of exogenous enzymes and probiotic supplementation on tilapia growth, intestinal morphology and microbiome composition. Tilapia (34.56±0.05g and Fawole et al. (2018). At the end of the feeding trial, blood from two fish per tank (n = 6 per treatment) was taken from the caudal arch using a 25-gauge needle and 1 mL syringe after the fish were anaesthetized with clove oil (100 mg L^{-1}). Blood samples were prepared and analysed as previously described by Adeoye et al. (2016) and Fawole et al. (2018). Two fish per tank were also sampled for histological appraisal of the mid-intestine (n = 6 per treatment) after the catfish were relived from the act with super dose of clove oil (300 mg L^{-1}). The samples were processed using standard histological procedure and the intestinal perimeter ratios (arbitrary units, AU) were assessed as described by Adeoye et al. (2016) as well as the numbers of intraepithelial leucocytes (IELs) and goblet cells in the epithelium, across a standardized distance of 100 μm.

All data are presented as mean ± standard deviation. Data were analysed using one-way analysis of variance (ANOVA). Multiple comparisons were performed using Duncan post-hoc test. Differences were considered significant at a value of $P < 0.05$. The statistical analysis was carried out using SPSS for Windows (SPSS Inc., 24.0, Chicago, IL, USA).

Results and Discussion

After seven weeks of feeding and weekly batched-weighing of the catfish, the growth indices (especially the final body weight and metabolic growth rate) of the African catfish fed diet supplemented with Leiber CeFi® Pro (at 0.3% inclusion level) performed significantly better ($P < 0.05$) than the control group fed diet containing 0% Leiber CeFi® Pro. This observation is within the optimum range of performance of African catfish of same size and age and compares favourably with the findings of Abdel-Tawwab et al. (2018) and Kemigabo et al. (2019).

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The haemato-biochemical parameters of the African catfish fed diets supplemented with Leiber CeFi® Pro showed that the fish are in good health and that Leiber CeFi® Pro had no deleterious effect on the catfish welfare. A significant change was however observed in the level of alanine transaminase (IU L^{-1}) of African catfish fed diets supplemented with Leiber CeFi® Pro; an indication that low dietary supplementation (0.3 – 0.6%) of Leiber CeFi® Pro in the diets of African catfish could help protect the membrane integrity of the liver cells.

The mid-intestine of African catfish from all treatments showed intact epithelial barriers with extensive mucosal folds extending into the lumen. Each fold consisted of simple lamina propria with abundant intraepithelial leucocytes and goblet cells. The perimeter ratio, goblet cells and intraepithelial leucocytes abundance of the African catfish fed the experimental diets are within the normal range for the catfish of same size and age under optimum condition and compares favourably with the findings of Onura et al. (2018). A significant elevation was observed in the abundance of goblet cells and intraepithelial leucocytes in the mid-intestine of the catfish fed diets supplemented with Leiber CeFi® Pro. The elevated proportion of mucus-producing goblet cells residing in the intestine of the catfish fed Leiber CeFi® Pro supplemented diets is likely to enhance the intestinal barrier function of the catfish. In similar vein, the significant increase in the abundance of intraepithelial leucocytes (component of gut associated lymphoid tissue) in the intestine of African catfish fed Leiber CeFi® Pro supplemented diets could be an indication of immune stimulation, protecting and preventing pathogenic invasion in the gut of African catfish *C. gariepinus*).

Conclusion

It could be concluded therefore that dietary supplementation (at 0.3%) of autolysed brewer's yeast is capable of improving growth performance, liver functionality and intestinal morphology in African catfish, *C. gariepinus*.

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EFFECT OF TEMPERATURE AND DIFFERENT SPAWNING AGENTS ON REPRODUCTION SUCCESS OF BURBOT IN CAPTIVITY

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Introduction

Since a few years burbot (*Lota lota*) has been introduced as a new aquaculture species in Belgium. However there is still some concerns regarding stable fingerling production. Latter can be improved by increasing control over the reproduction. Zarski et al. (2010) suggested that temperature is the major factor during final maturation and his study showed some variations in reproduction success under different thermal conditions. In this study three different temperature profiles were tested. This set-up could then be used to specify the better thermal regime that guarantees successful reproduction under controlled conditions. Kucharczyk et al. (2018) showed that the application of salmon gonadotropin releasing hormone analogue (sGnRHa) in burbot reproduction shortened latency time, synchronized final oocyte maturation and resulted in high embryo survival. Human chorionic gonadotropin (hCG) is another spawning agent that has been used successfully in the artificial reproduction of pikeperch (*Sander lucioperca*) (Zarski et al 2015). For this the effect of these two hormones were investigated in this experiment at two different application methods (one or two injections).

Materials and methods

In 2016 burbot larvae were transported from Germany to Aqua-ERF and the largest fingerlings were reared in RAS for almost three years. Just before their third winter 126 of these burbots (61 males, ABW: 594±168.3gram; 65 females ABW: 660.7±191.9 gram) were individually tagged and distributed over nine tanks (1m²; 0.4m³) connected to their own biofilte . Total biomass per tank was 8.78±0.63 kg m^{-1}. Three cooling chambers, holding three fish tanks each, were used to assure three different temperature profiles. Temperature was lowered gently from ±±10°C till ±±5°C, then dropped fast till 1.5°C and holding this temperature for a one or two weeks before increasing it till 2°C, 3°C or 4°C, depending on the treatment. Light conditions were modulated based on natural light regime in Germany and were the same for all cooling chambers.

Part of the fish population were given 1 or 2 injection (14 days interval) with either 10 µg kg^{-1} of sGnRHa or 500 IU kg^{-1} of hCG. The spawning agents were always diluted in saline solution so that each fish received 1ml of solution per kg. All fishes that did not receive the 1st or/and 2nd injection with spawning agent received an injection with a saline solution at 1ml kg^{-1}.

Cathetering of the females was repeated every week, starting at the moment of first injection, till oocyte showed to be ready for spawning. Females were then manually stripped to obtain the eggs. Obtained eggs were weighted and a working fecundity was calculated based on following formula: (Weight of eggs/Weight of fish) x100. Males were checked every week manually if they were given sperm.

Results and Discussion

The first female started to spawn ten days after the first injection with sGnRHa, while the latest spawned on day 40. Highest frequency of spawning females occurred from day 22 till day 40. In total 46 out of 65 females have spawned (71%) during the trail and although higher spawning percentage were obtained for fish treated with sGnRHa, no significant effect was observed between the five different spawning agent treatments. This was also the case for the three different temperature regimes and the interaction between temperature and spawning agents. Temperature, spawning agents and the interaction between those two did not influence significantly the working fecundity. In the males we observed that not all males spermiated and that even the same male did not spermiated during the whole 40 days period.

This study showed that burbot reproduction in RAS with 3-year old burbot, reared from larval size in RAS, is possible. The results indicates the possibility to obtain earlier eggs from burbot females with the use of sGnRHa and even a better synchronization than with hCG. Although working fecundity was not influenced by the treatments, egg quality and further larval quality could be influenced for which further study is needed

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PHENOTYPIC CHARACTERIZATION AND GENETIC VARIATION OF NILE TILAPIA *(Oreochromis niloticus)* FROM WILD AND CULTURE POPULATIONS

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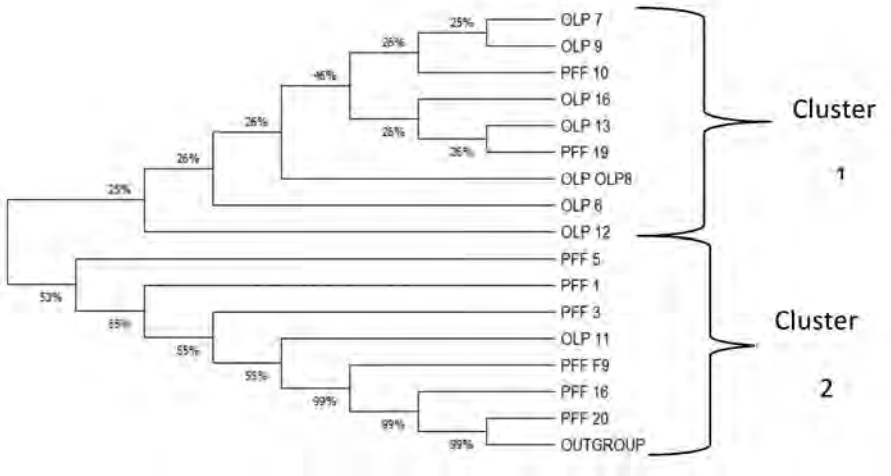
Introduction

The phenotype and gene diversity provide basic information of the species as it gives the level of diversity and population expansion within fish species. A notable genetic bottle-neck of fisheries enhancement programmes has been the discovery of genetic variation between wild and cultured stock (Islam and Alam, 2004). The Nile tilapia is a member of the cichlid family and notably one of the most important species in aquaculture worldwide (McCune, 1981). It is the most commonly farmed tilapia species in Nigeria owing to its good aquaculture qualities such as ability to withstand poor water quality and the acceptability to wide range of feed that support its cultivation. This study was designed to describe the phenotypic (morphological and meristic) characterization and genetic variations of wild and culture *Oreochromis niloticus* from different populations (Oyan dam and a private farm) in Ogun State, Nigeria.

Materials and methods

One hundred fish samples of were collected for the study comprising 50 samples each from Oyan dam being the wild population (consisting of 27 males (44.3%) and 23 females (59%)) and the private farm population situated at Ilaro, Ogun State (comprising 34 males (55.7%) and 16 females (41%)). Both populations were located within Ogun State, Nigeria. Twenty samples from each population were used for genomic studies. Morphometric and meristic variables were measured using simple standard procedures to minimize stress and precaution was taken not to compromise the fish welfare. The measured morphometric variables include: Weight, Total length, Head Length, Standard length, Preorbital length, Postorbital length, Predorsal fin length, Prepectoral fin length, Preanal fin length, Postanal fin length, Body depth, Eye diameter, Upper jaw length, Caudal peduncle depth. The meristic parameters taken were Dorsal fin spines, Anal fin ray, Number of the lateral line scales, Number of gill ray, Dorsal fin ray, Pectoral fin ray right, Pectoral fin ray, Number of ventral tail ray. The data on morphometric and meristic parameters were analyzed using descriptive statistics based on frequency and percentages. The data was also subjected to T-test and cluster analyses; significant differences were tested at $\alpha = 5\%$ using SPSS.



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Results

There were significant differences ($P < 0.05$) in the morphometric traits (except the Pre Orbital Length) and meristic parameters (except Dorsal fin spines, Pectoral fin ray right, Pectoral fin ray and Number of ventral tail ray) between the two populations. The cluster analysis based on the dendrogram of the morphological trait of *Oreochromis niloticus* from the two populations is divided into two main clusters, each with two sub-clusters. Cluster I comprised more of *Oreochromis niloticus* from culture population (Ilaro) with some from the wild (Oyan dam). Sub-cluster I.I had 31 samples from the culture with 7 samples from the wild. Sub-cluster I.II has 19 samples from the culture with 13 samples from the wild. Cluster II had all samples of Sub-cluster II.I (10 samples) and Sub-cluster II.II (20 samples) all from the wild. Both populations had high genetic variation among population and low genetic variation within species based on the distinctive classification from the cluster analysis. All sampled populations were polymorphic, but Nile tilapias from the wild were more polymorphic than the culture.

Discussion and conclusion

From the cluster analysis, higher morphometric differences were observed between the wild male and female *Oreochromis niloticus* than in the culture male and female population, though the numerical values were close. The similarity in Post Orbital Length (morphometric trait), Dorsal fin spines, Pectoral fin ray right and Number of ventral tail ray (meristic parameter) in the study species is an indication of relatedness between the two populations from different locations. The differences encountered between populations are attributable to the existence of heterogeneity between the two populations. Usually, meristic traits remain the same in the same species except otherwise conditioned by environmental pollution changing the phenotype. (Meyer, 1987; Gulliet *et al.,* 2003). *Oreochromis niloticus*, among the members of the Cichlidae family is among the fish species that is categorized in showing wide range of variations in body shapes and conformity (McCune, 1981). Although, the wild population could be a viable reservoir for high variability stock useful in fish breeding and future conservative programmes. The information obtained in this study could be useful in establishing phenotypic characterization and estimating genetic variability among other components of fish population dynamics

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CELL WALL DISRUPTION INCREASES THE NUTRITIONAL QUALITY OF MICROALGAE IN FISH: A COMPARATIVE STUDY BETWEEN NILE TILAPIA AND AFRICAN CATFISH

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Introduction

Microalgae are being considered as potential novel protein ingredients for aquaculture. This is coming at a time when the supply of traditional marine feed ingredients (fishmeal and fish oil) from capture fisheries are in decline (Hardy, 2010). The use of microalgae in fish feeds, is however, constrained by their rigid outer cellulosic cell walls. The cell wall prevents enzymatic hydrolysis of intracellular nutrients embedded with the microalgae, thereby reducing their nutritional efficacy in fish (Teuling et al., 2017). The current study was undertaken to examine the correlation between *in vitro* accessibility of nutrients and *in vivo* digestibility of nutrients from *Nannochloropsis gaditana* in fish. To discern if these effects were fish dependent, comparative analyses between Nile tilapia (an herbivorous fish) (Teuling et al., 2019) and African catfish (an omnivorous fish) (Agboola et al., 2019) was conducted using data of separate experiments

Materials and Methods

Nannochloropsis gaditana biomass was subjected to physical or mechanical treatments for partial or complete disruption of its cell wall. The cell disruption treatments are untreated – no disruption treatment (UNT), pasteurization (PAS), freezing (FRO), freeze-drying (FRD), cold-pasteurization (L40) and bead milling (BEM). Nutrient accessibility from the microalgae after the cell disruption treatments was determined by *in vitro* protein hydrolysis, nitrogen leaching, ion leaching, fat extractability and buffering capacity methods. In separate experiments, juvenile Nile tilapia and African catfish were fed identical diets containing six differently treated and untreated microalgae (at 30% diet inclusion level).

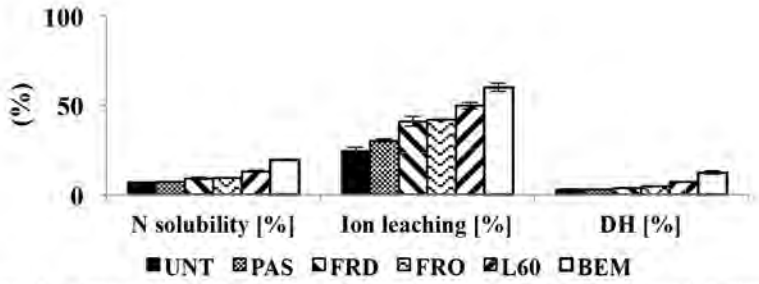

Fig. 1. Nitrogen solubility, ion leaching and protein hydrolysis of differently treated and untreated *Nannochloropsis gaditana*.

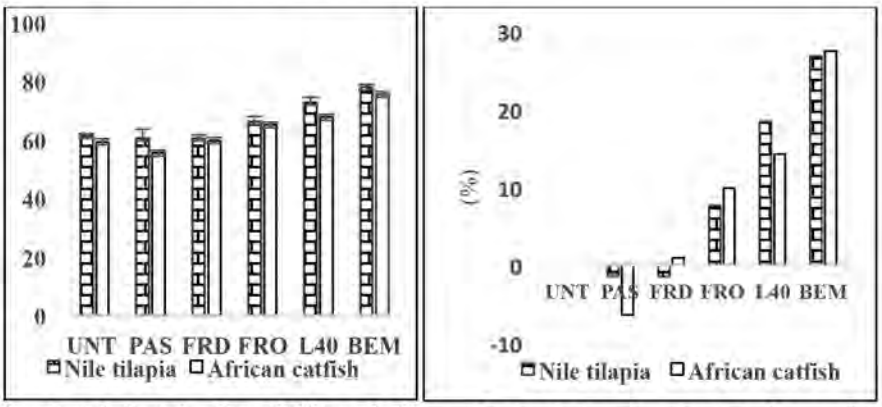

Fig. 2. (a) Protein digestibility of differently treated and untreated *Nannochloropsis gaditana* in Nile tilapia and African catfish. (b) Relative improvement in protein digestibility of differently treated *Nannochloropsis gaditana* compared to untreated. The presented values are relative to untreated microalgae (set at 0%).

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Results and discussion.

Freezing, bead-milling and cold-pasteurization cell disruption treatments resulted in an up to 4 folds increase in *in vitro* accessibility of *N. gaditana* nutrients, assessed from measurements of leaching and susceptibility to protein hydrolysis (Fig. 1). Cell disruption by FRO, L40 and BEM also improves apparent digestibility coefficient (ADCs) of nutrients from *N. gaditana* in both Nile tilapia and African catfish (Fig. 2a & 2b). In addition, there were significant interactions ($P < 0.05$) between cell disruption treatments and fish species on ADCs of crude protein and fats from *N.* gaditana, although the ADCs were higher in Nile tilapia than African catfish. This is as expected because Nile tilapia has more physiological capacity to hydrolyse plant ingredients than African catfish (Saravanan et al., 2013; Hlophe et al., 2014). In both trials, there is a strong correlation between accessibility of nutrients from the microalgae and its digestibility in fish

Conclusions

The study confirms that nutrient accessibility is a dominant limiting factor hindering nutrient digestibility of microalgae in fish. Furthermore, it was shown that freezing, cold-pasteurization and bead-milling cell disruption treatments is an effective strategy to increase nutrient accessibility and digestibility of microalgae in fish. The impact of cell wall disruption on nutrient digestibility of microalgae differs between Nile tilapia and African catfish

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ACUTE TOXICITY AND BIOACCUMULATION IN EELS (*Anguilla Japonica*) BY EXPOSURE OF WATERBORNE CADMIUM

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Introduction

Metals naturally exist in aquatic ecosystems, but side effects from industrialization have resulted in excessive concentrations. Cadmium is a particularly widespread and toxic example that is documented to accumulate in exposed organisms; it is used primarily in alloys, pigments, electroplating, and batteries. The purpose of this study is to investigate accumulation in muscle with determination of median lethal concentrations (LC_{50}) for Cd in adult *A. japonica*.

Materials and methods

Anguilla japonica specimens were acclimated for two weeks prior to experiments and food-deprived. An acute Cd toxicity test was conducted under laboratory conditions. Acclimated fish (n=90; average weight: 186.6±11.9g) were selected, divided into nine groups (10 per group). Water temperature was maintained with a heater at 29±1°C and the laboratory was kept in 24-h darkness to apply to environment of an eel farm. Experimental fish were exposed to waterborne $CdCl_2$ treatments of 0.25, 0.5, 1, 3, 5, 6, 7 and 9mg L^{-1} for 96h. A water-only control was also used. After a 96h Cd exposure, tissues (liver and muscle) of live fish were stored at -80°C until analysis. Tissues were analyzed using microwave with nitric acid to confirm Cd bioaccumulation

Results

LC_{50} of Cd in *A. japonica* after 24, 48, 72, and 96-h was 8.33, 6.60, 6.00 and 5.89 mg L^{-1}, respectively (Table I).

Cd exposure caused a net increase of Cd content in tested *A. japonica* tissues compared with the control (figure 1). As expected, accumulation rose with increasing exposure concentration. Significant differences were apparent at ≥ 0.25mg L^{-1} in muscle, ≥ 1mg L^{-1} in liver.

Table I. Estimated median lethal concentrations (LC_{50}) and confidence limits.

Period	Concentration ($CdCl_2$ mg L^{-1})	95% confidence limits	
		Lower	Upper
24h	8.33	7.13	11.16
48h	6.60	5.79	7.61
72h	6.00	5.31	6.69
96h	5.89	5.21	6.51

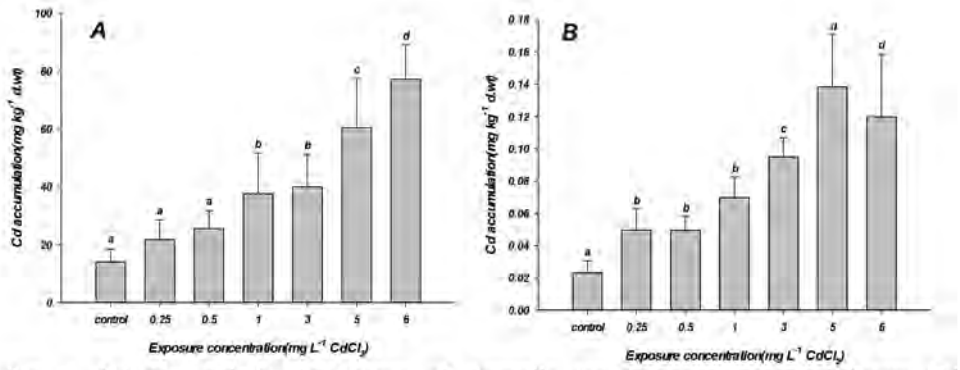

Fig. 1. Cd accumulation in tissues of *Anguilla japonica* exposed to different Cd concentrations. (A) Liver and (B) muscle. Superscript letters indicate significant differences ($P < 0.05$).

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Discussion and conclusion

This study demonstrated that *A. japonica* mortality increased with greater Cd concentration and exposure periods. Although previous research had attempted to examine acute Cd toxicity in freshwater fish, such studies were generally not sufficient for a full assessment. Nonetheless, we were able to compare our results to several reports to identify difference of Cd toxicity by experiment conditions. After 96h Cd exposure the LC_{50} in tilapia juvenile (*Oreochromis* sp.) were 0.7mg L^{-1} (Aldoghachi et al., 2016) and in adult guppies (*Poecilia reticulata*) was 30.4mg L^{-1} (Yilmaz et al., 2004). These data indicate that between-species differences in life history, genetic composition, and individual condition outweigh size and age in terms predicting fish tolerance to Cd toxicity (Rand et al., 1995)

Cd accumulation in the liver, considered the organ most sensitive to Cd toxicity, is higher than the muscle including control in results of this study. This result corroborates previous data; in *A. japonica* exposed to 50μg L^{-1} of Cd, the primary tissues that accumulated Cd were kidney and liver (Yang and Chen, 1996). Nonetheless, significant differences were indicated in the muscle at lower Cd concentration. It represent that liver is the most important organ for detoxification in acute exposure (Chowdhury et al., 2005). Despite Cd exposure at low concentration and during short period, significant increment in muscle can easily threaten to safety as food and health of consumer.

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FLESH QUALITY RECOVERY IN FEMALE RAINBOW TROUT (*Oncorhynchus mykiss*) AFTER EGG PRODUCTION

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Introduction

Large trout production is increasing in France especially for fillet smoking. Besides, trout eggs production involves obtaining large post-spawning fish, but their flesh displays poor quality and as such cannot be processed before a post-spawning rearing period. The present study aims to determine whether, in rainbow trout, the rearing period following egg production leads to the restoration of technological and organoleptic properties of fillets compatible with their processing and consumption.

Materials and methods

Two-years-old diploid female rainbow trout from the same autumnal strain cohort were reared in the INRA's experimental facilities (PEIMA, Sizun, France). Following spawning, nine groups of 25 trout were placed separately into nine 2m diameter circular tanks and fed ad libitum. Fish reared in the same tank were then sequentially sampled at 0, 1, 2, 4, 8, 13, 16, 24, and 33 weeks after spawning. Immature (no egg produced) female trout from the same cohort were also sampled at the beginning (C0) and at the end (C33) of the experiment. At slaughter, the body weight and Fatmeter® value were measured, then fish were fillete , and raw fillets (skinned and trimmed) yield was calculated. After two days of vacuum-packed storage in a cold room at 4°C, fillets were traditionally dry salted (4h) and cold smoked with green beech wood (5h). Smoking yield of fillets was calculated (dividing smoked fillet weight by raw fillet weight before salting and smoking). Instrumental measures of fillet color (Minolta Chromameter CR-400 with L*, a*, b* color space) and mechanical resistance were performed on raw and smoked fillets. Mechanical resistance of the anterior part of the fillet was measured using a penetrometer (a 15 mm diameter flat-ended cylinder probe) mounted to a force gauge of 100N (Andilog®), and a Kramer shear cell mounted on a static load cell of 2kN (INSTRON®). The total work done during the measurement was calculated and divided by the sample thickness.

Table I : Raw fillets quality parameters in rainbow trout slaughtered at different time following spawning.

		Time after spawning (week)									
	C0	**0**	**1**	**2**	**4**	**8**	**13**	**16**	**24**	**33**	**C33**
L*	42.4 ± 1.5***	47.7 ± 3.5 a	49.0 ± 3 .7a	48.9 ± 2.2 a	48.8 ± 2.6a	46.5 ± 1.6b	44.5 ± 1.9 c	43.5 ± 2.5 c	41.4 ± 2.0 d	41.9 ± 2.0 d	38.2 ± 2.2***
a*	11.3 ± 1.8***	7.9 ± 2.5d	7.8 ± 2. 8d	7.9 ± 3.0d	8.1 ± 2.7d	6.9 ± 2.8d	10.5 ± 3.3 c	12.4 ± 3.2 b	15.2 ± 1.7 a	15.8 ± 1.6 a	15.5 ± 1.7
Wtot/Th (a.u)	85.3 ± 11.7*	75.5 ± 16. 3b	89.4 ± 1 0.0a	89.9 ± 13. 7a	89.6 ± 12. 1a	88.6 ± 12. 4a	89.9 ± 10. 0a	84.7 ± 12. 9a	51.0 ± 4.3 c	49.1 ± 5.6 c	47.9 ± 7.0.

Mean ± standard deviation, n=20, 18 and 14, respectively in post-spawning group, C0 and C33.

Values in the same row with different letters are significantly different (Student–Newman–Keuls test, $p < 0.05$)

* and *** indicate significant differences between control and post-spawning trout ($p<0.05$ and $p<0.001$ respectively)

Wtot/Th: total work divided by sample thickness of raw fillet

a.u: arbitrary units

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Results

At spawning, trout showed significantly lower body weight, Fatmeter® value and raw fillets yield than trout from C0. Furthermore, raw fillets from these trout were less colored (lower redness a*) and with higher lightness L*, and exhibited lower mechanical resistance than raw fillets from C0 trout (table I). At 13 weeks, body weight and Fatmeter® value were significantly higher than at 1, 2, 4 and 8 weeks after spawning, and continued to increase till the end of the experiment. Raw fillets yield were found to increase only after the 16th week post-spawning. Lightness of fillet steadily decreased from the 4th week to the 24th week and did not change afterwards, whereas fillet redness increased from the 8th week to the 24th week after spawning (table I). Fillet mechanical resistance increased one week after spawning, remained high until the 16th week, sharply dropped between the 16th and 24th weeks and did not change afterwards (table I). Smoking yield of fillets from post-spawned trout was significantly lower than that of trout from C0, and significantly increased only at the 24th week. Post-spawning evolution of color and mechanical resistance of smoked fillets was similar to that of raw fillets, except for the fillet lightness. At the end of the experiment, body weight and Fatmeter® value of post-spawning trout and quality parameters of their fillets were found to be globally similar to those observed in C33

Discussion and conclusion

The lower body weight and Fatmeter® value observed in post spawning trout compared to immature trout is in agreement with existing data showing that egg production induces a decrease of somatic growth and a wasting of muscle fat stores. After spawning, trout substantially increased their body weight to eventually reach that of control trout. This muscle burst of growth along with the accumulation of muscle fat likely account for the increase of the fillet yield observed after spawning. At spawning, the flesh was strongly discolored resulting from the mobilization of muscle carotenoid pigments towards ovaries. After spawning, fillets progressively recovered their color. A feature in line with previous observations reporting that pigment concentration tends to increase rapidly in the fish muscle after spawning (Choubert, 1992). The decrease in the mechanical resistance of the flesh during the period that follows spawning may be explained by the increase in muscle fat content (Lefèvre et al., 2015) and as well as muscle fibers hypertrophy, an important determinant of flesh texture (Johnston, 1999). The increase in the smoking yield can be attributed to a lower water loss associated to increase in muscle fat content in the trout fillets (Mørkøre et al., 2001).On the whole, our results indicate that technological and sensory properties of the flesh are recovered in female trout 24 weeks after spawning. Further histologic and transcriptomic analysis would allow a better understanding of the biological process involved in this quality recovery.

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THE EFFECTS OF SBM AND RSM BASED DIETS ON THE GROWTH PERFORMANCES AND BODY COMPOSITION OF ZEBRAFISH (*Danio rerio*)

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Introduction

There have been significant research efforts at improving dietary composition of feeds and maximizing fish growth for sustainable aquaculture. This is achieved by identifying sustainable source of ingredients in order to reduce dependency on highly competitive and expensive fish by-products (Hardy, 2010). Although, zebrafish (Danio rerio) have an advanced impact in laboratories research for its utility in understanding developmental biology, genetics, toxicology, and recently nutrition (Ulloa *et* al., 2014; Khan and Alhewairini, 2018), the influence of diet composition on basic growth outcomes and body composition on the fish is less well demonstrated (Smith *et al*., 2013). The aim of the present study was to compare the acceptability and effect of SBM and RSM based diets with a commercial FM based diet, on growth parameters and fish body composition through repeated but non-invasive measurements on zebrafis

Material and methods

Two iso- nitrogenous (47% crude protein) and iso-calorific (11.8 MJ DE/kg) diets were formulated and compared with a commercial diet for zebra fish. This study employed a 3 x 2 factorial arrangement involving three diets at two stocking densities. The fish were fed three times daily at 5% of their body weight, for a period of eight weeks. Morphometric measurements, water quality and feed allowances were determined on weekly basis. After the experiment, fish were euthanized with tricaine methanesulphonate MS-222 (Sigma Aldrich), frozen at -20^{o}C, and later freeze dried until processed for various chemical analysis. The length-weight relationship, condition factor and the growth parameters of the fish were determined. Samples of feeds and fish were analysed for proximate (AOAC 2005), minerals, fatty acids, and amino

Table 1. Mean ± SE of the proximate composition, growth performance of zebrafish fed various diets

	Proximate composition (%)					Growth performance indices					
Items	DM	CP	EE	ASH	NFE	WG(g)	SGR	FCR	FI (g)	PI	PER
FFMD	25.92 ± 1.8	13.8 ± 1.0	8.5 ± 0.6^{a}	1.1 ± 0.3	3.1 ±0.3	2.0 ± 0.2^{a}	1.2 ± 0.1^{a}	0.1± 0.0	0.2 ± 0.0^{a}	1.5 ± 0.1^{a}	1.4± 0.1
FSBMD	23.1 ± 1.7	13.2 ± 1.0	5.7 ± 0.5^{b}	1.2 ± 0.3	3.0± 0.3	1.1 ± 0.1b	0.9 ± 0.1ab	0.1± 0.0	0.1 ± 0.0b	1.1 ± 0.1ab	1.0± 0.1
FRSMD	26.53 ± 1.36	14.5 ± 0.8	7.4 ± 0.6^{a}	1.6± 0.2	3.0± 0.4	1.0 ± 0.1b	0.8 ± 0.1b	0.1± 0.0	0.1 ± 0.0b	0.9 ± 0.1b	1.2± 0.2

FCMD; fish fed commercial FM diet, FSBMD; fish fed SBM diet and FRSMD; fish fed RSM diet.

Table 2. Fatty acids composition of the fish

Fatty Acid	Fish fed CFM diet	Fish fed SBM diet	Fish fed RSM diet
∑SFA	34.3 ±0.8^{a}	28.7±1.5^{ab}	22.2 ± 2.1^{b}
∑MUFA	39.1 ±0.4^{b}	45.7±0.4^{ab}	51.7 ± 2.0^{a}
∑PUFA	25.0 ± 0.5	23.1 ± 0.7	24.8 ± 0.1
∑n-3 FA	10.4 ± 0.3	11.8 ± 0.2	10.6 ± 0.2
∑n-6 FA	14.6 ±0.1^{a}	11.3 ±0.4^{b}	14.3 ± 0.3^{a}
∑n-9 FA	35.1 ±0.3^{c}	39.0±1.1^{ab}	46.3 ± 2.0^{a}
n-6:n-3	1.4:1.0	1.0:1.0	1.4:1.0

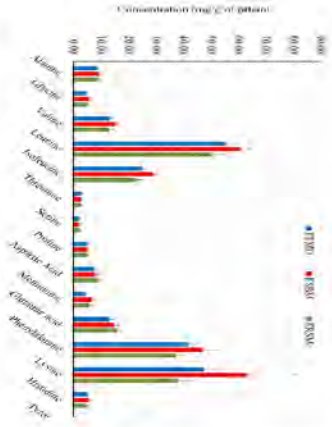

Figure 1.The amino acid composition of the fish

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acids compositions. The minerals were analysed by using ICP-OES technology following acid digestion while the fatty acid methyl esters were analysed by GC-MS following methylation and esterification, after fat extraction (using modified method of Folch *et al*., 1957). The amino acids was analysed by using EZ-Faast amino acid derivative technique for GC-MS after acid hydrolysis. Data were analysed using T-Test, ANOVA and GLM. Tukey's post-hoc test was used to compare means to observe significance at $p < 0.05$

Results

The crude protein, nitrogen free extract and ash contents on dry matter basis were not different ($p<0.05$) for the fish fed any of the diets (Table 1). Mean live-weight gain (WG), FCR and PI were higher ($p<0.05$) in the fish fed the CFM diet than the other diets. No differences were observed SGR, FI, and PER. Palmitic (C16), oleic (C18:0), and linoleic (C18:1n9) acids were prominent in all the fish. The total saturated and omega-6 fatty acids were highest in fish fed commercial diet (34.25 ±0.75, 14.57 ± 0.13) compared to the other diets (Table 2). The Ca, K, Mg, Na and P contents were more prominent in all the fish, with Na found to be highest ($p<0.05$) in fish fed RSM diet (31.220 ± 3.63 mg/l) than those fed commercial diet (21.510 ± 2.81 mg/l) and SBM diet (22.380 ± 1.24 mg/l) respectively. There were no significant differences in the contents obtained in the amino acid composition of the fish fed the diets (Figure 1).

Discussion and Conclusion

There was no adverse effects of the SBM or RSM based diets on fish in terms of the survival rates (>95%), chemical composition and growth observed. The effect of the diet x stocking density on the growth and performance of the fish was not significant. The omega-6 and omega-3 ratio (n-6: n-3) FA of the fish are within the lower ratio of n-6: n-3 fatty acid which is desirable in the preventing chronic diseases (Russo, 2009; Bogard et al., 2015). There was no significant effect of the AAs of the diets on the fish. The study showed that both SBM and RSM diets can potentially replace FM based commercial diet for zebra fish and other finfish to reduce cost and promote sustainability of aquaculture production

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ENTEROBACTER INFECTIONS OF CULTURED RAINBOW TROUT (*Oncorhynchus myk*

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Introduction

Rainbow trout (*Onchorhynchus mykiss*) culture was first begun in Turkey in 1970's and showed a great increase. The total production amount of Turkey has reached to 101 761 tons in 2017. Enterobacteriaceae is a large family of Gram-negative bacteria which includes the fish pathogenic genera, *Yersinia*, *Hafnia*, *Citrobacter, Klebsiella* and *Escherichia*. Upto date, various bacterial infections caused by Gram-negative pathogens were observed, but there is a limited information on the Enterobacteriaceae infections of rainbow trout except *Y. ruckeri* infections. The aim of this study is the determination Enterobacteriaceae infections of rainbow trout cultured in freshwater cage farms by using conventional bacteriologic, molecular and histopathological methods.

Materials and methods

With this aim, a cage facility located in a dam lake in our country was visited for a one-year period in April, June, July, September and December in 2017 and a total of 50 diseased rainbow trout samples were investigated. Bacteriologic inoculations from the visceral organs of moribund fish were streaked onto TSA (Tryptic Soy Agar) and bacteria were identified by conventional morphologic and phenotypic tests (Brenner and Farmer, 2009). Biochemical identification was confirmed with the PCR amplification of 16S/23S rRNA with the universal primers 27F and 907R (Lane, 1999). For histopathological examination, tissue samples were fixed in 10% formaldehyde, processed with routine methods and embedded in paraffin (Roberts, 2012). Histological sections were stained with Haemotoxylin – Eosin and analyzed under light microscope.

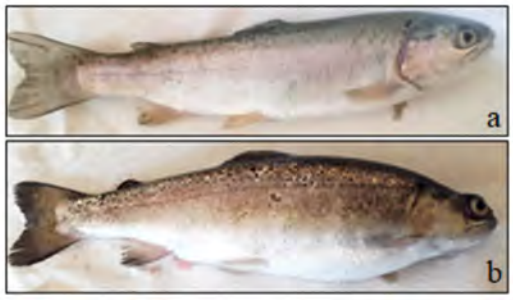

Figure 1.
a) Sloughing in the rainbow trout infected with *H. alvei*
b) Darkening of the skin and edema in the rainbow trout infected with *C. freundii*

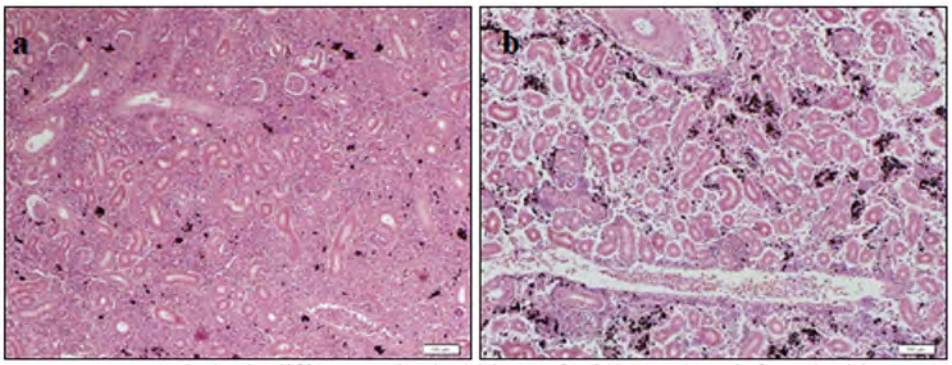

Fig 2. Pathologic differences in the kidney of rainbow trouts infected with *H. alvei* (a) and *C. freundii* (b)

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Results

In this study, of the members of Enterobacteriaceae, *Hafnia alvei* and *Citrobacter freundii* were recovered and identified from the infected fish samples. Biochemical identification was confirmed with 16S gene sequencing and blasting. Fish samples infected with *H. alvei* showed sloughing of the skin, swollen abdomen (Fig 1.a) and hyperemia in the visceral organs clinically. Histopathologically, they showed small melanomacrophage centers, hemorrhages and hyperemia in the visceral organs, and slight degenerations in the liver hepatocytes and kidney tubules (Fig 2.a). Fish samples infected with *C. freundii* showed exophthalmos, darkening of the skin, edema and accumulation of a transparent liquid in the peritoneal cavity (Fig 1.b) and splenomegaly clinically. Histopathologically, these samples showed slight necrosis in the hepatocytes but severe necrosis in the interrenal haemopoietic tissue, severe degenerations in the hepatocytes and kidney tubules and generalised melanomachrophage centers and hemosiderin accumualtions in the kidney and spleen (Fig 2.b).

Discussion and conclusion

Hafnia alvei and *C. freundii* are the members of Enterobacteriaceae, which can be mis-identified by biochemical profiling and may be confused with *Y. ruckeri*. Molecular studies and gene sequencing gives more reliable results for the identification (Austin and Austin, 2016). In this study, biochemical profiling was also misidentified our isolates but molecular studies provided the final identification of these bacteria.

The members of the Enterobacteriaceae previously caused infections in freshwater fishes after transportation, under stress or during cultivation in inappropriate conditions. Generally these bacteria cause hemmorhagic septicemia in fishes, but swollen abdomen is seen in *H. alvei* infections while *C. freundii* causes exhophthalmus and surface hemorrhages (Austin and Austin, 2016). In this study infected fish samples showed similar symptoms.

Despite these bacteria were recovered from rainbow trout previously by other researchers, there is a limited information on the histopathology of these infections. The results of our study showed that *C. freundii* infections cause more severe clinical and histopathological symptoms while *H. alvei* causes more mild pathologies in rainbow trout.

In conclusion, the results of this study revealed the need for the use of molecular methods in the identification of fish pathogenic Enterobacteriaceae species and the detailed differences of pathologies these emerging pathogens in rainbow trout were demonstrated by using histopathological methods.

Acknowledgements

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BEHAVIOURAL AND MORPHOLOGICAL ASSESSMENT OF PARAQUAT TOXICITY ON JUVENILES OF NILE TILAPIA, *Oreochromis niloticus*

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The impact of acute exposure of *Oreochromis niloticus* juveniles to a commonly used herbicides, paraquat was evaluated through changes in their behaviour and mortality. The fishes were exposed to lethal concentration of 0.00mg/l, 3.31mg/L, 3.86mg/L ,4.4mg/L and 4.97mg/L of paraquat for 96 hours. The toxicity bioassay procedure showed that the 96 hours LC_{50} was 3.85mg/l. Restlessness, erratic swimming, loss of equilibrium, discolouration and sudden fish death were observed in the exposed fish and these varied greatly with differences in concentration of the toxicant and this shows that mortality increases with an increase in concentration. The differences observed in the mortalities of *Oreochromis niloticus* at varying concentrations were significant ($p<0.05$), an indication that mortality could be a factor of concentration and time of exposure. Observation of Opercular Ventilation Count (OVC), Tail Fin Movemant Rate (TMR) and Air Gulping Index (AGI) showed a marked difference between control and exposed fishes, indicating that the herbicides negatively impacted these parameters. These behavioural and morphological anomalies became more pronounced with increasing concentrations of the herbicides. The physico-chemical parameters of the experiment were within the tolerated limit. The result further revealed that paraquat have the ability to induce unusual behaviours in juvenile fish and can therefore serve as reliable indicators of toxicity in our environment and hence, the volume of these herbicide that get into the environment should be regulated.

ASSESSING THE MAJOR CONSTRAINTS OF INTERNATIONAL AQUACULTURE CERTIFICATION FOR EXPORT FISH FROM BANGLADESH

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Introduction

Aquaculture has grown rapidly in Bangladesh that contributes 56% of the total production of 4.13 million MT of fish (DoF, 2017). The sharp rise of aquaculture production started from 1990s mainly due to technological development particularly, the development of artificial seed production in hatcheries and fish feed production. Pangasius (*Pangasianodon hypophthalmus*|) and tilapia (*Oreochromis niloticus*) have become two major fast-growing aquaculture species in Bangladesh. Although, globally Bangladesh is the 5th largest aquaculture producer but the fish export trade ranked feculences (FAO, 2018). However, the demand of pangasius and tilapia has continued to increase both in domestic and international markets (Moloko et al., 2013). The international export trade of Bangladeshi pangasius and tilapia is intangible and stuck only in domestic market due to absence of implementation of standard aquaculture certification. On the other hand, the largest Asian seafood exporters such as China, Vietnam, and Indonesia established aquaculture certification meeting international third party certifier guidelines. Therefore, the introduction of aquaculture certification in Bangladesh is an urgent need and demand of the time.

Materials and methods

The study was conducted based on multiple methodological approaches including literature review, secondary data analysis, primary data collection and analysis and laboratory analysis of fish, fish feed, pond wat , and pond sludge.

Results

Bangladesh has developed some rules and guidelines to comply with the international code of conducts for export on aquaculture governance such as water use, fish feed and hatchery rules, food hygiene and sanitation, etc. In contrast, a number of guidelines of aquaculture governance such as land use and zoning plans, responsible use of drugs and chemicals rules, disease control strategy, traceability procedure, etc. yet not been established and enforced at field level properly. As a result, the framers used indiscriminate aquaculture drugs and chemicals due to lack of knowledge regarding use of chemicals, application of chemicals and antibiotics, residual effect and expiry date and diagnostic facilities for proper disease diagnosis. Similarly, the farmers used commercial medicated feed without knowing the specification such as type and percentage of drugs in feed, name of veterinarian who prescribed the drug and date of manufacture. In case of farm-made feed, the farmer mixed-up drugs and chemicals with feed without authorized instructions. Therefore, the fish flesh have been contaminated by toxic heavy metals including lead, cadmium and mercury, where lead found around 5 folds higher in both pangasius and tilapia muscle compared to the acceptable limit. Consequently, the chromium level in fish flesh was 54 times higher in pangasius and 20 times in tilapia flesh, another constraint to export aquaculture products. Although, the socio-economic characteristics of farmers in Bangladesh are mostly complementary to adopt aquaculture certification but some of them are constraints to adopt certification particularly because a large number of farms are small in size having difficulty to comply with certification code of conducts. Nevertheless, the majority of the farmers was in middle age, educated and experienced which are technically efficient to adopt aquaculture certification

Discussion and conclusion

The aquaculture certification is based on international standards and complies with relevant local, national and international laws and regulations. However, the rules and guidelines on aquaculture in Bangladesh have remained in sporadic condition. In addition, as a larger seafood producer, it is a matter of regret that the code of conducts of top international third party certifier (e.g. ASC and GlobalGAP) are not followed or very seldom emulated by pangasius and tilapia farmers in Bangladesh such as site selection and management, reproduction system at hatchery, responsible drugs and chemicals use, water usage and disposal system, animal welfare and husbandry, sampling and testing of fish, feed use and management, pest control, environmental and biodiversity management, harvesting, handling and transportation, post-harvest - operations and traceability, farm operators occupational health and safety and social and welfare criteria. Regarding food safety issues, the produced fish was found contaminated by heavy metals being originated from fish feed. Therefore, it is important to establish clear and transparent policies and procedures in aquaculture sector in Bangladesh for environmental neutrality, social acceptability and ultimately helps to improve the sustainability (FAO, 2017). Compared to the regional and international trend, it is the time to take necessary steps by the government and non-government organizations addressing the issues of exporting commercially important farmed fish from Bangladesh. Otherwise, the growing aquaculture industry in Bangladesh would not sustain only based on the domestic market-based consumption.

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AMPHIPOD *Echinogammarus marinus* MEAL IN FORMULATED DIETS FOR JUVENILE TURBOT *Psetta maxima* – EFFECT ON GROWTH PERFORMANCE AND FATTY ACID PROFILE

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Introduction

Increasing fishmeal prices and the depletion of natural fish stocks drive demand for novel feed ingredients in aquaculture. Marine amphipods are a natural food source for many flatfis species and are rich in essential fatty acids hence strong candidates as fishmeal replacement in aquafeeds. Recent studies showed promising fatty acid profiles and fatty acid synthesis in marine amphipods (Alberts-Hubatsch et al. *under review*, Baeza-Rojano et al. 2014, Jiménez-Prada et al. 2018)Caprella equilibra, Caprella grandimana, Caprella penantis, Elasmopus rapax, Hyale perieri and Jassa sp. from marine water habitats and Echinogammarus sp. from freshwater habitats. Lipids, proteins, carbohydrates, ash and water contents were measured. Proteins and ash were the most abundant components in all the species, ranging between 37.9 and 44.6% and 29.3 and 39.7% dry weight, respectively. The lipid and carbohydrate contents showed lower levels (5.1-9.6% and 3.1-9.1% dry weight, respectively, which could lower the need for fish oil supplementation in finfis feeds. In this study, juvenile turbot *Psetta maxima* were fed with four different diets containing different levels of amphipod meal as fishmeal replacement: 0, 50 and 100 % of replacement and a commercial turbot feed as reference. The experimental diets were formulated to meet basic nutritional demands without added fish oil. Growth performance as well as lipid classes and fatty acid profile in muscle and liver tissue in response to di ferent feeding regimes were investigated.

Material and Methods

A 32-day feeding trial was conducted with juvenile turbot (1.43 ± 0.2 g), fed four different diets containing different levels of fish-meal replacement: (A) commercial turbot diet (BioMar Inicio) as reference diet, (B) control diet containing 100% fishmeal, (C) diet with 100% Amphipod meal, (D) diet with 50/50% Amphipod/fishmeal (Table 1). Each treatment was performed in four replicates of 20 juvenile turbot. After 14 days a subsample of five fishes per replicate was taken to show early changes in fatty acid profiles. At the end of the experiment, final growth parameters (weight, length) were recorded. Whole body as well as tissue samples of liver and muscle were taken, immediately frozen in liquid nitrogen and stored in -80°C until further analyses. Proximate analysis (crude lipids, crude protein, ash, energy) was performed using complete fishes. The lipid classes of muscle and liver were separated by thin layer chromatography and fatty acid methyl ester (FAME) of each class analyzed by gas chromatography.

Results and Discussion

Wet weight, length and SGR did not differ between replacement diets, but the commercial diet as reference exhibited higher growth rates (Table 1). However, survival rates did not differ between the treatments with 100% survival, with the exception of one fish in treatment C at day six.

Results for fatty acids and proximate analysis are still pending.

We expect differences in the fatty acids of liver and muscle, especially between diet B (100% fishmeal) and C (100% amphipod meal). We further expect differences in the lipid classes, also between the reference diet and the replacement diets. Natural feeds can exhibit more natural lipid classes that provide well-balanced lipid profiles, which often offer better palatability than processed lipid mixtures. Being part of the natural diet of flat fishes (Braber & De Groot 1973), we expect a better bioavailability of lipid classes and respective fatty acids then fishmeal diets

Generally, amphipods constitute a good source of high valued long-chain polyunsaturated fatty acids as well as other nutritional factors such as pigments. Growing interest in these species as feed ingredient in terms nutritional value and suitability as fish and crustacean feed calls for novel and sustainable culturing and processing methods for amphipods i.e. gammarids.

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THE AGROBIOTECHNOLOGY ECOLOGICALLY CLEAN PRODUCTS WITH THE USE OF NEW TECHNOLOGIES IN RAS

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Introduction
Biotechnology is a multicomponent system, the functioning of which is determined by three independently existing complementary components: the cultivated organism, the habitat and external influences that affect either only the organism or the habitat, or equally on both of these components. Exhaustion of opportunities for growth of fishing with a constant increase in demand for food from hydrobionts (one of the cheapest species of animal protein) has led to the rapid development of aquaculture. In the world, artificially diluted aquaculture products occupy, according to various estimates, about 40-45% of the industry market. The developing agrobiotechnology allows to obtain high-quality environmentally friendly fish products of high qualit , as well as crustaceans and plant crops.

Materials and methods
Experimental work on agro-biotechnology of the cultivation and production of environmentally friendly products were conducted over 5 years. During this time, a large amount of research work was carried out on the selection of optimal objects of cultivation. The research was carried out in the laboratory of aquatic bioresources and aquaculture on the basis of the experimental aquarium complex "Kagalnik" (Kagalnik, Rostov region) of the southern scientific center of the Russian Academy of Sciences using a unique collection of bioresources. The object of the study was agricultural crops, hybrid form of sturgeon, Clary catfish, tilapia. When conducting research using the fish-biological methods of production of experience, applied hydro-chemical, biochemical and biotechnical methods in accordance with conventional methods and the international standard using modern laboratory equipment.

Results
The technology of growing objects integrated installation for the joint cultivation of aquatic organisms and crops. In the integrated agrobiotechnological installation cultivation of such types of fish as: hybrid forms of sturgeon, African Clary catfish, tilapia, and also crops was carried out: salad, tomato, strawberry, pepper, dill. With the joint cultivation of hydrobionts, positive results were obtained. It was found that the growth of hybrid forms of sturgeon, tilapia and Clary catfish is 12 % higher when grown in agrobiotechnological plant than in the classical RAS. Earlier studies have led to the conclusion that it is advisable to grow the method of aquaponics culture with a short growing season (45-60 days.), and for the cultivation of crops with a longer period, it was decided to use a biological product based on bacteria of microbial origin for stimulation and better development. The volume of grown products is 24% higher than in traditional cultivation in RAS. The terms of growing aquaculture products have been reduced by 8%.

Discussion and conclusion
Agrobiotechnology for obtaining environmentally friendly products of aquaculture with the use of new technical means is promising at the present stage of aquaculture development. Allows you to combine several growing processes. The most important condition necessary to withstand the ever-increasing competition in the market of fish products is the excellent quality of products, both in terms of quality of meat and in terms of consumer preferences. Therefore, the results of these studies are the basis for further development of the production of Biosafety food products.

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THE USE OF MOLECULAR TOOLS TO IDENTIFY MARINE BIVALVE LARVAE IN ENVIRONMENTAL SAMPLES

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Introduction

Development of improved methods for identification and quantification of bivalve larvae in plankton samples can offer opportunities for improved understanding of temporal and spatial patterns of seed supply for aquaculture and also validation of hydrodynamic particle tracking models of larval dispersal and population connectivity (Robins *et al* 2013). Aquaculture production of mussels is dependent on reliable sources of wild seed, which can be a limitation to growth of the sector in some regions. However, effective monitoring of larval supply requires discrimination between planktonic larvae of many morphologically-similar bivalve species. The standard identification method is through taxonomic classification via direct microscopic observation, a particularly time consuming process which is severely hampered by a lack of experts in the field of plankton taxonomy, the accuracy of identification and human error. Polymerase chain-reaction (PCR) assays using species-specific primers present a powerful method to detect target species in environmental samples; in addition, the ability to gather quantitative real-time PCR data offers the potential for between-sample comparisons of relative larval abundance.

Methods and discussion

Species-specific assays were designed and optimised using primer pairs either gathered from published literature (Inoue, et al. 1995), generated using primer design software or manually designed using sequences aligned in MEGA7.

Blue mussel D-stage larvae were obtained from single species experimental culture and used to compare and optimise DNA extraction methods for larval samples (Figure 1).

Spawned larvae were also used to examine the quantitative potential of species-specific qPCRs, where data generated from differing concentrations of larvae were used to create
a model which predicts mussel larval numbers from real-time data.

Assays were used to analyse the bivalve content of vertical –tow plankton samples collected from multiple sites around North Wales over a two year period (Figure 2).

Main conclusions

These techniques represent a promising method for the detection and relative quantification of bivalve larvae in mixed plankton samples, which will shed new light on temporal and spatial patterns of spawning and larval transport. The methodology also has the potential to aid in aquaculture site selection and projections for optimal times when equipment deployment or installation can take place to maximise settlement potential.

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CONSERVATION OF NATIVE *Cyprinid* SPECIES IN HUNGARY – PRELIMINARY RESULTS ON THE GENETIC VARIABILITY OF CRUCIAN CARP (*Carassius carassius* L. 1758) AND TENCH (*Tinca tinca* L. 1758) POPULATIONS AND STOCKS

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Introduction

Crucian carp (*Carassius carassius* L. 1758) and tench (*Tinca tinca* L. 1758) are *cyprinid* fish species native to Central-Eastern-Europe including Hungary. They prefer to live in marshes, densely vegetated lakes and ponds, backwaters and horse-shoe lakes. Nowadays their role in aquaculture is not significant; however this may change in the future. They adapted well for higher temperatures and lower oxygen levels. The climate change may make these qualities more important and may make these species suitable for future aquaculture production. The populations of both species are on the decline in Hungary and in the neighboring countries. The main reasons of population reduction are habitat loss, human activities and in case of crucian carp the hybridization with the invasive gibel carp (*Carassius gibelio* Bloch, 1782). The conservation of the two species is on one hand important for the maintenance of biodiversity and on the other hand for the beginning of breeding work. The genetic information on natural populations in Hungary is limited so genomic and mitochondrial DNA markers are used to assess the genetic variability of the species. In case of crucian carp the extent of hybridization with gibel carp is also in the focus of our research. The future goal of this work will be the establishment and development of a live genebank and a cryobank of these species.

Materials and methods

Fin samples were collected from 310 crucian carp individuals representing 7 natural populations and 4 stocks from different fish farms in Hungary and from 160 tench individuals originating from a fish farm (Somogygeszti) in South-West-Hungary. Fin samples were stored in 70% ethanol until DNA extraction. The total DNA was extracted from the samples using the simple salting out procedure described by Miller et al. (1988). DNA qualities and quantities were measured by NanoDrop™ spectrophotometer. 5 microsatellite markers (MFW7, GF1, GF29, YJ0010 and YJ0022) were used to analyze the genetic variability of crucian carp so far. PCRs were carried out as it is described in the original papers. An automated ABI Prism 3130 Genetic Analyzer was used for the fluorescently labeled PCR products sizing. Genotyper 4.0 software package supplied by Applied Biosystems was used to estimate the length of fragments. GenAlEx6.501was used to calculate allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), number of private alleles, genetic variance (Fst), and heterozygote deficit. MICRO-CHECKER version 2.2.3 was used to detect possible genotyping errors, allele dropout and non-amplified alleles (null alleles). The genetic relationship between populations and individual assignments of fish was inferred via a Bayesian clustering analysis using the statistical program STRUCTURE version 2.3.3. The K with the greatest increase in posterior probability (ΔK) was identified as the optimum number of sub-populations using STRUCTURE HARVESTER. In case of the tench, the mitochondrial d-loop sequences were analyzed by PCR amplifi ation (primers Pro2:AACTCTCACCCCTGGCTACCAAAG and Phe2:CTAGGACTCATCTTAGCATCT TCAGTG), and BigDye Terminator sequencing (Applied Biosystems) from both ends. MEGAx and DnaSP 5.10 software were used for the analyses of sequences. A phylogenetic tree was constructed using the maximum likelihood method and Tamura-Nei model.

Results

A total number of 48 microsatellite alleles were described throughout the eleven populations and stocks of crucian carp. The lowest number of alleles was found on locus GF1 (3) while the highest number of alleles (26) was detected on GF29. The number of alleles ranged from 10 to 27 in the different populations. Altogether 10 private alleles were found with the frequency ranging from 0.017 to 0.093. The private alleles were found mostly in the wild populations. The average expected (H_e) and observed heterozygosity (H_o) for all populations and loci was 0.427 and 0.389 respectively. In case of most of the natural populations the H_e and H_o values were relatively close to each other while in case of two farmed stocks the Chi-square tests for Hardy-Weinberg equilibrium presented significant heterozygote deficit in four of the five loci. The AMOVA analysis showed that 34% of the whole molecular variance is among populations, while 12% is among

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individuals and 54% is within individuals. Genetic differentiation (pairwise Fst) between population pairs ranged from low to high values (0.022 and 0.592) supporting the existence of population substructure. The structure analysis supports the existence of at least two subpopulations in Hungary. The tench mitochondrial d-loop sequences contained 34 polymorphic sites among the 793 nucleotides and 14 haplotypes were identified in the population. Forty-four percent of the individuals carried the most frequent haplotype (Hap_8), while 5 moderately common (23-5.6%) and 8 rare haplotypes were also present. The phylogenetic tree shows two distinct groups of the haplotypes.

Discussion and Conclusions

Based on the so far limited microsatellite results one can say that the natural crucian carp populations in Hungary are genetically diverse and generally the natural populations have higher variability. There were only two exceptions found. These are small and totally isolated pond populations with small number of individuals. Some of the farm stocks have a very limited number of alleles and the proportion of heterozygotes is low. This phenomenon can be explained with a probable founder effect. The farm stocks were probably established with the use of limited number of broodfish. In the future process of the establishment of genebanks of the species, we have to practice extreme caution to avoid this mistake and collect the individuals with proper genetic background, representing the variability of the Hungarian populations. With the future mtDNA investigations and the analysis of diagnostic microsatellite markers, the potential crucian carp-gibel carp hybrids must be excluded from the genebank. The preliminary analyses of the Somogygeszti tench population shows high haplotype diversity, however the analyses of the nuclear genome is also necessary for conclusions. To build a tench gene bank, further wild and farmed populations should be analyzed and used to preserve proper genetic variability.

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β-GLUCANS, SODIUM ALGINATE AND THYROXINE HELP IN ENHANCED SURVIVAL RATE, STRESS TOLERANCE AND IMMUNITY IN BLUEFIN BREAM (*Sparidentax hasta*) LARVAE

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Introduction

Bluefin bream is one of the important commercial species in Kuwait and the Arabian Gulf. Reducing the mortality rates, in the larval rearing stage between day 7 and 35, caused by several types of pathogenic bacteria, is the aim of this study. One alternative method-gaining acceptance within the aquaculture industry is the use of immunostimulants. Because of the previous project FM074K results (Al-Gharabally H. et al., 2013) which showed that β-Glucans enhanced survival rate and disease resistance of sobaity larvae, ß-Glucans was chosen again along with Thyroxin (T4) and Sodium Alginate (SA) because both gave impressive results in enhancing immunity in general (Yamano 2005; Klaren et al., 2008; Schnitzler et al., 2011; Cheng et al., 2013; Liu et al., 2006). This study is aimed at evaluating the effects of the thyroxine (T4) and sodium alginate (SA), in comparison with β-glucans (BG), on the survival rate, stress tolerance, and immunity of Bluefin bream (*Sparidentax Hasta*) larvae.

Materials and methods

Dose optimization experiments must be carried out to identify the optimized doses of T4 and SA which will be applied with the already determined optimized dose of ß-Glucans in a dietary experiment which will continue for 35 days focusing on ages from 7th day post hatch (dph) till day 35 dph for improving the non-specific immune responses. Immune parameters will judged by haemagglutinin, bacterial agglutinin, lysozyme and phagocytic assays. Larval quality is evaluated by carrying out the following stress tests based on importance and frequently used methods in aquaculture: salinity, handling and formalin. For evaluating disease resistance, a sample from the immunostimulated and control larvae are challenged with pathogenic bacteria *Proteus vulgaris* spp at 10^7 colony-forming unit (CFU)/ml suspensions for 30 min, after which the challenged larvae will be transferred into fresh seawater aquariums of 30 l capacity to observe survival and mortality.

Results

The optimized doses for T4 and SA were best at 0.01 ppm and 3 g/l, respectively, in enhancing the overall survival for the first 7 d post hatching (dph). The evaluation of optimized doses of T4 (0.01 ppm), SA (3 g/l) and BG (45 mg/l) revealed that the best enhancement in growth and survival of larvae until 14 dph was with T4 treatment. However, BG was better than T4 at 21 dph. The stress test conducted at 35 dph showed that the immunostimulated larvae, irrespective of the stimulant used, were significant y better in their survival response after salinity and formalin stress. Immune parameters suggest a superior performance of the immunostimulated larvae in their phagocyte response, lysozyme activity, hemagglutinin, and bacterial agglutinin levels. The best survival in a challenge test of stimulated larvae against live *Proteus vulgaris* showed a higher survival response with SA and T4 (92 and 88% respectively) followed by BG (75%) and lastly 58% for the control group.

Discussion and conclusion

Immunostimulation of the larval stages of fish is an important step in the fish health management strategy as the fish at this stage of their life cycle are either immature histologically to handle antigens or immunologically to mount considerable defense to overcome the pathogens. Immunostimulation with T4, SA and BG was effective when applied to larvae from 14, 21 dph in case of enhancing larval growth. Immune response of immunostimulated larvae was also superior in the immunostimulated fish. Stimulation with T4 followed by BG can be helpful in enhancing survival and immunity.

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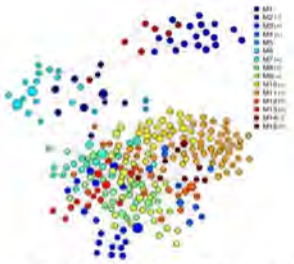

Figure 1. Bacterial co-occurrence network generated by SPIEC-EASI. Network modules detected by Louvain clustering are shown in different colours. Node size corresponds to the average sequence proportion operational taxonomic units in intestinal and faecal samples. No symbol indicates common modules, while bacterial clusters/sub-communities in healthy and diseased shrimps are indicated by (-) and (+), respectively.

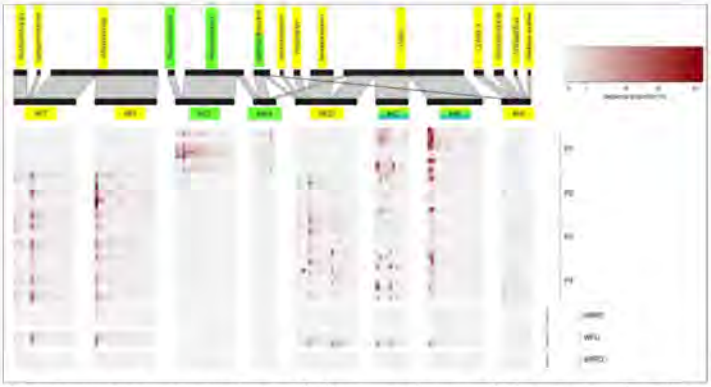

Figure 2. The sequence proportion of the members of the most dominant and most distinguishing network modules between healthy shrimps (P1 and green) and diseased shrimps (P2, P3, P4, and yellow) as well as their contribution to the particle-associated fraction from the respective ponds and sampling points. Their taxonomic affiliation is provided on genus level. Water samples were not used for the network construction.

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UNRAVELLING THE PATHOGENESIS OF SALMON GILL POXVIRUS IN FRESHWATER ATLANTIC SALMON

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Introduction: Salmon gill poxvirus is an emerging viral disease in the Scottish salmon farming industry and has been associated with high mortalities throughout the production cycle. Although severe gill pathology is generally associated with disease outbreak, little else is known about the pathogenesis of the disease. In order to better understand the transmission routes and potential outbreak triggers, two comparable cohorts of farmed salmon were studied over a 6 month period and through an SGPVD outbreak.

Methodology: Two cohorts of Atlantic salmon fry in a hatchery were studied concurrently in separate recirculation systems with varying histories of SGPV. Both systems share similar husbandry techniques, water source and fry intake, but only one has had SGPV recently. During the study period, one cohort became infected with the virus while the other remained naïve. The progression and effects of the disease were studied in order to better understand the prevalence of the virus and it's correlation to disease outbreak.

Blood, serum, histology and gill samples were taken from 14 randomly sampled fish from each cohort, at two week intervals, over a six month period. Once SGPV was detected, sampling was increased to twice weekly for two weeks in order to document the disease outbreak. As a result, the virus prevalence, spread and effects in various organs were documented throughout the outbreak.

Gill tissue was tested for viral load using qPCR, histopathology samples were screened for any pathology caused by the disease, blood samples were tested for red blood cell count, total and differential white blood cell counts and packed cell volume and serum was taken for serology and potential biomarker identification

Results: Results of the comparative study of naïve and infected cohorts will be presents in the final poster as analysis is ongoing.

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CHARACTERISTICS AND QUALITY OF PRODUCTION DATA IN AN ATLANTIC SALMON (*Salmo salar* L.) PRODUCER'S LEGACY DATABASE

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Intensive, commercial aquaculture production operations such as Atlantic salmon farming have more and more come to rely on a data driven and information intensive form of production management, facilitated by the acquisition of data from a multitude of sources such as electronic sensor devices and manually recorded observations (Aunsmo et al., 2008; Lee, 2000; Menzies et al., 1996). This data acquisition provides for the producers accumulating increasingly large and heterogeneous datasets, comprising numerous variables pertinent to production and stock management, including water quality measurements and indicators of the biological performance of the cultivated animal (Aunsmo et al., 2008; Lee, 2000). While typically being used to inform day-to-day management and for benchmarking purposes (Soares et al., 2011; Aunsmo et al., 2008; Lee, 2000), these datasets, given the appropriate analysis, may also carry the potential for discovery of new knowledge that can aid in better understanding and improving the production process.

To achieve a proper use and analysis of data, in which meaningful and interpretable results are to be obtained, an assessment of data quality with subsequent cleansing and pre-processing steps is necessary (Bocca and Rodrigues, 2016; Maletic and Marcus, 2009; Fayyad et al., 1996). Particularly, complex data sets require an assessment of which variables are available, their completeness, dependencies, geographic or temporal resolution, and what constitutes typical and atypical variation (Bocca and Rodrigues, 2016; Karr et al., 2006; Lacroix et al., 1997). Atlantic salmon producers' records are examples of such complex data sets given their size and heterogeneity, and with variables often being subject to temporal and spatial dependencies.

The objective of this study was to assess what characterises a typical production data set from Atlantic salmon farming, to establish which variables are available and to which extent they fulfil certain quality aspects, and to identify constraints to proper use and analysis and how data treatment can mitigate these. To achieve this, we obtained access to the legacy database of a large, vertically integrated, commercial Atlantic salmon production company, comprising production records from all the company's seawater cage sites in Norway. Data from full production cycles completed during the last 9 years were acquiesced from the database and subjected to an exploratory analysis and quality assessment, with a selection of variables chosen for further treatment.

This presentation will summarise the main findings of the exploratory analysis, demonstrating the database's adherence to relevant data quality principles, and review the procedures followed in data cleansing and pre-processing. Key challenges, particular to the analysis of aquacultural production data, will be highlighted.

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NILE TILAPIA GROWTH PERFORMANCE USING XYLANASE AND BETA GLUCANASE

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The purpose of this study was to evaluate the production performance of Nile tilapia *Oreochromis niloticus* when supplemented with a commercial beta xylanase and beta glucanase enzymes. Two practical basal tilapia diets were formulated to contain 32% protein and 6% lipids. One basal diet formulated to contain a low level of fiber (LF) based on soybean meal, was modified by top coating liquid enzyme to produce five levels of enzyme inclusion (0.00, 0.015, 0.030, 0.045, and 0.060 g/100g). A second basal diet was formulated to contain a high level of fiber (HF). To increase the fiber content, 30% distillers dried grains with solubles were used as a replacement for soybean meal. This basal diet was modified by top coating liquid enzyme to produce five levels of enzyme inclusion (0.00, 0.015, 0.030,0.045, and 0.060 g/100g). The test diets were offered to sex reversed juvenile tilapia (mean initial weight 10.31 ± 0.31 g) over a 70 - day growth trial. Four replicate groups of 20 fish per aquaria were offered the test diets at near satiation levels. At the conclusion of the growth trial, survival was near 100% and weight gain was around 600%. The inclusion of beta xylanase and beta glucanase resulted in significan differences in final mean weight ($P = 0.0029$), percent weight gain ($P = 0.0128$), thermal unit growth coefficients ($P = 0.0046$) with no change ($P > 0.05$) in feed conversion ratio ($P= 0.2153$), apparent net protein retention, apparent net energy retention, hepatosomatic index and intraperitoneal fat index. In general, fish maintained on the high fiber diet performed better to the addition of the enzyme. The use of beta xylanase and beta glucanase showed clear advantages, improving growth performance of Nile tilapia.

FISH ENERGY BUDGET OF THE ZEBRA SEABREAM (*Diplodus cervinus*) UNDER OCEAN WARMING AND ACIDIFICATION

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Introduction

Climate change is one of the greatest environmental challenges that cause profound impacts on marine organisms and ecosystems (IPCC, 2014). According to the latest report of the Intergovernmental Panel on Climate Change (IPCC, 2014), it is predicted seawater pH decrease (0.2-0.6 units) and seawater surface temperature increase (0.3–4.8°C) in estuarine and coastal areas by the end of the 21st century with strong local variations. Indeed, ocean warming and acidification represent the major threats to many marine organisms, particularly fish, deeply affecting their physiological responses in ways that often compromise their energy budget, i.e. the energy intake and expenditure within the whole organism (Jobling, 1994; Yurista, 1999). The impacts of these environmental stressors on fish bioenergetics remain unclear and still require further understanding. In this context, the main goal of this work was to study, for the first time, the effects of ocean warming (+4°C, i.e. 23°C) and acidificat on (ΔpH=−0.4 units equivalent to ΔpCO_2~1000 µatm) on the energy budget (i.e. growth, excretion, metabolism and food consumption) of juvenile zebra seabream (*Diplodus cervinus*).

Materials and methods

Juvenile zebra seabream (*Diplodus cervinus*) specimens (4.8±0.9 g total weight) were exposed to four scenarios during 61 days to understand the potential consequences to organisms under current and future expected conditions (i.e. seawater warming, Δ=4°C and ΔpCO_2~1000 µatm equivalente to ΔpH=−0.4 units according to IPCC projection scenario RCP8.5; IPCC, 2014): i) Scenario 1 – Control, i.e. seawater temperature set at 19°C and pH at 8.0 (pCO_2~405 µatm; current conditions used in juvenile zebra seabream rearing in Iberian Peninsula); ii) Scenario 2 – Warming – seawater temperature set at 23°C and pH at 8.0 (pCO_2~405 µatm); iii) Scenario 3 – Acidification – seawater temperature set at 19°C and pH set at 7.7 (pCO_2~1000 µatm) and; and iv) Scenario 4 – Warming + Acidification – seawater temperature set at 23°C and pH set at 7.7 (pCO_2~1000 µatm). During the experimental period, specimens were fed with 3% of the average body weight (divided in two meals per day). Four specimens per replicate/tank (n=12 per treatment) were randomly weighted and sampled at the end of the experiment, freeze-dried (whole fish) and kept at -80°C until further analyses. Biochemical analyses (dry matter, ash, total lipids, crude protein and gross energy) were determined in the diet and fish body according to the Association of Official Analytical Chemists methods (AOAC, 2005). For calculation of energy budget equation (C=G+F+U+R), growth (G), excretion (faecal, F and nitrogenous losses, U), routine metabolism (R) and food consumption (C) were determined. For growth, fish were weighted every week and energy content in fish and faeces was measured by combustion in an adiabatic calorimeter pump. To calculate energy loss through excretion, faeces were collected daily, oven-dried at 65ºC to constant weight and stored at -20°C for analysis of energy. Nitrogenous compounds (ammonia-N) were determined at the end of the experiment using 12 individually fish enclosed in 500 mL glass bottles during 1h for each treatment. Regarding respiration, routine metabolic rates (RMRs) were determined at the end of the experiment according to previously established methods (Anacleto et al., 2018) with some modifications.

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Results and discussion

Energy proportion spent for growth dominated the mode of the energy allocation of juvenile zebra seabream under the combined effect of ocean warming and acidification. Acidificati n alone strongly decreased the ammonium excretion rate (AER), but increased fat and protein contents, as well as the viscerosomatic index, VSI. On the other hand, under warming conditions, an increase in metabolic rates (RMRs) was registered, likely since temperature directly affects the rate of all biological processes. Nevertheless, the combined effects of both stressors promoted an increase of fish growth, but a decrease of the hepatosomatic index (HSI).

Conclusions

Overall, such extreme conditions of ocean warming and acidification, is expected to greatly affect the energy budget of marine fish, leading to impacts on fish communities and ecosystems. Such studies are extremely relevant to unravel the partitioning of energy in the different physiological processes of fish species. Further research combining other stressors (e.g. hypoxia or presence of contaminants) are needed to better understand and forecast fish ecological effects, in order to develop potencial mitigation measures.

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MONITORING IMPACTS OF MACROPLASTICS IN MADEIRA ISLAND'S OFFSHORE FISH FARM OF *Sparus aurata*

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Introduction

Madeira is a small Portuguese Island in which the aquaculture industry started developing fish farms in 1996. Currently the island has three *Sparus aurata* offshore fish farms, all located in the south coast. The most recent one, MARISMAR, located in Calheta, is where this study took place.

Plastic debris imposes a real threat to the marine ecosystem, approximately 60 to 80% of the marine debris in the world is plastic (Derraik, 2002) and almost 10% of the annual production ends in the ocean where degradation can take several hundred years (Avio et al., 2017). The threats to marine life are primarily mechanical due to ingestion of plastic debris and entanglement (Laist, 1987, 1997; Quayle, 1992).

A previous preliminary study of this thematic was done last year in the same fish farm and showed that further work was needed.

The aim of this study is to monitor macroplastics in the fish farm cages and its impact on the livestock

Material & Methods

In order to avoid the need and costs of diving to identify and quantify plastics in the exterior side of the cage's nets, a camera attached to an extensible tube was used to film the cage's perimeter on the surface, and in depth in 4 areas (north, east, south and west).

S. aurata stock analysis was made by examining the gut content of dead individuals in the cages. This methodology allowed to quantify the mortality due to plastic ingestion and the types of plastics that were most often ingested. It was also possible to analyze the gut content of individuals in good health that were sampled once a month by the company.

In addition, weather conditions and ocean currents were also monitored in order to determine its influence on the presence of plastics and beach surveys were made to compare the plastics that were present on the coast with the ones that were found at the cages.

Results

Preliminary results showed that out of a total of 62 video samplings that were analyzed, we were able to identify in 12 videos, plastic fragments. Most of the plastics were plastic bags, but also ropes and fragments of the cage's structure. The weather conditions, particularly, the rain seems to affect the presence of plastics on the cages, as well as on the beaches surveyed.

Regarding the stock analysis, we analyzed gut content of 157 fish, of a sample of 289 dead fishes (some were unfit for gut analysis). Regardless, all fish had their morphological data taken (weight and standard, fork and total length). From the 157 fish suitable for gut analysis, 9 had plastics (4 in the intestine and 5 in the stomach), all the same type- seem to be fragments of plastic bags. The analysis of the monthly sampled fishes in good health (total of 166) did not show any plastics so fa .

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Discussion & Conclusion

The study is still ongoing so further analysis regarding the influence of weather and ocean currents is still needed. Most of the plastics found in the sea cages seem to be from domestic disposal (shopping/garbage bags), agriculture activities (plastic bags used in banana plantations) and the aquaculture itself (ropes and pieces of the cages). According to Li et al. (2016), plastic debris originates from two major sources, land-based and ocean-based, with domestic, industrial and fishing activities being the most important contributors.

The stock analysis showing the same type of plastic being ingested could mean the individuals are ingesting plastic selectively because more than one type of plastic was observed in the video samplings. Although, this seemingly selectiveness could be due to the thin nature of plastic bags, making it easier to enter the cages. Carpenter et al (1972) examined several species of fish with plastic debris in their gut and observed that only white plastic fragments where ingested, indicating that the individuals were feeding selectively. So far most of the plastics found in fish have been white, however we also found a blue one and two black ones.

Plastic pollution in the marine environment is now recognized as a real threat with a global-scale distribution and adverse effects. Due to the long-life of plastics on marine ecosystems, harm to marine life would continue for many decades even if the production and disposal of plastics suddenly stopped (Avio et al., 2017).

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DIETARY TAURINE INCLUSION IN HIGH PLANT BASED-DIETS HAS POSITIVE EFFECTS ON SENEGALESE SOLE (*Solea senegalensis*) PERFORMANCE

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Introduction

Aquaculture has grown at a very fast rate during the last years and this growth has been supported by a significant research effort directed to increase the industry´s sustainability. During the last two decades, intense research efforts have focused on the potential of plant-derived ingredients as an alternative to substitute fishmeal and fish oil in aquafeeds formulation. However, dietary replacement of fishmeal by plant sources may result in an unbalanced supply of selected nutrients, among which taurine has been identified. Taurine is known to be involved in many important physiological processes (such as bile salt synthesis, control of plasma cholesterol levels, cellular osmoregulation, control of endocrine tissues physiology, among others), and it is considered an essential nutrient for marine fish. But taurine is virtually absent in terrestrial plant ingredients, while it is particularly abundant along the marine food chain. Thus, when feeding marine fish with terrestrial plant protein-based diets taurine requirements may not be fulfilled

This presentation will review the work developed during the last years to understand the importance of taurine for a marine fish species important for Southern European aquaculture – the Senegalese sole (*Solea senegalensis*). Senegalese sole is a high value flatfis species, which requires the incorporation of particularly high levels of proteins in the feed (60% DM crude protein) to reach maximum protein accretion during their juvenile stage. The main objectives of these studies were: to evaluate the effects of dietary fishmeal replacement by plant protein-sources on growth and metabolism of juvenile Senegalese sole; and to understand if dietary taurine supplementation had beneficial effects on fish physiology and performance.

Materials and Methods

The different experiments performed used two basal diets: a fishmeal-rich diet (FM), with a formulation similar to a commercial diet for Senegalese sole, and a plant protein-based diet (PP), in which plant protein sources replaced 85% of proteins from marine origin. Taurine content in PP and FM diets were 0.09% and 0.5% (dry matter basis), respectively. Based on PP and FM formulations, experimental diets diets were further supplemented with different taurine levels. All diets were isonitrogenous and isoenergetic.

Senegalese sole (*Solea senegalensis*) juveniles were used as model species for all experiments (13-150g, depending on the experiment).

Due to the passive feeding behaviour of Senegalese sole juveniles, the first experiments were developed to guarantee a reduced leaching of dietary taurine and other amino acids to the environment and to ensure an efficient and reliable delivery at fish tissues. Therefore, taurine encapsulation was tested and dietary taurine leaching in water was analysed and post-prandial taurine kinetics in fish plasma was monitored.

Growth trials using the two basal diets and diets supplemented with taurine were performed, and the impact on Senegalese sole growth performance, feed consumption and utilisation, and several physiological indicators were analysed. Furthermore, short-term experiments using radiolabelled nutrients were performed in order to analyse the effect of dietary taurine levels on nutrient (such as protein and lipids) absorption and metabolism.

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Results and Discussion

Strategies to microencapsulate taurine proven to be successful. Growth was impaired in Senegalese sole juveniles fed diets with high levels of fishmeal replacement by plant protein-sources and detailed analysis revealed negative repercussions at physiological and metabolic level. Negative effects were detected, for instance, on intestinal epithelia, on ion transport at intestinal level, on bile salt synthesis and transport, on lipid digestion and absorption, and on protein metabolism. Al least part of these negative effects could be reversed by an appropriate level of dietary taurine supplementation to plant-based diets. The results obtained in the several studies indicate that taurine should be included in high plant-based diets has a strategy to mitigate the negative effects of these diets on Senegalese sole performance and metabolism.

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EVALUATION OF MUSSEL (*Mytilus galloprovincialis*) SPAT COLLECTOR ROPES IN LONGLINES FROM THE SE BAY OF BISCAY

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Introduction

In the Basque Country (SE Bay of Biscay) the interest for offshore aquaculture has increased considerably during recent years. In 2012 AZTI set up an experimental facility at 2km off the coast of Mendexa (Biscay), to test the feasibility of culturing shellfish in open waters. This facility is installed at a deep of 45-50m and consists of longlines; the organisms cultured are mainly mussels (*Mytilus galloprovincialis*). However, the supply of mussel seed is critical for the development of industrial mussel production. Small mussel populations can be found along the Basque coast (Azpeitia et al., 2017); yet, the spatial and temporal dynamics of mussel spat arriving to this coast are highly variable and poorly understood. Therefore, the aims of the present study are i) to evaluate the settlement and recruitment seasonal patterns of mussel seed from natural resources using five different collector types and ii) to assess the effect of collector type, to make recommendations on the most efficient collectors to producers.

Material and methods

In March 2018 five different spat collectors were deployed in the experimental longline in Mendexa. The collector ropes were 10m long and were suspended at a water depth of 1m within 1m of distance between each other. The collectors, provided by a local supplier, were namely: P ("peluda"), R ("rizada"), G ("grope"), L ("loch") and B ("brae") (Figure 1). The different collector designs offer different textural properties and structural complexity which may enhance the strength of seed attachment.

With a sampling frequency between 45 to 60 days, spat attachment was assessed in May, July, August and October 2018 and January 2019. In each sampling, 3 replicates of 10 cm of each collector type were retrieved. Additionally, in order to characterize the experimental zone, environmental parameters were analyzed monthly. For the determination of settlement and recruitment patterns, the abundance of individuals per rope meter and the length of mussel seed were estimated. The procedure to evaluate larval settlement and recruitment was adapted from the methodology described elsewhere (Filgueira et al., 2007; Fuentes-Santos and Labarta, 2015).

Results

Results showed that the highest seed settlement (*i.e.*, mussels passing through a 5 mm sieve) occurred in July 2018 in all collector types. However, the most filamentous ropes (namely Loch and Brae) were the ones that presented the highest abundance, showing values up to 25,000 ind/m of rope (Figure 2). In August, as mussels continued growing, the abundance of settlers decreased while the first recruits (*i.e.*, mussels retained on a 5 mm sieve) appeared in the collectors, especially in G and B ropes. The highest recruitment was detected in October 2018 in G, L and B ropes. However, in January 2019, the recruitment decreased but the collectors with the highest abundance were L and B ropes.

Discussion and conclusion

The highest seed settlement that occurred in July 2018 corresponded with the spawning season of natural mussel populations which takes place mainly in spring in the Basque coast. In turn, this temporal settlement variation could be partially explained by seasonal fluctuatio s in environmental factors, such as temperature and some nutrients In both, settlement and recruitment phases, G, B and L rope types presented the highest mussel seed density followed by R rope type and to a less extent by P. Other authors have obtained similar results in Galicia (NW Spain) indicating that filamentous ropes possess a higher surface area for seed attachment than non-filamentous ropes (Filgueira et al., 2007).

In conclusion, results showed that collector type had an effect on the seed abundance, either at settlement or recruitment phase. Filamentous collectors (G, B and L ropes) were the ones in which a higher quantity of seed was settled and in which higher recruitment abundance was recorded in January. Thus, filamentous ropes are the one recommended for natural seed collection to producers.

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Fig. 1: Photo of the five different collector types

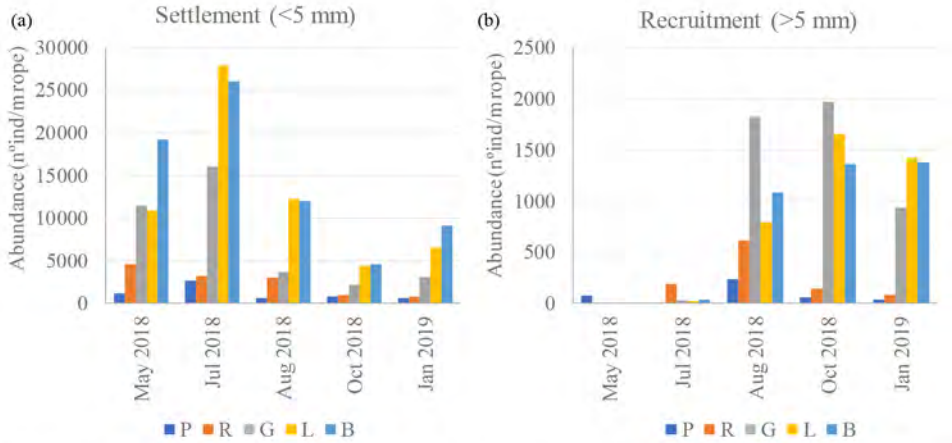

Fig. 2. Seasonal pattern of mussel seed (a) settlement (1000µm, 2400µm) and (b) recruitment (5mm, 10mm, 15mm, 20mm) in five different collector ropes (P: "peluda"; R: "rizada"; G: "grope"; L: "loch" and B: "brae") at 1m depth in the experimental longline culture system in Mendexa.

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THE USE OF DIATOM *Skeletonema costatum* (BACILLARIOPHYTA) ON PURPLE SEA URCHIN (*Paracentrotus lividus*) LARVAE AND POST-LARVAE DIET

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Introduction

The sea urchin *Paracetrotus lividus* is a valuable fishing resource due to its gonads ("roe"), greatly appreciated in Europe as a seafood delicacy, where local populations have been heavily exploited. The interest on echinoid aquaculture has been increasing in the last two decades. The production of echinoderms constitutes a challenge mainly due to the existence of different states over its lifecycle and the complex biotic relationships that occur during phase transitions larval and post-larvae. The quality of the microalgae provided is a primordial factor having a direct (nutritional properties) and indirect (water quality) impact on growth, competence (capacity for settlement) and survival. In the last years several microalgae species have been tested in order to optimize the larvae culture. *Skeletonema costatum* is a diatom commonly used in the bivalve larval cultivation. Nutritionally it is characterized by a relatively high levels of EPA and DHA in its lipid profile. It is also notorious for the high antioxidant and antiflammatory activity (Franca, 2019). However, the use of this diatom in *P. lividus* larval cultivations is poorly known. The main aim of this works was to test the use of *Skeletonema costatum* on *P. lividus* larvae and post-larvae cultivation.

Materials and Methods

Three diets were tested: *Rhodomonas* spp. (D1), *Skeletonema costatum*, (D2) and a mix (50/50) of *Rhodomonas* and *S. costatum* (D3). Four 6L glass balloons were used for each treatment. 15,000 larvae with 2 days after hatching (DAH) were introduced in each ballon. Daily microalgae were provided in order to obtain a concentration of 3.000 cell mL^{-1}, 10,000 cell mL^{-1}, 15,000 cell mL^{-1} and 20,000, depending on the development phase. Survival and biometric sampling were performed at 2, 4, 8, 12 and 15 DAH. Settlement trial was performed on 20 L fibe glass tanks (2 replicates each diet) with an initial n=15,000 larvae each. At 50 DAH biometric sampling and survival rate was assessed. Microbiological quality of the water was analyzed using TSA (non-selective medium) and TCBS (*Vibrio* selective medium).The inoculated media were incubated at 25 oc for 48 – 96hrs, then isolated bacteria were subjected to taxonomical analysis according to Bergey's manual of Determinative Bacteriology (1994) and isolated bacteria were identified by using API20 E system.

Results and Discussion

During the larval cultivation assay it was verified that the larvae fed with the D2 diet (55.8%) and D3 (39.9%) had a survival at 15 DAH substantially higher than D1 (5.5%). When compared with similar studies, the survival obtained with diets D2 and D3 were similar or even higher in some cases (Ahmed et al., 2016; Carboni et al., 2012; Castilla-Gavilán et al., 2018). The low survival in D1 was possibly due to the higher microbiological load (*Vibrio alginolyticus* and *Vibrio pectenicide*) that apparently develops more easily in a *Rhodomonas* spp culture environment. It was observed, however, that larvae fed only with *S. costatum* (D2) showed a relatively lower development than D1 and D3 diets (table 1). It was also found that the competency index used (rudiment length/body length) was significantly lower for larvae fed with the D2 diet ($P < 0.001$). In general, these results are in accordance with other similar studies, showing that the microalge diversified diets contribute to a better development of *P. lividus* larvae. During the settlement and post- settlement phase there was also a lower growth of the sea urchins fed with the D2 diet ($P < 0.001$) and a relatively higher survival for the ones fed the D3 diet (42.5%).

Conclusions

Through this study it was found that the diatomaceous *S. costatum* is an adequate food for the larval cultivation of sea urchins *P. lividus*, providing a high survival of larvae and post-larvae. Associated with other microalgae, as in this study, the development, settlement and post-larva development may be further potentiated.

Acknowledgment: Project DIVERSIAQUA Mar2020, 16-02-01-FEAM-66 and Project OURIÇAQUA

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SEED CONDITION AND DIETARY N CONTENT AFFECTS C/N HOMEOSTASIS IN TISSUES OF JUVENILE CLAMS *(Ruditapes philippinarum)*

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Introduction

Different physiological strategies leading to differential growth encompass endogenous (i.e. inter-individual and inter-familiar) variability in energy demands (Bayne, 2004). Dietary composition, in addition to gross energy income are determining of growth trajectories (Kreeger et al, 1996). Therefore, in this work we analyzed the effect of N content of diets on elemental (C, H, N) composition of seed tissues of *Ruditapes philippinarum* as well as the differential effects resulting from inter- and intra-familiar differences.

Materials and methods

Fast (F) and slow (S) growing seeds of the Manila clam *Ruditapes philippinarum* were selected from each two families (1 and 8) created in hatchery. Two aliquot subgroups from each of the four groups (F1, F8, S1 and S8) were conditioned to either a N-rich (N+) or N-poor (N-) diet for 30 days. These diets consisted of the microalga *Rhodomonas lens* at either exponential (N+) or stationary (N-) growth phase of culture, the latter presenting a C:N ratio that was 2.5 times the C:N ratio of the former. After determining elemental balances under both diets (following Tamayo et al., 2014), tissues were dissected separately and elemental composition of each tissue was analyzed by means of an Euro EA Elemental Analyzer (CHN) from EuroVector, using acetanilide as standard. Finally, dry and organic tissue weight was recorded for biometrical analyses.

Results

%N content of tissues was significantly higher when conditioned to N+ diet, which promoted significant reductions of %C and C:N ratios (Table I). No significant differences were found for growth category (F vs S) in any of the parameters while differences between families were only significant for %C. ANOVA interaction terms among factors only showed significant differences when tissue was involved, reflecting the high impact of tissue composition above the rest of factors.

C:N ratio of the digestive gland was, among the different tissues, the most affected by diet and seed condition, and reflected the net balances of C:N (see figure 1 left and center)

On the other hand, C:N ratios of the whole tissues exhibited a different behavior according to growth category (Figure 1, right): While F seeds maintained a rather constant ratio, C:N of S seeds tended to decline with N+ (but only in Family 8) and to increase with N-.

Finally, growth of clams (in terms of live weight, see Table I) was affected by diet, and especially by growth category. Furthermore, condition indices only showed differences between families, where family 8 reached values > 30% than family 1, in which shell weight was the responsible of these differences.

Discussion and conclusion

Diet composition was able to promote changes in soft tissues of clams after a month of exposure, although effects differed among selection groups: While whole body composition of slow growing seeds fluctuated according to diet composition, F seeds were able to maintain C/N homeostasis; this differential behavior did not occur in the digestive gland. On the other hand, S seeds behavior differed among families, with Family 1 showing a high degree of homeostatic regulation of C/N composition.

Additionally, N+ diet enhanced growth rates, which suggests that nutritional limitations would come from N content rather than energy content of the food (Kreeger et al, 1996). Finally, differences in condition index between families could be indicative of different allocation profiles: The rate of energy assignment to soft tissues growth would be higher in Family 8 while Family 1 would invest comparatively more energy into shell thickening.

(Continued on next page)

Table I. Pooled means (SD) of %N and C, C:N ratios, growth (live weight, mg d^{-1}), dry weight of tissues (DWt, mg) and shells (DWs, mg), and condition index (CI) of the four factors studied (A: diet acclimation, G: growth category, F: family, T: tissues; in which: Gi: gill, AM: adductor muscle, DG: digestive gland, GMF: gonad, mantle and foot). Significant differences ($p < 0.05$) are highlighted in bold

		N%	C%	C:N	Growth	DWt	DWs	CI
A	**N+**	**12.8 (0.1)**	**49.2 (0.1)**	**3.9 (0.0)**	**7.4 (0.6)**	87.0 (8)	709.3 (66)	12.2 (0.3)
	N-	**12.3 (0.1)**	**49.6 (0.2)**	**4.1 (0.1)**	**5.3 (0.7)**	81.2 (8)	673.5 (66)	11.8 (0.3)
G	**F**	12.6 (0.1)	49.4 (0.1)	3.9 (0.1)	**8.9 (0.7)**	**141.1 (5)**	**1150.9 (36)**	12.3 (0.3)
	S	12.5 (0.1)	49.4 (0.2)	4.0 (0.1)	**3.6 (0.3)**	**27.0 (2)**	**231.4 (12)**	11.7 (0.3)
F	**1**	12.6 (0.1)	**49.3 (0.1)**	3.9 (0.0)	6.4 (0.7)	84.0 (8)	**783.8 (70)**	**10.3 (0.2)**
	8	12.5 (0.1)	**49.6 (0.2)**	4.0 (0.1)	6.2 (0.6)	84.0 (8.7)	**610.3 (60.4)**	**13.5 (0.3)**
T	**Gi**	**12.8 (0.1)**	**48.4 (0.1)**	**3.8 (0.0)**				
	AM	**13.6 (0.1)**	**48.4 (0.1)**	**3.6 (0.0)**				
	DG	**11.5 (0.2)**	**51.1 (0.2)**	**4.5 (0.1)**				
	GMF	**12.4 (0.2)**	**49.6 (0.2)**	**4.1 (0.1)**				

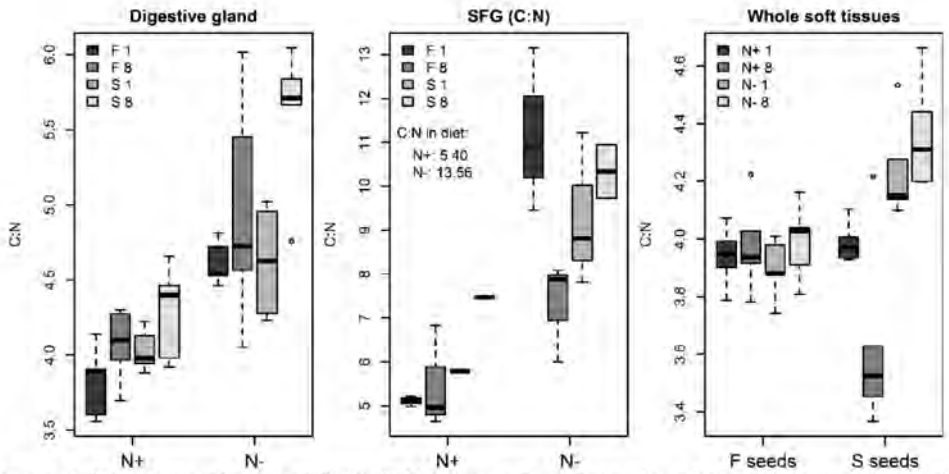

Figure 1. C:N of digestive gland (left), C:N balances (center) and the whole soft tissues (right), of F and S seeds from families 1 and 8 under both diets (N+ and N-)

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IDENTIFICATION OF SUCCESS FACTORS FOR ANTIPARASITIC TREATMENTS AGAINST THE SALMON LOUSE *Caligus rogercresseyi* IN CHILE THROUGH AN EXPERT PANEL

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The sea louse *Caligus rogercresseyi* is an insidious ectoparasite that affects farmed salmonids in Chile. Effective control of the sea lice requires the use of antiparasitic drugs, which are administered to fish as immersion treatments. These are complex procedures in which a drug solution is poured into the fish cage. Salmon companies have developed their own strategies to perform immersion treatments. These practices have not been fully described nor evaluated in terms of treatment success. The objective of this study was to identify and quantify the effect of different practices on treatment success, through an expert elicitation process. This process consisted in the design of a questionnaire, its application to experts through personal interviews and a group discussion. We organized the relevant factors using a causal diagram (Fig. 1) and the expressed associations as likelihood ratios (LRs). Experts indicated that the most important factors impacting treatment success were: 1) treatment duration, 2) drug concertation in water, 3) preparation of the drug solution, and 4) distribution of the drug solution. This study constitutes the first effort made in Chile to identify the best practices for performing immersion treatments across the salmon industry.

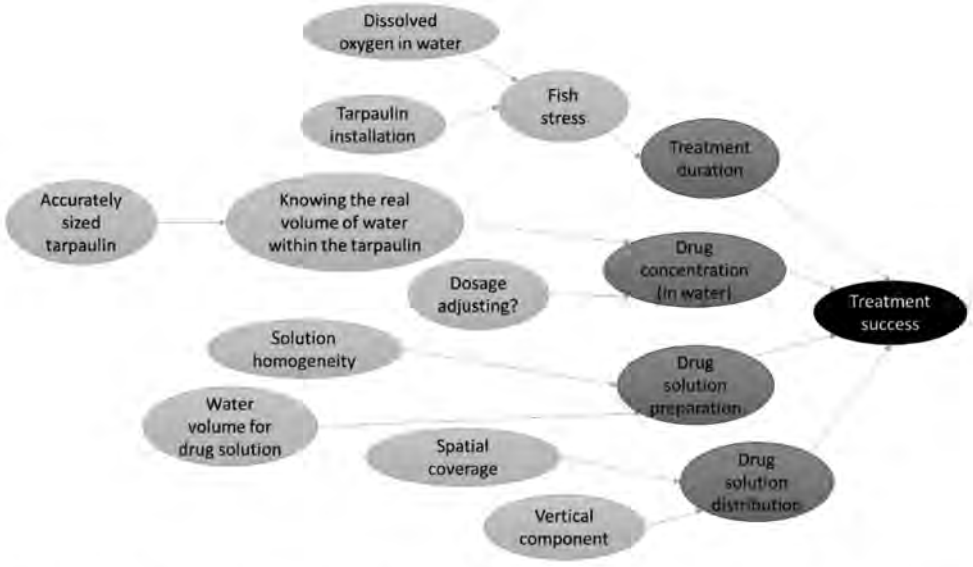

Figure 1. Final causal diagram of antiparasitic treatment success, based on expert opinions. Direct factors are presented in dark gray, while indirect factors in light gray.

THE ROLE OF EPIDEMIOLOGY IN THE SURVEILLANCE AND CONTROL OF PREVALENT FISH DISEASES – THE CASE OF SEA LICE IN FARMED SALMONIDS IN CHILE

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Chile is the second larger producer of farmed salmon in the world. The sea lice *Caligus rogercresseyi* is one of the most challenging fish health problems for this industry because of its complex control (as the sea lice quickly develop tolerance to chemotherapeutants), and due to the fact that it is ubiquitous in sea water. In Chile, sea lice is under an official surveillance and control program administered by the health authority SERNAPESCA.

The aim of this abstract was to highlight the role of epidemiology in surveillance and control of prevalent aquatic disease, such as the case of sea lice.

Surveillance of sea lice in Chile involves weekly reports of sea lice levels for every single farm rearing Atlantic salmon or rainbow trout. The amount of surveillance data makes it difficult to appreciate spatial and temporal distribution patterns of sea lice. Using quantitative epidemiological approaches such as linear mixed effects models it was possible to observe that sea lice: 1) has a clear east-west spatial pattern (Fig. 1); 2) increases over the salmon production cycle (Fig. 2A); and 3) has decreased over the last 6 years (Fig. 2B). Epidemiological methods have been also used to investigate the recent expansion of sea lice to regions where it was thought that environmental conditions were not optimal for their development.

In terms of control, epidemiological quantitative methods have been successfully used to: 1) evaluate the field efficacy of antiparasitic drugs on different sea lice developmental stages (i.e. juvenile, adult males, gravid females); 2) to provide the first evidence of the sensitivity loss of sea lice to the organophosphate azamethiphos; and 3) to demonstrate the beneficial effect of the synchronization of antiparasitic treatments among farms within an area.

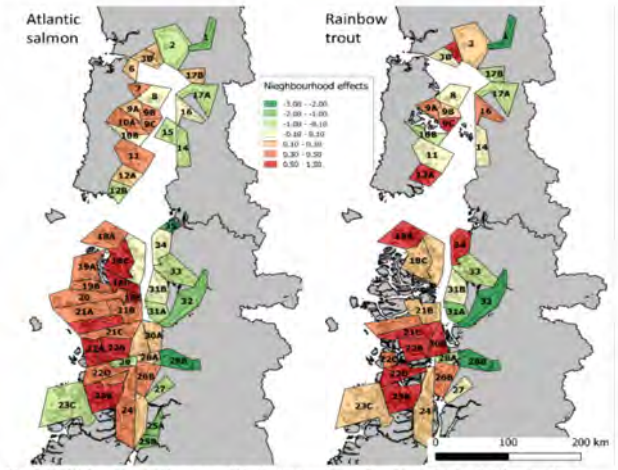

Fig. 1. Neighbourhood effects on adult *C. rogercresseyi* abundance for both Atlantic salmon and rainbow trout in Los Lagos and Aysén regions, Chile. Period 2012-2017.

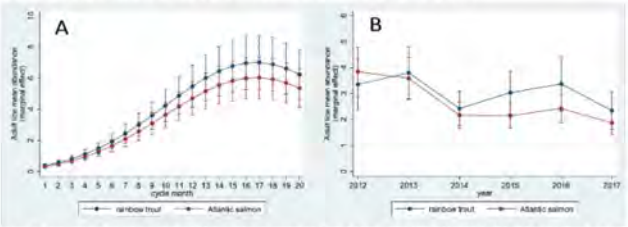

Fig. 2. Adjusted predictions of the cycle month (A) and the year (B) effects (with 95% CI) on adult *C. rogercresseyi* abundance in Atlantic salmon and rainbow trout in Los Lagos and Aysén regions, Chile. Period 2012-2017.

Caligus rogercresseyi INFESTATION IS ASSOCIATED WITH PISCIRICKETTSIA SALMONIS-ATTRIBUTED MORTALITIES IN FARMED SALMONIDS IN CHILE

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Infectious diseases are major challenges for the sustainability of the farmed salmon industry worldwide. Particularly in Chile, Caligidosis and Piscirickettsiosis are the diseases with the greatest biologic and economic impacts. Caligidosis is caused by the ectoparasite *Caligus rogercresseyi*, commonly called sea lice, provoking extensive skin damage, chronic stress and worsen feed conversion efficienc . Piscirickettsiosis is caused by *Piscirickettsia salmonis*, an intracellular facultative Gram-negative bacterium and is recognized as the most important bacterial disease for the salmon industry in Chile, generating high mortality rates. The relationship between *C. rogercresseyi* and *P. salmomis* is unknown, but it may help explain the high prevalence of this two pathogens in Chile. The objective of this study was to determine if *C. rogercresseyi* infection is associated to Piscirickettsiosis mortality early in the production cycle of Atlantic salmon and rainbow trout in Chile.

Data on Caligidosis and Piscirickettsiosis was obtained from the respective Official Programs for Surveillance and Control (OPSC), administered by the Chilean Fisheries and Aquaculture Service (SERNAPESCA). Caligidosis data consisted in weekly *C. rogercresseyi* mean abundances, classified by juvenile (chalimii stages), mobile adult (male adults plus non-gravid females) and gravid female stages, recorded at the farm level. Data on Piscirickettsiosis corresponded to weekly specific mortality due to *P. salmonis*, recorded at the farm level as well. Mortality was attributed to Piscirickettsiosis if fish presented clinical and/or pathological signs, and the presence of *P. salmonis* was confirmed by IFAT or RT-PCR. Our study was restricted to data collected from 2014 to 2016. We modeled Piscirickettsiosis mortality as a function of mobile *C. rogercresseyi* abundance, using a linear regression model. Other predictors included in the modeling process were the salmonid species (i.e. Atlantic salmon or rainbow trout) and the administrative region where fish farms were located (i.e. Los Lagos or Aysén).

The final model showed a positive and significant association between the log mobile *C. rogercresseyi* mean abundance and the log Piscirickettsiosis cumulative mortality early in the production cycles of both Atlantic salmon and rainbow trout (Table I).

Our study provides evidence, for the first time, that the first Piscirickettsiosis outbreak in the production cycle of Atlantic salmon and rainbow trout in Chile could be caused to some extent by *C. rogercresseyi*.

Table I. Final model showing the effect of mobile *C. rogercresseyi* infestation on Piscirickettsiosis mortality early in production cycles of Atlantic salmon and rainbow trout in Chile.

Variable name	Coefficient estimate	Standard error	*p*-value*	95% confidence interval	
Intercept	0.509	0.188	**0.007**	0.138	0.879
Log mobile *C. rogercresseyi* mean abundance	0.226	0.078	**0.004**	0.073	0.379
Salmonid species (rainbow trout as reference)					
Atlantic salmon	-1.197	0.209	**<0.001**	-1.607	-0.786

* Bolded *p*-values indicate significant variables at $p \leq 0.05$.

GENETICS OF LICE EATING ABILITY IN LUMPFISH (*Cyclopterus lumpus)* USING ddRAD SEQUENCING

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Introduction

Lumpfish, *Cyclopterus lumpus* is a high value aquaculture species which is widely distributed in the North Atlantic Ocean. Juveniles of lumpfish are mainly produced and used as a biological (green) control against sea lice (*Lepeophtheirus salmonis*) in Norwegian Atlantic salmon production. Sea lice infestation is a major problem for the aquaculture industry in Norway, causing production and economic losses estimated at 5 billion NOK in 2015 [1]. As a result of the large economic losses, the use of lumpfish as a cleaner fish has increased dramatically in aquaculture with nearly 24 - 25 million juveniles produced and sold as cleaner fish in Norway in 2016 [2]. Lumpfish is also appreciated for meat, caviar and roe production, and is considered highly nutritious. The emerging importance of lumpfish demands development of economical genetic resources along with the exploration of potential for selection methods (classical vs. advanced) for effective breeding programs to efficiently improve traits of economic importance

Material and Methods

A lumpfish population of ca. 60 full-sib families with an average of 25 full-sibs per family (ca. 1500 offspring in total) derived from the lumpfish breeding program established by Nofima AS was used for this study. Individuals of this population with an average weight of 54.75 g (10-154 g) were cohabitated with an Atlantic salmon population at the rate of 1 lumpfish per 4 salmon individuals. Cohabitation was kept for a total of 21 days, and the lice eating ability of each lumpfish was recorded at day 21 by the use of gastric lavage. The fish for which lice was present in the stomach were considered to be lice eaters (trait recorded = 1) and those without lice in the stomach were considered non-eaters (trait recorded = 0).

Tissue samples were collected from all PIT-tagged individuals for DNA extraction, library preparation using ddRAD and genotyping by sequencing. Libraries were sequenced using Illumina Hiseq 4000 platform (100bp paired-end sequencing), and the sequence data were used to generate genome-wide SNP genotypes for all the samples. The individual specific quality reads were aligned to the reference sequence which was developed using genomic TruSeq libraries sequenced at ~100X depth. The mpileup function of SAMtools version 1.2 was used to call variants and the call option of bcftools [3] was used to call the genotype at each variant site for each animal.

Genotypic data and the pedigree information were further used to estimate genetic variation for the trait, develop a linkage map, perform genome wide association analysis (GWAS) and for the estimation of breeding values. The heritability estimates for the binary trait were obtained using ASReml 4.0 with a pedigree (A) or genomic (G) relationship matrix. Linkage map construction was conducted using Lep-Map v2 [4] and GWAS was performed using the GCTA program with "–mlma-loco" function to detect marker ~ trait associations. Pedigree vs. genomic breeding values for lice eating ability were estimated to compare selection accuracy. The predictions were performed using PBLUP, GBLUP, BayesB, BayesC, and Bayesian Lasso models using the R/BGLR [5] program. The following linear mixed model was used for the estimation of heritability and predictions, where is a vector of phenotypic records on lice eating ability on each individual, is an overall mean, is a vector of additive genetic effects distributed as , or , where and are genomic and pedigree relationship matrices, respectively; is the corresponding incidence matrix for additive effects, and is the vector of random residual

Table1: Heritability estimates for lice eating ability using genomic vs. pedigree information.

Model	Liability Scale	Observed Scale
PBLUP	0.16 (0.07)	0.07 (0.03)
GBLUP	0.16 (0.08)	0.06 (0.03)

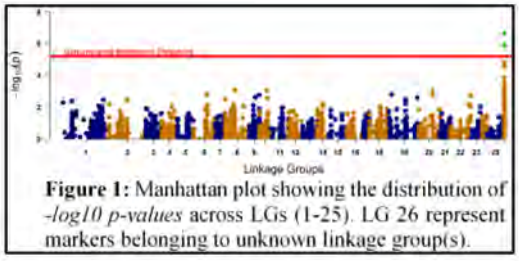

Figure 1: Manhattan plot showing the distribution of *-log10 p-values* across LGs (1-25). LG 26 represent markers belonging to unknown linkage group(s).

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effects with . GWAS was performed by applying the same above model with a parameter included to obtain SNP effects. Accuracy of prediction was computed using validation scheme by random masking the phenotypes of ~ 20% of the offspring. The accuracy was computed as the correlation of the estimated breeding value (pedigree/genomic, PEBV/GEBV) with the phenotype which was scaled by the square root of heritability.

Results and Discussion

The genotyping process using ddRAD sequencing resulted in a dataset containing 7,729 quality SNPs. Linkage mapping resulted in 3,474 markers grouped into 25 linkage groups (LG) which are consistent with the karyotype of this species. Heritability estimates for lice eating ability computed on observed and liability scale using pedigree vs. genomic information are detailed in **Table 1**. These estimates show that the lice eating ability has significant genetic variation. GWAS analysis revealed two low frequency SNPs presenting significant association (**Figure 1**) to the trait with *p-value* significantly lower than the genome-wide Bonferroni corrected threshold (*p-value* = 6.47×10^{-6}). Average accuracies of prediction by applying prediction models with pedigree vs. genomic information were 0.395 ± 0.199 and 0.420 ± 0.227 respectively.

In conclusion, results revealed the existence of low but significant genetic variation for lice eating ability and estimated breeding values using genomic (GBLUP) vs. pedigree (PBLUP) information presented 6.3% higher accuracy, which should ultimately be reflected in overall genetic gain

Acknowledgement

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RELATIONSHIP BETWEEN MODELS OF DISPERSION OF ORGANIC WASTE FROM FISH FARM AND THE VARIATION OF THE ISOTOPE NICHE AND BIOACCUMULATION OF TRACE ELEMENTS IN BIOFILM

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Introduction

In ecological studies, stable isotope analyses is a reliable tools for elucidating the trophic niche and inferring pathways of energy flow in food webs (Cifuentes et al., 1988). This method involves the comparison of stable isotope ratios between consumers and food supplies (Deegan and Garritt,1997). While $\delta^{13}C$ allows the carbon source to be differentiated, $\delta^{15}N$ permits the relative trophic position of an organism to be assessed. Hence, by using carbon isotopes, it is now possible to infer the relative importance of each carbon source to organisms in food webs (DeNiro and Epstein, 1981), since carbon isotope ratios remain relatively unaffected by trophic transfer. By using nitrogen isotopes, on the other hand, it is possible to accurately determine the trophic levels occupied by organisms (DeNiro and Epstein, 1981). The consistency of nitrogen enrichment at each trophic transfer provides a convenient quantitative measure of relative trophic position within a food web, and may be correlated with contaminant concentrations to estimate metal concentrations and biomagnification rates (Hoekstra et al., 2002).

Like sediments, organisms may show concentrations of metallic elements up to several thousand times those found in the water column, with the advantage that these levels reflect the availability over time of these metals to the biota (Wilson and Elkaïm, 1992). Therefore, the metal content in the biota may be analyzed to establish spatial and temporal variations in the bioavailability of heavy metals in the marine and estuarine environment, offering time-integrated measures of ecological relevance (Rainbow, 1995).

This study aims to assess the potential impacts of trace elements on marine benthic environments and to assess accumulation in biofilm. For this, a monitoring study was performed and a two models used to predict trace elements emissions (MAMPEC) and wastage marine dispersal (MERAMOD) were parameterized and evaluated with the comparative study of isotopic niche and trace elements composition of biofilm.

Materials and methods

The Western Mediterranean coast is currently one of the most aquaculture intensive areas in Spain, focused on the production of gilthead sea bream and sea bass, with an average production of around 11.000 metric tons per year. The farming is exclusively carried out in open marine water cages with an average depth of 37 meters. Samplings were carried out during the end of summer of 2018 at 2 fish farms. The farming is carried out in open marine water cages 6 km from the coast. The field assays were performed using glass slides as the artificial substrate for the biofilm community to attached to them. Glass slides were supported by slide holders. The slide holders, in turn, were maintained 3 m below the water surface by an anchoring system and a buoy. Slides were deployed from a fish cage located at the edge of the fish farm facility along a horizontal transect at 0, 25, 75, 175 and 650 m from the cages.

The model used in the present study was MAMPEC (Marine Antifoulant Model to Predict Environmental Concentrations). It is a steady-state 2D integrated hydrodynamic and chemical fate model with a user friendly interface. MAMPEC is based on the Delf3D-WAQ and Silthar model.

The released trace elements from the marine fish farm wastes was estimated with MERAMOD model, a particle tracking model containing grid generation (bathymetry, cage layouts), particle tracking, resuspension and benthic impact response modules. This model estimates the flux or total deposition of waste material (faeces and feed) at the sea bed discharged from mariculture operations.

(Continued on next page)

Results

The isotopic signatures clearly differentiated the biofilm community along the environmental gradients. The $\delta^{13}C$ content was significantl lower at 0 than at 650 m from the fish farm. The $\delta^{15}N$ content was significantly lower at 0 than at 650 m from the fish farm. Isotopic niche of biofilm showed higher similarity to fish feed $\delta^{13}C$ and $\delta^{15}N$ composition at the sampling stations located near of cages. The PCA analysis indicated a higher metal bioaccumulation in the sampling stations with an isotopic niche influence by fish farm waste

The results of this work indicated that biofilm was a useful and effective bioindicator in terms of both community composition and metal bioaccumulation. It present discernible differences for the majority of measured parameters on spatial scales and hold a key position within the food-web. The isotopic niches and metal bioaccumulation were correlated with the results of the two models used to predict trace elements emissions (MAMPEC) and wastage marine dispersal (MERAMOD).

Discussion and conclusions

The field results confirm that there are clear accumulation of metals and other trace elements in biofilm along environmental gradients, which suggest that waste dispersion influence on biofilm accumulation. However, the field study indicated a higher metal accumulation in biofilm close to the fish cages as it was predicted by the MERAMOD mode

The results from the present study showed that metal concentrations varied between both fish farms studied in function of fish farmed annual production. In the fish farm 1, with high annual production, metal concentrations were significantly higher at 0, 25, 75, m for biofilm samples. However, there were only significant differences in fish farm 2 (lower annual production) at 0 and 25 m. MAMPEC model indicated that the metal contamination due to antifouling paints could be negligible due to the low concentrations. The results from MERAMOD model indicated that fish food could be the main source of metals in biofilm

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ECOINTENSIFICATION OF AQUACULTURE: INNOVATIVE MORTALITY DISPOSAL IN A CIRCULAR ECONOMY PERSPECTIVE

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Among the main side streams from intensified aquaculture are mortalities and discarded fish. Statistics shows a typical loss of 15-20 % of the fish from sea cages, which equals 6-9 % of the biomass. This represents an annual cost of more than €100 million for the Norwegian aquaculture industry.

GAIN (Green Aquaculture Intensification in Europe), a recently awarded Horizon 2020 project, aspire to deliver services and technologies to market within the project period to contribute to the ecointensification of European aquaculture production. Resource efficienc , reduced environmental impact, increased precision and valorisation throughout the production chain are all key elements in the approach to improve seafood self-proficiency and regional stabilit .

Current method for mortality disposal in Norwegian aquaculture is facing challenges for health, safety and environment, as well as representing a costly handling. Waister has investigated an innovative method of mortalities disposal based on drying at site and transport of the dried and sanitized product.

In accordance with facilitation of a circular economy, Waister will make necessary valorisation of the dried product for use in in compliance with government regulations in EU and Norway. By proving safe application of the dried and sanitized product, further applications may emerge, requiring adaptations or amendments to current government regulations.

A GAIN demonstration facility has been established at a Norwegian Atlantic salmon smolt producer, for evaluation, documentation and professional training on relevant substrate in industrial scale. A dryer system for mortalities will be demonstrated at this site with processing of the amount received from the smolt production.

We will present a comparison of the current method and the innovative method and point out potential increase of value of the dried product in compliance with government regulations.

ZEBRAFISH EMBRYOTOXICITY OF OLIVE OIL MILL WASTEWATER AND CONSIDERATION OF IT'S RENEWABLE POTENTIAL

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Introduction

Olive mill wastewater (OMW) is a by-product from the olive oil production. In a short period of time, an amount of 6 x 10^6 of OMW is annually produced mainly in the Mediterranean basin. Its pollution load is mainly related to OMW's high biological and chemical oxygen demand, but also with a high content of phenolic compounds, organic and solid matter, metals, mineral substances, and acidic character (Pardo et al., 2017; Caporaso et al., 2018). Since no dedicated EU legislation exists on OMW management, the OMWs are usually directly spread into sewer systems, lakes, sea, etc. thus posing threat to the aquatic ecosystem. The present study was undertaken to evaluate the environmental impact of OMW to living organisms, to identify the OMW components among phenolic group which are directly correlated with OMW's toxicity and to complete this research with pointing out a huge potential of their use as a renewable resource.

Materials and methods

Fresh OMW was obtained from olive oil production plant located in Slovenian Istria, which uses a traditional discontinuous press for the extraction of olive oil. Prior to the extraction, an initial chemical and physical determination of OMW main constituents was performed. Polar fraction was obtained by solid-phase extraction (SPE) of raw OMW sample (De la Torre-Carbot et al., 2005). Analysis of phenolic compounds in polar fraction was carried out using gas chromatography-mass spectrometry (GC-MS).

Zebrafish embryotoxicity test (ZET) was performed on raw OMW and its polar fraction using zebrafish *Danio rerio* embryos, in accordance with OECD 236 Guidlines (2013). Lethal and sub-lethal effects were estimated up to 96 h of exposure.

Results

The Folin-Ciocalteu procedure (Vitali Čepo et al., 2017) revealed tested OMW sample as highly enriched in phenolic compounds (9.22 ± 0.17g GAEa L^{-1}). The identification of individual phenolic compounds by GC-MS pointed out tyrosol and catechol as the most abundant polyphenolic compounds.

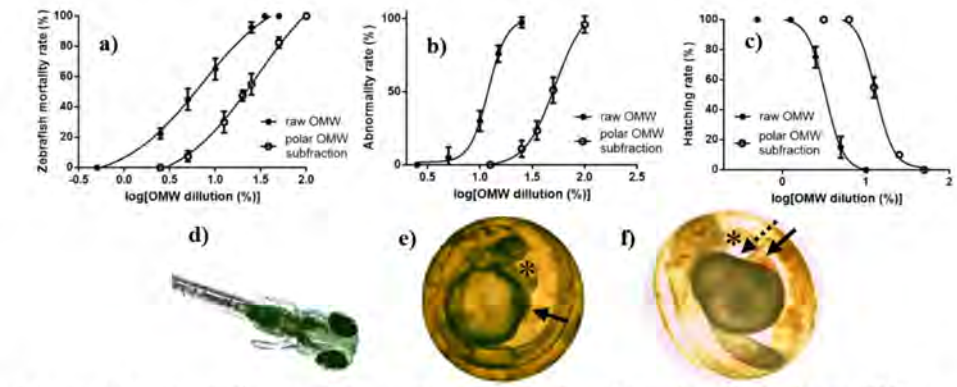

Figure 1. Concentration-response curves representing the toxic effect of raw OMW and polar fraction on 96-h old *D. rerio* a) survival, b) developmental abnormality, c) hatching. Raw OMW (e) and polar fraction (f) caused pericardial edema (asterisk), blood accumulation at the yolk sac (arrow), yolk sac edema (dashed arrow), lack in pigmentation formation. Picture d) represents control larvae on artificial water.

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Both, raw OMW and polar fraction induced negative impact on zebrafish ontogenesis, which manifested through high acute toxicity (Fig. 1; a) with LC_{50} values of 7.05 and 28.96%, respectively. Interestingly, almost the same abnormalities (pericardial edema, blood accumulation) were observed on both tested samples (Fig 1; e, f). Among observed endpoints, hatching appeared as the most sensitive (LC_{50}= 3.28% of the raw OMW).

Discussion

Although the chemical characterization of OMW gave us an insight into the chemical composition of this complex matrix, accurate assessment of OMW toxicity was obtained by determining its impact on zebrafish embryos. Similar toxicity trend was observed during exposure to raw OMW and polar fraction, which emphasizes an important role of polyphenols in OMW overall toxicity. According to the Urban Water Directive (Directive 1991), OMW tested within this study should not be released directly into the sewer systems or water bodies because the amount of phenols in wastewater exceeds the value of 1 mg/L. At the same time, it should be pointed out that the most abundant polyphenols in tested OMW were tyrosol and catechol, which are beside their toxic potential, well known for their antioxidant, anti-allergic, anti-inflammator , anticancer and antihypertensive activities (Caporaso et al., 2018). For that reason, an approach for using OMWs as a renewable resource is of global interest. Polyphenols from olive oil mill wastewaters can be converted into a valuable source of antioxidant compounds, that can be added to food in order to improve the nutritional properties and preserve their quality. Such exploitation of waste resources will not only prevent environmental pollution but also provide an economic opportunity and enable sustainable environmental management.

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1,25-DIHYDROXYCHOLECALCIFEROL-GLYCOSIDES OF NATURAL ORIGIN IN AQUACULTURE

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Introduction

Land animals can produce vitamin D3 (VD3) in skin under the influence of UV light. However, under intensive agricultural conditions endogenous VD3 production is not sufficient and additional VD3 has to be supplemented with the diet. Even though some fish can also produce VD3, most VD3 is ingested through the nutrition. Most of the aquatic VD3 is produced by plankton near the surface. Nevertheless, similar to farmed land animals, fish and shrimp in aquaculture also needs VD3 supplementation.

Whereas the physiological role of VD3 in birds and mammals is quite clear, there are still many open questions when it comes to fish. In terrestrial animals VD3 is converted in the liver to 25-hydroxyvitamin D3 (25D) which acts as storage form. In a next step, the metabolic active form 1,25-dihydroxycholecalciferol (1,25D) is produced in the kidney. This metabolite binds in the target tissue to a VD receptor (VDR) and the formed complex in turn binds to the responsive gene and triggers the biological action. Primary function is regulating calcium and phosphor uptake and bone mineralization. 1,25D has also beneficial e fects in muscle and immune functions as well as in cell differentiation and proliferation.

In fish the same VD metabolites as in terrestrial animals are formed and VDR is also present in many tissues, however, there are quantitative differences whose underlying reason are not yet clear. In terrestrial animals 25D acts as storage form and are present in the blood in a 1000-time higher concentration than 1,25D. This is mainly because of a tight regulation in formation and a fast elimination via 1,24,25D. Different to mammals and birds, 1,25D seems to be the predominant metabolite in fish

Early experiments in VD-depleted aquatic animals demonstrated the essentiality of VD3 in fish by finding reduced growth parameters in low VD3 diets.

Dose 1,25D (µg/kgf)	0	1	2	10	50	100
Start biomass (g)	663	658	663	662	662	663
Average body weight (g)	28.8 ± 2.6	28.6 ± 2.3	28.8 ± 2.8	28.8 ± 2.2	28.8 ± 2.6	28.8 ± 2.6
Final Biomass (g)	1,260	1,248	1,304	1,494	1,410	1,301
Average body weight (g)	54.8 ± 6.8[a]	54.3 ± 7.6[a]	56.7 ± 5.6[a]	65.0 ± 7.0[b]	61.3 ± 5.5[b]	56.6 ± 6.0[a]
Survival rate (%)	100	100	100	100	100	100
Weight gain (g)	597	590	642	832	749	638
Average weight gain (%)	190	190	197	226	213	198
gain in %		100%	104%	115%	94%	93%
Ca content (g/fish)	0.42	0.39	0.43	0.54	0.46	0.44
P content (g/fish)	0.42	0.4	0.42	0.56	0.49	0.43

Superscripts denote a significant difference (95%)

Indice	control	Low FM	Low FM + 1,25D
fish meal (%)	40	25	25
Dose 1,25D (µg/kgf)	0	0	10
Initial biomass (g)	1,048	1,034	1,033
Average body weight (g)	26.2±1.9	25.9±2.4	25.8±2.0
Final biomass (g)	3,247	3,058	3,444
Average body weight (g)	81.2±17.0[a]	76.4±19.5[b]	86.1±17.1[a]
Survival rate (%)	100	100	100
Weight gain (g)	2,199	2,023	2,411
Average weight gain (%)	310	296	333
change in %	**100%**	**95%**	**113%**

Superscripts denote a significant difference (95%)

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As mentioned above, farmed fish and shrimp rely on dietary VD3 supplementation, which is 1500 to 3500 IU/kg feed for fish (DSM Vitamin Supplementation Guidelines 2016). Given the dominant content of 1,25D in fish, it is possible that supplementation of this specific metabolite has positive effects. Here, we describe first experiments in farmed fish with a standardized product of the plant *Solanum glaucophyllum* which naturally contains 1,25D in a glycosidic form.

Material and Methods

Test article is a product, based on *Solanum glaucophyllum* and standardized to an analytical content of 10 µg/g dry mass of analytical determined 1,25D. In both experiments described in detail below, the test product was given on top of the commercial supplementation of VD3 (2500 IU/kg).

In both trials rainbow trouts (*Oncorhynchus mykiss*) of mixed sex were used. The fish were kept in tanks at 14°C with twice daily feeding to apparent satiation.

Experiment 1 (dose finding): 35 animals per tank in 6 replications for 32 days. Diet in %: **Fish meal 25**; Wheat flour 17; Vegetable protein 44; Vitamin premix 0.72; Mineral premix 1.2; Others 4.94; Defatted rice bran 1; Fish oil 6.5; ad 100%.

Treatments: G1: control; G2: 1 µg 1,25D3/kg feed; G3: 2 µg 1,25D3/kg; G4: 10 µg 1,25D3/kg; G5: 50 µg 1,25D3/kg; G6 100 µg 1,25D3/kg.

Experiment 2 (fish meal replacement): 40 animals per tank in 6 replications for 62 days. Control diet (T1) in %: **Fish meal 40**; Wheat flour 19; Vegetable protein 35; Vitamin premix 0.35; Mineral premix 0.8; Fish oil 6.5; Others 0.36; Defatted rice bran 0. Diet low fish meal (T2): **Fish meal 25**; Wheat flour 14.6; Vegetable protein 44; Vitamin premix 0.72; Mineral premix 1.2; Fish oil 8.5; Others 4.8; Defatted rice bran 1.14. And Treatment T3: low fish meal with 10 ug 1,25D3/kg diet

Results and Discussion

The result of experiment 1 is shown below. The product was well tolerated, and survival rate was 100% in all treatments. Best performance and optimal Ca and P retention were achieved with 10 µg 1,25D3/kg feed.

The aim of experiment 2 was to see whether the addition of 1,25D from plant would compensate for reduced fish meal content in the diet. It was shown, that average body weight under 1,25D supplementation was numerically over-compensating the reduction in fish meal from 40 to 25%. Statisticall , it was equal to the control diet T1.

Conclusion

Application of a standardized herbal product containing 1,25-dihydroxycholecalciferol-glycosides to young trout on top of the usual vitamin D supplementation is able to improve Ca and P utilization. This may allow a P reduction in feed without reducing growth performance. It could also been shown that 1,25D can compensate for a reduced fish meal content. Further trials are planned to expand the application to other fish species and other stages of development

COMMERCIAL AQUAPONICS: TWO ECONOMIC SCENARIOS FOR A DOUBLE CIRCUIT SYSTEM

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Introduction

Coupling the production of aquatic animals (e.g. fish) and plants (e.g. vegetables) in greenhouses forms the basis for aquaponic systems. Despite the fact aquaponics as a nearly zero-waste food production method has an important potential in terms of sustainable development in the food sector it is still a niche market. An aquaponic research facility in Waren, Germany operating with a controlled-environment agriculture (CEA) approach served as basis for the economic evaluation. Its recirculating aquaculture system (RAS) and its hydroponic system (HS) are unidirectional coupled on demand but maintain separate water cycles in both production units. A one year full operation period delivered the initial situation dataset.

The present study had three goals: First, to provide a short report about the aquaponic facility within its distinct boundary conditions. Economic calculations were done as an Ex post analysis based on the data recorded over the course of twelve months. Second, to present a scenario using the assumption of an explicitly improved productivity of the facility. Third, to develop a scenario as a generalisable example that emphasises the core idea of aquaponics and maximises the transfer of the RAS waste water to the HS. To examine the profitability of the facility an Ex ante cost benefit analysis (CBA) was performed on both scenarios.

Material and Methods

At the initial situation (InitS) the RAS unit for rearing African catfish (*Clarias gariepinus*) consists of 12 fish tanks with a productive water volume of 26.4m³. The HS in Waren uses 320m² net-acreage of the 352m² greenhouse area. No artificial light was employed for plant cultivation; therefore a production break of two months (winter break) took place which was used for disinfection and maintenance work. Energy was provided by a heating demand controlled combined heat and power unit (CHP) which was supported by photovoltaic power.

The factors describing the changed boundary conditions of the cases are given in Tab. I.

Tab. I Overview on the boundary conditions factors concerning the three cases

		InitS	ScenA	ScenB
RAS	Volume	1	1	1
	Yield	1	**1.64**	1.64
	Investment	1	1	1
HS	Area	1	1	**3.44**
	Yield	1	**2.46**	**8.46**
	Investment	1	1	**2.66**
SUM	Investment RAS/HS	1	1	**1.38**

Tab. II Profits and losses per functional unit

		InitS	ScenA	ScenB
Product		per kg	per kg	per kg
	Fish	-0.28 €	0.31 €	0.31 €
	Tomatoes	-2.74 €	-0.21 €	1.29 €
Functional unit (FU)		per FU	per FU	per FU
	Tomatoes per kg fish	0.4 kg	0.61 kg	2.09 kg
	FU incl. Energy sales	**-0.45 €**	**0.76 €**	**3.59 €**

Concerning the cost structure, fish feed had the main cost share in all cases in RAS whereas labour had the main cost share in HS. For the InitS 35% of the RAS were attributed to fish feed and 61% of the HS costs to labour. In ScenB these shares increase to 42% (fish feed) of the RAS and respectively 66% (labour) of the HS costs.

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Based on the existing aquaponic configuration of the InitS, a scenario A (ScenA) was conducted assuming to reach the full productivity potential of the RAS as well as of the greenhouse. This scenario is based on the existing aquaponic configuration and serves as a direct comparison to the initial situation. A second scenario B (ScenB) seeks to maximise the utilisation of the fish waste water for plant irrigation and to minimize the environmental impact of the aquaponic facility waste water by a greenhouse extension.

Results

The factors describing the changed boundary conditions of the cases are given in Tab. I.

The economic results of InitS showed that the initial operation of the facility caused overall net debts. The profits and losses per functional unit for the three cases are given in Tab. II. The first scenario (ScenA) covered the operation costs of the facility and made a bargain with the sold electricity, generated by the CHP, but failed to return the investment within a reasonable time span. The second scenario (ScenB) extended ScenA by featuring a more than threefold enlarged HS unit. For this aquaponic configuration it could be shown that it was possible to reach a level of economic viabilit .

Concerning the cost structure, fish feed had the main cost share in all cases in RAS whereas labour had the main cost share in HS. For the InitS 35% of the RAS were attributed to fish feed and 61% of the HS costs to labour. In ScenB these shares increase to 42% (fish feed) of the RAS and respectively 66% (labour) of the HS costs

Discussion and Conclusion

InitS does not achieve profitability even if due to a stable, regional market a high tomato price could be achieved. The main reasons were (1) the low productivity concerning both fish and plants, (2) the inexperienced staff, and (3) the ongoing research which consumed additional efforts.

According to the scenario calculations ScenB is economical feasible even with a winter-break production schedule. Main credits based on local funding opportunities have a period of 15 years, the assumed operation life of the facility. The operation balance of 78k€ surpasses the annual annuity payment of 61k€ and thus ScenB achieves a positive outcome of 17k€ per year. The Waren aquaponic facility was built within a research project and therefore with a limited financial budget. ScenB was invented to mitigate the weaknesses of the InitS, but even being feasible, it has still optimizing potential.

Despite the extension of the greenhouse within ScenB, the facility is only mid-size in the rural context where rather larger facilities make sense. In contrast in urban application cases also smaller facilities may be of importance due to the competitive pressure concerning space and locations. Thus it is encouraging to learn that even mid-size aquaponics may be economical and thus can be implemented into the urban fabric.

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INTEGRATION OF A NEW PHOTOSYNTHETIC BIOFILM FILTER IN RECIRCULATING AQUACULTURE SYSTEMS AND DIRECT BIOMASS RECOVERY

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Introduction

The treatment of nitrogen compounds in recirculating aquaculture systems (RAS) is a rigorous task to ensure optimal water quality and fish production. In nature, the concentration of different species of nitrogen is controlled by photosynthetic organisms and by different microbial metabolisms, including nitrifying bacteria. The traditional treatment of RAS water is to separate the solid matter and the metabolic waste to avoid the decomposition of this material which increases the concentrations of total ammonia nitrogen (TAN). Even at low doses, these nitrogen species are toxic to fish. Despite this first treatment, a non-negligible fraction of TAN is found in water and requires a second treatment by nitrification. Ammonium will be oxidized to nitrite and nitrate. Nitrite is also toxic to fish and nitrate, although more stable, does not enter the fish diet but may cause growth inhibition beyond a certain concentration (vanRijn et al., 2006, Davidson et al. 2014). As a result, nitrate-rich waters are regularly renewed and are released into the environment, polluting lake and river ecosystems through eutrophication.

Nitrification is a widespread method for eliminating TAN because, thanks to the acidifying power on the medium, this process allows a good control by preventing the new formation of ammonia nitrogen and in particular volatile ammonia. However, the acidification due to the increase of CO_2 dissolved in the environment is very problematic because it affects the quality of water for fish. This CO_2 must be removed before recirculation in the fish pond by a fairly energy-consuming system requiring an oxygen supply. A TAN treatment system based on microalgae would be very advantageous because it directly assimilates ammonia nitrogen and CO_2 to transform it into biomass while producing oxygen. This biotechnology offers the possibility of directly exploiting the algal biomass produced, for example, by processing into a food packaging medium or as a feed for fish farming

Methods

The research project starts with the assembly, the setting of parameters and finally the operation of the photosynthetic biofilm reactor to allow biofilm development. Then, it is intended to operate two tests: one for the treatment of the final effluent leaving the RAS and the other, for the treatment of the RAS water simulating as much as possible the volume of water retained and the daily treatment of a biological filte .

Fig. 1. System for water treatment from RAS by a photosynthetic biofilm culture reactor and generation of bioproducts based on algal biomass.

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Preliminary results

A previous project called AlgOnfilm allowed to validate under industrial conditions (Wastewater Treatment Plant of Yverdon-les-Bains) and for 6 months, the pilot biofilm photobioreactor system. The main goal was to treat the daily water volume attributable to 1 EH (equivalent-inhabitant) on a ground surface of 1 m^2. The most important results were a total N reduction around 60% while for P it was 100%. For ammonium the reduction was 100%. The concentration of heavy metals in water was also reduced on average (on all metals) by 20%. Another meaningful result was the production of a fairly homogeneous bioplastic resin by mixing 30% lyophilized algae and 70% biodegradable polyester.

Scenario analysis of nutrient removal from an industrial RAS facility:

The water analysis allows establishing the stoichiometric reaction of biomass production in relation to the reduction of nitrates and the production of oxygen. With a N: P ratio of 36:1 and considering that all the inorganic nitrogen is in the form of nitrate, it is predicted that photosynthetic activity assimilates N and P in this way:

$$CO_2 + 0.72\ H_2O + 0.36\ NO_3^- + 0.01\ H_2PO_4^- \rightarrow CH_{1.78}O_{0.36}N_{0.36}P_{0.01} + 1.56\ O_2 + 0.37OH^-$$

Oxygen production based on the ground surface will be the result of the quantum yield (moles of O_2 released per mole of absorbed photons) multiplied by irradiance (which is the surface density of flux of light energy). The quantum yield is a hypothetical value based on a theoretical maximum efficiency of 0.1 and which takes into account several losses (reflection, respiration, photo-saturation or -inhibition), while the irradiance can be estimated for any location and for any period of the year. Oxygen production based on the ground surface for an installation in the Jura-Nord Vaudois District between the months of April and October = 2.08 moles $m^{-2}\ d^{-1}$ (with a quantum yield of 0.06 and an average irradiance of 34.66). This O_2 production will therefore be accompanied by a production of 1.33 moles $m^{-2}\ d^{-1}$ of algal biomass, which from the elementary recipe of algal biomass, means an estimated production of 33 $g^{-2}\ m^{-2}\ d^{-1}$.

According to this production, the NO_3^- consumption of the system can also be deduced. Following the elemental molar composition of the algal biomass (defined from these culture conditions), the fraction of nitrogen corresponds to 20% of the total biomass. Thus, the rate of nitrogen consumption will be 6.6 g $m^{-2}\ d^{-1}$.

Expected impact

The project idea is to generate a synergistic approach for the integration of microalgae production in the aquaculture context in order to develop an economic model taking into account its transformation to a bioproduct. For modern aquaculture systems in particular, the technology offers the advantage of reducing the budget for water consumption (water renewal today is 33% while it could be 5%) as well as the power consumption costs when compared to filters based on nitrification which require constant aeration. Additional revenues can be expected from the recovery of algal biomass as a feed for fish farming or as a raw material for the production of bioplastic materials.

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MESOCOSM EXPERIMENTS AS A TOOL FOR CYANOBACTERIAL BLOOMS DYNAMICS RESEARCH

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Cyanobacterial blooms, especially of *Microcystis spp* have become a widespread phenomenon in aquatic systems, where they profoundly affect many properties of ecosystems causing decrease in dissolved oxygen and pH increase (rev. Havens, 2008; Huisman et al, 2018), which in turn may have significant impact to other aquatic organisms. More detailed studies of algal populations in cyanobacterial blooms dynamics are needed. To address this question, we developed FlowCam-based imaging flow cytometry approach to differentiate *Microcystis spp*. morphoforms (Malashenkov et al., 2019; Zhantuyakova et al., 2019) and used it to carry out mesocosm experiments in May-October 2018.

Shortly, mesocosm samples were collected from longest-running lake mesocosm experiment in the world (Lake Mesocosm Warming Experiment (LMWE), Lemming, Denmark; established in 2003 (Liboriussen et al, 2005), and water parameters, turbidity, pH, nutrient composition (TN, TP), oxygen, conductivity, and chlorophyll-a were recorded. We selected three replicates of the enriched nutrient treatments differed in their temperature scenarios. Groups 1 represents the tanks with enriched nutrients (N:P) and unheated ambient temperature, whereas group 2 and group 3 represents an IPCC climate scenarios X2 and X2 + 50% (Intergovernmental Panel on Climate Change). Samples were fixed with glutaraldehyde 1% and acquired using FlowCam VS-4 imaging flow cytometer instrument (10x objective). Obtained data were further classified and quantitatively analyzed using VisualSpreadSheet software (Fluid Technologies Inc. USA) and statistical software. Analysis by FlowCam instrument allowed to differentiate and quantitate various phytoplankton groups, such as colonial cyanobacteria (*M. wesenbergii, M. novacekii, M. smithii, M. aeruginosa, M. ichthyoblabe),* green algae, dinoflagellates, different groups ofdiatoms, cryptomonads etc. The spatiotemporal distribution of major phytoplankton taxa and different *Microcystis* morphophorms were quantified to estimate the relative contribution of di ferent groups and bloom dynamics.

Colonial cyanobacteria (*Microcystis spp*.) were prevalent during summer months and made a large contribution to phytoplankton biomass in tanks with enriched nutrients. Increase in the temperature (tanks groups X2) led to early cyanobacterial bloom formation and colonies size growth. Cryptomonads blooms were observed later in the season in tanks with unheated ambient temperature and in X2-tanks. However, further increase in temperature (tanks X2+50%) led to suppression of colonial cyanobacteria and cryptomonad dominance which was not associated with a particular season. During peak and blooms dying-off *M. wesenbergii* samples include a “half-empty” phenotype colonies; i.e. sheets filled with occasional empty spaces (Fig. 1) or even without any cell inside (“empty” sheets). There is an increase of “empty sheets” as well as “half-empty” colonies of *M. wesenbergii* among group X2 tanks (higher temperature) in August when the abundance of *M. wesenbergii* is decreasing significantly (dying-off blooms). The potential scenario is that during cyanobacteria bloom development, *M. wesenbergii* colonies are growing in size, and some of them losing cells and sharply changing density that leads to increase in buoyancy. We hypothesize that it may contribute to *Microcystis* colonies re-distribution in water column and facilitation of wind-forced migration of colonies in surface layer of water even against flow stream (rivers).

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EFFECT OF MANGROVE DEFORESTATION AND ITS CONSERVATION

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Introduction

Mangrove is a small tree that grows in coastal saline or brackish water, which serves as natural barrier against ocean dynamics along the shoreline. Mangrove refers to a tidally influenced wetland ecosystem within the intertidal zone of tropical and subtropical latitudes. Healthy mangrove forests are key to a healthy marine ecology which serve as nursery ground for many of fishes and habitat for birds and other reptiles. The mangroves make an enormous contribution to the food chain that supports the coastal fisheries and also involving in treating the polluted ecosystems. However, more than 35% of the world's mangroves are already gone. The figure is as high as 50% in countries such as India, Philippines, and Vietnam, while in the Americas they are being cleared at a rate faster than tropical rainforests. This article reviewed the importance, impacts and conservation of mangroves.

Mangrove deforestation

Reduction/destruction of mangrove forest is called mangrove deforestation. According to United Nations Environmental Program (UNEP) about half of the world's mangrove areas have been destroyed. The reason is over exploitation for firewood, poles, and charcoal production; conversion to agriculture, salt plane development, and coastal aquaculture & urbanization. As per the Network of Aquaculture Centre for Asia Pacific (NACA) report about 20–50% of all current mangrove deforestation is due to shrimp farming or coastal aquaculture practices.

Mangroves are naturally destroyed by flood, hurricane, cyclone, insect pests such as woodborers, caterpillars, drying of mangrove trees and fire accidents. Mangroves are naturally destroyed by anthropogenic threats such as tree felling for fuel wood and wood products, reclamation for agriculture and aquaculture, urbanization, industrialization and pollution.

Conservation of the Mangrove ecosystem:

- Afforestation
- Strict implementation of legislation (including laws and policies)
- Monitoring and Surveys (MCS) - land and aerial, etc.
- Protection (including conservation, parks and reserves development, etc.)
- Creating awareness among the stakeholders

Mangrove ecosystem can be utilized for culture (Agriculture, Aquaculture -capture fisheries, culture fisheries), natural products useful for medicinal purposes, drugs, etc.), other products (timber, salt production, honey, etc.), socio-economic aspects, tourism and traditional medicines.

If deforestation of mangroves will continue, it could lead to severe losses of biodiversity and livelihoods, in addition to salt intrusion in coastal zones and the siltation of coral reefs, ports and shipping lanes, with consequent losses of income from tourism and the loss of knowledge of mangroves. To overcome the problems, government and stake holders should take necessary actions like proper monitoring, planning and policy making to protect the mangrove ecosystem for upcoming generations.

IN VITRO OVULATION METHOD CAN BE USED TO MONITOR THE PRE-SPAWNING STATE IN STURGEON FEMALES

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Introduction

It is often not known in which physiological condition surgeon females are during the hibernation and pre-spawning period status changes. The present study shows that the injection of an emulsion of fatty acids improved reproductive properties of Siberian sturgeon females and that this improvement was accompanied with an increase of the part of oocytes ovulated *in vitro*. Thus, in vitro ovulation method can be used to monitor the pre-spawning state of females and to accurately predict their reproductive qualities.

Material and methods

The work was carried out in 2019 during winter and spring. Twenty females of Siberian sturgeon from the stock of Konakovo Sturgeon Breeding Farm (Tver Oblast)were used. All females were kept at a temperature of 2-8°C, i.e. they were at a pre-spawning hibernation state.

All females in the wintering period were subjected to two biopsies for isolating ovarian follicles which then were incubated *in vitro*. The 1st biopsy was performed on February, 5, a second on March, 5. After the 1st biopsy, a half females received an intraperitoneal injection of an experimental drug. The drug contained high purified fish oil -5 g; wheat germ oil – 5 g; soybean lecithin – 2 g and up to 100 ml purified distilled wate . The dose injection was of 1ml per kg of body weight.

After biopsy follicles were placed into 55 mm Petri dishes, 33±2 follicles per dish. 5 replicates per females. Each Petri dish contained 10 ml RMS with adding 0,75 g/l of sodium bicarbonate and antibiotics (Goncharov, 1978; 2009). After placed of follicles was added progesterone to concentration 333 ng/ml. Follicles were incubated at 16°C.

To obtain eggs, females received the pituitary injection in the period 12-26 April. The quality of the eggs was an assessment by fertilization and hatching rates (%).

Results

In females whose oocyte responded to progesterone stimulation after the first biopsy, the percentage of ovulation *in vitro* increased considerably after the second biopsy (fig.1a). In treated females, these differences were significantly higher than in intact females (fig.1 b)

The difference between treated and intact females *in vivo* fertilization rate was not found. However, an effect fatty acids drug manifested in increase hatching rate. It was significantly higher in treated females (fig. 2)

The results obtained during the experiment were used for correlation analysis at the different pair variable (table 1). The table shows that the *in vitro* ovulation rate after 2nd biopsy has a significant positive relation with *in vivo* fertilization and hatching rates.

Discussion and conclusions

In the present study was shown that a single injection of the drug consisted of fatty acids can increase the *in vitro* ovulation rate in treated females. It shall be noted that the increasing percentage of ovulation oocytes *in vitro* under the influence of the drug affected on the prognostic potential in relationship to reproducing characteristics of females in vivo. Thus, we have demonstrated the ability to monitor the physiological state of females during the pre-spawning period using the method of incubating ovarian follicle in vitro.

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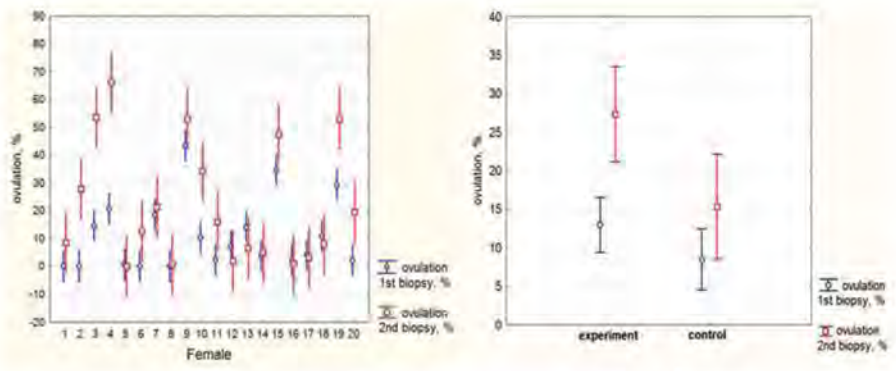

a b

Fig.1. *In vitro* ovulation rate (a) Data of two biopsies; (b) Influence of experimental drug on the ovulation percentage (ANOVA)

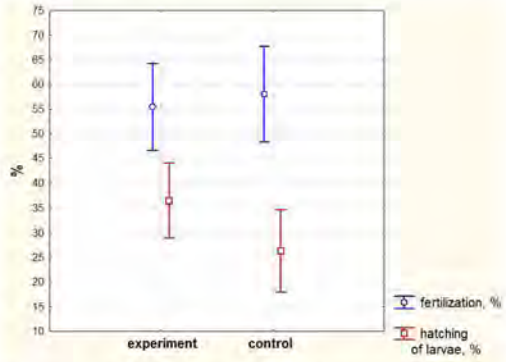

Fig.2 Analysis of *in vivo* fertilization and hatching rates in dependence on the experimental drug (ANOVA)

Table 1. Correlation analysis in different pair variables, obtained in incubation in vitro and in vivo

Pair variables	Valid	Spearman	t(N-2)	p-level
% ovulation 1st biopsy in vitro & Female	100	**0,232450**	**2,3659**	**0,01995**
% ovulation 1st biopsy in vitro & % fertilization in vivo	100	0,001494	0,0147	0,98823
% ovulation 1st biopsy in vitro & % hatching of larvae	100	0,187839	1,89321	0,06128
% ovulation 2nd biopsy in vitro & Females	100	-0,12723	-1,2698	0,20714
% ovulation 2nd biopsy in vitro & % fertilization in vivo	100	**0,323550**	**3,3850**	**0,00102**
% ovulation 2nd biopsy & % hatching of larvae in vivo	100	**0,407930**	**4,4230**	**0,00002**

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POPULATION GENETIC ANALYSIS OF WILD COMMON CARP (*Cyprinus carpio*) POPULATIONS FROM HUNGARY WITH A NEWLY DESIGNED MULTIPLEX MICROSATELLITE SET

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Introduction

Common carp (*Cyprinus carpio*) is a widespread freshwater species in Eurasia with high importance in aquaculture and fisheries. However, the native wild populations of common carp are considered vulnerable to extinction by the International Union for Conservation of Nature (IUCN). In Hungary common carp is produced in the highest amount in aquaculture and different genotypes ("landraces") were developed during the last centuries. Microsatellite markers are particularly suitable for numerous applications including population genetics, parentage analysis or marker assisted selection, nevertheless, no proper, cost-efficient, and rapidly applicable multiplex microsatellite set is available at the moment for common carp. During this study a novel multiplex microsatellite set was developed and tested on three different common carp strains.

Materials and methods

The available common carp genome was investigated with *in silico* analysis by the GMATA (Genome-wide Microsatellite Analyzing Tool Package) software (Wang and Wang, 2016) in order to obtain tetranucleotide microsatellite markers genome-wide. Primers were designed for the flanking region of the detected 64000 microsatellites. The number of potential markers was restricted to 36 by the annealing temperature, specificit , primer-dimer formation and genomic location of the primers. The amplification reactions (PCR) for the selected markers were optimized individually and their polymorphism was checked by capillary electrophoresis on 3100 Geneteic Analyzer (Applied Biosystems) using Gene Scan LIZ 500 size standard (Applied Biosystems). Alleles were identified by GeneMapper 4.0 software. Four different pentaplex sets were assembled from the most congruous markers designed with tailed forward primers and tail specific fluorescent primers (PET, VIC, NED, FAM) suitable for simultaneous fragment analysis. The utilisation of this newly designed microsatellite multiplex set was tested on 75 individuals from three Hungarian common carp populations: 1. the broodstock of Velencei landrace from Kajászó, 2. a population of Balatoni Sudár landrace from Irmapuszta, 3. a natural population of lake Hévíz (which is a thermal lake). The first two landraces belongs to the *Cyprinus carpio morpha accuminatus* form, while the third belongs to the *Cyprinus carpio morpha Hungaricus* form of the wild carps. Data analysis was carried out by Genealex ver. 6.5, Microsatellite Toolkit ver. 3.1.1 and RStudio ver: 1.0.143 software.

Results

Half of the detected 64000 tetranucleotide markers located on chromosomes, the others on genomic scaffolds with unknown locations. For further analysis 32000 microsatellites were used which located on chromosomes and 36 ideal markers were selected thus every one of them located on different chromosomes. Monomorph markers and microsatellites that amplify alleles from more homologue locus (more than two alleles – consequence of ancient carp genome duplication) were excluded from further investigation. Four different pentaplex sets were developed according to the annealing temperature and primer-dimer formation and tested with 75 individuals with the following results: the average number of alleles (Na) per locus was 5.75±3.77 with a range from 2 till 16. The mean Simpson diversity index (1-D) for the alleles of these marker sets was 0.414, showing relatively high number of alleles with even distribution (evenness= 0.629). We calculated the PIC (polymorph information content) numbers for these markers and 6 of them were highly polymorphic (PIC>0.5). For population genetic analysis, we calculated the observed (average Ho= 0.188±0.01) and the expected heterozygosity (He= 0.42±0.06), which indicated heterozygote deficiency in all three populations. Deviations from Hardy-Weinberg Equilibrium were tested by Chi^2 test and it was significant ($p<0.05$) with 14, 3, and 1 markers in the Kajászó, Hévíz, and Balaton populations, respectively. The AMOVA (Analysis of Molecular Variance) analysis revealed that the overall genetic variance is explained in 42 % by the genetic variance among populations, which was confirmed by discriminant analysis of principal components as well.

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Discussion and conclusion

We conclude that this novel multiplex microsatellite set is a powerful tool for fast and cost effective analysis of both natural stocks and farmed populations of common carp. The markers were appropriately polymorph and informative to perform genetic evaluation within and between populations. Our study proves that the investigated populations differ from each other significantl , implying no panmixis among the populations. Deviations from Hardy-Weinberg Equilibrium suggest inbreeding, especially in the farmed population from Kajászó.

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FATTY ACID PROFILES OF GREEN, BROWN, AND RED SEAWEEDS AS A CHEMOTAXONOMIC TOOL

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Introduction

Seaweeds are still a not very well-known marine resource. Namely, there is a deficit of studies on their lipid fraction, which may be partly due to the very low fat level. However, this fat may be rich in bioactive fatty acids (FA), such as the n-3 polyunsaturated FAs (n-3 PUFA), which are known to play an important role in the prevention of cardiovascular and some autoimmune diseases, possessing anti-tumoural and anti-inflammatory properties. Furthermore, the study of its FA profile may convey meaningful insights. A possible application is the utilization of the knowledge regarding the seaweed FA profiles as a chemotaxonomic tool. In particular, though seaweed FA profiles are usually rich in polyunsaturated FAs (PUFA), there is a very significant variability in the quality and quantity of these FAs. For most species, n-3 PUFA are predominantly composed of shorter chain FAs, such as C16 n-3 and C18 n-3. However, there are also some species with significant amounts (on a dry matter basis) of eicosapentaenoic acid (20:5 n-3, EPA). This study was based on a hierarchical cluster analysis (HCA) of the FA profiles of many different European green, red, and brown seaweed species determined by IPMA team.

Materials and Methods

The following European green, red, and brown seaweed species were studied: *Alaria esculenta*, *Chaetomorpha linum*, *Gelidium sesquipedale*, *Halopteris scoparia*, *Osmundea pinnatifida*, *Petalonia binghamiae*, *Pterocladiella capillacea*, *Rhizoclonium riparium*, *Ulva* spp. The fatty acid profile was determined by Gas Chromatography- Flame Ionization Detector as described by Bandarra et al. (1997). An internal standard was used in order to attain the absolute content of each fatty acid.

Results and Discussion

It was observed that red seaweed (e.g. *Osmundea pinnatifida*, *Pterocladiella capillacea*) could reach very high EPA contents (> 12 % of total FA), rare in green and brown seaweeds. However, there were red seaweed species poor in EPA, for instance, *Gelidium sesquipedale*, whose n-3/n-6 ratio was only 0.61 ± 0.02. This ratio also varied widely among brown and green seaweeds, reaching a value near 2 in *Alaria esculenta* (brown) and near 4 in *Chaetomorpha linum* (green). While these high ratios in green seaweeds were mainly supported by C16 n-3 and 18:3 n-3, in brown seaweeds this role was taken by 18:4 n-3 and EPA. In addition, the dichotomy between genetic influence and the action of environmental factors was shown to be relevant. Though HCA did not substantiate a phylogenetic relationship for the orders of seaweeds investigated, differences at the specific level seem to exist and be consistent. Indeed, the case of these seaweeds raises questions on the relative importance of the phylogenetic proximity *vs* environmental and other factors in determining a specific FA profile. On the one hand, FA signatures are claimed to differentiate among marine seaweeds, at least among ordinal and family ranks, on the basis of a thorough multivariate statistics analysis (Galloway et al., 2012). On the other hand, it is known that the FA profile and total A amounts of seaweed vary significantly throughout the year (Manivannan et al., 2008)

On the whole, taking into account the various seaweed taxonomic groups, a balanced assessment must highlight the complexity of interacting seasonal, spatial and species-specific drivers a fecting the FA composition of seaweeds.

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THE IMPORTANCE OF MACROALGAL BIOMASS IN THE DIET OF CULTURED SEA URCHIN (*Paracentrotus lividus*): GROWTH AND FATTY ACID PROFILE

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Introduction

Sea urchins are a marine resource whose demand has been growing worldwide. In particular, sea urchin gonads, the edible part, are considered a *delicatessen*, whose size, texture, and taste are valorized. In Europe, *Paracentrotus lividus* is the most relevant. Over the last two decades, interest in echinoid aquaculture has been increasing. Used diets are critical. Though macroalgae may be better for the development and growth of sea urchins, they entail logistical and economic difficulties, namely, the availability and stocking of good quality macroalgae. Hence, combining with ingredients from terrestrial plants, such as maize, is important. The objective of this work was to feed captivity born juveniles during 4 months with macroalgae and maize, thereby evaluating growth, survival, and fatty acid (FA) profile of *P. lividus*.

Materials and Methods

Accordingly, each of two alternative diets was fed to sea urchins in four replicate tanks: diet U - *Ulva* sp. and diet M+U - 40 % of maize grains + 60 % of *Ulva* sp., w/w. The fatty acid profile was determined by Gas Chromatography- Flame Ionization Detector as described by Bandarra et al. (1997). An internal standard was used in order to attain the absolute content of each fatty acid.

Results and Discussion

Concerning the results, survival during the trial period was 100 %. At the end of the trial, sea urchin fed diet U showed better somatic growth than M+U animals. The substitution of maize in the diet formulation altered its FA profile. The most impressive change was the reduction of n-3 PUFA level and increase of n-6 PUFA level with the replacement of macroalgae by maize, thus leading to a decrease of the n-3/n-6 ratio from 13.2 ± 0.4 to 1.1 ± 0.2 (Table 1).

The observed FA profiles of the U and M+U diets largely reflect the usual composition of *Ulva* sp. and maize. With respect to the FA profiles of the gonads of farmed urchins fed the two alternative diets, they were similar to the FA profiles of the diets in the proportion of the saturated, monounsaturated, and polyunsaturated FAs, as well as in the relative importance of almost all main FAs. However, there were departures from the diet's FA profile. The eicosapentaenoic acid (20:5 n-3) and arachidonic acid (20:4 n-6) were remarkably higher in the urchin gonads than in the U and M+U diets, respectively. These comparisons strongly suggest that *P. lividus* is able to self-synthesize long chain PUFA (with 20 carbons) by incorporation of precursors (C16 and C18) of these FAs directly from their diet, as previously observed in another study with *P. lividus* (Prato et al., 2018) and for other sea urchin species (Chen et al., 2013).

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Table 2 – Fatty acid profile (%) of the alternative diets (U and M+U) given to the farmed sea urchins and in the gonads of farmed urchins fed with the alternative diets.

Fatty acid	Diet		Urchins	
	U	M+U	U	M+U
14:0	1.58 ± 0.01^{A}	0.95 ± 0.00^{B}	4.73 ± 0.71^{a}	8.31 ± 1.89^{a}
16:0	20.70 ± 0.23^{A}	18.60 ± 0.18^{B}	15.84 ± 0.41^{a}	14.80 ± 2.35^{a}
Σ SFA	28.72 ± 0.23^{A}	24.35 ± 0.18^{B}	26.60 ± 0.08^{a}	26.42 ± 4.36^{a}
18:1 n-9	1.36 ± 0.07^{A}	11.54 ± 0.12^{B}	0.75 ± 0.09^{a}	8.33 ± 1.91^{b}
18:1 n-7	11.98 ± 0.11^{A}	7.33 ± 0.06^{B}	6.53 ± 0.42^{a}	2.07 ± 0.51^{b}
20:1 n-9	0.12 ± 0.01^{A}	0.15 ± 0.00^{B}	2.29 ± 0.39^{a}	4.39 ± 0.16^{b}
Σ MUFA	17.34 ± 0.04^{A}	21.41 ± 0.08^{B}	19.66 ± 0.28^{a}	22.84 ± 2.48^{a}
18:2 n-6	2.55 ± 0.01^{A}	22.79 ± 0.06^{B}	0.75 ± 0.04^{a}	20.41 ± 5.69^{b}
18:3 n-3	8.96 ± 0.06^{A}	6.00 ± 0.03^{B}	3.68 ± 0.26^{a}	1.20 ± 0.08^{b}
18:4 n-3	15.64 ± 0.18^{A}	9.38 ± 0.11^{B}	10.39 ± 0.26^{a}	1.40 ± 0.39^{b}
20:4 n-3	1.14 ± 0.07^{A}	0.68 ± 0.04^{B}	4.06 ± 0.94^{a}	0.40 ± 0.11^{b}
20:4 n-6	0.19 ± 0.02^{A}	0.12 ± 0.01^{B}	1.03 ± 0.13^{a}	6.45 ± 1.24^{b}
20:5 n-3	1.35 ± 0.11^{A}	0.81 ± 0.06^{B}	7.48 ± 0.06^{a}	1.66 ± 0.44^{b}
22:6 n-3	1.47 ± 0.11^{A}	0.88 ± 0.07^{B}	0.27 ± 0.00^{a}	0.20 ± 0.17^{a}
Σ PUFA	44.93 ± 0.27^{A}	48.84 ± 0.11^{B}	40.20 ± 1.08^{a}	36.75 ± 5.59^{a}
Σ n-3	41.56 ± 0.32^{A}	25.55 ± 0.19^{B}	36.31 ± 1.39^{a}	6.74 ± 2.04^{b}
Σ n-6	3.15 ± 0.07^{A}	23.15 ± 0.08^{B}	2.71 ± 0.38^{a}	29.80 ± 7.32^{b}
Σn-3/Σn-6	13.19 ± 0.39^{A}	1.10 ± 0.23^{B}	13.48 ± 1.39^{a}	0.25 ± 0.13^{b}

Values are presented as average±standard deviation. Different uppercase letters within a row correspond to statistical differences ($p<0.05$) between diets. Different lowercase letters within a row correspond to statistical differences ($p<0.05$) between gonads of farmed urchins fed with the alternative diets.

GENOMIC SELECTION TO IMPROVE WHITE SPOT SYNDROME VIRUS (WSSV) RESISTANCE IN A *Litopenaeus vannamei* BREEDING PROGRAM

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Introduction

The Pacific white leg shrimp, *Litopenaeus vannamei*, is one of the widely cultured shrimp species in the world with an annual production exceeding 3 million tonnes (FAO, 2017). The shrimp farming sector is often affected by disease outbreaks. White spot syndrome virus (WSSV) is still among the most dangerous pathogens affecting shrimp worldwide (Escobedo-Bonilla et al., 2008; Sánchez-Paz, 2010). Selective breeding for WSSV resistance is a sustainable way of supplying robust seed to the shrimp sector (Gjedrem and Rye, 2018; Nguyen, 2016). The heritability of WSSV resistance in *L. vannamei* has been estimated to be low (Gitterle et al., 2006, 2005) to moderate (Trang et al., 2019), and the trait shows an unfavourable genetic correlation with growth (Gitterle et al., 2005; Trang et al., 2019). Genomic selection is expected to increase the selection accuracy for resistance traits (Vela-Avitúa et al., 2015), and hence to be the optimal selection method for WSSV resistance.

The *L.vannamei* Cartagena (C) family based breeding program operated by Benchmark's Genetica Spring at the Atlantic coast in Colombia is currently at 17th generation, applying selection for growth, high pond survival, robustness and resistance to acute hepatopancreatic necrosis disease (AHPND) and Taura Syndrome (TSV) under strict biosecurity protocols. In parallel, Genetics Spring runs an additional breeding program for *L. vannamei* in Tumaco (T) at Colombia's Pacific coast, mass selected for 10 generations for increased resistance to WSSV. The aim of this study was to improve WSSV resistance in C (nucleus) and commercial multiplier lines through genome enabled selection. Here, we test the accuracy of genomic selection and present results from our genome-wide association study (GWAS) looking for loci associated with WSSV resistance.

Material and methods

Forty GWAS families were created for GWAS study by crossing 10 pure sires each from C and T with 20 pure dams each from C and T where 1 sire was mated with 2 dams, resulting in; 10 pure C (CC), 10 pure T (TT) and 20 crossbred (CT) families. A separate experimental training population, consisting of 58 families with pure T (TT) and crossbred (CT), was created for genomic selection to improve WSSV resistance as described by Lillehammer et.al. (2019). Challenge tests were conducted separately on shrimp from the GWAS families (n=200 pure TT, 200 pure CC and 400 crossbred CT) and training data families (n= 751 pure TT and 696 crossbred CT) in four-ton tanks, using artificial seawater at 30ppt salinity and 26^{o}C temperature. In brief, juvenile shrimp of average weight 3g were infected with WSSV using minced infected tissue fed at a rate of at 3% biomass. Mortalities were recorded each hour for the duration of the test. WSSV infection was confirmed by PCR and histopathology. Selective genotyping (by choosing early and late dying animals within the families) was performed on the GWAS family animals (CC=144, CT=190 and TT=147) to increase the power of detecting rare alleles

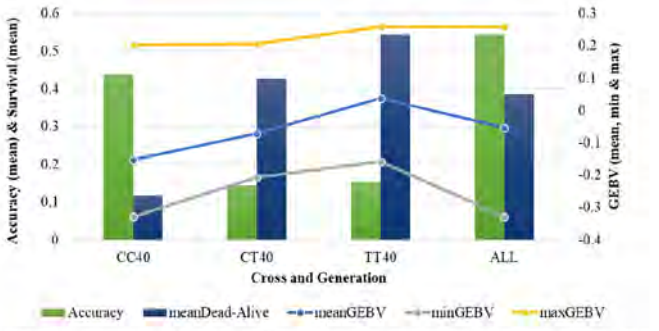

Figure 1. Accuracy of GEBV prediction (mean), phenotype dead/alive (mean) and GEBV prediction (mean, min and max) for the GWAS families for the trait dead or alive using linear model

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of large effect (Xing and Xing, 2009), whereas, all animals from the training families were genotyped using an Illumina ~19K SNP chip. GWAS was performed for two traits; binary survival ("dead or alive") at end-of-test, and "hours survived". A genomic relationship matrix was used for estimation of variance components and to predict genomic breeding values (GEBVs) using genomic BLUP (GBLUP) methodology. The accuracy of the genomic prediction (correlation between estimated genomic breeding values and phenotype, divided by the square root of the heritability) was evaluated using genomic data from the training family as training data and the GWAS family as validation data.

Results and Discussion

Accumulated mortality for the duration of the challenge test for GWAS and training families was 64% and 61%, respectively. Among the GWAS families, the pure C (CC) had highest mortality (89%) followed by crossbred (CT; 58%) and pure T (TT; 46%). Among the training families the highest mortality was found in crossbred (CT; 76%) compared to the pure T (TT; 46%). The heritability estimates for the binary dead or alive trait was moderate irrespective of whether a linear model (0.26 ± 0.04) or threshold model was used (0.27 ± 0.04). The estimated heritability for hours survived was 0.38 ± 0.04. These heritability estimates are higher than reported for a previous study on the Cartagena population (Gitterle et al., 2005), as well as for an un-related population (Trang et al., 2019), most likely due to the high level of resistance present in the combined C and T stocks studied here. In the previous study by Gitterle et.al. (2006), only the pure C (CC) population was used, and average mortality rates were much higher than seen for the TT and CT families in the present study.

The GWAS for WSSV resistance for both traits in GWAS families showed polygenic inheritance with large number of SNP markers explaining genetic variance (Figure not shown). This suggests that a genomic selection approach, exploiting both within-and between-family genetic variation, will be more suitable than conventional family-based selection. The accuracy of genomic prediction for the binary trait ranged from 0.14 (pure TT) to 0.43 (pure CC) with an overall accuracy of 0.54 (ALL) (Figure 1). In contrast, Lillehammer et al. (2019) reported relatively high cross validation accuracy (0.64 to 0.69) using the training population alone. Slightly lower overall accuracy found in this study for GWAS families is most likely due to the relationship between training and validation data; the validation data did not consist of direct full-sib relatives of the training population. In addition, lower accuracy observed among CC, CT and TT GWAS families was most likely because of the limited data available due to selective genotyping followed within these groups. High Pearson correlation (~0.98) was observed between mean GEBV prediction and mean observed survival (dead-alive) in GWAS family groups (Figure 1) possibly indicating accurate GEBV predictions in the absence of relevant phenotypic information: a scenario for the breeding candidates not exposed to WSSV. Though the observed mean survival and mean GBEV prediction for example for pure C (CC) was low, it was still possible to identify animals with high GEBVs for WSSV resistance (max GEBV, Figure 1), like what we can find in highly resistant pure T (TT).

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WHAT SEPARATE WINNERS FROM LOSERS? EVIDENCE FROM A CROSS-COUNTRY INVESTIGATION ON CONSUMER ACCEPTANCE OF NEW AQUACULTURE PRODUCTS

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Introduction

An increasing number of new food products (including new aquaculture products) fail in the marketplace (Fuller, 2016). Even though, it is already established in the literature that having an value-added, superior product in the eyes of the consumers makes a significant advantage on the marketplace, still new product developments are often company and not consumer-driven (Banovic et al., 2016). Strong market and consumer knowledge, as well as labelling schemes are key success factors to obtain the product-market fit and assure acceptance of new aquaculture products (Banovic et al., 2019). The present study uses a cross-cultural context to investigate European consumers' acceptance of new aquaculture products and define the relative perceived value consumers place on different labelling schemes, such as nutrition and health claims, country of origin (COO) label and ASC eco-label, thus separating winners from losers. Besides investigating new aquaculture product acceptance, the current study further uncovers consumer segments which would be more willing to accept new aquaculture products.

Materials and methods

The consumer acceptance of new aquaculture products (i.e., fresh/chilled, smoked, and canned) was modelled by using conditional logit and latent class analysis to investigate aquaculture products' choice and consumer segments in the five European countries (France, Germany, Italy, Spain and the UK). The selection of attributes and their levels has been based on previous studies (Banovic et al., 2016; Reinders et al., 2016) and secondary data on recently launched fish products, used labelling schemes (e.g., health and nutrition claims), and prices for the countries under the study. Based on the above, consumer perceived value of health and nutrition claims, COO and ASC eco-label, as well as consumers' willingness to pay was investigated in the context of new, aquaculture products (for more information see Banovic et al., 2019). Data collection has been undertaken through on-line choice experiments (30 minutes per study) in each of the five study countries and for

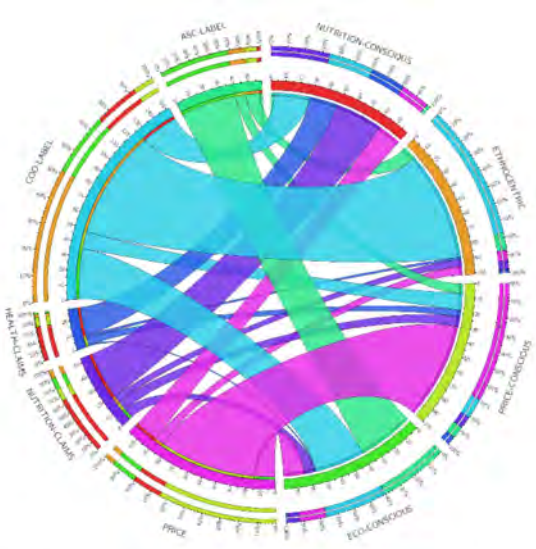

Figure 1. Importance of health and nutrition claims, country-of-origin (COO) and eco (ASC) label per consumer segment: "nutrition conscious", "ethnocentric", "price conscious" and "eco-conscious"; graphical abstract from Banovic et al. (2019).

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each of the three selected products (i.e. fresh/chilled, canned, and smoked). In total, one thousand five hundred and ninety eight participants participated in the study with approximately 500 participants per selected product. Quotas on age, gender, and income have been applied across countries and products to avoid sampling bias (for three selected products: mean age = 40.8 years, 50% male, ~ 65% of participants had average income).

Results

What separate "winners" from "losers", on the general level, in the eyes of these consumers when choosing aquaculture products, is COO label, i.e., aquaculture products produced or sourced from the own country in combination with ASC eco-label (see Banovic et al., 2019). However, consumers from different segments are willing to accept new aquaculture products depending on their own personal interests and relative importance of the different attributes, see Figure 1. Thus, the results further show presence of similar consumer segments across all five investigated European countries, named "nutrition conscious", "ethnocentric", "price conscious", and "eco-conscious" consumers and their criteria for acceptance of new aquaculture products.

Conclusions

What makes the aquaculture product a success is providing added-value (in terms of communicated attributes) that deliver unique benefits to the "right" consumer. A segmented view of the European consumer in this study gives guidelines that could facilitate development of new aquaculture products for improved targeting to further marketing effort and to generate better competitive advantage. This is particularly appropriate now in light of the upsurge of the new aquaculture products on the market.

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HOW TO GROW A RHODOPHYTA: SEEKING AN EFFICIENT AND PROFITABLE NUTRIENT SOURCE FOR *Gracilaria gracilis* (RHODOPHYTA, GRACILARIALES) START-UP CULTURES

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Introduction

To protect wild seaweed species and populations that are often overexploited due to their known value, humankind has cultivated seaweed as an alternative source. Seaweed culture optimization is thus essential to achieve profitable growth and production rates, with at least the same, if not better, biochemical value as their wild counterparts. Hence nutrient composition of any given formula is one of the many elements that may be manipulated within a seaweed culture system, playing a key role in seaweed functional composition.

One of the most well-known medium in seaweed culture is the Von Stosch Enriched (VSE) formula; its modified version is highly beneficial to the growth of the agarophyte *Gracilaria gracilis*, with reckonable success due to its high content in ammonia (NH_4^+) and phosphate (PO_4^{3-}), among other essential nutritive elements. However, VSE formulation is both laborious and costly and thus, the present work aims to seek out a cheaper, easier-to-make alternative, among other culture media and commercial fertilizers. Our goal is to increase the overall profit by decreasing both cost and labour in choosing and formulate this substitute, while avoiding to compromise *G. gracilis* growth rates and biochemical value, which are partially shaped by its nutrient source.

Materials & Methods

Specimens of *G. gracilis* were selected and harvested during low tide in Lagoa de Óbidos (39°24'01''N, 9°13'10''W), Portugal, and transported to the lab inside dark cooler boxes. All specimens were individual and thoroughly washed, and all necrotic parts removed. The cleaned seaweeds were then placed under acclimatization for one week in 60 l plastic open dark containers provided with clean seawater (20ºC, 30-35psu, 16:08 Light:Dark). Afterwards, carefully selected *G. gracilis* tips, were cut, cleaned, and distributed across 250 ml flat-bottom flasks with constant aeration, under a grolux plus daylight combo (1500lux), in a climatic room at 20ºC. All individuals were provided with sterilised seawater at 35psu, supplemented on a fortnight basis with a nutrient source. Culture media and fertilizers tested were f/2, FloraNova Bloom (FNB), FloraNova Grow, and Nutribloom, while Von Stosch Enriched (VSE) medium modified for red seaweeds (Redmond et al., 2014) was kept as control. Both FloraNova and Nutribloom formulas were used both unchanged and boosted with a NH_4^+ source. All assays were performed in triplicate and individuals were weighted prior to culture media renewal. Daily growth rates were measured after 28 days, according to Hayashi et al. (2011).

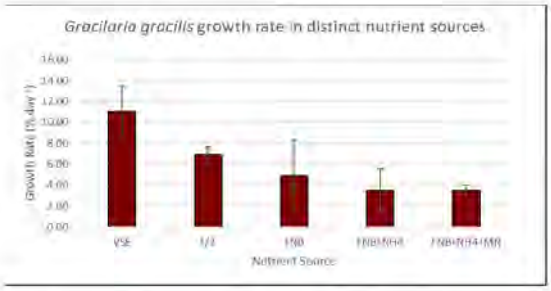

Fig. 1. *Gracilaria gracilis* growth rate under controlled conditions, and supplied with distinct nutrient sources. VSE: Von Stosch Enriched nutrient medium; f/2: f/2 medium, FNB: FloraNova Bloom; FNB+NH4: FloraNova Bloom supplied with ammonia; FNB+NH4+MN: FloraNova Bloom supplied with ammonia and manganese.

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Results

Results show that the best overall growth was observed for *G. gracilis* tips supplied with VSE medium (11.3%.day^{-1}), followed by those grown with f/2 medium (6.96%.day^{-1}) (Fig. 1). The commercial fertilizer and both its modified formulations show lower growth rates than the above mentioned (ranging from 3.51 to 4.89%.day^{-1}, the latter from the standard FNB formula with no modifications). Work is still in progress regarding the remaining mentioned fertilizers, to determine whether it is relevant to supply the standard nutrient media formulas with extra elements relevant to *Gracilaria*, while also taking into consideration the nutrient composition and concentration already present in each formula.

Discussion

According to Fig. 1, VSE still stands as the best overall nutrient source. However, the standard FNB formula may also be considered a viable alternative in the long term, provided its lower cost and still noticeable effectiveness in growing *G. gracilis*. Curiously, adding extra nutrients to this fertilizer showed no improvement to *G. gracilis* growth, when comparing these results to those from the standard formula. The f/2 medium is a commonly used nutrient source for algae, having however NO_3 as the main source of ammonia, instead of NH_4^+; although results show a positive effect in *G. gracilis* growth, this nutrient source also shares the same drawbacks as VSE's in terms of costs and easiness to prepare, and would thus be an alternative worth exploring only if it had presented growth rates even greater than those obtained with VSE.

In all assays, *G. gracilis* showed no visible signs of stress, keeping its natural dark red colour throughout the 28 days, which stands as an evident sign of healthy individuals. Most thalli also presented active growing along the tip, as well as a number of ramifications developing and expanding across the thallus length

Conclusion

Similarly to other seaweeds, *G. gracilis* requires several key macro and micronutrients to grow and thrive, such as ammonia, phosphates, iron, manganese, as well as the vitamins biotin, thiamine, and vitamin B12. These elements are also required by several land plant species and are thus present in commercial fertilizers tailored for them, which are also a cheaper alternative to culture media designed and formulated from scratch. However, not all fertilizers have the ideal nutrient set for seaweed growth; thus it is necessary to assess whether it is sensible and cost-effective to further enrich and tailor the nutrient source with additional nutrients to respond to any given species needs, and thus promote growth rates and quality of the end product.

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ANTIBACTERIAL ACTIVITY OF THE RED SEAWEED *Gracilaria gracilis*

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Introduction

Aquaculture production systems present a set of limitations and constraints, which hinder the health and welfare of fish and shellfish. High density populations, regular handling, poor water quality, improper feed quality and the presence of pathogens, can trigger stressors that favour the emergence of pathogenic outbreaks. The presence of pathogens causes low food conversion rates, reduced growth and ultimately low survival rates, leading to significant economic losses. One of the ways of preventing the occurrence of disease, particularly of bacterial origin, can be the supplementation of the fish feed with bioactive compounds with antimicrobial activity. The use of seaweeds in the fish feed, either directly or in the form of extracts, can be an asset, providing not only compounds of nutritional value, but also several bioactive compounds, with known benefits. In general, seaweeds present antimicrobial and immunostimulant effect, but also anti-viral, prebiotic and antioxidant activities, among others. Recently there has been a growing interest regarding the potential of seaweeds as a source of nutritional and health related compounds, and the genus *Gracilaria*, a renowned agarophyte extensively cultivated worldwide, is no exception. The objective of the study was to evaluate the *in vitro* antimicrobial activity of crude extracts of *Gracilaria gracilis* against XX pathogens of bacterial origin, common in aquatic environment, seeking to assess the value of these extracts as additives in fish feed.

Materials & Methods

Gracilaria gracilis (Stackhouse) Steentoft, Irvine & Farnham, was collected from Lagoa de Óbidos (39º24'01''N 9º13'10''W), Caldas da Rainha, Portugal. The ethanolic or aqueous extracts were prepared using a 1:10 ratio of biomass: solvent (w/v), with agitation at room temperature, and different extraction times were tested (Vlachos et al. 1996, Chan et al. 2015). Extracts were filtered, centrifuged and concentrated in a rotary evaporator (ethanolic extracts) or freeze-dried (aqueous extracts). All extracts were tested against several bacterial fish pathogens: *Vibrio anguillarum*, *Vibrio alginolyticus*, *Photobacterium damselae* subsp. *piscicida*, *Photobacterium damselae* subsp. *damselae*, *Aeromonas hydrophila* subsp. *hydrophila*, *Aeromonas salmonicida* subsp. *Salmonicida* and *Edwardsiella piscicida*. For the evaluation of the antibacterial activity, the Agar Disk Diffusion Method (Bauer and Kirby, 1966), was used, according to the National Committee for Clinical Laboratory Standards. Briefl , target strains were prepared to obtain a working culture containing approximately $1x10^8$ cells ml^{-1}. Aliquots of each bacterial suspension, were inoculated using the spread plate method on Muller-Hinton agar plates with 1.5% NaCl (Thanigaivel et al. 2015). To perform the assays, the crude extracts (20µl) were dissolved in 1ml of the respective solvent, and used to soak sterile disks (6mm in diameter, Oxoid), that were applied to the inoculated plates. Disks containing 30µg of chloramphenicol were used as a positive control, disks soaked with 20µl of the respective solvent were used as a negative control. Plates were incubated 24h-48h at ideal temperature of each strain and the inhibition zone was measured (mm). All tests were performed in triplicate.

Results

In general, ethanolic extracts present a superior antimicrobial activity to aqueous extracts, thus revealing ethanol as the most suitable solvent for extraction. The results also indicate that extraction times of 30 minutes and 1 hour show the best results. Inhibition halos were recorded against *Vibrio anguillarum* (8mm), *Aeromonas hydrophila* subsp. *hydrophila* (9mm), *Aeromonas salmonicida* subsp. *salmonicida* (8mm), *Photobacterium damselae* subsp. *damselae* (9mm) and *Photobacterium damselae* subsp. *piscicida* (10mm).

Discussion and conclusion

Gracilaria gracilis is a natural source of antimicrobial compounds with potential value to human or animal health and can be used in food or feed to increase resistance to several pathogenic bacterial strains, as shown by the results. The effects observed under *in vitro* conditions are quite interesting in view of the devastating effects some of these pathogens may have on several aquaculture fish species. The effects of dietary seaweed biomass supplementation with *Gracilaria* sp. were previously assessed in meagre (*Argyrosomus regius*) subjected to infection with *Photobacterium damselae* subsp. *piscicida* (Peixoto et al. 2017), indicating that the inclusion of seaweeds may confer advantages in coping with biotic stressors, as oxidation damages were reduced. Based on the current research, seaweed extracts show promising properties as antimicrobial agents and health promoting agents, but further research is needed. The use of *G. gracilis* extracts, and their effects on mortality, as well as in the immune response parameters, is currently being tested in order to understand the *in vivo* effects of this seaweed extracts.

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Acknowledgements

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DIFFERENTIAL IMMUNE RESPONSES IN GILTHEAD SEABREAM AND MEAGRE JUVENILES INFECTED WITH *Photobacterium damselae* SUBSP. *piscicida*

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Introduction

Aquaculture plays an important role in food production worldwide. However, the presence of pathogens is currently a major constraint for its development, leading to disease outbreaks and therefore causing severe economic losses (Romalde, 2002; Essam *et al*., 2016). *Photobacterium damselae* subsp. *piscicida* (*Phdp*) is the causative agent of photobacteriosis, formerly known as fish pasteurellosis, a bacterial septicaemia. *Phdp* is able to infect a wide variety of marine fish with economic relevance, including the yellowtail (*Seriola quinqueradiata*) in Japan and gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), sole (*Solea senegalensis* and *Solea solea*) and meagre (*Argyrosomus regius*) in Europe (Romalde, 2002; Essam *et al*., 2016; Costa *et al*., 2017).

The aim of this work was to compare cellular and humoral immune responses of gilthead seabream and meagre against *Phdp*.

Materials and methods

A time-course study was performed at CETEMARES (Instituto Politécnico de Leiria) facilities, with gilthead seabream (mean wet weight 40 ± 7.3 g) and meagre (mean wet weight 65 ± 11.9 g) juveniles obtained from the Aquaculture Research Station-EPPO (IPMA, Olhão, Portugal). Among each population, 12 fish were randomly selected and sampled before infection (time 0 h). Thereafter, the remaining animals were randomly selected and intraperitoneally injected (IP) with 100 µl of 1 10^5 CFU ml^{-1} of *Phdp*, while control fish were sham-injected with 100 µl a phosphate buffered saline solution. Afterwards, fish were randomly distributed as a completely randomized design into 12 tanks of 60 l closed recirculation systems (i.e. triplicates per experimental condition, for each studied species). Two animals per tank (n=6) were randomly selected and sampled at 3, 6, 9, 24 and 48h after IP injection. At each sampling point, fish were anaesthetized with 2-phenoxyethanol and blood samples were collected for haematological procedures such as total, differential counting of peripheral leukocytes and total circulating erythrocytes. The remaining blood was centrifuged and plasma was collected for the assessment of innate humoral immune parameters. Every recirculation system was kept at 25°C for 21 days and pathological signs and mortality were observed and recorded.

Results

Infected meagre mortality started at 24 h and exhibited a mortality rate of 100% at 48 h post infection. The individuals showed reduced appetite, lethargy and abnormal swimming behaviour, whereas no mortality was observed in infected seabream. Regarding white blood cells both species presented a similar response, since total cell counts increased in infected groups across time. However, peripheral lymphocytes showed a differential response depending on the host, since those numbers increased over time in infected seabream, whereas meagre dropped lymphocyte counts for both infected and sham injected treatments. In contrast, neutrophil counts decreased in infected meagre, while gilthead seabream did not show any changes in this phagocyte population. In both seabream and meagre juveniles, circulating thrombocytes increased over time in infected individuals compared to sham injected controls. Plasma peroxidase activity increased in infected meagre and seabream but seemed to be particularly high in infected seabream. Plasma bactericidal activity increased in infected meagre with no changes in seabream.

Discussion and conclusion

In the case of the infected seabream individuals it was observed a migration of lymphocytes to the bloodstream in response to this pathogen, while in meagre the lymphocytes decreased. These distinctive immune responses may indicate immunogenic stimulation or immunosuppressive conditions, resulting in lymphocytosis or lymphocytopenia for the same

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pathogen (Clauss *et al*., 2008). These results can be directly related with the specific response of each specie. For the white blood cells, the levels increased as early as 3 h post-infection for both species, revealing a mobilization of these cells to the infected tissues (Chaves-Pozo *et al*., 2005). The fish innate immune response is considered to be an important component to rapidly avoid the proliferation of a pathogen (Whyte, 2007). In this study, the innate immune response to *Phdp* infection lead to an increased bactericidal activity levels in infected meagre. Neutrophils are regarded as part of the innate immune response; however, they can interact using cytokines and chemokines with some cell-mediated responses, making them a bridge between defence systems (Rosales *et al*., 2017). It is already established that some pathogens, overcome the neutrophils defence, by means of blockage of chemotaxis and phagocytosis (Rosales *et al*., 2017), being *Phdp* capable of extensive apoptosis of macrophages and neutrophils that results in lysis of these phagocytes by post-apoptotic secondary necrosis (do Vale *et al*., 2005). This effect was especially relevant in meagre, occurring a neutrophil decrease in the infected treatment relatively to the control, reaching almost zero 24h post-infection. Although the infected seabream did not perish, the studied immune parameters did not show the reason behind the seabream ability to survive to this pathogen. Therefore, different parameters should be analysed to reach a conclusion regarding these results. A deeper study about the reaction of the immune parameters to the same pathogen in different fish species may provide valuable biomarkers to identify and avoid the proliferation of bacterial pathologies.

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EUGENOL INDUCES A SEAZURE-LIKE STATE DURING SHORT-TERM EXPOSURE IN FISH

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Introduction

Eugenol is one of the most commonly used and commercially available plant extractives for anaesthesia/immobilisation of fish. However, stress, ventilatory failure and medullary collapse have been reported in fish exposed to this compound (Sladky et al., 2001; Davidson et al., 2000; Readman et al., 2013). Many of the current drugs or alternative products recently put forward as novel anaesthetics are regarded as general anaesthetics based on the premise that loss of reaction to visual or mechanical stimuli after exposure to the drug is accompanied by unconsciousness (Ross and Ross 2008). Yet, the use of behavioural markers alone, such as the observation of latency to reaching a stage of general immobilisation, does not prove general anaesthesia with unconsciousness or analgesia. Animals may reach a stage of sedation and be completely immobilised but not unconscious or pain free. The aim of this study was to evaluate through electroencephalographic (EEG) recordings the extent to which eugenol promotes depression of the central nervous system (CNS), acting as a general anaesthetic for fish.

Materials and methods

Tambaqui, *Colossoma macropomum* were acclimated for 15 days in semi-static systems prior to the beginning of the experiment. Eugenol (Quimidrol™, Joinville SC – Brazil) was purchased from a commercial establishment. A stock solution of eugenol was prepared by diluting it in commercial alcohol (96%) at a ratio of 1:9, and stored in an amber glass bottle at 4°C until use. Eugenol baths at 65 µL L^{-1} sufficed to promote a fast induction (< 3 min) to "anaesthesia", *ie*., absence of or minimum opercular beating with loss of reaction to tail pinch stimulus as reported by Roubach et al. (2005) for this species. For the design of appropriate equipment and recording of electrophysiological data, the methodology of Pineda et al. (2011) was used. Fish [3.4 g ± 0.58 (SD); 5.5 cm ± 0.8 (SD), total length] were assayed in three groups as follows: a) sham control; b) fish exposed to eugenol and c) fish in recovery. Nine fish per group (n = 9) were used; each individual was considered a replicate, and used only once. For the recording of EEG, fish were individually netted from the maintenance tanks and equipped with the electrode set, after which fish were placed into the experimental chamber, inside a 1–L fish tank, previously filled with the same aerated water (0.5 L) from the maintenance tank and added by eugenol at 65 µL L^{-1}, or anaesthetic-free water, accordingly. During induction, recordings of the field potential in brain, including baseline profile in sham control were performed in the course of 5 min. EEG during recovery was also registered in anaesthetic-free water immediately after the anaesthetic exposure (10 min recordings).

Results and Discussion

EEG in sham control showed low amplitude of tracings (Fig. 1A, left) and the spectrogram indicated that energy intensity was concentrated in frequencies below 10 Hz (Fig. 1A, right). Mean basal amplitude recorded in the mesencephalon of tambaqui was 0.0220 ± 0.0007 mV^2 / Hz x 10^{-3} (Fig. 2) whereas the induction process was characterized by intense brain activity with excitability, which evolved to a seizure-like state showing amplitude recordings of 0.0824 ± 0.0078 mV^2 / Hz x 10^{-3} (Fig. 2). During induction, elevations in frequencies were observed as shown in Fig. 1B (left). A 10s magnification of the tracings within the first third of the EEG recordings showed the occurrence of action potential bursts (Fig. 1B, centre), being the spectrogram of frequency in line with the tracing pattern attained, showing high intensity of energy throughout the record (Fig. 1B, right). During recovery, fish resumed normal brain activity and excitability gradually decreased (Fig. 1 C, left), with mean amplitude of 0.0181 ± 0.0030 mV^2 / Hz x 10^{-3} (Fig. 1C, left). The fragment amplification of the EEG during recovery revealed a similar pattern of tracings compared to that of sham control (Fig. 1A and C, centre). At the beginning of the record, the spectrogram showed that energy consumption was higher, indicating an extra effort for recovery. However, the intensity of the signal decreased over time, and fish were fully recovered at the end of the EEG recording (Fig. 1C, right, and 2).

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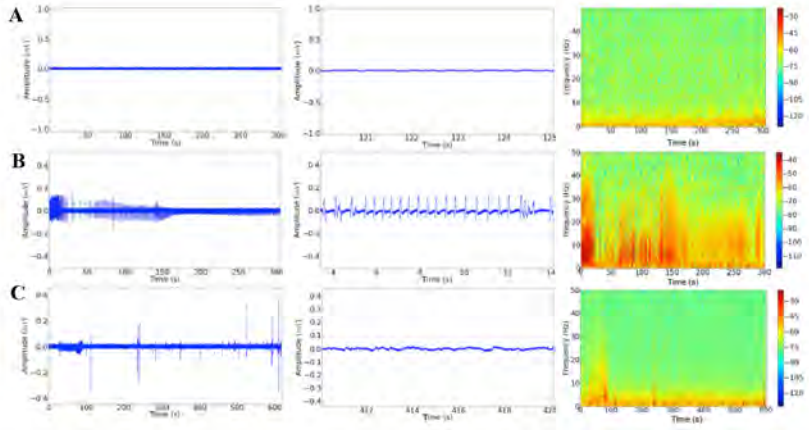

Fig. 1. Electroencephalogram (EEG) recordings of the mesencephalon region in juvenile tambaqui, *Colossoma macropomum*. A – Recordings in sham control in 300s (left), fragment amplification of EEG (10s) (centre) and spectrogram of frequency (right). B – EEG in fish exposed to eugenol at 65 µL L^{-1} for 300s, fragment amplification of EEG (10s) (centre) and spectrogram of frequency (right); C – EEG recordings during recovery in eugenol-free water 600s (left); fragment amplification of EEG (10s) (centre) and spectrogram of frequency (right).

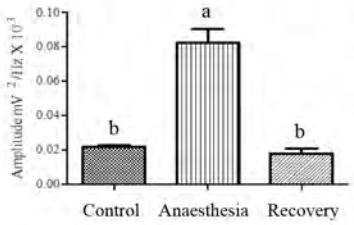

Fig. 2. Mean amplitude (± SE) recordings from the mesencephalon region of juvenile tambaqui, *Colossoma macropomum* in frequencies of up to 50 Hz. Electroencephalogram (EEG) recorded in sham fish (Control) (300s), during exposure to eugenol at 65 µL L^{-1} (Anaesthesia) (300s) and after eugenol exposure (Recovery) (600 s). Distinct letters above columns denote significant differences after ANOVA and Tukey's test, $p < 0.05$, $n = 9$.

During exposure to eugenol, although complete body immobilisation was attained, fish presented an intense neuronal excitability, which was consistent with a seizure-like event. Recovery was gradual albeit it required twice the time used for induction. Our results showed that eugenol did not adequately anaesthetize fish during short-term exposure, for it failed to promote depression of the CNS and therefore was not effective as a general anaesthetic and not compatible with a deep anaesthesia state in tambaqui, *C. macropomum*.

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OCEAN ACIDIFICATION AND MULTIPLE STRESSORS AFFECT LARVAL BUT NOT JUVENILE STAGES OF THE EASTERN OYSTER (*Crassostrea virginica*)

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Introduction

Ocean acidification (OA) is considered to be a major threat to the future of our oceans. The altered seawater chemistry associated with OA is predicted to be particularly threatening for calcifiers such as sensitive bivalve species, particularly during early life stages. This study was designed to assess and compare the effects of OA on larval and juvenile eastern oyster (*Crassostrea virginica*) viability, growth, and immunity, and evaluate the impact of food availability on resilience to OA.

Materials and Methods

For larval studies, adult oysters were spawned and larvae were cultured under ambient (400ppm) or elevated (1000ppm) pCO_2 conditions. Viability and growth were assessed after two weeks. Larval susceptibility to bacterial pathogens (cocktail of *Vibrio coralliilyticus*, *V. alginolyticus*, and *Listonella anguillarum*) was evaluated on 7-day-old oysters. The effect of trophic resources on viability was assessed following a diet of high (100% recommended agal quantity) or low (10% recommended) food for a period of 24h.

For juvenile studies, oysters (shell length 5mm ± 1.5mm, n=1,600) were procured from hatchery stocks and transferred to ambient or elevated pCO_2 conditions. Following an acclimation period, oysters were exposed to fed or completely starved conditions for 10d, then exposed to *Roseovarius crassostreae* (etiological agent of Juvenile Oyster Disease) for 4 weeks.

Results

Overall larval mortality was highest under OA after a two-week exposure. Larval growth was lowest under OA. Under combined OA and food limitation mortality was higher than all other conditions (Fig. 1). Under OA, larval oysters are significantly more susceptible to bacterial pathogens (Fig. 2)

Juvenile mortality was highest under ambient pCO_2 and starvation conditions (Fig. 3). OA or OA and starvation did not significantly affect the survival of juvenile oysters. Starvation significantly reduced the length of oysters within their relative pH treatment, and overall length was smallest in juveniles under OA and starvation. Starvation of juvenile oysters increased susceptibility to ROD, however, susceptibility was highest in oysters under ambient pCO_2 conditions.

Discussion

Results suggest that larvae are more susceptible to the effects of OA with regard to viability, susceptibility to infection, and viability under food limitation as compared to juvenile oysters. However, both larvae and juveniles face reduced growth under OA conditions suggesting shell building and maintenance are particularly challenging for oysters under acidified conditions.

Ongoing work seeks to further determine the molecular basis for resiliency to OA and identify specific molecular features associated with survival. These findings provide greater insight into the specific effects of OA on *Crassostrea virginica* physiology and how multiple stressors may work synergistically to affect the resiliency of oysters to environmental stress.

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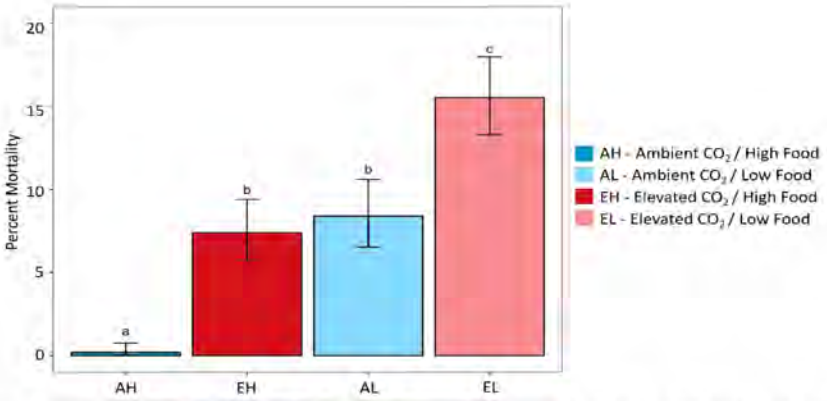

Fig. 1. Percent mortality for larval eastern oysters (mean ± SD) under ambient and elevated pCO_2 and two different food concentrations. Different letters indicate significant difference at $p<0.05$.

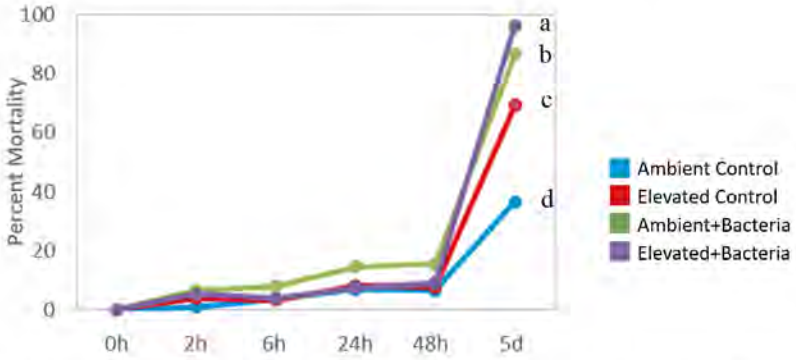

Fig. 2. Percent mortality data for larval oysters exposed to pathogens at multiple time points following exposure. Different letters indicate significant differences between different conditions within each sampling time (ANOVA followed by Holm-Sidak post-hoc test, $p<0.05$).

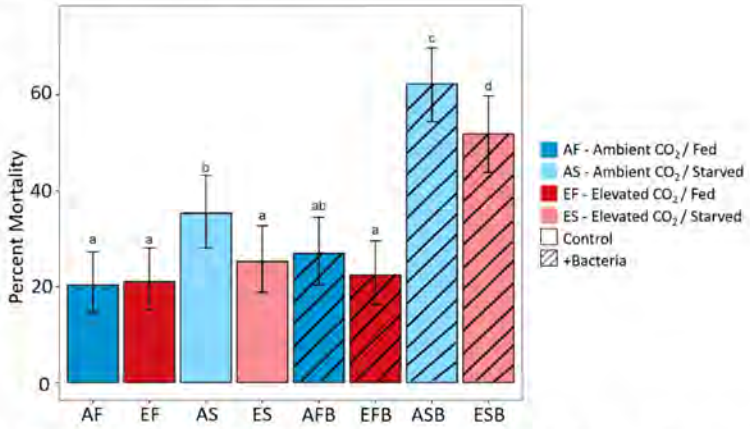

Fig. 3. Juvenile Food Limitation Experiment and pathogen challenge percent mortality. Significant differences between treatments represented as different letters with a significance level of $p<0.05$.

EFFECTS OF DIFFERENT SOURCE AND LEVELS OF OIL ON LIPID METABOLISM RELATED GENE EXPRESSION IN JUVENILE TAMBAQUI (*Colossoma macropomum*)

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Introduction

Tambaqui (*Colossoma macropomum*) is a native fish species of the Amazon basin, being one of the main species in Brazilian aquaculture. LC-PUFA can be obtained through the diet or endogenously produced (biosynthesis) through the combined action of two enzymes, namely fatty acyl elongases (Elovl) and desaturases (Fads). Studies have established that tambaqui is an herbivorous fish that can fulfil its essential FA requirements with dietary provision C18 PUFA LA and ALA. The functional and molecular characterization of Fads2, elovl5 and elovl2 established that they can operate towards a variety of PUFA substrates with chain lengths ranging from 18 to 22 carbons (Ferraz et al. 2018). These enzymes have been extensively studied in several aquaculture species in an effort to understand the ability of fish species to utilize dietary vegetable oils (VO) replacing fish oils (FO) in current aquafeed formulations indeed. To elucidate these questions in tambaqui, we combined different sources of oil and levels to investigate lipid metabolic pathways. The present study aimed at understanding the ability of tambaqui to satisfy its LC-PUFA requirements when fed on VO-rich diets commonly; gene expression of key enzymes involved in LC-PUFA metabolism (FADS2, ELOVL5 and ELOVL2) in the liver and the brain were evaluated.

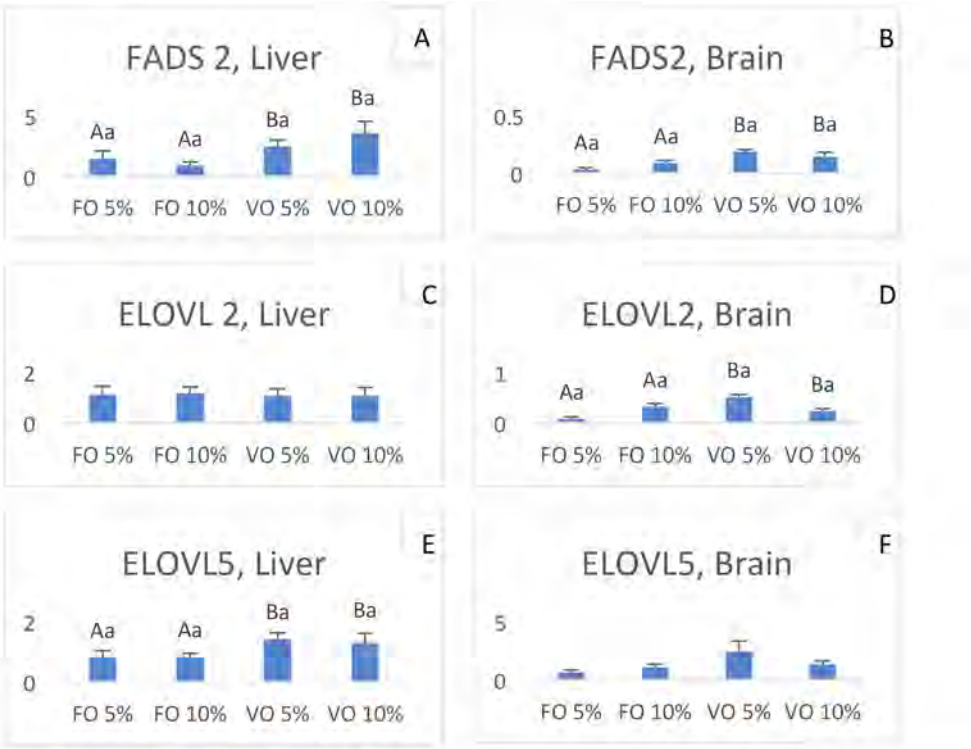

Fig. 1. Expression of genes involved in long-chain polyunsaturated fatty acid (LC-PUFA) biosynthesis. Expression of Fads2 in liver (A) and brain (B), Elovl 2 in liver (C) and brain (D), Elovl 5 in liver (E) and brain (F) determined by real-time quantitative PCR. Different letters represent significant different between diets (ANOVA, $P<0.05$) where the fast letter is the effect of font od oil and second is about percentage of oil.

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Materials and methods

The present study was conducted to evaluate the influences of different dietary fatty acid profiles on the biosynthesis of LC-PUFA in juvenile tambaqui. Four diets were prepared (FO5%, FO10%, VO5% and VO10%). A 9-week feeding trial was conducted using juvenile fish (8.61 ± 1.38g). Fish were dissected and samples of liver and brain were preserved in an RNA stabilization buffer. Total RNA was extracted using the "illustra RNAspin Mini Kit" (GE Healthcare). cDNA was synthesized from 1000 and 500 ng total RNA, respectively from the liver and brain, using the "NZY Fist-Strand Cdna Synthesis Kit" (NzyTech) following the protocol. Fluorescence-base quantitative PCR was performed with the NZYSpeedy qPCR Green Master Mix (2x) (nzytech gene and enzymes). Relative gene expression was calculated with the ΔΔCt method including the PCR efficiencie of the target and reference gene. Target gene expression was normalized utilizing the geNorm software, using a multiple reference gene approach (normalization with β-actin, tubulin and microgobulin).

Results

The present study demonstrated that, in the period of 9 weeks, no difference in body weight gain (BWG), feed conversion ratio (FCR) and survival were observed between treatments with dietary vegetable oil (5 and 10 %) and dietary fish oil (5 and 10 %). Expression of LC-PUFA biosynthetic pathway genes (Fads2, Elovl5 and Elovl2) was determined in tambaqui liver and brain using qPCR (Fig. 1). The expression of Fads2 and elovl5 in the liver increased in fish fed the VO source (VO5%, VO10%) (Fig. 1, A and E). Effects of VO on the expression of Elovl2 in liver was not detected (Fig. 1, C). Similarly, the brain presented high expression of Fads2 and Elovl5 in VO (Fig. 1, B and D), and no difference in the elvol5 (Fig. 1, F).

Discussion and conclusion

In this study, the expression of fads2 and elovl5 in the liver was up-regulated when the fish were fed by the VO dietary source. A similar profile was observed in the brain. These results suggest that fish fed high dietary VO have more available C18 substrates such as LA (18:2n–6) and LNA (18:3n–3) for elongation and desaturation. Additionally, in the brain the higher expression levels of elovl2 suggests that a greater contribution of this metabolic step to the production of docosahexaenoic acid (DHA). In conclusion, these results suggested that fish oil can be replaced by vegetable oil for tambaqui and that the percentage of 10% is not high to the point of disrupting the animal's metabolism.

Acknowledgment

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EXPLORING MACHINE LEARNING METHODS FOR GENOMIC PREDICTION OF DISEASE RESISTANCE AND BODY LENGTH IN THE GILTHEAD SEA BREAM

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Introduction
The use of machine learning (ML) methods is rapidly increasing in genomic science (Schrider and Kern 2018, Ho et al. 2019, Telenti et al. 2019). ML methods have also been proposed as alternatives to statistical methods for predicting breeding values in the context of genomic selection. Although in particular scenarios ML approaches are reported to outperform parametric methods for genomic prediction (Howard et al. 2014), increasing evidence suggests that relative performance largely depends on the analysed data sets (*e.g.* González-Camacho et al. 2018, Montesinos-Lopez et al. 2019). Here we evaluate ML methods applied to predicting phenotypes for two traits, body length and resistance to fish photobacteriosis in the gilthead seabream, one of the most important species for European aquaculture.

Methods
Two published data sets (Palaiokostas et al. 2016, PAL2016; Aslam et al. 2018, ASL2018) were re-analysed starting from pre-processed sequence data to obtain a common SNP data set for nearly 2,000 individuals. After quality filtering, raw sequence reads were mapped against the draft sea bream genome (Pauletto et al. 2018) using BWA. SNP variants were called using SAMTOOLS. Loci showing high missing data rates (>30%) and minor allele frequency lower than 0.05 were filtered out. Genotype imputation was carried out using BEAGLE yielding over 80,000 SNPs were obtained. Two traits were recorded in the original experiments: survival time after infection with *Photobacterium damselae piscicida* and total body length. Individual survival data were transformed into binary data (dead or alive at day 10 after challenge) or continuous data (age-normalized length). Parentage relationships were reconstructed using CERVUS on five random subsets of 1000 SNPs. For ML, support vector machine (SVM) and linear bagging (LB) were implemented either as classification or regression algorithms. For parametric methods, Bayes B, Bayes C,Bayes Lasso, Bayes Ridge Regression, which was shown to be equivalent to GBLUP, were used. The ASL2018 experimental population was used for training and cross-validation, while PAL2016 was used as an independent test set. Accuracy and Matthews correlation coefficient (MCC) were calculated and compared across methods.

Results
Higher accuracy was observed when predicting survival than body size. Cross-validation showed higher performance for ML methods, particularly for LB (MCC=0.48) than Bayesian regression methods, which had comparable performance (MCC=0.38-039). Accuracy for body size prediction was lower for all methods (Pearson correlation<0.25). Using trained algorithms on ASL2018 to predict the independent data set (PAL2016) show consistently poor performance.

Discussion and conclusions
Revisiting two existing data sets allowed us to demonstrate genotype data imputation using genomic information increased the number of genotyped loci. These expanded data sets were then used to compare parametric and ML methods in their ability to correctly predict two traits: survival after experimental challenge with *P. damselae piscicida* and age-corrected body length. Accuracy estimated through cross-validation was extremely high especially for disease resistance using ML methods. However, the ability to predict either phenotype on independent samples was much less promising. Several factors might have led to reduced predictive power in the present case such as different age of animals and different protocols for experimental infection. It is possible, however, that estimating accuracy using a single population might fail to consider that in other genetic backgrounds the genomic basis for the same trait is different.

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MUSCLE TRANSCRIPTOME RESPONSE TO FASTING-REFEDING IN FAST-GROWING COMPARED TO AGE- OR SIZE-MATCHED SLOW GROWING INDIVIDUALS IN THE GILDHEAD SEA BREAM

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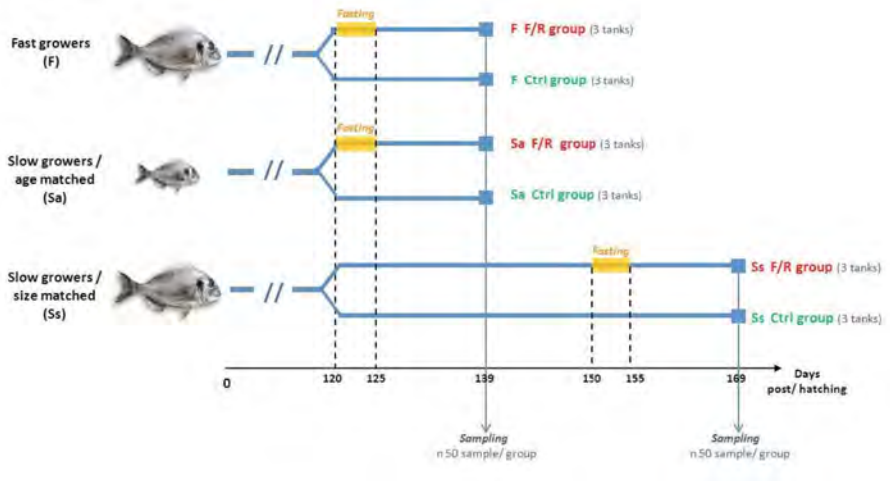
Introduction

Compensatory growth is a phase of rapid growth, which can triggered by refeeding animals after a period of prolonged fasting. period. In fish, compensatory growth has been related to increased feed intake and possibly in feed conversion efficiency (Ali et al. 2003). As skeletal muscle accounts for over 60% of total body mass in fish, understanding how muscle gene expression profile changes during compensatory growth is quite interesting especially in farmed species, where body and fillet size represents a key trait. Fish also show large within-species genetic variation in growth rates, particularly in unselected populations. Contrasting the transcriptomic basis of compensatory growth in fast- and slow-growing animals might then reveal the genomic basis of differences in muscle physiology between individuals with divergent growth performance.

Methods

Juvenile sea breams all originating from a single spawning event across unselected parents were raised in a single tank until they reached 120 days of age. Fish were then selected by size (weight and length). The upper 10% of the population was considered as "fast grower" (F), while the lower 10% as "slow grower". The latter was subsequently divided into two sub-groups (Sa and Ss). Groups F and Sa were subjected to six days of fasting and then fed ad libitum for 14 days until sampling. Group Ss was farmed under identical conditions until the average size reached that of group F at the start of the fasting experiment (Figure 1). Group Ss was then subjected to the same fasting-refeeding protocol described above. For each group, control-fed fish were kept under identical conditions except for the fasting part. White muscle tissue samples were collected as shown in Figure 1.

RNA was extracted from individual samples for each condition (F-F/R, F-ctrl, Sa -F/R, Sa-ctrl, Ss-F/R, Ss-ctrl). Four biological replicates per condition (each made of a pool of three individuals) were used to construct indexed cDNA libraries. All libraries were sequenced on a Illumina Hi-Seq 2500 (SE). Quality-filtered reads were mapped against the sea bream assembled genome (Pauletto et al. 2018) by means of STAR aligner following the two-pass mapping mode. Read counts for each sample, at the gene level, were extracted by GeneCounts quantification while running STAR. Analysis of differential gene expression was conducted in EdgeR. For each sample, the sum of raw cpm (count per million) of all genes was calculated by means of aggregate function on R. Genes showing a cpm value lower than 1 in more than half of the samples for each species were filtered out. Remaining genes were normalized with the Trimmed Mean of M-values (TMM) method and, after estimating common and tagwise dispersions, Likelihood Ratio Test (lrt) as provided in EdgeR was employed to assess differentially expressed genes (DEGs) between conditions.



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Results

A large number of genes were found to be differentially expressed when using a threshold of logFC>1 or FC<1 and FDR<0.05 between F and S control groups. Groups F and Sa (age-matched slow growers) showed much greater similarity in muscle transcriptome profile than size-matched slow growing animals. However, when the transcriptomic basis of compensatory growth was compared across groups, the list of differentially expressed genes between groups was very short. A single gene (pyruvate dehydrogenase kinase 2, PDK2) stands out showing a much greater down-regulation (logFC=-2.7) in fast growing animals during compensatory growth.

Discussion and conclusions

Muscle transcriptome in age-matched slow growing animals appears to be closer to fast growers in size-matched animals suggesting an ontogenetic shift in expression profiles. The high similarity in transcriptome profiles of compensatory growth, on the other hand, seems to indicate that the potential for muscle growth is quite similar for either fast- and slow-growing fish. However, a key gene in the regulation of mitochondrial metabolism (PDK2) showed a consistently divergent pattern between fast- and slow-growing animals during compensatory growth. Fasting induces the repression of pyruvate dehydrogenase (PDH), which mediates the switch between carbohydrates and lipid oxidation in the mitochondria, favoring the consumption of lipids during starvation (Gudiksen and Pilegaard 2017). Significant down-regulation of PDK2, which inhibits PDH, during compensatory growth compared to control-fed animals suggests that in fast-growing fish mitochondrial metabolism shows a marked preference toward carbohydrate oxidation.

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SATELLITE-MAPPED BIOLOGICAL POTENTIAL FOR MOVING PACIFIC OYSTER AQUACULTURE OFFSHORE

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Introduction

On the French Atlantic coast, the possibility of expanding shellfish aquaculture, which traditionally takes places in intertidal areas, to offshore locations is considered to be a real possibility to overcome problems of carrying capacity, water quality and ecological impacts issues. Finding new sites into which to expand oyster aquaculture offshore relies on the co-occurrence of a combination of different requirements, but a basic prerequisite is to be able to obtain significant growth, survival, and quality of the product. This work is based in Bourgneuf Bay, south of the Loire estuary (France), where offshore experiments have been carried out since 2008 by a regional organization that supports innovation in the shellfish industry (Syndicat Mixte pour le Développement de l'Aquaculture et de la Pêche en Pays de la Loire (SMIDAP)). The main objective of this study was to highlight the potential for offshore Pacific oyster (*Crassostrea gigas* (Thunberg)) cultivation, and to investigate spatial patterns therein using satellite image time series data.

Materials and methods

Bourgneuf Bay is highly turbid, with annual mean Suspended Particulated Matter (SPM) concentration ranging from 27 to 129 mg L^{-1} from south to north, and maximum values exceeding 1000 g L^{-1} during spring tides (Gernez et al., 2017). This high turbidity has a negative impact on oyster growth and, which in turn contributes to the relatively slow growth of oysters in the bay (Gernez et al. 2014). A simple technique using bottom-cages was tested to collect off-shore growth data. These were used to calibrate a Dynamic Energy Budget (DEB) model of Pacific oyster growth, which incorporates SPM as a forcing variable in order to take the influence of high SPM concentrations on the bivalve ingestion (Thomas et al. 2016). The model was run with satellite-retrieved chlorophyll-a (Chl-a) as a proxy for food availability (mostly phytoplankton), SPM, and sea surface temperature (SST) as the driving variables. SST data were obtained from the Advanced Very High Resolution Radiometer (AVHRR) operated by the US National Oceanic and Atmospheric Administration (NOAA). SPM and Chl-a concentrations were obtained from the European Space Agency's Medium Resolution Imaging Spectrometer (MERIS) in full spatial resolution (300 m), with existing algorithms to obtain SPM and Chl-a concentrations locally-tuned for Bourgneuf Bay specifically (i.e., calibrated and validated using separate matchups between in situ and satellite datasets for the bay). All SST, SPM, and Chl-a products were aggregated to ten-day averages from 2003-2011 to create the regular time series data needed to run the model, given irregular overpass frequency (2-3 days) and gaps due to cloudiness of the original data. Oyster growth indicators were then mapped and compared at existing intertidal and hypothetical offshore farm sites within Bourgneuf Bay.

Results

The model output comprises oyster growth (shell length, transformed allometrically to total weight as described above, and dry flesh mass) maps at the same timestep as the input data (i.e., every ten days). Growth of total weight over time was then further transformed into several industry-meaningful growth performance indicators, using key market timings and market weight thresholds identified through consultation of producers and professionals from Bourgneuf and the neighbouring Marennes-Oléron Bay (Fig. 1).The results confirmed that offshore sites have better potential for oyster growth than the traditionally oyster-farmed intertidal sites overall, but that this is highly spatially variable. The current work uses higher spatial resolution input data than has previously been used, allowing corresponding higher spatial resolution model outputs, coherent with the farm-scale and therefore more applicable and relevant to the end-users considered. The output maps of growth over time can be used in Spatial Multi-Criteria Evaluation together with layers of other spatialized data that may influence whether production may be possible (e.g., bathymetry and distance from harbours, which determine feasibility) or how favourable it may be at a given site (e.g., presence of conflicting uses, such as fishing or tourism

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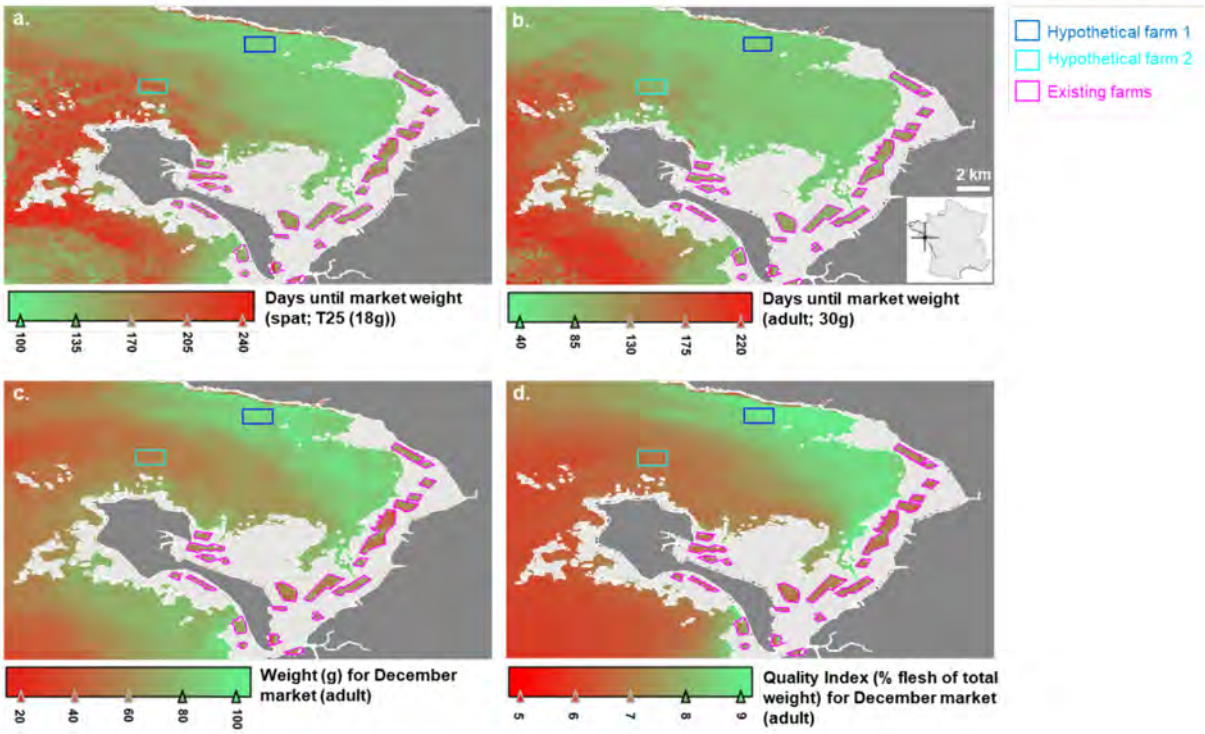

Figure 1. Maps of select oyster growth performance indicators for Bourgneuf Bay, with locations of existing farms in the intertidal zone, as well as those of the two hypothetical offshore farms. (a) Days until T6-T8 spat reach target market size to sell to other producers (size T25; approximately 18g); (b) days until adult minimum market size (30g) is reached; (c) weight (g) obtained for the (main) December market; and (d) Quality Index (drained flesh weight/total weight (%)) obtained for the (main) December market. Maps are of the mean indicator values for the full nine-year time series.

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BLOOD BIOCHEMISTRY DURING SEAWATER TRANSFER: INVESTIGATION INTO THE CAUSES OF FAILED FISH SYNDROME IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

The term failed fish (or runt) syndrome is a synonym for a fish with impaired growth or substantially slower growth rate after sea-transfer (Skjelstad and Gu, 2016). Although observed throughout salmonid aquaculture, (typically ~10%) it is greatly increased for rainbow trout (*Oncorhynchus mykiss*) reared in full salinity where failure levels of >30% are often encountered. Rapid acclimation to increasing salinity through physiological modifications is crucial in euryhaline teleost (Guner et al., 2006). Thus, this study objective was to compare the clinical chemistry and tissue structure differences in rainbow trout pre and post seawater transfer to investigate the impacts and potential causes of this syndrome.

Materials and methods

The study took place in a fish farm, under aquaculture conditions. Blood samples were initially taken from random pit-tagged fish 4 weeks before seawater transfer. Second blood samples were taken 8 weeks post seawater transfer. Fish condition was measured and tissue samples were taken for supportive histopathological analysis in both time points. All serum samples were analysed within 3 months of freezing on the clinical chemistry analyser Daytona RX (Randox Laboratories Ltd., Crumlin, UK) using commercial kits. Routine histology was used to produce tissue sections for histological inpection of trout tissues. Statistical analysis was performed using the General Linear Model (GLM) and Linear Discriminant Analysis (LDA) to classify the two groups according to their biochemical profiles

Results

Fish serum biochemical analysis showed significant increase activity ($p<0.05$) of ALP (alkaline phosphatase) and concentration of ammonia, recorded in the fish with good condition after the transfer to seawater. In comparison, fish with retarded growth had significantly higher activity of LDH (lactate dehydrogenase), together with decreased activity of amylase and concentrations of potassium, while concentration of chloride was higher after the transfer to seawater. Interestingly, despite no statistical differences could be found for the majority of measured endpoints in freshwater environment, it is noteworthy that the parameters with potential effect on osmoregulatory balance and nutritional deficit, chloride, sodium and zinc concentrations, respectively, were identified as the variables that distinguish best these two groups at initial stage of growth. Moreover, no significant differences in fish condition were observed. Histopathological evaluation of stunted fish liver showed hepatocyte shrinkage along with marked glycogen reduction. Furthermore, tissue damage to the eye chamber was evident in form of exfoliation of cornea.

Discussion and conclusion

Results of the present study indicate that initial fish size does not have any particular role in predisposition to runt syndrome. Runt fish showed signs of poor nutritional status that was observed through serum metabolic endpoints, but also some evidence of osmoregulatory dysfunction. Moreover, there was a proof that fish from the freshwater, that later became failed in seawater, had disturbed osmoregulatory function and zinc deficienc . Cataracts induced by zinc deficiencies have been described wild and farmed salmon (Bjerkås et al., 2004). A study by Bjerkås and Sveier (2014) demonstrated that fluctuation in water salinity caused higher intraocular pressure. This could be due either to increased influx through the damaged cornea, or as increased water uptake in the whole body due to poor fluid regulation through periods of osmotic stress. In addition, another possible cause for this syndrome in trout could be reduced vision due to cataracts that prevents normal feeding and eventually led to trout emaciation and mortalities. Blood biochemistry shows excellent potential to investigate fish health status and was associated with organ/tissue damage. Further research to evaluate the genetic aspect of this syndrome and better bloodstock selection could add to improvements in sea trout aquaculture.

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PHYTOPLANCTON AND LARVAL PATHOGENS IN BIVALVE HATCHERY: *Chaetoceros-vibrio*

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Introduction

Outbreaks of disease caused by some *Vibrio* species represent one of the main production bottleneck in shellfish hatcheries (Prado, 2006). Monitoring the routes of entry of bacteria should be the first step to prevent proliferation of these opportunistic pathogens in the bivalve cultures and the subsequent detrimental effects.

Phytoplancton, used as food and produced by mass culture, is one the main sources of bacteria. Previous studies have demonstrated that the marine heterotrophic bacteria were at level $\approx 10^6$ cfu/ml in the mixture of microalgal species utilized in the hatchery (Dubert et al., 2015). Vibrios were present, but not the main component of the population of culturable bacteria. In this work, we studied specifically the interactions between vibrios and active microalgal cultures of *Chaetoceros* spp., to deepen in the knowledge of the dynamic of the pathogens in the hatchery.

Materials and methods

The larval pathogens assayed were (P1) *Vibrio neptunius* PP-145.98, (P2) *V. ostreicida* P-203, (P3) *V. europaeus* PP-638 and (P4) *V. bivalvicida* PP2-605.

Two different species of *Chaetoceros*, *C. calcitrans* and *C. muelleri*, were cultured. In each experiment, one stock of microalgae in exponential stage and vibrio-free was distributed in sterile glass carboys with 2 l. of phytoplancton. The pathogens (suspensions in sterile seawater) were inoculated to reach final concentrations of 10^3-10^4 cfu/ml. Cultures without bacterial addition were maintained as control.

Cultures were performed following the routine in the hatchery of the Centro de Cultivos Mariños. They were kept for 7 days in the isothermal chamber, at 19±1ºC and under constant illumination. Each *Chaetoceros* species were assayed in two experiments, with two replicates.

Samples were collected in sterile containers and immediately processed *in situ*, following Prado et al. (2005) for enumeration of vibrios in Thiosulphate-Citrate-Bile-Sucrose (TCBS) plates. Results were expressed as cfu/ml. Microalgal cells were counted in cell chamber, with a light microscopy, expressing the results as cel/µl.

Results

Chaetoceros calcitrans

The growth of the microalga *C. calcitrans* was similar, independently of the presence of pathogens in the culture. Regarding to the pathogens, all of them decreased until low levels or even no detection at the end of the experiments.

Chaetoceros muelleri

The two stocks of *C. muelleri* showed different initial density, higher in the second experiment. Independently of this fact, the pattern of growth of the microalga was not completely similar between control and some inoculated cultures. In particular, P3 and P4 reached higher densities than control at the end of both experiments.

The results of the pathogens differed between experiments. In the first one, all the strains decreased since the initial samples, being below the detection threshold at the end. In the second one, P1 and P2 showed a similar pattern to the first challenge, while P3 and P4 increased their levels in the first 24 h, and decreased later but still found at the end

Discussion and conclusion

The experiments were performed under "real" conditions, with the aim of study the behaviour of the pathogens in the hatchery, specifically their ability to survive in phytoplancton cultures, as well as their possible pathogenicity for microalgae.

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As a first conclusion, this pathogenicity was discarded in all the cases. Regarding to the survival of the pathogens in *Chaetoceros* active cultures, at the end of the experiments all of them were at very low levels or even under the detection threshold, meaning an important decrease in relation to the initial concentrations. In contrast with these results, in laboratory assays the algal cells and the supernatant of *Chaetoceros* cultures did not display inhibition against any of the pathogens, suggesting that the active culture is an essential condition in the interaction.

These results could explain the low frequency of detection of vibrios in phytoplancton cultures. However, the survival of some larval pathogens together with the lack of pathogenicity for microalgae, also imply that the phytoplancton may be an "asymptomatic carrier" of vibrios.

Therefore, routine microbiological controls should be established in the hatcheries to prevent the possible transmission of vibrios from food to the larval cultures.

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Vibrio bivalvicida, A REAL THREAT FOR *Ostrea edulis*. THE NEED FOR RESEARCH ON THE DYNAMIC OF PATHOGENS IN HATCHERY

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Introduction

Bivalve hatcheries are currently the only viable solution to fulfil the spat demand for shellfish aquaculture. This fact is particularly relevant in species like the flat oyster, *Ostrea edulis*. The decline of natural beds and natural recruitment has led to the need of hatchery production of seed not only to sustain the aquaculture, but also, and not less important, to restore the original populations. Unfortunately, episodes of larval and post-larval mortalities compromise this production worldwide, being the aetiological agents of these episodes mainly bacteria belonging to some species of the genus *Vibrio*. A wide knowledge about the pathogens which affect the production of flat oyster hatcheries is the first step to develop innovative procedures to solve the problem.

In this work, we report a heavy outbreak of disease which affected the flat oyster cultures in a Galician hatcher .

Materials and methods

A'Ostreira is the only hatchery in Galicia, and one of the few in the world, that produces flat oyster spat. Recently, heavy mortalities were recorded simultaneously in the larval cultures, once they reached 180µm. The batches were spawns from different broodstocks, obtained from natural beds (wild oysters) and from rafts ('batea'), respectively. Given the gravity of the situation, microbiological analyses were performed to elucidate the origin of the problem. Samples of larvae, seawater of culture tanks, and phytoplancton used as food were processed and spread on Thiosulphate-Citrate-Bile-Sucrose (TCBS) plates. Bacterial isolation, preservation and identification of isolates followed the methodologies described in Prado et al. (2014). In addition, virological analyses were performed to detect the possible presence of ostreid herpesvirus OsHv-1 or OsHv-1 µVar by Realtime-PCR using the specific primers OsHV1BF/B4 and B probe (Martinot et al. 2010; OIE, 2018)

Results and discussion

The pathogen *Vibrio bivalvicida* was isolated from diseased larvae, regardless the broodstock, as well as from the seawater of culture tanks. The microbiological sampling of phytoplancton cultures used as food (*Tisochrysis*, *Isochrysis*, *Diacronema*, *Chaetoceros* and *Tetraselmis*) allowed to rule out the microalgae as the source of the problem. Similarly, the seawater used to fill the tanks was also discarded

All the samples analysed were negative for the detection of OsHV-1 or OsHV-1 µVar.
This is the first report of the pathogen *V. bivalvicida* in an episode of mortality of *O. edulis* in hatchery. All the oyster cultures were affected, with acute signs when they reached 180µm, which indicates that this pathogen is a real threat.

Regarding the origin of the pathogen, in this case, phytoplancton and seawater was discarded, while the broodstocks seemed highly unlikely as the source, considering the simultaneous mortalities in all the larval batches. Considering the previous detection of this pathogen in the hatchery, the risk of its persistence associated to the surfaces should be considered. The biofilm formation may confer important ecological advantages (resistance to antibiotics, bleach or desiccation, acquisition of genetic traits...) to the pathogen and imply the risk of re-emergence. Therefore, this approach should be introduced in the hatchery studies and management.

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NOVEL LARVAL REARING TANK DESIGN: CAN A NEW APPROACH TO HYDRODYNAMICS CHANGE THE WAY WE PRODUCE MARINE FISH LARVAE?

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Introduction

For a successful expansion of the Aquaculture industry, the production of robust, high quality juveniles with high growth potential needs to be enhanced, so the numbers of fish that eventually reach the market can suppress the consumer needs. However, fish larviculture is often considered a bottleneck in fish farming due to the variable quality of juveniles produced, with direct impact on survival and growth performance results during ongrowing. The mass mortality of larvae is one of the most significant problems that occur in the initial stages of production and can be due to small flow modifications in the larval rearing tanks caused, for example, by inadequate handling of the aerators or the inlet flow rates. Frequently, the management of the water flows in the larval tanks is based on the empirical knowledge of the growers (Sumida et al., 2013). Excessive flow can hinder larval feeding and force them to spend too much energy to maintain its position (Tucker, 1988), which can lead to mass mortality. On the other hand, if the flow is too weak, it usually induces the formation of "dead zones", with inadequate water quality for the larvae (Shiotani et al., 2003). However, not many studies have analyzed the influence of the flow pattern in aquaculture tanks, and those that focus on tanks for larval culture are even more scarce (Sakakura et al., 2007 and 2018, Blanco et al., 2014, Moorhead 2015).

In larval culture systems, tank geometry, water inlet and outlet devices, as well as aeration systems when they exist, play a key role in the distribution of the water velocities generated (flow pattern) and condition: 1) larval swimming activity, 2) buoyancy or sedimentation of food and microalgae, and 3) cleaning of solid waste generated (feces and food not ingested). In the last decade, significant research efforts have been made in the development of inert microdiets that can replace live feeds in larviculture, as early as the onset of the larvae exogenous feeding. However, the introduction of inert microdiets at first feeding can strongly deteriorate water quality in rearing tanks since the larvae have poor locomotory capabilities and feed needs to be given in excess so it is constantly available and within reach. As the tanks currently used are designed for live feeding, this excess accumulates at the bottom of the tanks and its removal is a difficult and a time-consuming task

Therefore, the objective of this study is to design a larval culture tank, which allows the generation of an effective water flow pattern, adjustable according to the size of the larvae. The maintenance of a uniform flow will be prioritized, favoring the homogeneous distribution of the larvae in the volume of the tank, avoiding relevant velocity gradients, which cause the larvae to collide with the walls of the tank and with the devices installed inside. Additionally, tank design will consider cleaning efficienc , aiming at ensuring excellent water quality and reducing labor associated with tank cleaning.

Methods

After analyzing the advantages and disadvantages of different tank geometries a first prototype of semicircular vertical section and rectangular horizontal section was selected. A second tank prototype was also constructed with circular horizontal section and rounded vertical section at its bottom. The capacity of each of the prototypes is about 90 liters and have air-lift systems that control the flow velocity. In addition to the hydrodynamic conditions, the specific design of each of them considered the ease of cleaning, the oxygen supply and the ability to control the water velocity. Moreover, both tanks have transparent sections that allow the characterization of the water flow using tracer particles that, properly illuminated, allow to register their trajectories and the distribution of speeds in their interior.

After tank hydrodynamics characterization, larval rearing success will also be assessed.

In the end of the study, one of the prototypes will be selected and real scale models will be produced and tested.

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Results

At the moment of this submission, the characterization of the relationship between the water flow induced by the air lift system and the angular velocity of the water in the first tank prototype has been accomplished. The water flow pattern obtained was, as intended, capable of homogenously distributing the particles in the water column. The efficiency of the cleaning system designed has also been demonstrated.

At poster submission deadline, it is expected to have further results on the hydrodynamic characterization of the second prototype, as well as, data that prove the adequacy of both prototypes for larval rearing.

Acknowledgements

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MICRODIETS SUPPLEMENTED WITH NUTRITIONAL ADDITIVES AS A TOOL TO IMPROVE IMMUNE CONDITION AND ENHANCE GROWTH PERFORMANCES OF WHITE-LEG SHRIMP (*Penaeus vannamei*) POST LARVAE

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Introduction

The white-leg shrimp (*Litopenaeus vannamei*) produced in aquaculture is a highly valued commercial product whose demand has substantially increased in recent years. To meet the market demands, larvae and post-larvae yields in hatcheries increased intensively, globally originating around 4.2 million tonnes of adult shrimp per year (FAO, 2018).

Nevertheless, initial developmental stages are critical and frequently associated with sub-optimal growth and low survivals due to opportunistic pathogens, often originating poor quality post-larvae with high disease susceptibility. Therefore, there is room for the creation of innovative microdiet solutions that can enhance development during the initial stages and consequently improve the shrimp quality in the posterior phases, increasing their resistance to stress and pathogenic factors.

In fact, industrial shrimp farming is extremely susceptible to pathogenic episodes resulting in disastrous consequences to production (Flegel, 2012). The use of antibiotics in the aquaculture industry is limited due to inherent environmental issues and the increased resistance between pathogens. Furthermore, shrimp depend uniquely on their innate immune system and cannot be vaccinated, which makes immune stimulation an extremely important strategy, with some authors already verifying an increase in survival of immune stimulated shrimp (Smith et al., 2003; Yogeeswaran et al., 2012). Since the modulation of the immune system through nutrition is currently possible the potential of innovative nutritional solutions that improve the immune condition of shrimp is foreseen as tremendous.

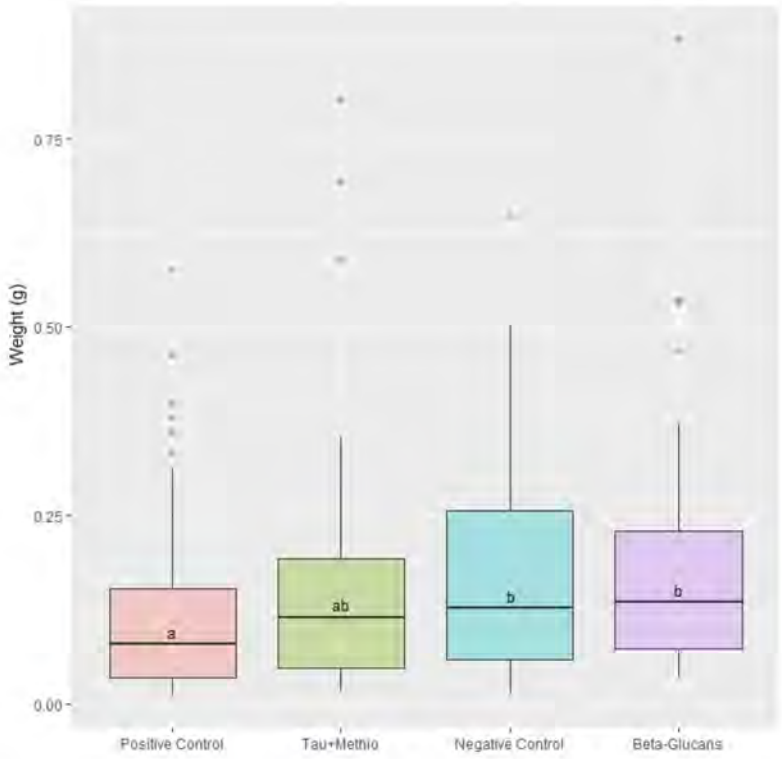

Figure 1 - Final body weight of white-leg shrimp post-larvae at the end of the trial (n=3 experimental units). Different subscript letters indicate statistical differences ($p<0.05$) between treatments.

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This study aimed to evaluate the effects of several health promoting nutrients/additives (i.e. vitamins C and E, beta-glucans, taurine and methionine) supplemented in microdiets on the growth performance, oxidative status and immune condition of white-leg shrimp post-larvae.

Methods

White-leg shrimp post-larvae (mean wet weight 5 mg) were fed isonitrogenous and isocaloric diets supplemented with different feed nutrients/additives. A commercial diet was used as Positive control whereas a Negative control diet was considered with vitamin C and E supplementation levels below than those used in the commercial diet. The two remaining diets consisted on the commercial diet supplemented with beta-glucans or taurine plus methionine. Each diet was tested in triplicate tanks. Shrimp were kept at around 28 ºC and fed *ad libitum* for 23 days. At the end of the trial, shrimp were individually weighted for growth performance determination and samples were collected for oxidative stress parameters and immune status assessment.

Results and discussion

Significant differences in growth performances between treatments were observed. Shrimp from the Negative control and Beta-Glucans dietary treatments achieved a higher final body weight than those from the Positive control at the end of the trial (Figure 1). However, no significant differences between treatments were observed in feed conversion ratio, relative growth rate and survival. These results will be further discussed in light of the oxidative stress and immune status assessment.

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DIETARY INFLUENCE OF YEAST AS A SOURCE OF NUCLEOTIDES ON TILAPIA *Oreochromis niloticus*

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Introduction

The use of nucleotides in fish nutrition has several benefits including, increased food intake, rapid intestinal repair, improved mucosal gut flora and mucosal surfaces (Li and Gatlin III, 2006). Nucleotides supplementation in salmonids diet increased resistance to infections caused by bacteria and viruses, and increased the efficacy of vaccination and osmoregulatory capacity (Burrells, *et al*., 2001a; 2001b). Literature approved the growth promoting effect of MOS and Immune-stimulatory power of β-glucan (Gu *et al*., 2011; Murthy *et al*., 2009).

The objective of this study was to evaluate the effect of yeast as nucleotide source on growth performance, oxidative stress, immune response, expression of some immune related genes and mortality of Tilapia (*Oreochromis niloticus*) challenged with *Aeromonas hydrophila* and *Lacococcus gravaeie*.

Material and Methods

A total of 270 *O. niloticus* (50.7 ± 0.8g of body weight) were distributed in a completely randomized design, with 3 treatments: 1- Control; 2- 0.2% of *Saccharomyces cerevisiae* as a source of nucleotides (YNU - Hilyses®, ICC Brazil Company); 3 – 0.2% of YNU, with 3 replicates each (glass aquaria of 40x30x100cm with 20 fishes each). The fishes were fed with 3% of its total biomass during 3 months and, the acclimation period was 2 weeks. The growth parameters measured were body weight (BW, g/ind.), body weight gain (BWG, g/ind.) and feed conversion ratio (FCR) at 1 and 2 months. Also the clinicophatological, oxidant parameters, relative quantitative PCR of immune gene expression, phagocytic activity (%) and index, lysozyme activity (µg/mL) were evaluated at 2 months, as weel the histological examination of liver, spleen, kidney and intestine. After 2 months (after the end of feeding trial), a challenge test was carried out with 10 fishes/group replicate inoculated intra-peritoneal with Gram positive bacteria *Lacococcus gravaeie* (0.2mL containing $3x10^8$CFU/mL) and Gram negative *Aeromonas hydrophila* (0.2mL containing $1.5x10^8$CFU/mL). Also, 10 fishes per each microbieal chellenge were injected with saline solution (Negative Control). The mortality rates were observed during 1 week. The challenge tests were executed two times. Un-paired one-way ANOVA test was used to test significance between groups at $p \leq 0.05$ after that post hoc test (Tukey) was used for pairwise comparison

Table I. Growth performance, oxidative stress, expression of immune related genes, innate immunological and mortality parameters, after challenges 1 and 2 with *L. gravaeie* and *A. hydrophila*, of Nile tilapia

Parameters	Control	0.2% YNU	0.4% YNU
BW (g) 2 months	97.86^{a}±1.18	123.6^{b}±2.54	130.5^{b}±4.08
BWG (g)	48.1^{a} ±1.96	71.3^{b} ±2.14	80.5^{b} ±2.04
FCR	2.1^{a}±0.26	1.65^{b}±0.11	1.5^{b} ±0.07
Catalase	268.73^{a}±43.8	354.87^{b}±39.6	402.27^{c}±25.4
G-redutase	142.7^{a}±3.5	160.76^{a}±2.34	269.93^{b}±20.6
IL1-β	0^{b}	0.6^{b} ± 1.9	4^{a} ± 2.2
TNF-α	0^{b}	3.07^{a}±.2	4.28^{a}±0.3
Phagocytic activity (%)	57^{b}	66^{a}	68^{a}
Phagocytic index	1.8^{b}	2.3^{a}	2.1^{a}
Lysozyme activity (µg/mL)	435.8^{b}	466.1^{a}	481^{a}
Mortality (%) *L.gravaeie*[1]	90^{b}	30^{a}	10^{a}
Mortality (%) *A. hydrophila*[1]	100^{c}	40^{b}	0^{a}
Mortality (%) *L.gravaeie*[2]	80^{b}	47^{a}	30^{a}
Mortality (%) *A. hydrophila*[2]	97^{b}	53^{a}	43^{a}

*Different letters in the same line indicate differences by Tukey test ($P<0.05$).

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Results

YNU supplementation improved the growth performance in relation the control group ($P<0.05$). Similary, YNU in both levels increased catalase and G-redutase activities, and expression of immune related genes parameters ($P<0.05$). Also, the phagocytic and lysozyme activity were increased ($P<0.05$). After the challenge with the both bacteria's, mortality decreased ($P<0.05$) in both groups with YNU (Table I).

There was no significant difference in clinicopathological parameters between 0.2% and 0.4% of YNU supplemented groups versus control group ($P<0.05$).

The spleen of YNU supplemented Nile tilapia showed focal wide area of melano macrophage aggregation in the white pulps. The YNU groups showed the intestine with mild hypertrophy in the lining mucosal epithelium with wide area for the crypts. Also, focal lymphoid cells proliferation was detected in the mucosa and submucosa. There was no histopathological alteration in the liver, the greater vaculation of the hepatocytes indicated well nourished fish. The kidney showed mild vacuolization in the tubular lining epithelium, focal lymphoid cell aggregation was detected between tubules in the fished of the group supplemented with 0.4% of YNU.

Conclusion

In general, the inclusions of 0.2 and 0.4% of YNU has proven to improve the growth performance of Nile tilapia, boost the immune response and increase the resistance against diseases.

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THE MULTIPLE FUNCTIONS OF PISCIDINS IN THE EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Introduction

Antimicrobial peptides (AMPs) are one of the host's first line of defenses against a wide range of infectious agents. Apart from the antimicrobial activity, AMPs are known to influence other biological processes, such as immunomodulation and iron metabolism (Chaturvedi et al, 2018; Neves et al, 2015). Fish present a specific group of AMPs, the piscidins. These peptides have been characterized in several fish species, being altered when fish are subjected to an infection, and showing antimicrobial activity against multiple pathogens. Furthermore, several studies have shown the potential of using synthetic peptides to promote fish survival upon infection (Katzenback, 2015). However, in the European sea bass (*Dicentrarchus labrax*), a commercially important fish produced in aquaculture, the identification of the several piscidin types and their biological roles remains poorly explored. Here, we characterize the piscidin family in sea bass. The expression of piscidin genes is evaluated under different stimuli, as well as the antimicrobial activity of piscidin peptides against several fish pathogens.

Material and Methods

European sea bass *(D. labrax*), with an average weight of 50 g, were subjected to different experimental conditions and piscidin expression was evaluated. Infection: different groups were i.p. infected with 1.0×10^5 CFU of *Photobacterium damselae* spp. *piscicida (Phdp)* or *Vibrio anguillarum* and samples collected at 24, 48, 72 and 96 hours post infection. Iron overload: sea bass were i.p. injected with 2 or 5 mg of iron dextran and samples collected at 1, 4, 7, 10 and 14 days post iron administration. To determine the antimicrobial activities of piscidins, 1.0×10^6 CFU of different bacterial strains were incubated for 24 hours with serial dilutions of the synthetic peptides. The following bacteria were used: *P. damselae* spp. *piscicida*, *P. damselae* spp. *damselae*, *V. anguillarum*, *V. alginolyticus*, *Aeromonas salmonicida*, *A. hydrophila*, *Edwardsiella tarda* and *Yersinia ruckeri*. Healthy sea bass were used for piscidin characterization at genomic and protein levels and basal expression.

Results

We identified seven different piscidins in sea bass, with different amino acid sequences and antimicrobial activities, depending on the pathogen and piscidin peptide. Most of piscidin genes present the highest basal expression in the intestine, except for piscidin 2, that is highly expressed in the gills. Our data show an up-regulation of piscidin genes after infection with *Phdp;* however, after infection with *V. anguillarum*, the expression of piscidins is, in general, down-regulated. Furthermore, preliminary results show that piscidin gene expression increases in fish that received a high dose of iron dextran, in a dose-dependent manner.

Discussion and conclusions

Our findings indicate that, *in vitro*, piscidin peptides have a direct effect against pathogens. However, different outcomes are observed for the piscidin genes during experimental infections. While infection with *Phdp* promoted an increased regulation of these genes, *V. anguillarum* seems to suppress the expression of piscidins. Furthermore, modulation of piscidin expression in our experimental models of iron overload shows that piscidins also respond to iron modulation, indicating that these AMPs may have other yet undisclosed functions asides antimicrobial activity. It is known that iron is also essential for bacterial progression during infection. Thus, iron is in a continuous regulation to be available for body processes, being also modulated to ensure that is inaccessible to pathogenic microorganisms. Further studies will focus on the administration of peptides in fish, to understand the role and mechanisms of action of piscidins under the context of immune response and iron metabolism regulation, and to possibly uncover a novel function for these particular peptides in fish

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CYCLOPTERUS-STR: DEVELOPMENT AND VALIDATION OF A GENOME- AND TRANSCRIPTOME-DERIVED MICROSATELLITE DATABASE OF *Cyclopterus lumpus*

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Introduction

Short Tandem Repeats (STRs) or microsatellites are molecular markers harbouring tandemly repeated oligonucleotide sequences (one to six nucleotides) that are ubiquitously distributed in eukaryotic genomes (Vieira et al. 2016). Based on their location in the genome, STRs are classified as Type I or Type II, where the former is located within functional genes and the latter within non-coding intergenic regions (Selkoe and Toonen 2006). Type I (transcriptome-derived, EST-STRs) and Type II (genome-derived, g-STRs) STRs are widely used in molecular ecology studies, conservation initiatives and marker-assisted breeding programs of numerous plant and animal species (Selkoe and Toonen 2006; Vieira et al. 2016). Maduna et al. (in revision) used an *in-silico* approach to develop a total of some 232,000 genome-wide STR from genome and transcriptome sequence data for the commercially important lumpfis *Cyclopterus lumpus* (L. 1758). Out of these genome-wide STR, multiple primer pairs per locus were successfully designed for 6926 STRs. The main objective of this study was (i) to construct and catalogue an online database of microsatellites and primers called *Cyclopterus*-STR for the commercially important lumpfis *Cyclopterus lumpus* (L. 1758), and (ii) assess the amplification efficienc , allele size, and level of polymorphism (i.e., number of alleles) of a small subset of primers reported by Maduna et al. (in revision).

Material and methods

The user-friendly interface for the *Cyclopterus*-STR database was developed using HTML, PHP language, JAVA scripts and MySQL database. The database has six tabs (Home, Tutorial, STRs, About, Contact and Related) and provides different search criteria for EST-STRs and g-STRs, primers, amplification efficienc , flanking sequences and physical map information (Fig. 1B). A total of 80 STRs (33 g-STRs and 47 EST-STRs) were selected for small-scale validation in a panel of 36 lumpfish individuals collected along the Norwegian coastline. Total genomic DNA was extracted from finclips using the *DNeasy*® Tissue Kit following the manufacturer's instructions (Qiagen). We amplified loci following the standard amplification reaction and cycling conditions outlined in Skirnisdottir *et al*. (2013) with modifications inherent to the present study, which involved optimizing the annealing temperature for each locus. Genetic diversity descriptors per locus, which include the number of alleles per locus (A_N), observed heterozygosity (H_O), and unbiased expected heterozygosity (H_E) were calculated in GENALEX v.6.503 (Peakall and Smouse 2006).

Results

Cyclopterus-STR web application provides the first repository of genome-wide STR markers for *C. lumpus*, which allows users to search for their desired marker properties such as type of repeat motif, repeat number, amplification success and level of polymorphism (if *in vitro* validated). Moreover, the user is presented with several primer sets per locus for *in vitro* testing. Fig. 1 shows a preview of the *Cyclopterus*-STR database, as well as different search options for STRs. Out of 33 g-STRs, 22 (67%) loci successfully amplified, of which, 17 were found to be polymorphic with A_N, H_O and H_E, ranging from 2–10, 0.012–0.821 and 0.012–0.829, respectively. While 37 out of 47 (79%) EST-STRs successfully amplified with 18 loci found to be polymorphic with A_N, H_O and H_E, ranging from 2–15, 0.040–0.923 and 0.040–0.918, respectively.

Fig. 1. Schematic representation of screenshots of the *Cyclopterus*-STR database. (a) Search for EST-STRs (applicable to g-STRs too) by repeat motif length and frequency or repeat sequence, and by linkage group (if available). (b) Search results along with primer information.

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Discussion and conclusion

The present study presents the first user-friendly web based molecular marker repository for *C. lumpus*. The molecular resource reported in *Cyclopterus*-STR are freely available to the scientific community to aid in constructing linkage maps and mapping loci involved in quantitative trait loci (QTL) for foraging-related traits in *C. lumpus*. Moreover, the genome-wide panel of STR will enable the use of marker assisted selection of desired traits in breeding programs of *C. lumpus*.

The overall amplification success rate was 73.75% from the 80 primer sets selected for in vitro validation, with the EST-STRs having a slightly higher success rate. The level of polymorphism per locus were higher for EST-STRs, indicative of substantial genetic variation in functional gene regions in the wild. Therefore, the reported EST-STRs will indeed aid in assessing adaptive genetic diversity which will provide valuable insight into the impact of escapees from aquaculture farms on the genetic patterns of functional variation in wild population (*sensu* Jónsdóttir et al. 2018).

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EFFECT OF PLANT DENSITY IN COUPLED AQUAPONIC SYSTEMS ON THE WELFARE STATUS OF AFRICAN CATFISH (*Clarias gariepinus*)

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Introduction

Very little is currently known about the effects of aquaponic systems onto fish welfare (Yildiz et al., 2017). However, Baßmann et al. (2017) described that African catfish (*Clarias gariepinus*) reared in a coupled aquaponic system had significantly fewer external injuries of skin, fins and barbels than fish reared in a control RAS without plant cultivation. Wounds i.a. facilitate pathogenic entry into the fish body, thereby impairing fish welfare (Huntingford et al., 2006). So, there is evidence that aquaponics not only supports sustainability and is resource-conserving technology, but also has advantages for fish welfare. Fish react to environmental influences through various physiological and behavioural adaptations (Barton, 2002) that allow conclusions to be drawn about their well-being. In this study, different welfare indicators were used to assess the welfare status of African catfish reared in coupled aquaponic systems with different numbers of basil plants (*Ocimum basilicum*) to determine the effects of the plant density.

Material & Methods

Three small-scale RAS were compared over 85d, each including fish tanks and hydroponic units in triplicates with a varying number of basil plants (control: n=0; moderate plant density, mpd: n=48; high plant density, hpd: n=144). African catfish were reared in an extensive stocking density (11.0 – 21.7 kg/m^3). Water changes and additional plant fertilisation were not conducted. Water quality and plant growth was documented; fish welfare indicators such as growth performance, mortality, plasma cortisol, glucose, lactate, biting wounds, and different behavioural patterns (agonistic behaviour*, air breathing*, curiosity, escape attempts*, stereotypy*, and swimming activity*) were analysed by visual and video observations (*ethology after van de Nieuwegiessen et al., 2008; 2009) in regular intervals. Prior to blood sampling, stress was induced by 10min air exposure and subsequently 1h confinement (after van de Nieuwegiessen et al., 2009) to observe potential alterations of the endocrine stress response. The data were statistically verified (p at 0.05). The results of different fish welfare indicators were evaluated using a newly developed ranking system, where the actual values were replaced based on their magnitude by numbers of 1-9. The higher the total sum of a treatment group, the lower the fish welfare was considered relative to the other groups.

Results

Most water parameters did not differ significantly between the groups; merely oxygen showed significant differences, with the highest mean concentration in the control (8.4 mg/L) and the lowest in the hpd (8.2 mg/L). pH slowly decreased from approx. 7.5 at the beginning to 5.3-6.1 ($p > 0.05$); on day 38 this was adjusted by application of an alkaline buffer, which raised the pH up to 8.3-8.4. This acted as an additional stressor and led to temporal alteration in the cortisol responses of the control and mpd group. Both showed elevations, whereas the hpd group showed an elevation priorly and lagged this acute response. Due to this progression cortisol showed significant differences (control: 18.8 ng/mL, mpd: 19.9 ng/mL, hpd: 25.8 ng/mL). Glucose and lactate levels did not differ significantly (control: 5.5, 2.6 mmol/L; mpd: 5.6, 2.7 mmol/L; hpd: 5.3, 2.6 mmol/L). The hpd fish showed the least activity (control: visual 77.8% / video 81.6%; mpd: 74.6% / 82.6%; hpd: 63.2% ($p < 0.05$) / 78.8%). High agonistic behaviour (control: 5 / 131; mpd: 4 / 57; hpd: 1 / 45) and the highest number of skin lesions (control: 3.9; mpd: 2.9; hpd: 3.4) were observed in the control. Growth performance and mortality of the fish did not differ significantl .

Discussion

Compared to literature data, water quality results were adequate for African catfish in each system; also growth and survival rate were regular. Some differences in welfare indicators between the groups were detected. For instance, in the hpd group an acute cortisol response reaction to the pH adjustment was suppressed. Based on the multivariate evaluation method, and considering the behavioural patterns, wounds and physiological indicators, the fish welfare inside coupled aquaponic systems was nevertheless assessed as high compared with the control. The presence of the plants as well as the increasing plant density proofed a positive effect on the fish. A possible explanation could be an allelopathic influence of either the plants or the associated microorganisms, in particular from the rhizosphere (Yildiz et al., 2017).

(Continued on next page)

Conclusions

Although welfare indicators do not always present a uniform picture and inter-individual differences may occur, the results demonstrate a visible effect of the aquaponic cultivation on fish welfare. According to the newly developed evaluation method (Baßmann et al., 2018), the fish associated with a high number of plants inside the same system showed the comparatively highest level of well-being.

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EFFECTS OF MONO AND MIXED BLOOMS OF COSMOPOLITAN HARMFUL ALGAE *Alexandrium, Karenia,* and *Chattonella* ON THE REPRODUCTION OF JAPANESE PEARL OYSTER *Pinctada fucata martensii*

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Several species of harmful algal blooms (HAB) cause mass mortalities of fish in aquaculture farms, including the cosmopolitan PSP- and non PSP-producing *Alexandrium catenella* and *A. affine*, ichthyotoxic dinoflagellate, *Karenia mikimotoi*, and the raphidophyte, *Chattonella marina*. Several aspects of the toxinology of these harmful algae remain intractable due to the complexity of the toxicity mechanism underlying the fish- and shellfish-kills. In addition, several harmful algal species have been recently shown to affect the reproduction of shellfish at very low cell densities. The effects of mixed blooms of harmful algae on aquatic organisms, however, have not been explored.

The Japanese pearl oyster was used as a model bivalve. Under laboratory conditions, the gonads of adult pearl oyster, *Pinctada fucata martensii*, were stripped of spermatozoa and oocytes, and artificial fertilization was carried out. Early developmental stages and larvae were used for experimental exposure to *K. mikimotoi*, *A. catenella*, *A. affine*, and *C. marina* in mono-specifi and mixed bloom experimental frames. The quality of gametes, fertilization success, embryogenesis as well as larval survivorship, activity and anti-oxidant enzymes were assessed during the exposures.

Differential negative effects on gametes, fertilization, embryogenesis, as well as larvae were found. Several interactions in mixed blooms of the harmful algae were found. The results of the current study, along with our recent findings, stress the effects of harmful algal species on the reproduction of bivalve molluscs and the importance of monitoring mixed harmful algal blooms in coastal areas and shellfish aquaculture farms

THE IMPACT OF GENOMIC SELECTION IN COMMERCIAL BREEDING PROGRAMMES FOR FOUR IMPORTANT SPECIES IN EUROPEAN AQUACULTURE

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Background
Genomic Selection has transformed animal breeding over the last two decades, especially for the major agriculturally important species. In aquaculture the implementation of genomic innovations in breeding are mixed. Genomic tools, such as SNP genotyping arrays, have been available for Atlantic Salmon and rainbow trout, and genomic selection is widely applied in these species. For European seabass and gilthead seabream the genomic tools are coming commercially available currently. Optimization of family structures to maximize benefits from genomic selection has been described (Nielsen et al. 2009; Sonesson & Ødegard 2016). The impact of genomic selection in practical breeding programmes needs to be demonstrated to support further implementation by the breeding industry. The application of genomic selection for quality and disease traits and also the use of partial datasets needs practical methods to be developed. For some species the design of genomic selection programmes with group matings needs to be investigated.

Materials and methods
Parameters from the designs of current commercial breeding programmes are obtained. These parameters include the number of parents and selection candidates, mating designs, as well as types of traits to be measured and their genetic parameters. The value of adding genomic data is assessed using deterministic predictions (Dekkers 2007; Rutten et al. 2002) when the designs allow for this, such as programmes where family designs are implemented. For other designs, such as group mating designs, the use of stochastic simulation is implemented in R software (R). In addition to phenotypes measured on selection candidates, including traits in the breeding programme that can only be measured in the commercial environment will be specifically ta geted.

Results
The impact of using genomic information within the current designs of commercial breeding programmes will be presented. The important measures of comparison are genetic progress over time and the rate of inbreeding under the alternative selection scenarios, with and without the use of genomic information. An assessment of implementation cost is made in terms of the number of genotypes required. Causes for differences between the impact of genomic information between the targeted species will be discussed.

Future work
Genomic selection is implemented in practical breeding programmes of four species that are important to European aquaculture as part of the European research project AquaIMPACT. In this project the application of genomic selection is focused on traits that need to be recorded in commercial conditions.

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DEFATTED YELLOW MEALWORM (*Tenebrio molitor*) LARVAE MEAL: A PROMISING FISHMEAL SUBSTITUTE FOR EUROPEAN SEABASS

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Introduction

Defatted yellow mealworm (*Tenebrio molitor*, TM) larvae meal is already industrially produced at a large scale. TM is a rich source of protein (up to 70%) with a well-balanced amino acid and fatty acid profile. This study evaluated the feasibility of replacing fishmeal (FM) by this sustainable ingredient in European seabass (*Dicentrarchus labrax*) juveniles. The growth performance, nutrient utilization, intestinal morphology, tissue composition, humoral innate immunity and flesh quality was evaluated in fish fed th TM as a partial and total substitute of FM.

Materials and methods

Four extruded isonitrogenous diets (48% crude protein on a dry matter basis) were formulated to replace increasing levels of FM by TM meal: 0 (CTRL), 40 (TM40), 80 (TM80) and 100% (TM100) replacement. Each diet was assigned to triplicate homogeneous groups of 25 fish (initial average body weight 55 ± 5 g) fed until apparent satiation for 10 weeks. Fish were subjected to a 12-hour light/12-hour dark photoperiod regime and kept in a recirculating saltwater system (35‰, 22±1°C). At the end of the growth trial, fish were individually weighed and measured. Five fish from each tank were sampled for whole body composition. Muscle was collected for total lipid content a fatty acid (FA) profile analysis and colour and texture measurements. A portion of intestine was sampled, fixed and processed for histological analysis. Plasma was collected to analyse alternative complement pathway (ACH50), lysozyme and peroxidase activity. The apparent digestibility coefficients of the experimental diets were also determined, according to Cho & Slinger (1979), after including 1% Cr_2O_3, as inert marker, in the experimental diets.

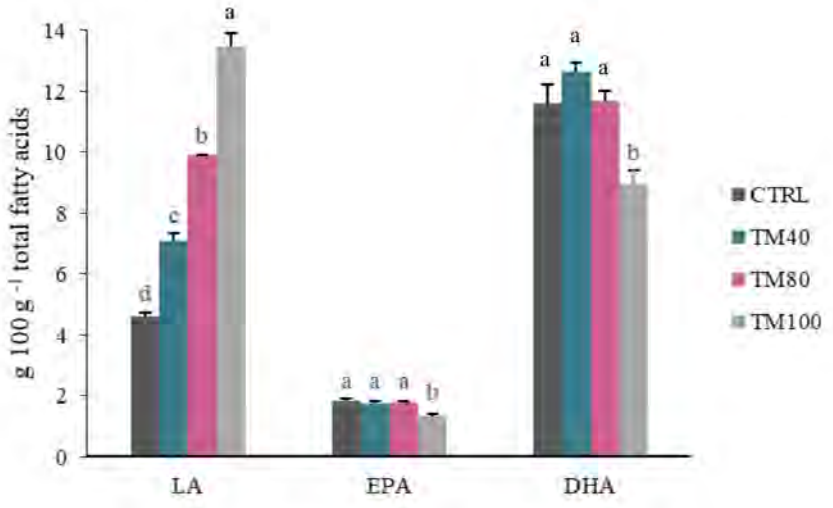

Fig. 1. Muscle level (g 100 g $^{-1}$ total fatty acids) of linoleic (LA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids of fish fed experimental diets. Values are presented as mean ± standard deviation. Different superscript letters differ significantly ($p < 0.05$).

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Results

Total replacement of FM by TM meal significantly reduced the protein digestibility of European seabass juveniles compared to the CTRL diet. The voluntary feed intake also decreased in fish fed TM100. But these fish had the best feed conversion ratio resulting in similar final body weight among treatments. Despite the significantly decrease of protein digestibility in fish fed TM100, the morphologic integrity of the intestine was maintained. Although whole body lipid content and the hepatosomatic index increased in fish fed TM100, flesh total lipid content, colour and texture remained similar among dietary treatments. The inclusion of TM meal resulted in a concomitant increase in the muscle level of linoleic acid (LA, 18:2n-6), whilst the eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids remained unaffected in fish fed diets up to 80% replacement level (Fig.1). The inclusion of TM meal did not alter the ACH50 and lysozyme activity but increased plasma peroxidase activity.

Discussion and conclusion

The present study shows that it is possible to replace up to 80% FM by TM meal without impairing European seabass juveniles' growth performance, nutrient utilization, flesh quality, intestinal morphology and humoral innate immunity. In fact, up to 80% of replacement of FM by TM meal fish resulted in fair levels of EPA and DHA in muscle (0.46 g 100 g $^{-1}$), which was above the recommended level for human consumption to decrease the risk of cardiovascular diseases (0.25 g 100 g $^{-1}$ portion of fish; EFSA, 2010). Altogether, these results evidence a great potential of TM to replace FM in diets for European sea bass juveniles.

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PARTIAL REPLACEMENT OF FISHMEAL BY DEFATTED YELLOW MEALWORM (*Tenebrio molitor*) LARVAE MEAL IMPROVES EUROPEAN SEABASS FEED EFFICIENCY

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Introduction
Defatted yellow mealworm (*Tenebrio molitor*, TMd) larvae meal can be an interesting alternative to fishmeal (FM), due to its high content in protein (up to 70%), well-balanced amino acid profile and eco-friendly features. Besides, its industrial production is already well established. This study assessed the feasibility of replacing FM by this sustainable ingredient in European seabass (*Dicentrarchus labrax*) from juveniles to market size. The growth performance and nutrient utilization of fish fed TMd as a partial and total substitute of FM was compared to that of fish fed a control diet

Materials and methods
Three extruded isonitrogenous and isolipidic diets (47% crude protein and 20% crude fat on a dry matter basis) were formulated to substitute 50% (TM50) and 100% (TM100) of fishmeal by TMd. Each diet was assigned to quadruplicate homogeneous groups of 15 fish (initial average body weight 68.6 ± 5 g) fed until apparent satiation for 16 weeks. Fish were subjected to a 12-hour light/12-hour dark photoperiod regime and kept in a recirculating saltwater system (35‰, 22 ± 1 °C). At the end of the growth trial, all fish were individually weighed and measured and four fish from each tank were collected for whole body composition analysis and nutrients balances calculations. Additionally, 30 fish per treatment were taken after a 48-h fasting period for the evaluation of fish slices by a sensory panel

Results
The experimental diets were well accepted by fish and no mortality occurred during the experiment. Fish fed TM50 had the lowest voluntary feed intake but attained similar final body weight, resulting in the best feed conversion ratio (FCR) and highest protein efficiency ratio (PER) among treatments. Fish fed TM100 had higher whole body fat content than fish fed CTRL, but the hepatosomatic and viscerosomatic index (HSI and VSI, respectively) remained unaffected by TMd inclusion.

The inclusion of TMd to replace FM resulted in a significant increase in fat and phosphorus retention, without affecting nutrients' gain. The sensory panel could not perceive any significant differences in the slices global acceptance from fish fed the different experimental diets; all the samples were positively characterized by their good aspect, odour, texture and flavou . Textural properties were also similar among fish, but fish fed with TMd showed a higher flesh juiciness than those fed the CTRL diet.

Discussion and conclusion
The present study shows that the substitution of 50% FM by TMd significantly improves FCR and PER, without affecting fish growth. Likewise, total replacement of FM by TMd didn't impair European seabass growth performance, HSI and VSI, but increased the whole body fat content, suggesting a strong impact on lipid metabolism. On the other hand, the increased fat retention in fish fed diets with TMd may explain the higher juiciness of its flesh.

These results evidence the great potential of TMd to replace FM in diets for European seabass, but the total substitution of FM has to be addressed with caution in a long term trial.

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LIGHT RESPONSES OF SALMON LOUSE (*Lepeophtheirus salmonis*) COPEPODITES

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Salmon louse (*Lepeophtheirus salmonis*) infection is still a large problem for salmon aquaculture in Norway as well as in other salmon-producing countries, although knowledge about this species is increasing. To be able to find solutions to this problem, more knowledge is needed on the basic biology and behaviour of the species. Investigating the light response behaviour of the salmon louse infectious stage, the copepodite, is important regarding responses to both natural and artificial light in the ocean, as well as in exploring the potential of using light as remedy to decrease infection rates.

Light responses of copepodites were investigated using a laboratory experimental setup modified after Miljeteig et al. (2014). The light sources used were the wavebands white, blue, and green, and each of these were tested at different irradiance levels and pulsation settings. The first set of experiments (E1) was performed in a 40x12cm aquarium with 150-200 copepodites per trial, to investigate the movement of the copepodite group relative to the light stimulus. The second set of experiments (E2) was performed in a 20x12cm aquarium with 12 copepodites per trial, to track individual copepodites and investigate parameters like swimming speed and directionality, using the same light stimuli as in E1.

The movement of the copepodites were registered using an infrared-sensitive camera with adjustable frame rate, and data was extracted from the videos using image processing. For E1, positions of the copepodites, and thus distance from the light stimulus, were detected throughout the experiments. For E2, an additional tracking algorithm was developed and applied.

Results will be presented at the conference.

The experimental setup and the data processing methods developed in this study can be adjusted to application on many different planktonic species and with other types of stimuli, and has thus the potential to be used for a wide range of studies.

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IN VITRO AND *IN VIVO* ASSESSEMENT OF THE SUSCEPTIBILITY OF RAINBOW TROUT (*Oncorhynchus mykiss*) TO THE VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS UNDER ALTERNATIVE FEEDS (*Hermetia illucens*, *Arthrospira platensis*)

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Introduction

Genetic Adaptions to new environmental conditions are crucial in the evolution of fish populations. Especially in aquaculture facilities, sudden changes in nutrition can occur, due to changes in the composition of the feed. The use of fishmeal is being controversially discussed due to overfishing of the oceans. Therefore, the replacement of fishmeal with alternative protein sources is a main and timely scientific focus. Supplementations might lead to slower growth or affect fish health or animal welfare.

"Sustainable Trout Aquaculture Intensification - SusTAIn" is a cooperative project that explores the genetic variability of trout to adapt to novel resources in order to use these for a sustainable aquaculture. The impact of feeding substitute protein sources on the health of fish is a main focus of the project. This aspect is examined by evaluating chronic stress and analysing gene expressions in order to assess immunological responses and the susceptibility to trout specific pathogens.

Materials and methods

Eight local strains of rainbow trout and a commercially available strain were fed with differently supplemented feeds (control feed, feed supplemented with *Hermetia illucens* or *Arthrospira platensis*) and compared in growth performance and susceptibility to trout-specific pathogens. The susceptibility of trout to the viral haemorrhagic septicaemia virus (VHSV) was evaluated by *in vitro* and *in vivo* studies for the best and worst performing strains. For the *in vitro* evaluations, fin tissue and primary cell cultures from scales from the different trout strains, which were fed with the differently supplemented feeds were infected with VHSV. The grade of replication of VHSV in the *in vitro* infections was evaluated using titration on RTG-2 cell cultures with determination of the 50 % tissue culture infective dose ($TCID_{50}$). For the *in vivo* studies, fish from each feeding group and strain were infected with VHSV. Mortality was evaluated on day 29 post infection.

Results

The analysis of *in vitro* infection of fin tissue of parents of eight trout strains under control feed did not show any significant differences in virus replication.

The analysis of *in vitro* infection of fin tissue of the three feeding groups of best and worst performing strains did not show any significant di ferences in virus replication.

Results of *in vivo* experiments indicated that the some trout strains showed significant differences in mortality rates with over 75 percent difference between most susceptible and resistant strain. The mortality was independent from growth performance of given strains of fish, also feed supplements had no impact on mortality under VHSV-infection.

Conclusion

The preliminary results indicate, that the survival of rainbow trout from VHSV-related disease under severe disease pressure, is predominantly influenced by the genetic background. These results proved to be independent from feeding of fishmeal substituted feed and growth performance. Summarising: Fish meal protein replacement with *Hermetia illucens* or *Arthrospira platensis* should not influence the susceptibility of the rainbow trout to VHSV.

IDENTIFICATION AND PATHOGENICITY OF *Vibrio* spp. ISOLATED FROM RECIRCULATING AQUACULTURE SYSTEMS (RAS) STOCKED WITH PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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Introduction

Some *Vibrio* sp. play an important role as pathogens in aquaculture of shrimps (e.g. *V. parahaemolyticus*, *V. harveyi)*, others can be the cause of wound or diarrhoeal infections in humans (e.g. *V. parahaemolyticus*, *V. vulnificus*). Still, these bacteria are ubiquitous in marine and brackish aquatic environments and a part of the normal flora of shrimps´ digestive tract and skin. For the detection of *Vibrio* spp. a reliable method of identification is necessary and for evaluation of the potential risk that derives from these isolates the analysis of different pathogenicity factors is possible.

Materials and methods

Different methods of identification, which are suitable for standard diagnostics in laboratories, were compared. Biochemical identification and identification using mass spectrometry (MALDI-TOF) were conducted. Furthermore, parts of the *16S* rRNA gene with two different sequence lengths (V1-V5, V1-V8) and parts of the housekeeping gene *pyrH* were sequenced.

Known pathogenicity factors were analysed that induce an elevated pathogenicity in certain *Vibrio* spp.. These include factors that elevate the pathogenicity of *V. cholerae* (VPI, ToxR, ToxS), as well as different haemolysins (*vhh*, *vfh, tdh, trh*). The motility of the isolates was analysed and the presence of different flagelline gene loci was investigated, as these contribute to motility in bacteria and can promote the adhesion on mucous membranes.

Results

The identification of *Vibrio*- isolates from RAS and type strains using different methods of identification proved to be highly divergent. Sequencing of the variable regions V1-V8 of the *16S* rRNA gene and MALDI-TOF mass spectrometry proved to be the most reliable methods. Identification using MALDI- OF was especially efficient for pathogenic species.

Analysing pathogenicity factors showed that RAS are a habitat for potentially pathogenic *Vibrio* spp.. Even in RAS with healthy shrimp potentially pathogenic *Vibrio* spp. could be found. Therefore, the analysis of pathogenicity factors can only give hints toward a risk of infection.

Conclusion

The identificati n of *Vibrio* spp. using standard diagnostic methods was difficult and for many isolates, the identification results were divergent. The investigations on isolates from different systems showed that potentially pathogenic *Vibrio* spp. can be found in RAS. Missing entries of sequences in the databases, low numbers of comparable isolates and high interspecific similarities of biochemical characteristics or nucleotide sequences are the main reasons for the difficulties in reliably identifying some *Vibrio* sp.

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DEVELOPMENT OF OFFSHORE BIVALVE SHELLFISH PRODUCTION IN THE EASTERN IRISH SEA

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Blue mussel (*Mytilus edulis*) is an important aquaculture species in the UK and particularly Wales (Ellis et al, 2015). The traditional method currently utilised in the Menai Strait involves collecting and placing wild seed mussel on the sea bed, followed by management to market size. The Menai Strait production area supports harvests of 7-10,000 tonnes of mussels per annum, accounting for 30-50% of all UK mussel production (Blue New Deal, 2018).

Reliance on wild mussel seed is a limiting factor to industry expansion (Çelik et al, 2016). In Wales recent observations have suggested that mussel seed beds have been in decline with inconsistent seed availability potentially affecting volume and profitability of current mussel production. The use of longlines and raft systems for seed collection as well as mussel grow-out are commonplace globally (Kammermans and Capelle, 2018) and represent a potential alternative for sourcing mussel seed.

The Menai Offshore Subsurface Shellfish Systems (MOSSS) project is a collaboration between Bangor University and the Menai Strait mussel industry. The aim is to develop innovative offshore rope systems to ensure a reliable supply of good quality mussel seed. Research is focussed on assessment of the site as a suitable area for the production of mussels and assessment of the collection seed on artificial substrates. Despite the failure of intertidal and benthic seed settlement plankton surveys indicate that bivalve larvae remain highly abundant in coastal and offshore areas in the eastern Irish Sea.

Within MOSSS mussel seed collection is being developed using a longline system at a pilot commercial-scale offshore site in north Wales. The site was selected based on key physical and biological parameters. For example, we used results from biophysical modelling of mussel larval dispersal from known adult populations, observations and simulations of waves, tides and winds, seabed survey data and analysis of vessel traffic in the area. Furthermore, we conducted planktonic monitoring including; genetic analysis to accurately quantify bivalve larvae at species level and initial technical trials have tested different collection materials and practical operation of mussel seed lines in an area with a large tidal range.

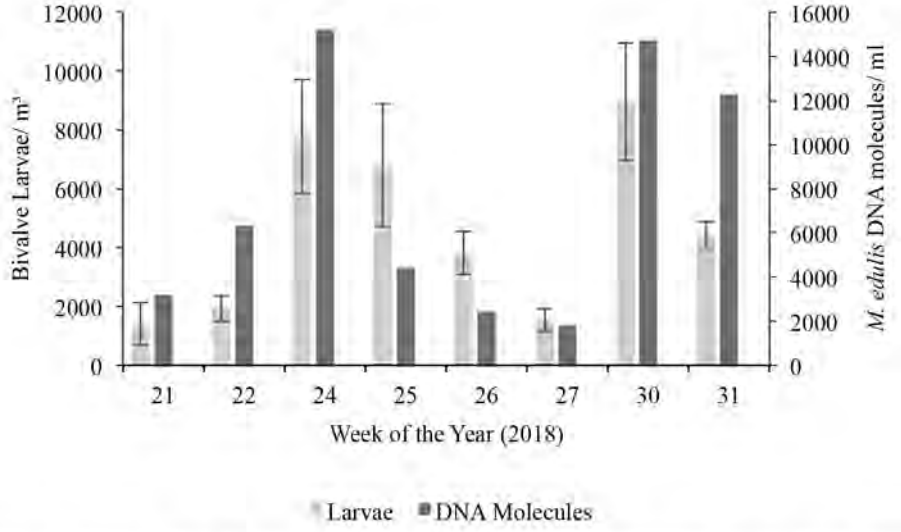

Figure 1. Mean bivalve larval abundance (light grey) and concentration of *M. edulis* DNA molecules (dark grey) in plankton samples collected from the experimental longline site between week 21 and 31, 2018.

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Regular monitoring of the plankton during the summer of 2018 demonstrated high quantities of bivalve larvae, with peaks in bivalve larval abundance coinciding with peaks in *M. edulis* DNA, indicating a high supply of mussel larvae at the experimental site (Figure 1). Settlement of spat during the summer of 2018 was successful, and after a three-month growing period, five header lines, each holding 1500 m of rope yielded 30 tonnes of seed, with the most successful lines carrying a maximum of 3.6kg m^{-1}.

This initial investigation into the potential for suspended mussel seed collection has been a success, though consistency of seed supply and settlement still needs to be evaluated. The integrity of the rope system over the following extreme winter of 2018/2019 also demonstrated the possibility for offshore grow-out cultivation even in such a high energy location with extreme wind and wave events and a tidal range of >7m.

In the summer of 2019, the MOSSS project will trial methods for assessing the potential for grow-out of mussels at the experimental site. The aim is to extend the existing site by considering operational experience gained thus far along with hydrodynamic (wave and tide) modelling. The project will outline the development of a multi-criteria assessment of potential areas for offshore mussel production in the Eastern Irish Sea for use by stakeholders to identify expansion of the aquaculture industry in this region.

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ASSESSING THE EFFECTS OF GAS INFUSION SYSTEMS TECHNOLOGY TO PRODUCE HIGH DISSOLVED OXYGEN FRESHWATER SATURATION ON ATLANTIC SALMON (*Salmo salar*) GROWTH AND OVERALL HEALTH WITHIN A SIMULATED COMMERCIAL HATCHERY SETTING

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Technological advances that promote optimal growing conditions for enhanced fish performance and fish health are necessary to ensure RAS systems are operated in a cost-effective manner. Dissolved oxygen is an essential and costly component for RAS but having the ability to supersaturate water with elevated infused dissolved oxygen concentration while not increasing total gas pressure presents interesting possibilities to explore optimal aeration for RAS performance.

GIS Gas Infusion Systems Inc., has developed a unique oxygen infusion system based on the principal of “mass gas transfer”. This involves a one to one molecular exchange in water between nitrogen (removed) and oxygen (infused) resulting in no increase to total gas pressure.

The current study objective was to assess the effect of the GIS technology on performance, growth, survival and overall fish health over a period of 264 days. A total of 13,500 Atlantic salmon first feeding fry were equally distributed into nine study tanks. The experimental setup involved 3 tanks for each treatment: DO ambient (90-100%), DO medium (140-160%) and DO high (190-210%). A density reduction plan was designed to simulate commercial stocking densities and all fish were fed to satiation. Bulk weights for each tank occurred monthly, in addition to individual fish weights and body condition scores at cutbacks. Dead fish were removed daily, recorded and necropsied. Serum samples were collected pre and post smolt to assess Hct levels. At 520-degree days post vaccination, further serum samples were collected and analyzed for IgM titer levels using ELISA.

Major study outcomes include the following: 1) both high and medium DO groups demonstrated a 168% increase in weight over ambient, 2) high and medium DO groups had a minimum 50% increase in survivability over ambient, 3) there was no statistical difference in precocious male percent for all treatment groups, 4) no acute or near-term mortalities (60 days post smolt) were associated with smoltification from high/medium DO freshwater directly into ambient saltwater, 5) median IgM optical density increased by 0.06 (doubled) for high/medium DO groups compared to ambient.

The study results give a clear indication that the GIS infused dissolved oxygen levels at 150% provided enhanced fish performance levels, improved fish health for an optimal economic return. RAS operators now have the technology to achieve “faster growth – better health” with a unique, scalable oxygen infusion system.

SENSITIVITY OF NORTHERN SHRIMP *Pandalus borealis* TO COMMERCIAL FORMULATIONS USED AS ANTI-PARASITIC DRUGS

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Introduction

The ecto-parasitic salmon louse (*Lepeophtheirus salmonis*) is one of the main challenges in marine aquaculture. Chemical delousing is used in addition to alternative methods like cleaner fish and mechanical removal of the crustacean parasite. The use of chemicals as medicine has raised concerns over the impact on non-target crustaceans like Northern shrimp *(Pandalus borealis),* a keystone species in the marine ecosystem.

Materials and methods

A series of laboratory experiments has been performed, exposing shrimp to environmentally realistic exposure concentrations of four commercial formulations used as anti-parasitic drugs; 1) the in-feed drug Releeze, containing the chitin synthesis inhibitor diflubenzuron (DIF); the bath treatments Alpha Max (2) and Salmosan (3) with the neurotoxic compounds deltamethrin (DEL) and azamethiphos (AZA), respectively, as the active ingredients; and the bath treatment Paramove (4) containing hydrogen peroxide (H_2O_2). Shrimp were exposed to DIF medicated feed under current (C: 7.0 °C, pH 8.0) and future global change conditions (Ocean Acidificatio and Warming; OAW: 9.5 °C and pH 7.6) in flow-through exposure systems. DEL, AZA and H_2O_2 were only tested at current conditions. To document potential delayed effects, a recovery period in clean water was included in all experiments.

Results and discussion

Effects of DIF on shrimp larvae. Two weeks exposure to DIF medicated feed caused significantly increased mortality. The effect of OAW and DIF on mortality of shrimp larvae was additive; 10 % mortality in C, 35 % in OAW, 66 % in DIF and 92 % in OAW+DIF. In OAW+DIF feeding and swimming activity was reduced for stage II larvae and none of the surviving larvae developed to stage IV. Due to shorter intermoult period at elevated temperature, the OAW+DIF larvae were exposed throughout two instead of one critical pre-moult period. This can explain the more serious sub-lethal effects for OAW+DIF than DIF larvae. A single day exposure at 4 days after hatching did not affect DIF larvae, but high mortality was observed for OAW+DIF larvae, possibly because they were exposed closer to moulting when crustaceans are more sensitive to chitin synthesis inhibitors. The additive effect of DIF (local driver) and OAW (global drivers) on survival of shrimp larvae emphasize the importance of managing the local drivers (reducing pollution) to slow down the detrimental impact of future global environmental changes

Effects of DIF on adult shrimp. Ovigerous shrimp were exposed to DIF medicated salmon feed for two weeks before their post-hatch moult. The shrimp were exposed to DIF under the same current and future environmental conditions as the shrimp larvae. The mortality of DIF exposed shrimp was approximately 60 % higher than mortality of control shrimp, and while more than 60 % of the control shrimp moulted successfully, only 2 - 7 % of DIF exposed shrimp survived moulting. Most of the mortality occurred during moulting in the depuration period, and even after four weeks of depuration, when the mean tissue concentration was still 33 ng g^{-1} w/w, mortality was higher for DIF exposed shrimp. High mortality was observed for adult shrimp after consumption of approximately 0.1 g of DIF medicated feed. Mortality was also higher in OAW than in C, but the combined effect of DIF and OAW was less than additive. In an additional experiment, where adult shrimp without eggs were exposed to DIF medicated feed, high moult-related mortality was observed already after four days exposure. Some exposed shrimp did, however, moult successfully after two-three weeks of depuration.

Effects of H_2O_2. The Paramove treatment solution for salmon contains 1500 mg/L H_2O_2. Increased mortality and reduced feeding rate were observed after exposure to three 2-hour pulses of 1.5 mg/L. Increased mortality was also observed after one 2-hour pulse of 15 mg/L. Mortality occurred around three days after the first pulse (delayed effect). Gill damage and evidence of lipid peroxidation in the hepatopancreas was documented after one hour exposure to 1.5 mg/L and 15 mg/L H_2O_2, and the effects were more evident at the highest concentration.

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Effects of DEL. The Alpha Max treatment solution for salmon contains 2 µg/L DEL. When adult shrimp were exposed to seven 2-hour pulses of 2 ng/L DEL they survived, but the feeding rate was significantly reduced during and after the exposure. Tissue damage in the hepatopancreas was also observed. Continuous exposure to 2 ng/L DEL for up to 24 hours was lethal to adult shrimp. Shrimp larvae were even more sensitive to DEL than the adult shrimp. High mortality of larvae was observed after one 2-hour pulse of 2 ng/L DEL, and few of the survivors were able to swim.

Effects of AZA.The Salmosan treatment solution for salmon contains 100 µg/L AZA. When adult shrimp were exposed to seven 2-hour pulses of 100 ng/L AZA there was no effect on survival, swimming activity or feeding rate, but indication of tissue damage was observed in the hepatopancreas. One 2-hour pulse of 100 ng/L AZA did not affect survival of shrimp larvae.

Conclusions

High mortality of shrimp was observed after exposure to Releeze medicated feed (DIF) and after a few hours of exposure to Alpha Max (2 ng/L DEL) or Paramove (1.5 mg/L H_2O_2). This means that the shrimp were affected at 1000 times diluted salmon treatment solution for these bath chemicals. Similar dilutions of Salmosan (AZA) had much less effect on the shrimp. The results from these experiments combined with results from dispersion modelling (e.g. Refseth et al. 2016) indicate that the use of chemicals against salmon lice is a risk to shrimp stocks living in areas with frequent use of chemical delousing.

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FACTORS DETERMINING THE CONDITIONS OF THE MARINE AQUACULTURE (CAGE CULTURE) DEVELOPMENT IN THE SOUTHERN CASPIAN SEA

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Introduction

The Caspian Sea is currently the largest internally drained lake in the world, with a total area of 375.000 km2, and is located in south-central Eurasia, between Iran, Azerbaijan, Russia, Kazakhstan and Turkmenistan. It is situated at 28 meters below from world ocean level. Classified as a deep inland sea for its size, depth, chemical properties, and peculiarities of thermohaline structure, water circulation the Caspian Sea conditionally divided into three parts: South Caspian, Middle Caspian and North Caspian (Aladdin and Plotnikov, 2004). Due to its closed ecosystem, the Caspian Sea has a high potential and high risk in non-native aquatic species introduction. The Caspian Sea, as one of the most important water resources in Iran, has good weather and maritime aquaculture abilities. Many breeding species have the ability to breed in this region for half a season or all-round, with the depths of the Caspian Sea in the southern region and a wide range of temperature variations throughout the year (6 to 32 ° C) and average salinity of 12.5 ppt.

More than 62 countries are currently active in cage aquaculture. Total crop production in cages in 2005 was about 3.4 million tons and the total aquaculture production in the world in 2006 (with aquatic plants) was about 66.7 million tons worth 85.9 billion dollars and the share of aquaculture was cultivated 19.3 million tons and marine fish were 2.2 million tons. By 2025, the forecast of marine fish production will be about 10 million tons, and Malaysia, Thailand, Vietnam, China, Norway and Iceland are the leading countries for fish farming in this way (Halwart et al., 2007)

Cultivation of fish in Iran with rainbow trout has been started in recent years with floating cages at depths of 30-50 m in the southern Caspian Sea.

Materials and methods

Physico-chemical parameters data were collected via water bottle sampler along regions which the fish cage culture site located in Nowshar off the Iranian coast of the southern Caspian Sea in the bottom depths of 30 m in years of 2013-18. Economic and Social data were also collected through library archives via relevant experts in the department of aquaculture of the Iranian Fisheries Organization as well as Rainbow trout fish cage culture site in Mazandaran province, Iran. In this study, the growth of salmon fish was evaluated based on the production input of each floating cage with a diameter of 20 meters and a height of 8 meters in the southern part of the Caspian Sea during autumn and winter and based on the initial inputs (fish and food costs). Also, the study of efficiency (production to cost), net worth value (NPV), internal rate of return or guaranteed rate of return (IRR), investment evaluation through descriptive statistics was analyzed.

This study, based on the assumptions about the production of 25-20 tons per cage and the experiences of fish breeding in cages in 2013 (autumn and winter) based on the amount of final produce and food consumed and the purchase of fry fish between the two initial storage weights of 150 and 250 g has come to fruition.

In this study, fish prices and food prices in 2014 were used. Therefore, the price of pre-paid fish for each kilogram of 5 USD and the price of food per kilo of 1 USD and the forecast of the price of fish in the harvest of 4 USD were used

Table 1: Statistical data of the average profit from fish farming in marine cages

Profit (%)	Sale (USD)	Food cost (USD)	Fry fish cost (USD)	Initial weight (g)	Fish Cage#
15.1	117	54	21	500	5 site

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Results

The purpose of this study was to evaluate the physico-chemical parameters effective on water quality as well as the economical and social feasibility of cage fish farming in the southern Caspian Sea.

Results showed that the amounts of water temperature, transparency, pH, dissolved oxygen, BOD5, total alkalinity and TSS ranged from 9.00 to 29.00˚C, 0.50 to 12.00 m, 8.05 to 8.74, 5.76 to 12.85, 21 to 195 and 0.00 to 0.12 mg/l, respectively which could be favorable for fish rearing in the sea. The proper temperatures for cultivation of salmonids species in this area were begun in October and ended in March. In the current study, results of pH and dissolved oxygen were consistent with the standard range of pH (7.80-8.50) and dissolved oxygen (>5 mg/l) for cage fish farming.

The results of rainbow trout breeding in cage culture in the Caspian Sea in 2013 showed the range of the number of fry stocks were 8500 to 58500 individuals with an average weight of 35 to 250 g, that the average profit during the 4 months was 7.3% (Table 1).

Discussion and conclusion

One of the most important factors in fish breeding in cages is the use of appropriate species in terms of adaptation to the biological conditions of the region and its economic significanc . In the current situation, rainbow trout can be one of the options, given the outstanding features of aquaculture and adaptation to the environmental conditions of the South Sea region. Therefore, in this study rainbow trout as an appropriate breeding species in the southern part of the Caspian Sea was used in this assessment. This region with a coastline of 250 km from a depth of 20 to 100 m and 2027 km^2 has the cage aquaculture capacity. Mazandaran with a population of more than 3 million and about 4.9 percent of the population has an area of 1.46 percent of Iran (23,842 km^2). Considering the potential of the aforementioned region in the coastal strip of the southern Caspian coastline, at least 200 cage-producing sites could be expected with 40 cages per site and produce 160,000 tons, and generate employment of 1,000 directly and 2000 indirectly with 2000 Billions income per year and average net profit of 40% can be expected to be 800 billion, which producing part of the country's protein requirement, will create jobs, prosperity, prosperity and prosperity of the region's economy.

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INDIVIDUAL PHOTO - IDENTIFICATION OF FISH WITH VISIBLE SKIN PATTERNS (SUMATRA BARB *Puntigrus tetrazona)* USING COMPUTER VISION

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Introduction

Nowadays increasing the consumption of fish production and aquatic organisms pushes aquaculture to produce fish more intensively. The trend of increasing aquaculture production leads to automation of processes for increasing the profits and facilitate the cultivation process. Automation of fish cultivation process helps to control general issues of aquaculture. The idea of automatization of fish production is the general aim of precision fish farming concept where control – engineering principles are applied to fish production process, increase the possibilities of fish farmers to monitor, control and document all biological processes of fish cultivation process (Fore et al., 2017). One of the main parts of automation of the process of fish cultivation are fish identification and tracking the fish trajectory in tank. Individual identification of fish is mostly based on fish marking and tagging (Pine et al., 2003). These methods are invasive and negatively effect on a fish, increase the mortality risks, injuring and highly stressful for the fish (Ombredane et al., 1998). Despite, today studies about non–invasive identification of individuals in fish groups are not described and fully studied. There are just few papers dealing with identificati n of individual fish with limited the number of individuals (Hirsch et.al., 2015; Al – Jubouri et al., 2018). Non-invasive method of fish identification such as image-based identification is significantly important because it can reduce the different negative effects on a fish welfare. The aim of this paper is proving the concept of non-invasive image-based fish identification of individuals using the visible patterns on a fish body and stability of these patterns during cultivation period. For identific tion of individuals we were used visible features on a Sumatra barb *Puntigrus Tetrazona* body represented by black stripes along the body.

Material and methods

Sumatra barb (25 fish individuals) small ornamental fish was used in this study. The fish was chosen for the study because of unique black vertical stripes on their body. These patterns can be used as visible features for individual identification of commercially important fish of this kind of patterns, for example pike-perch *Sander lucioperca* and European perch *Perca fluviatilis*.

The digital camera (Nikon D90), with controlled lighting, the background and the fish position, was used for data collection. Data were collected under different angle and position, images were taken from one side view of all fish. Data were collected two times during two weeks for fish inside the aquarium. Tree images of each individual were taken in every data collection. Fish detection procedure consists of standard image segmentation based on the color subtraction, object detection, filtration and parametrization. Different texture descriptors were tested for image parametrization. The best result was achieved using histogram of oriented gradients (HOG) (Dalal et.al., 2005) descriptor. First the specific subpart of the fish body was localized based on the fish length and height. The selected region (central part of the body) was parametrized and as used as feature vector.

Each fish was represented by three images for one data collection. The identification was then tested on two datasets. The classification was based on the simple nearest neighbor classifier where the measure of the similarity was based on HOG descriptor.

Results

The first step of the identificatio was the image processing. The use of standardized background and illumination enables to easily detect the fish in the image and do the parametrization. Fish object localization worked for all images without any significant problem (fish body was localized correctly). The identification of 25 individuals was tested as the classification within one data collection and between two data collections (test of pattern stability). The overall accuracy of classification based on the selected part of fish body was 100% for data collection in one day and 88% in between two data collection.

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Discussion and conclusion

This study successfully shows that fish individuals of the same species can be automatically identified based on the visible pattern on the body using computer vision during the time of the fish cultivation. We proved that the selected pattern can be used for individual identification because the classification accuracy within the same day data collection was 100%. The test of the pattern stability for long term identification was represented by the identification between two data collections with the accuracy 88% (3 fish were identified incorrectly). The lower classification accuracy is caused by the not optimal quality of the collected images. The classification accuracy clearly show that the fish pattern is unique to distinguish between 25 individuals of the same species. The small-scale research studies usually use comparable number of the fish; therefore, the method is promising to substitute conventional procedure of fish identification (e.g. tagging or marking). The restriction in the data collection (illumination, background) does not allow to generalize the results to the real conditions. To fully automatize the identification based on the visible pattern, more fish should be involved in the study and the data should be collected under the real conditions of the experimental studies or fish cultivation. Data collection is very important part of the experiment, better data collection will enable better results. The visible patterns, in our case it is vertical stripes, can be used for identification of Sumatra Barb (*Puntigrus tetrazona*) and these patterns are stable during the growing of the fish. However, this is not just limited to Sumatra Barb, the approach can be applied to any fish species with visible pattern on the body (stripes or dots). The approach could be beneficial for commercial fish species and even for pike-perch and European perch.

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USE OF THE PROBIOTIC, PREBIOTIC AND SYMBIOTIC AS GUT PRIMERS IN EUROPEAN EEL LARVAL CULTURE

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Introduction

European eel is a targeted, high-value species for aquaculture, which utilizes resource-efficient recirculating aquaculture systems (RAS). Recently, efforts to establish a closed cycle production have been intensified. Thus, European eel research via assisted reproduction has recently succeeded in producing viable larvae and currently focuses on establishing first feeding larval culture and development of methods facilitating metamorphosis into leptocephali (Butts et al., 2016, Politis et al., 2018). The necessity to fill gaps in knowledge relating to nutritional requirements and digestive capacity of European eel larvae drives the focus on molecular and physiological mechanisms regulating feeding, digestion, and growth. Numerous studies have shown that administration of probiotics (live microbial feed supplements that modulate gastro-intestinal microbial communities) and prebiotics (non-digestible feed additives, which stimulate the abundance or activity of beneficial gastro-intestinal microbes) can help shaping the gut microbial community in fis larvae (Egerton et al., 2018). These "biotics" beneficially affect host welfare and survival by driving and improving colonization of the gut microbiome (Daniels et al., 2010). Thus, the aim of this study was to test gut-priming probiotics (*Pediococcus acidilactici*), prebiotics (mannan-oligosaccharides) and synbiotics during development of European eel yolk-sac larvae to evaluate potential improvement of ingestion and digestion capacity at first-feeding

Material and methods

All fish were handled in accordance with the European Union regulations concerning the protection of experimental animals (EU Dir 2016/63). The experiment was conducted at the EEL-HATCH facility in Hirtshals (Denmark) where broodstock maturation, gamete production and embryonic rearing were performed according with the procedures described in Politis et al. (2018). Two days post hatching, ~400 larvae were gently transferred into 24 acrylic 2L jars (drz400sm hank, JugDesk Type, Taipei, Taiwan), divided in four groups and connected to four RAS units, representing the 4 treatments (control, pro-, pre- and syn-biotics).

Each RAS unit consisted of a 50 L capacity wet biofilter filled with RK bio-element, a protein skimmer (Wavereef, China), a 100 L reservoir hosting the main pump and a 180 L header tank. Six jars were connected to each system and water flow was set to ~0.2 L/min. Within the jar, an upwelling flow created enough turbulence to keep the larvae in suspension and maintaining optimal oxygen levels for the larval rearing. The outlet was placed on the top part of the jar, where a 250µm filter enabled a good water exchange. In order to keep the bacteria level under control, each system was connected to a UV filter (active from 9 pm to 9 am). Water temperature was kept at 19 ± 1 °C and salinity was progressively reduced from 36 to 18 psu over a period of 4 days according to Politis et al. (2018) in order to improve larval survival. Probiotics (Bactocell, Lallemand SAS, France), prebiotics (AgriMOS, Lallemand SAS, France) and synbiotics (Bactocell + AgriMOS) were added to the corresponding RAS according to the company's recommendations (Table 1). One RAS received no additives (control). When larvae reached 9 dph, 0.3 ml of food was added to each jar five times a day (at 9,11,13,15 and 17) with the water flow on in order to get the larvae used to the feed and the feeding regime. After first-feeding (13 dph), 0.5ml of food was provided at the bottom of the jara and the larvae allowed to feed for 30 minutes, with no water flow present, 5 times a day. At the end of each feeding session, the flow was turned on in order to flash out excess food. Temperature, salinity and larval mortality were recorded daily. To evaluate bacterial density water samples were taken at 2, 4, 8 13 and 18 dph using the Bactiquant method (Mycometer A/S, Denmark). At the beginning of each trial, ~15 larvae per batch (3×) were randomly sampled and photographed. Additionally, on 4, 8, 13, and 18 dph, from each batch (3×), treatment (4x) and replicate (3x), randomly selected i) ~15 larvae were sampled and photographed using a zoom stereomicroscope for assessment of larval morphometrics, ii) ~30 larvae stored in RNAlater for molecular analysis, iii) ~10 larvae, washed with osmosis water and stored in -20 °C freezer to assess the enzymatic activity and iv) ~10 more larvae stored in neutral 10% formalin buffer (Hounisen, Denmark) for histological analyses.

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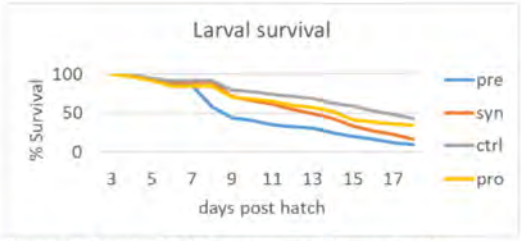

Figure 1 Survival of European eel larvae under different gut-priming treatments until 18 days post hatch

Results and Discussion

We hypothesized that administration of probiotics, prebiotics or the combination of the two (synbiotics) to eel larvae during early ontogeny, would cause an earlier activity of digestive enzymes (and related gene expression) towards better ingestion and digestion capacity, survival and growth. Preliminary results showed that prebiotics and synbiotics did not promote survival of European eel during early life history compared to the control, while survival was slightly higher when probiotic supplements were provided, similar to, larvae reared with no additives in the water. Regarding ingestion rate, we could not observe any effects of the use of pro-, pre- or synbiotic during the feeding period. Further analyses will investigate the influence of these gut-priming supplements at a molecular level combined with the detection of main digestive enzymatic activity (amylase, protease, lipase, phosphatase, trypsin) during the experimental period.

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SPATIAL CONFLICTS OF COASTAL FISHERIES WITH LARGE SCALE SALMONID AQUACULTURE IN A NORWEGIAN FJORD ENVIRONMENT ANALYZED BY GIS AND STAKEHOLDER SURVEYS

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Introduction

Farming of salmonids has grown rapidly in Norway since its onset around 1970, and in 2017, the total production of salmonids was 1 303 352 tonnes, of which about 95% was Atlantic salmon, *Salmo salar*, and 5% rainbow trout, *Onchorhyncus mykiss* (Directorate of Fisheries, Bergen Norway). Today, fish farming is the major part of the Norwegian fisheries and aquaculture sector, which together constitutes the second largest industry of Norway, as measured in terms of export value. This growth has not come without conflicts with other users of the coastal zone, in particular with wild salmonids and salmonid fisheries due to proliferation of sea lice and genetic influence of escapees (Taranger et al. 2015), but also with other wild fish and invertebrates.

In particular saithe, *Pallachius virens* has been demonstrated to aggregate in close vicinity of salmonid cages (Bagdonas et al. 2012, Dempster et al. 2010). Farms are also interconnected due to migration by wild fish moving among farms. Excess fish pellets have been shown to a fect the fatty acid composition and taste of wild saithe (Skog et al. 2003).

The impact of delousing agents to wild crustaceans has caused great concern. Flubenzurons are orally administered agents that act by interfering with the synthesis of chitin in the salmon lice. They are effective against all crustaceans that undergo moulting, including the larval and pre-adult stages. Since the bioavailability of tefluorobenzuron in Atlantic salmon is low (approximately 10%) and the metabolism is minimal, most of the drug will be released from the fish as parent compound via faeces. Solubility of diflubenzuron and teflubenzuron in water has been demonstrated to be low, the substances associate readily with particles rich in organic content, and degradation time is slow (Samuelsen 2016). This has caused concerns on effects of in particular crustacean benthic biota, but the compounds are toxic to any species that undergo moulting in their life cycle including lobsters, crabs and shrimps.

Recently, the effects of another delousing agent, hydrogen peroxide, H2O2 has come into attention (Fang et al. 2018). It has long been viewed environmentally friendly, because it is degraded to water and oxygen. The acute effects of high concentrations of H2O2 are largely unknown, however, major differences in sensitivity between species are seen (Fang et al. 2018 and references therein).

Materials and methods

Systematic stakeholder interviews with saithe and shrimp fishermen, representatives from the aquaculture industry and managers in the public sectors were carried out. A GIS based conflict analysis (GRID, GeoReference Interactions Database, developed within COEXIST (www.coexistproject.eu) - an EU funded project) was carried out. The case study area is identical to Production Area 3 of the Norwegian coast, mainly constituted by the Hardanger Fjord and areas in its vicinity. There is a large salmon farming industry in the area, presently with 169 sites. Furthermore, the hydrography is significantly influenced by hydroelectric power plants surrounding the fjords. Three Marine Protected Areas are present.

Results and discussion

The purpose of this study was to assess the impact of fisheries and aquaculture on coastal ecosystems, as well as synergies and conflicts between these and other human activities in a Western Norwegian area with high concentration of industrialized aquaculture.

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The GIS based GRID system could be used to score conflicts and synergies among fisheries, aquaculture and environmental protection associated with geographical location. It aims to analyze and locate conflicts as well as synergies between several coastal activities. GRID calculates conflict scores and provides interaction matrices and maps, in a static vie .

The stakeholder survey demonstrated that in particular shrimp fishermen were affected by environmental changes believed to be associated with fish farming. Shrmp fishing grounds inshore were little used. The saithe fishermen gave more diverse responses. In general they reported reduced quality, in particular when large numbers of farmed salmon were present in the vicinity. Others also reported that the catches were generally high, probably due to increased biomass.

The aquaculture industry stakeholders all acknowledged that environmental constraints hampered growth, and they emphasized the need for technological development. In general farmers operating inshore considered that closed systems were the best solution, whereas some farmers believed more in offshore systems. Most companies were involved in tecnological development projects.

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CORDINET AND COPERNICUS MARINE – ON THE USE OF EARTH OBSERVATION DATA FOR MARINE ENVIRONMENTAL MONITORING

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Introduction

Copernicus is the European Earth Observation infrastructure. As partners in the Cordinet project (Horizon 2020) we at IMR focus on: promoting and facilitating Copernicus Marine observations, particularly in the High North, and along the Norwegian coast. Aquaculture, fisheries and other marine activities are examples of users

Copernicus Marine

is a "one-stop-shop" providing freely available operational data on the state of the marine environment for use by marine managers, advisors, and scientists, as well as intermediate and end users in marine businesses and operations. The Copernicus Marine offers operationally updated and state-of-the-art products that are well documented and transparent. The European Commission's long-term commitment to the Copernicus program offers long-term visibility and stability of the Copernicus Marine products. Furthermore, Copernicus Marine offers a dedicated service desk, in addition to training sessions and workshops.

The service addresses four segments:

- Coastal and marine environment
- Marine resources
- Marine safety
- Weather, climate, and seasonal forecasting

Some examples of users:

- Ship-routing in ice-covered areas
- Support to seismic ship operations
- Ice information service
- Monitoring potential oil spills
- Predicting man-overboard drift trajectories

Parameters of particular relevance to aquaculture:

- Algal blooms, including harmful algal blooms
- Surface temperature
- Surface currents
- Seawater pH.

The CoRdiNet project is:

- An open network with five Copernicus Relays with a coordinating function on local, regional, cluster and national levels

- Supporting, promoting and stimulating digitalization and new business solutions based on earth observation data from the Coperniocus project.

- Bundling the local expertise in the Civil use of Earth observation close to the needs and offers of citizens, administration and businesses, and will share them with other Copernicus Relays, Academies and new Earth observation players.

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Three of the Copernicus Relays have a regional focus (Basilicata, University of East Midlands, Bavairia, one has a national focus (IMR) and the fifth contributres the expertise of a space application company (GMV

NEREUS the network of European Regions using Space Technologies comprises 26 regions. NEREUS is active in exploiting the benefits of space technologies while supporting European regional space policies

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Watch out for the open calls for Expression of Interest on theCoRdiNet´s website www.cordinet.net

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CONSTRAINTS AGAINST GLOBAL DEVELOPMENT OF AQUACULTURE: COMPETITION FOR SPACE, RESOURCES AND SOCIETAL COMMITMENT

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Introduction

Aquaculture is essential to contribute to unravelling and solving the climate-food-ocean challenges and to unlock the economic and Food and Nutrition Security (FNS) potential of the oceans. In this context, developing aquaculture is as a means to build resilience for food security, and thus, even peace. (Buck and Langdon 2017, SAPEA 2017).

The oceans cover around 70% of total Earth surface This invaluable source of area can contribute much more to both the blue economy and FNS in a changing world[15]. Aquaculture is specified as a core sector in EU's Blue Growth strategy (European Commission 2012, 2014, European Union 2014). Realizing this potential requires higher levels of economic productivity through diversific tion, technological upgrading and innovation, while simultaneously promoting societal equality and sustainable management of land, soil, inland and marine waters and engaging all food system actors. This requires the systematic utilization of knowledge, technologies and innovation throughout the aquaculture value chains. Not the least challenge is to allocate space for aquaculture activities, which was the core problem studied by the EU project Aquaspace.

Methods

Based on 17 Case studies in Europe, Asia, Australia, New Zealand, China, Canada and the USA, we studied the constraints as well as the drivers of the development of aquaculture activities. The case studies were highly different in terms of size, species and development. Some were highly industrialized and significant contributors to food security as well as local and national economy, whereas others were of smaller, and essentially local scale (Strand and Bergh 2017). Stakeholder workshops and investigations were arranged in all case studies,

Results and discussion

Today, more than 95% of the global food production comes from agriculture. Available arable land is limited, and future needs will put a strong pressure on the world´s remaining rain forests and other natural environments. The sea supports at least 50% of primary bio production, and we cannot afford to leave its resources underutilized. This situation demands increased understanding and improved management of marine ecosystems and increased efforts on sustainable aquaculture (SAPEA 2017).

Marine Spatial Planning was a key issue overarching all cases. Conflicts with other users of the coastal zone is limiting aquaculture development. The other users include (amongst others) tourism, nature protection, fisheries, energy production, and transport. The importance of different competing users is highly variable among the case studies. A general observation is that development of aquaculture is dependent on a willingness to allocate area at the expense of other users, that has to be politically and societally accepted (Galparsoro et al. 2019).

Furthermore a general observation was that co-use for different purposes, notably energy, but also tourism and marine protection areas, could reduce the level of conflict, thereby allocating more space for aquaculture, as was also discussed by the previous COEXIST project Stelzenmüller et al. 2013).

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CLIMATE CHANGE AND MITIGATION OF ITS IMPACTS OF NORTH EUROPEAN AQUACULTURE

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Introduction

Global warming will gradually increase the temperature of Norwegian and North European coastal waters and have significant effects on the aquaculture industry. Unlike wild fish stocks, farmed organisms are quite literally locked into specific localities, and conditions may be less suitable for the species´ physiological demands. Thus, optimal farming conditions for the species common in European and Norwegian aquaculture today are expected to gradually move northwards in a parallel shift.

In many places, fish farms will have to be relocated and/or farming technology modified in order to reduce the undesirable effects of higher temperatures. Current sites could in turn be suitable for other species than those farmed today, and cultured species that are presently farmed in Southern Europe could gradually enjoy better conditions in Norway than further south. Examples of species that may be more important in aquaculture along the North Atlantic shores are turbot, sea bass, sea bream, oysters and scallops.

Aquaculture is a relatively new industry in Norway and Northern Europe, and in contrast to the fisheries, it lacks the historical reference to, and experience with, natural changes in climatic conditions. The fisheries have adapted to natural climate cycles for centuries. (Stenevik and Sundby 2007) In contrast, the cold winters along the Norwegian coast during the early part of the 20th century would have caused severe problems for the salmon farming technology of today, if it had been present.

Furthermore, despite the dramatic global context, most models of global warming point out that the expected changes will be relatively slow in the context of industrial life-cycles. Although there is every reason to expect, and plan for, a gradual movement of the aquaculture industry northwards, there is no reason to quit salmon farming in Southern Norway tomorrow.

What can be expected?

Present-state studies of the future climate show that air temperatures will rise by 2-4°C during the 21st century. In the seas off the coast of Norway, the temperature will raise by 1.5-2.0°C (Stenevik and Sundby 2007). This implies that the temperatures presently found in Southern Norwegian coastal waters will be common along the coast of Northern Norway in a 50-100y perspective. The change in the mean temperature is however not a major obstacle for farming of the presently most important species in Southern Norway, Atlantic salmon, rainbow trout and Atlantic cod. On the contrary, culture of cold-blooded animals would in principle benefit from higher temperatures up to the point where they exceed or compromise the biological limits of the organisms.

In a biological context, the extreme temperatures may be more important than just the average temperatures, especially considering the impact of high temperatures on the immune system and the proliferation of pathogenic agents. Periods with high temperatures suboptimal to the fish will be longer, and events with temperatures higher than the biologically safe limits of the farmed species will be more common. It can be assumed that this will alter parasite-host-dynamics in a negative way for aquaculture.

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Other major effects of the expected climatic changes along the Norwegian and North European coast the next century will be increased intensity and frequency of storms and increased rain, plus a moderate increase of high tidal level. Such effects will cause a need for improved technological solutions, and significant investments may be needed. However, they do not constitute “impossible” biological or technological problems. Intense storms may, however cause damages to fish farms, and increase the with high numbers of escaped fish. However, as escapees are considered a major environmental problem, improved technological solutions must be developed and implemented, as they presently are.

Diseases

Many (but not all) diseases may occur more frequently in warmer weather, particularly bacterial infections with bacteria or parasites adapted to relatively high temperatures (Johansen et al. 2011) . Just as important as the temperature range of bacteria and parasites, is the temperature range of the cultured fish. Rearing a species at too high or low temperatures inevitably may compromise the immune system, leading to increased disease problems. For instance, important diseases such as francisellosis, vibriosis, furunculosis, as well as several viral diseases and parasites are typically associated with high water temperatures.

On the other hand, winter ulcers and cold water vibriosis are typical examples of diseases occurring in cold waters. In this context, the extreme temperatures are much more important than the middle temperatures.

Reproduction of parasites like salmon lice is temperature dependent, thus shorter generation times, and thereby increased infestation rates may be expected with higher average temperatures. Spreading of salmon lice may also be altered by increased freshwater along the coast, particularly in the fjords. Improved modelling of water movements in fjords and coastal environment in various climatic scenarios may be a useful tool for predicting changes, as is the case for predicting salmon lice migration today Asplin et al. 2014, Johnsen et al. 2016). It should be noted however, that hydroelectric power plants may have a greater impact on hydrography than the anticipated climatic changes in this century.

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SALMON CAGES SUPPLIED FRESHWATER FOR CONTROL OF SEA LICE

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Introduction

Reduced salinity has demonstrated significant effect to control infestation of two major parasites in seawater salmon cages, sea lice and the amoeba causing amoebic gill disease (AGD). Efficient treatment of AGD is achieved in low-saline water of less than 3ppt, while survival and infectivity of mobile sea lice decrease stages at < 27ppt (Powell & Clarke, 2002; Bricknell et al., 2006). The chalimus and adult lice stages are relatively resistant to low-saline water; however, freshwater treatment in well-boats for 3-8 hrs has turned out to efficient y remove lice (e.g. Haarsaker, 2013). The present study describes introduction of enclosures in salmon cages supplied freshwater as an protective attempt to control sea lice.

Materials and methods

Circular enclosures with circumference of 50m were put in large commercial net cages. Tarpaulin-covered sides from surface to 7m depth excluded horizontal mixing of water between enclosure and cage. Thus, only vertical intrusion of seawater through the open bottom area impacted the salinity of the enclosure's water column added desalinated water at the surface. The adjacent desalination unit (reverse osmosis), kept on a platform raft, produced 30 – 50 m^3 water/h and supplied two enclosures, i.e. each enclosure received 15 – 25 m^3/h. Salinity, temperature and dissolved oxygen were frequently monitored at different depths. Fish abundance measurements were performed within and outside the enclosures for 3 weeks with an echosounder system (type: CageEye).

Results and comments

The salinity was strongly fluctuating within the enclosures due to vertical flow motion and unstable production of desalinated water (Table 1). Several technical attempts, such as optimization of tarpaulin design, buoyancy capacity, oxygen injection and better power supply of the desalination process should be tested to improve the stability of the brackish water layer.

During the 3 week study period, the fish density was consistently lower inside the enclosures compared to outside. On average, the fish abundance was reduced to 31±4% in the enclosures and the fish stock also seemed to avoid the layers beneath the enclosures (14 – 18m depth). However, a notably increased abundance throughout the study may indicate that the fish gradually get used to such installations in the cage

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Table 1. Salinity in an enclosure at 1 – 5 m depth. February – May 2018

	1m	3m	5m
Average (ppt)	21.7	27.4	31.4
Max	33	33	33
Min	0	11	28
N	33	39	37

FARMING WARMWATER FISH ON LAND IN A COLD COUNTRY – A CRAZY IDEA OR A NEW SUSTAINABLE SEAFOOD SUPPLY CHAIN?

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Introduction
Landbased aquaculture of warm water species such as tilapia *Oreochromis niloticus*, clarias *Clarias gariepinus* and shrimp *P. vannamei* constitute an emerging food sector in Sweden. The heated sustainability debate related to both wild-caught and conventionally farmed seafood, in particular shrimp, has likely been a major driver. Several small, but fast-growing companies who profile themselves as more sustainable than conventional aquaculture have started producing seafood in recirculating aquaculture systems (RAS). Their products are marketed to environmentally conscious Swedish consumers as alternatives to salmon (tilapia), tuna (fresh clarias), eel (smoked clarias) and also to imported shrimps (*P. vannamei*)

Methods
In this study, we used Life Cycle Assessment (LCA) methodology to analyze environmental pressures of the seafood products from one of these companies. LCA is widely used to characterize the environmental impacts of product supply chains and identify improvement options and been used to evaluate numerous aquaculture production systems.

Results
We present results on environmental pressures (e.g. greenhouse gas emissions and eutrophication) for the product supply chain of tilapia and clarias farmed in Sweden and identify environmental hotspots and improvement options. We found that the feed is of paramount importance, also for this type of infrastructure- and maintenance-intensive aquaculture system. Inclusion of resource-intensive feed ingredients such as animal by-products increase the environmental footprint considerably. We also illustrate how the source of electricity for water heating and recirculation affects results. The importance of the contribution from the farming system in relation to feed production is presented. Our results identify which aspects are most important to consider in the future development. Finally, comparisons with previous LCA results for the farmed fish these products are intended to replace are presented

Landbased tilapia and clarias farm in southern Sweden.
Photo: Gårdsfisk

THE METHODICAL IMPROVEMENT OF SPERM CRYOPRESERVATION IN VOLGA PIKEPERCH (*Sander volgensis*)

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Introduction

Volga pikeperch is an economically important gamefish and has a great industrial potential in Central and Eastern Europe. According to former studies the meat quality is similar to the pikeperch (*Sander lucioperca*). However Volga pikeperch is less sensitive for stress and tolerate more the reduced oxygen level as well than the mentioned relative species. Its propagation has been already published (Bokor et al. 2007). However the efficiency of commercial production is needed to be improved. Cryopreservation allows the simplification of the broodstock management and can reduce the costs of the propagation and rearing process (Bokor et al. 2019, Cabrita et al. 2010). The technique also allows to help the reintroduction of natural populations of the species and to satisfy the increasing angling demand. Limited information is available regarding to sperm cryopreservation in Volga pikeperch (Bokor et al. 2007).

Materials and methods

In our study, 3 different experiments were carried out to improve the sampling process and sperm cryopreservation methods in Volga pikeperch males (*N*=39). The spermiation was hormonally induced using 3 mg bodyweight kg^{-1} of carp pituitary 24 hours prior to sampling. Sperm was collected 4 different methods according to the experimental design. The progressive motility (pMOT) in both fresh and cryopreserved samples was recorded with a CASA system (Computer-assisted Sperm Analysis, Sperm VisionTM v. 3.7.4., Minitube of America, Venture Court Verona, USA) using 50mM NaCl solution (buffered with 30mM Tris, pH: 8, Lahnsteiner et al. 2011). In our study, 3 different extenders was compared (modified Tanaka extender: 137 mM NaCl, 76.2 mM $NaHCO_3$, Bernáth et al. 2015; Pike extender: 150 mM glucose, 75 mM NaCl, 30 mM KCl, 1 mM $Na_2HPO_4 \times 12H_2O$, 1 mM $MgCl_2 \times 6H_2O$, 1 mM $CaCl_2 \times 2H_2O$, 20 mM Tris, and 0.5% BSA, pH: 8.0 ± 0.2, Várkonyi et al. 2018; Glucose extender: 350 mM glucose, 30 mM Tris, pH: 8.0 ± 0.2, Bokor et al. 2007). Diluted sperm (1:9) was loaded into 0.5 or 5mL straws. In all experiments, cryopreservation was carried out in a Polystyrene box at 3 cm above the surface of liquid nitrogen for 3 or 7 minutes according to the size of the straw (Bokor et al. 2019, Horváth et al. 2003). As cryoprotectant, 10% of methanol was used. The 0.5 mL (13 s) and 5mL (35 s) straws were thawed at 40 °C using a waterbath (Bokor et al. 2007 and 2019).

Experiment 1. The comparison of 4 different sampling methods
Sperm from 5-5 males was collected using regular stripping methods, catheter, stripping into Pike extender and squeezing of testis. Motility was compared in all groups.

Experiment 2. The comparison of 3 different extenders
The testis of 7 males was squeezed and the sperm was diluted in modified Tanaka, Pike and Glucose extenders. Samples were cryopreserved using 0.5 ml straws.

Experiment 3. The comparison of 0.5 and 5 mL straws and 2 different sampling methods at hatchery conditions
Samples were collected using squeezing and regular stripping (6-6 males). Motility was compared in both groups. Item, squeezed sperm from 5 males was loaded into 0.5 and 5 mL straws and cryopreserved in P. box.

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Results

In *Experiment 1.* the highest motility was measured in the case of the catheter (92±5%). A significantly lower movement was measured using the regular stripping method (5±7%) and using the Pike extender (8±11%) in comparison with the catheter and the squeezed testis (63±12%). In *Experiment 2.*, a significantly higher pMOT was measured in the case of modified Tanaka (40±15%) and Pike extender (25±16%) compare to the Glucose extender (2±1%). A similar movement was recorded in the control group (49±9%) and modified Tanaka extender. In *Experiment 3.* a similar pMOT was recorded with squeezed testis (68±21%) and the regular stripping method (65±35%). No significant difference was observed between the 0.5 (42±8%) and 5 (28±12%) mL straws. A significant reduction in pMOT was measured in both cryopreserved groups following thawing in comparison with the control (73±19%).

Discussion and conclusion

According to our results both the regular and catheter stripping of sperm, as well as squeezing of testis can be applicable during the propagation process. However the volume of sperm can be obtained is the highest by the squeezing method. Modified Tanaka extender and the two sizes (0.5 and 5 mL) of straws is applicable for Volga pikeperch sperm cryopreservation at hatchery condition.

Acknowledgements

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UNRAVELLING CHANGES DURING INDUCED SEXUAL MATURATION OF FEMALE EUROPEAN EEL (*Anguilla anguilla*) THROUGH RNA-SEQ: WHAT HAPPENS TO THE LIVER?

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Introduction

For the aquaculture industry, closing the life cycle of European eel in captivity is of interest, given the critical status of the stock. In captivity, sexual maturation can be successfully induced by hormonal treatments (Pedersen, 2003). At a biological level, sexual maturation involves the hypothalamus-pituitary-gonadal-liver axis, which generate massive changes of the physiology of the animals, also considering that the females do not eat during the maturation process. The development of high-throughput sequencing technologies can provide useful tools to investigate those changes at a whole and deep scale allowing an increasing understanding of the major biological and molecular pathways that are altered during induced vitellogenesis by determining how the expression of thousands of genes changes from immature stage to pre-spawning stage. In this work, we will focus our attention on one of the main organs involved in the maturation process, the liver.

Material and methods

The study included a total of 16 female farmed eels: 8 immature eels and 8 receiving a constant weekly dose of CPE (Carp Pituitary Extract; 20mg/body weight) to induce hepatic vitellogenesis and follicular development. Immature eels were sampled before maturation at week 0 (average weight ±standard deviation: 862 ±107.41g) and at week 9 of maturation (average weight: 901.25±50.94g). Liver and ovaries were sampled. Gonadosomatic index (GSI) and liver index (LI) were calculated and maturational state was confirmed by histological analyses.

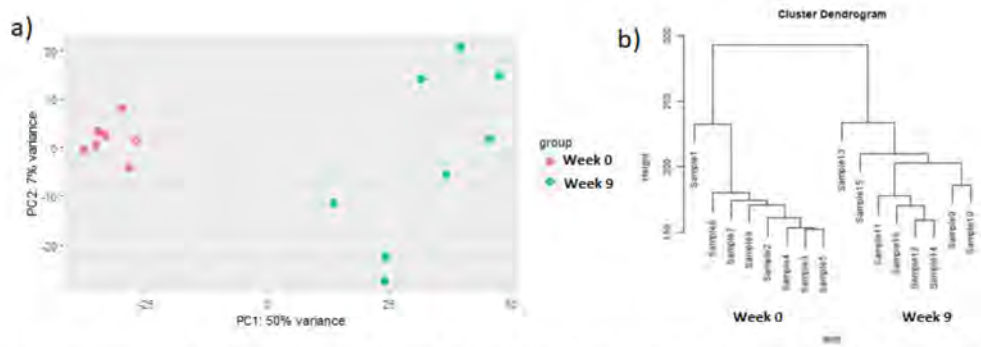

Fig 1 Principal component analysis (PCA) plot and cluster analysis of all RNA-seq samples. (a) PCA plot of all RNA-seq samples. (b) Cluster dendrogram of all RNA-seq samples. The log2-nomalized values of all the genes were used for PCA and cluster analyses.

Gene symbol	lipid oxidation	fatty acid beta-oxidation	lipid catabolic process	fatty acid catabolic process	cellular lipid catabolic process	lipid modification	fatty acid metabolic process
ABCD2	■	■	■	■	■	■	■
ABCD3	■	■	■	■	■	■	■
ABHD4			■				
ACOX3	■	■	■	■	■	■	■
ACSL1							
AMACR	■	■	■	■	■	■	■
APOA4			■				

Fig 2. Gene enrichment results of the lipid related GO terms. Green boxes represent the involvement of that gene in the function. All genes related to the GO terms were upregulated.

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RNA was extracted from liver samples, RIN (RNA integrity number) was calculated to check the integrity and the samples were sequenced with the Illumina HiSeq 2000 platform. Trimmed reads were aligned to the European eel reference genome (Henkel et al 2012) with Hisat2 (Kim et al. 2015), retaining only uniquely mapped reads. The software StringTie (Pertea et al. 2015) was used to predict transcripts, using the *in silico* genomic annotation file to increase the accuracy of the transcript prediction. Then, only transcripts with an overlapping >70% with the in-silico gene annotation were used for reading count and differential expression. Read count was performed with HTSeq (Anders et al. 2015) with intersection-strict as intersection option, after sorting the reads by name as recommended in the manual. Differential expression analysis was performed with Deseq2 R package (Love et al. 2014). Transcripts were considered differentially expressed with a Padj<0.01 and a log2fold ratio >|1.5|. Enrichment was performed with the differentially expressed genes with the tool Gorilla (http://cbl-gorilla.cs.technion.ac.il/), retaining GO (Gene Ontology) terms with $P<10^{-3}$.

Results and discussion

As an effect of hormonal treatment, average GSI increased from 1.46±0.27 in immature females to 16.75±7.04 in mature females, concurrently the LI increased from 0.78±0.11 to 1.25±0.27, showing high correlation (0.90). *RNA-seq* on liver generated approximately 20 million of paired-end reads each sample For each sample, app. 65% (±2.49 standard deviation) of the reads generated from the Illumina sequencing uniquely mapped the reference genome. The StringTie analysis identified 71,756 different transcripts and 53,319 predicted genes. Here, only the transcripts that overlap with those previously detected in silico where considered, retaining 31,632 genes. This is done because the current annotated version of the Eel genome is composed by more than the 75% of the scaffold below 1Kb, and therefore may overestimate the number of genes. With the expression level (normalized read count) of the retained genes it is possible to clearly clusterize the two groups (pre-maturation and close to spawning), as shown by both the PCA (Fig1a) and the dendrogram (Fig 1b). For the PCA analyses, the variance on the PC2 is higher across the week 9. This may indicate that, despite the same treatment and conditions at week 9, there is a higher individual variability in terms of overall gene expression.

The differential expression analyses revealed 916 differentially expressed genes (595 upregulated and 321 downregulated). Among the upregulated genes, the highest score was detected (8.09 log2fold ratio) for the vitellogenin gene (VTG1). While the expression level of this gene was anticipated to increase in the liver during eel ovarian development (Parmeggiani et al., 2015), it is interesting to notice that this gene had the highest upregulation score among the 595 upregulated genes. Furthermore, the enrichment analysis showed upregulated genes involved in lipidic metabolism, as shown in Fig 2. Other enriched pathways were mainly allocated in GO terms related with the transport of molecular components, biogenesis and multicellular organism development that includes the progression from immature stage to mature stage, where mostly of the genes detected were downregulated.

Conclusions

These results provide new insight of the molecular changes and genes/biological pathways involved at the molecular level in the liver, which help to understand the massive phenotypical changes that occur during induced maturation, where European eel do not eat but reallocate several metabolic resources.

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MONITORING SHORT-TERM MICROBIOLOGICAL DYNAMICS WITH FULLY AUTOMATED ONLINE FLOW CYTOMETRY IN REALTIME

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Introduction

Microorganisms are omnipresent and crucial players in all aquatic systems and hence also in aquaculture. Mostly, they are harmless but occasionally they can have negative effects on water quality or the infrastructure itself. However, bacteria and algae are also actively used in production processes as well as water treatment. In all cases it is crucial to closely monitor these organisms and to make informed choices about whether they need to be managed and what the best options would be for that. One critical aspect in this is the tracking of microbial dynamics at short timescales (seconds to days) given that those systems are usually highly variable rather than stable over time.

Tracking of these dynamics requires sampling and analysis at very short intervals and ideally in real-time allowing for immediate interpretation/reaction. For decades this was impossible with conventional cultivation-based methods but also with advanced molecular methods, which are still too labour-intensive, time-consuming, and costly for such applications. One promising approach is the use of flow cytometry. This detection method has been adapted from medicine and has specific advantages including: rapidness, sensitivity, reproducibility, accuracy in quantification, and differentiation of total and intact cells.

Material and methods

We have developed a fully automated online flow cytometry system that overcomes the tedious and restricting practice of grab-sampling and subsequent cultivation on agar plates Besmer et al. (2014). In short, water samples were drawn directly from the water resource of interest every 15 min, mixed with a fluorescent stain, incubated, and then measured with the flow cytometer. Rinsing and extended cleaning were performed regularly and periodically respectively. The resulting large sets of flow cytometry data were batch processed with custom software.

Results

Over the last years we discovered a myriad of short-term microbial dynamics in engineered and environmental aquatic ecosystems. The high frequency, in situ measurements allowed for the detection of subtle changes in cell concentrations during measurement periods ranging from weeks to months. We detected interesting and often unexpected microbial dynamics in every investigated system at time scales from hours to weeks and bacterial concentrations levels between 10^4 and 10^7 cells ml^{-1} (Figure 1). For example, clear diurnal fluctuat ons in bacterial concentrations were linked to photosynthesis in rivers (Figure 1A), to intermittent water extraction in groundwater (Figure 1C), to varying water production rates in a drinking water treatment plant (Figure 1D), and to daily water usage in a building (Figure 1E). In addition, the influence of precipitation events and increased discharge on bacterial concentrations over time was accurately quantified in rivers (Figure 1A), springs (Figure 1B), and groundwater (Figure 1C).

Discussion and conclusion

These results boost the idea of advanced monitoring of microbial dynamics, which is critical for a better understanding of underlying causes of fluctuatio s as well as the ecological and operational consequences thereof. While originally applied for bacteria in drinking water, applications of both fresh- and saltwater are in progress looking at bacterial and algae concentrations and viability in parallel.

We expect that these findings will massively stimulate improvement of process monitoring, water treatment design and improvement (e.g., disinfection in RAS/CSS, ponds), optimisation of feed and other production processes, and conceptual approaches to smarter sampling schemes, but also open up new applied and fundamental research directions.

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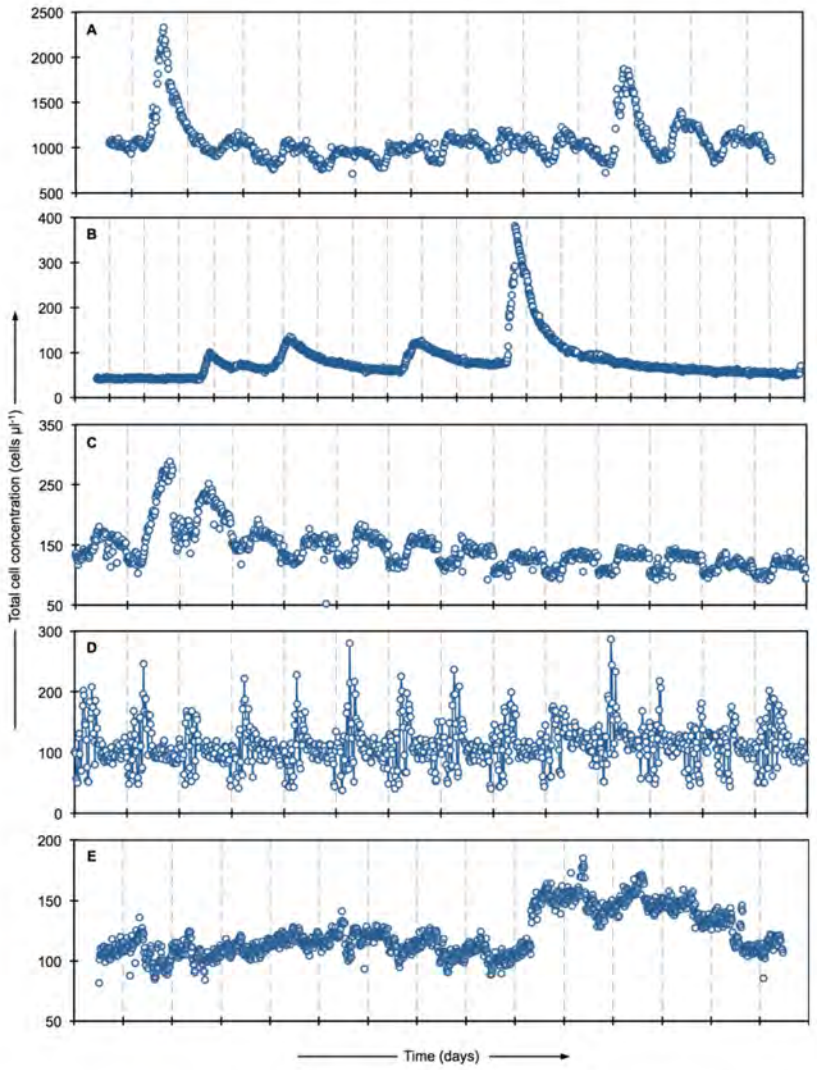

Figure 1: Temporal dynamics of total cell concentration in river water (A) Besmer et al. (2014), spring water (B), pumped groundwater (C) Besmer et al. (2016), treatment plant outlet (D) Besmer and Hammes (2016), tap water (E) Besmer et al. (2014).

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DEVELOPMENT OF FEEDS FOR JUVENILE ATLANTIC BLUEFIN TUNA (*Thunnus thynnus,* L): EFFECT OF LIPID LEVEL AND SOURCE

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Introduction
The development of formulated diets and feeds is essential to increase production of farmed tuna species. Although there is limited knowledge in this area for Pacific Bluefin tuna (*Thunnus orientalis*) in Japan, no major attempts have been made with Atlantic Bluefin tuna (*Thunnus thynnus*; ABT). In the present study, two trials were performed using inert formulated diets as juvenile feeds for weaned ABT in order to establish adequate dietary levels of both lipid and omega-3 long-chain polyunsaturated fatty acids (LC-PUFA).

Materials and methods
The nutritional trials consisted of two consecutive 10-day feeding trials (A and B) with weaned 41 dah ABT juveniles, from two different batches of spawned ABT eggs. Each trial investigated two experimental extruded feeds in comparison to the commercial reference feed (MGK) to bench-mark the growth performance of the experimental feeds. In the first trial, ABT (initial weight = 2.9±0.9g) were fed for 10 days with either MGK or two experimental feeds, differing in dietary lipid levels (15 or 20%), using krill oil (KO) as the sole lipid source in order to estimate the optimal lipid content. In the second trial, fish (initial weight = 3.3 ± 0.6g) were fed either MGK, 15KO or a feed containing 15% lipid with a combination (1:1, v/v) KO and rapeseed oil (RO) (15KORO). At the end of each experimental trial samples of liver were collected for lipid and molecular analysis.

Results
In Trial A, fish fed MGK displayed the highest growth, followed by 15KO, with 20KO displaying the lowest growth although no differences were found in terms of fish survival. Thus, a lipid content of 15% was considered better than 20% for ABT juveniles. In Trial B, fish fed 15KO and 15KORO showed the highest growth in terms of weight and fork length (including weight gain and SGR). Increasing dietary lipid level or adding RO to the feeds did not increase liver lipid content as compared to 15KO. The liver fatty acid profile largely reflected dietary intake confirming very limited LC-PUFA biosynthetic activity for this teleost species. Consequently, the liver of fish fed 15KO and 20KO displayed the highest contents of docosahexaenoic acid (DHA). The hepatic expression of genes for lipid and fatty acid metabolism, transcription factors, and antioxidant enzymes was investigated with many of the genes showing regulation by both dietary lipid and LC-PUFA contents.

Discussion and conclusion
The present study showed promising results that suggest ABT juveniles can be grown on inert dry feeds that support good fish growth and the accumulation of the health-promoting fatty acid DHA. Results from the trials indicate that diets with relatively high protein to lipid ratios of between 3 and 4 could support good growth of juvenile ABT. Diet had no significant impact on survival, partly due to high variability among treatments. In the present study, liver fatty acid profiles generally reflected those of diets, as shown previously in other fish studies (Betancor et al. 2014; Araújo et al. 2017). This clearly indicates that, despite being relatively short, the feeding trials were sufficiently long in these very fast growing animals to result in changes to the biochemical composition that would be expected in fish doubling their weight

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In conclusion, the present study suggests that ABT juveniles can be grown on inert extruded dry feeds that result in good fish growth and accumulation of the health-promoting fatty acid DHA. Furthermore, a blend of VO and KO could be used as the dietary lipid source up to a dietary lipid level of 15 % without affecting fish performance. The expression of lipid metabolism genes in ABT liver showed a different response to dietary lipid level/fatty acid profile, consistent with previous data indicating limited n-3 LC-PUFA biosynthetic capability in ABT. However, gene expression showed some differences between the two trials, which highlight how the genetic background of different batches of ABT juveniles could affect the regulation of metabolic gene expression and thus be a factor in weaning success. The expression of antioxidant enzymes was also altered by diet, related to dietary contents of antioxidant nutrients. Thus, further studies are required in order to fully elucidate the lipid and fatty acid requirements of this iconic species in relation to dietary sources and production costs.

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FEASIBILITY OF AN OIL DERIVED FROM A GM-OILSEED CROP AS A SUBSTITUTE FOR FISH OIL IN FEEDS FOR EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Introduction

There is a growing gap between the supply and demand of omega-3 (n-3) long chain polyunsaturated fatty acid (LC-PUFA)-rich fish oils (FO) for use in aquaculture, and so sustainable alternative supplies of these health-promoting fatty acids are required. Although vegetable oils (VO) have been used, they are characteristically rich in C_{18} PUFA but lack the n-3 LC-PUFA, eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively), which compromises the nutritional quality of the farmed product. One viable alternative is to use oilseed crops that have been metabolically engineered with the capacity to synthesize EPA and DHA. One such crop, *Camelina sativa*, has been genetically modified using algal genes in order to produce seed oils containing EPA+DHA. The aim of the present was to evaluate the new GM Camelina-derived oil as substitute for FO in feeds for sea bass (*Dicentrarchus labrax*), known to have a very limited capacity to biosynthesize LC-PUFA.

Materials and methods

The EPA+DHA Camelina oil was evaluated in sea bass (16.7 ± 0.09 g, initial weight) in a 16-week feeding trial in triplicate tanks (3 treatments x 3 tanks = 9 tanks, each with 50 fish). The transgenic Camelina oil was compared with a commercial control and a diet containing levels of EPA+DHA similar to those found in the GM-Camelina derived oil diet (3 treatments). Diets were isolipidic and isoproteic using non-defatted fishmeal and plant meals as the protein source which fulfilled fish nutritional requirements. Fish were acclimated to the experimental tanks for two weeks and fed a commercial feed prior to the onset of the experimental feeding period. Fish were kept at a light cycle of 12 h light and 12 h dark and hand-fed to satiety. At the end of the dietary trial, a total of 8 fish per tank (n = 24 fish per treatment) were sacrificed. Two fish were sampled from each experimental tank (2 fish x 3 tanks = 6 fish per treatment) for whole proximate analysis. Muscle, intestine, brain and liver were removed from another six fish per dietary treatment (two per tank; n = 6 fish per treatment) and frozen for fatty acid analysis.

Results

All the experimental feeds were accepted by the fish, more than doubling their weight at the end of the experimental trial. No differences were observed in fish total or fork length ($p = 0.088$ and 0.117, respectively), weight ($p = 0.179$) or specific growth rate ($p = 0.204$). Similarly, no differences in survival were observed ($p=0.867$) at the end of the experimental trial, being over 95%. Tissues generally reflected the fatty acid composition of the diets, with liver showing certain biosynthetic capacity that was also reflected in the gene expression analysis

Discussion and conclusion

Previous trials in marine fish that completely replaced FO with VO in feeds resulted in reduced growth that was probably related to a reduced intake of essential LC-PUFA, which are not found in VO (Fountoulaki et al., 2009). In the present study we evaluated the complete substitution of FO by an oil obtained from a GM-oilseed crop in sea bass feeds. The GM-derived oil proved to be an effective substitute for FO, displaying growth rates that were similar to those achieved by control fish fed different diets containing blends of FO and VO. Similarly, recent studies employing GM-derived oil as substitutes for FO in both Atlantic salmon (*Salmo salar*) (Betancor et al., 2015; 2016a; 2017; 2018) and gilthead sea bream (*Sparus aurata*) (Betancor et al., 2016b) feeds showed that fish fed these oils were as successful as those fed FO. In summary, the genetically engineered Camelina oil was shown to be a viable source of n-3 LC-PUFA and a potential candidate to replace FO in feeds for sea bass.

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TAURINE METABOLISM IN ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*, L.) LARVAE AND EFFECTS OF INCLUSION LEVEL VIA ROTIFER (*B. rotundiformis*)

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Introduction
In order to optimize Atlantic bluefin tuna (*Thunnus thynnus*, L.; ABT) production, further knowledge of the nutritional requirements of the species is pivotal, and understanding biological mechanisms of nutrient assimilation in larvae is a key area. Taurine appears to be a crucial nutrient for teleosts, especially top predator species such as ABT. While dietary taurine supplementation has been highly recommended, studies on taurine assimilation and biosynthesis are lacking for this iconic species. The present study aims to provide insight into the molecular mechanisms involved in taurine biosynthesis and transport in ABT by determining tissue distribution and ontogenetic development of expression of enzymes involved in taurine synthesis and metabolism: cysteine dioxygenase (*cdo*), cysteine sulfinic acid decarboxylase (*csad*), 2-aminoethanethiol dioxygenase (*ado*) and taurine transporter (*tauT*). Additionally, the expression levels of these genes in response to graded levels of dietary taurine supplementation was also studied.

Materials and methods
Samples of ABT tissues including brain, gills, heart, kidney, spleen, liver, intestine, white muscle, red muscle, adipose tissue, ovary and testis were obtained from broodstock tuna for tissue distribution of taurine metabolism genes. ABT larvae in the standard production protocol were sampled at 1, 13, 15, 18, and 25 days after hatch (dah) to determine the expression of taurine metabolism genes during early ontogenesis. Tissue and larvae samples were collected and processed in RNALater®. For the taurine nutritional trial, ABT larvae were fed from 2 to 14 dah with rotifers enriched with either 0.0 g (tau0), 0.5 g (tau05), 1.0 g (tau1) or 2.0 g (tau2) taurine per 10^6 rotifers. Samples of ABT larvae and tissues (n=6, 50 ABT larvae per replicate) were used for molecular analysis. Sequences of genes encoding for taurine metabolism (*tauT*, *cdo*, *ado* and *csad*) were obtained by identifying their sequences on SRA and primers designed for quantitative analysis (qPCR) plus antioxidant enzymes, digestive enzymes and housekeeping genes. Taurine and amino acids were determined in rotifers and ABT larvae by the Waters UPLC® AccQ-Tag Ultra Method®.

Results
Rotifers effectively accumulated taurine with ABT larvae fed treatment tau2 attaining the highest concentration of taurine. However, ABT larvae fed tau1 (3.7 mg taurine g^{-1} rotifer) displayed higher growth, survival and flexion index at 14 dah, than larvae fed the other taurine levels. The full open reading frame (ORF) for *cdo* and partial sequences for *csad*, *ado* and *tauT* were obtained, with the translated polypeptides being 202, 176, 166 and 324 amino acids, respectively. All three showed characteristics such as cupin motifs in Cdo and predicted N-glycosylation sites in Taut that are common to these genes in other species. Phylogenetic analysis showed that the ABT sequences clustered with sequences of other teleosts, and separately from mammals and molluscs. Tissue distribution varied, with adipose tissue, kidney, white muscle and testis/brain showing highest expression of *cdo*, *csad*, *ado* and *tauT*, respectively. Whole larvae expression of *csad* peaked at 15 dah, whereas the other genes generally increased throughout development to show highest expression at 25 dah. Larvae fed tau1 also showed generally higher expression of *tauT* and *cdo* and digestive and antioxidant enzyme genes.

Discussion and conclusion
In summary, the present study indicated that ABT larvae possess enzymes necessary for the biosynthesis of taurine through the two main pathways. The three enzymes and the taurine transporter showed differential tissue expression and could be detected before the onset of external feeding. Expression of the biosynthesis enzymes was not obviously regulated by dietary taurine level, possibly indicating a nutritional requirement for this nutrient. In contrast, *tauT* expression was upregulated when dietary levels of taurine were low, indicating a role for this gene in maintaining taurine levels in muscle and taurine homeostasis in ABT. Rotifers supplemented with taurine at 1 g per 10^6 rotifers improved the growth of ABT larvae, without affecting final survival. In conclusion, despite the presence of taurine biosynthesis genes, ABT larvae required a supply of dietary taurine at around 3.7 mg g^{-1} feed (rotifer; tau1) in order to ensure adequate growth and development.

Acknowledgements
We wish to thank the technical staff at Laboratory of Marine Aquaculture (IEO), Puerto de Mazarrón (Murcia, Spain) and Nutritional Analytical Services (NAS; Institute of Aquaculture, University of Stirling, UK) that contributed to this work. This work was supported by the Programa Estatal de Investigación del Ministerio de Economía y Competitividad, Spain [Ref. AGL2014-52003-C2-1-R, 2014].

METHANOTROPH (*Methylococcus capsulatus)* MEAL AS AN ALTERNATIVE PROTEIN SOURCE FOR YELLOWTAIL*, Seriola quinqueradiata*

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Although fish meal (FM) has been considered the most preferred protein source in aquafeeds because of its high degree of utility by fishes, intense focus has been given on the reduction and/or elimination of FM protein over the past several decades due to its high price and insufficient supply. FeedKind (FK, Calysta, Inc., CA, USA), which is primarily comprised of the methanotroph, *Methylococcus capsulatus,* is considered as a promising alternative due to its high protein content (~70%). Its utility was determined in yellowtail, *Seriola quinqueradiata*, which is one of the most important marine species in Japan.

Two trials were carried out in this study to find out optimal replacement level of FM by FK. In Trial 1, six iso-energetic diets were prepared as follows: control diet (C) with FM as protein source, and FM of diet C was replaced at 25, 50, 75 and 100% by FK and subsequently referred to as FK25, FK50, FK75 and FK100, respectively. In another diet, 3% of FK in diet FK100 was replaced by enzyme-treated FM (EFK). Fifteen fish of mean weight ca. 126g were randomly distributed into each of eighteen 500 L circular tank and set in triplicate for each treatment. Fish were fed to apparent satiation with the experimental diets twice per day at 09:00 and 15:00, 6 days per week, for 8 weeks. In Trial 2, seven iso-energetic diets were prepared as follows to further confirm the optimal replacement level: diet C similar to Trial 1, and FM of diet C was replaced at 20, 25 and 30% by FK and referred to as FK20, FK25 and FK30, respectively. FK from diet FK25 was replaced either by further grinding FK (FK25J) or slightly lower digestible FK (FK25L). Furthermore, 3% of FK in diet FK25 was replaced by enzyme-treated FM and referred to as EFK. Ten fish of mean weight ca. 80 g were randomly distributed into each 500 L circular tank in triplicate for each treatment and fed to apparent satiation with the experimental diets twice per day at 09:00 and 15:00, 6 days per week, for 8 weeks.

In Trial 1, all growth parameters and nutrients retention efficie cy showed similar pattern and no significant differences were observed between fish fed with diets C and FK25 ($P>0.05$). However, other experimental diets showed significantly lower growth performance compared to diet C, and enzyme-treated FM could not stimulate the performance ($P<0.05$). When differently processed FK was used and feed formula was slightly adjusted in Trial 2, even diet FK30 (30% of FM by FK) together with all other experimental diets produced similar results compared to the control group and there were no significant differences in the growth performance, nutrients digestibility and retention efficienc , except for lipid retention. Again, enzyme-treated FM could not significantly improve the growth performance ($P>0.05$). Overall, the results suggest that 30% of FM can be replaced by FK used in this study without compromising the growth performance of yellowtail.

LARGE-SCALE CULTIVATION OF SEAWEED IN WEST CORK: AN IRISH SUCCESS STORY

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Background
Rich in bioactive compounds including antioxidants, soluble dietary fibers, proteins, minerals, vitamins, phytochemicals, and polyunsaturated fatty acids, the potential of seaweed as food stretches beyond its use in Japanese cuisine. Seaweed has played a role in Irish cuisine for centuries; ranging from the use of carrageen (*Chondrus crispus*) in jelly-like puddings, to the use of dulse (*Palmaria palmata*) in baking (Indergaard and Minsaas, 1991). In the past, the seaweed biomass was primarily sourced from beach-cast seaweed, an unreliable method for any large-scale production of seaweed-based foods .

Globally, over 95% of all harvested seaweed is sourced from seaweed farms, with China producing approximately 47% of all seaweed biomass (FAO, 2018). However, the seaweed farming industry in Europe is still in its infancy, with less than 3% of the seaweed biomass produced in Europe coming from cultivation activities (Skjermo *et al.*, 2014). Although small in comparison to the global market, Europe has a unique set of assets envied by market leaders such as China, including an Atlantic coastline that harbours more than 3 000 seaweed species in nutrient-rich and clean waters, providing a stockpile of high diversity and high quality biomass.

Seaweeds can be produced in mono-culture or integrated aquaculture systems, on-land (using tanks or raceways) and at-sea (using long-line structures) (Pereira and Yarish, 2008). In Europe, the production of kelp (brown seaweeds e.g. *Alaria*, *Saccharina*, *Laminaria*, etc.) follows the same techniques used in Asia. Basically a) collection of fertile sporophytes; b) production of microscopic gametophytes in land facilities and seeding of lines/ropes with fertilized zygotes; c) germination of the plants until a certain size and transfer of these substrates with young blades to open-water sites; d) grow out phase at-sea in long-lines until harvest.

Globally, the seaweed bioeconomy is expected to rapidly increase to an estimated US$17.6 billion by 2021. In Ireland, the Sea Change Strategy stated that they aimed for the seaweed sector to reach a market size of €30 million by 2020 (Heffernan, 2006). However, wild seaweed stocks simply will not be able to fulfil the demand. Therefore, we must turn to cultivated or "farmed" seaweed.

Bantry Marine Research Station Ltd (BMRS), based in the south-west of Ireland, is one of the only institutions with a large-scale at-sea seaweed farm in Europe. Based on a 6-hectare site, BMRS currently cultivates over 10 tonnes (wet weight) of *Alaria esculenta* on long-lines in Bantry Bay annually. *A. esculenta* contains various high-value compounds, including fucoxanthin and phlorotannins, with the potential to enhance both human and animal nutrition and general well-being. However, optimal deployment and harvesting dates of the long-lines must be identified in order to optimise the cultivation of *A. esculenta* for these high-value bioactives.

Methods
Long-lines were deployed in a staggered manner in the autumn/winter of 2017 (year 1) and 2018 (year 2), and sampled at intervals during the spring of 2018 and 2019. Morphological and biomass data was collected for each sample point. Fucoxanthin was extracted and characterised using UHPLC methods (completed for year 1 only). Antioxidant activities of both compounds were also determined (completed for year 1 only).

A method for analysing phlorotannins (membrane-bounds and cytoplasmic) is being developed.

Results
Highest biomass was found in the ropes deployed last (09/12/2017; year 1) and harvested on 28/05/2018. The average weight was 12kgs per 1m of line (wet weight) when harvested. A general observation was that the weight increased over time, but the length of the blades reached their maximum at the beginning of May (2.25m), after which they became broader rather than longer.

(Continued on next page)

Initial analysis of the biomass in year 2 indicates a higher yield overall, with the highest biomass found in the ropes deployed last (11/12/2018). In general, the *A. esculenta* showed a faster growth rate in year 2 with longer blades on average than those in year 1.

For year 1, fucoxanthin levels were highest in the rope deployed last (09/12/2017) and harvested on 05/05/2018, three weeks before final harvest.

The results also indicated that freeze-dried samples had a more consistent moisture content and had a higher fucoxanthin content when compared to oven-dried samples.

Conclusions

Based on the results of year 1, delayed deployment of the long-lines provided better overall yields. Furthermore, the fucoxanthin levels observed were comparably higher than those reported.

The project is in its second year, and analysis on the biomass from the year 2 sampling timepoints are due to be completed in July/August of 2019.

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WATER QUALITY AND MICROBIAL COMMUNITY IN A SOLE HATCHERY RAS FOR ENVIRONMENTAL SUSTAINABILITY AND FISH WELFARE

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Introduction

Recirculating aquaculture systems (RAS) are a promising technology of fish production to reduce aquaculture environmental impact by reducing water usage, optimizing waste management and nutrient recycling (Martins et al., 2010). Water recirculation relies on the stability of physical, chemical and biological processes to improve biosecurity. Disruptions can cause fish disease outbreaks by opportunistic pathogenic bacteria. The beneficial bacterial community used to treat fish metabolic waste that is released into the water is still a challenging area to monitor. Next Generation Sequencing (NGS) technologies have permitted a deeper understanding of its diversity and abundance (Martínez-Porchas & Vargas-Albores, 2017). The aim of this study was to characterize the water microbiota across the different sectors of a sole (*Solea senegalensis*) RAS unit, and understand its relation with the water quality parameters.

Materials & Methods

Samples were collected from an established aquaculture production unit with four main systems: Open System inlet (OS), Breeding Stock (BS), Weaning (W2) and Pre-Ongrowing (PO). The later three are independent systems; BS and W2 are kept at salinity 35 while PO is kept at salinity 15. Water was filtered into Sterivex™ Filter Units with a 0.22µm Millipore Express (PES) membrane for DNA extraction. DNA extraction was performed with PowerWater Sterivex™ DNA Isolation Kit, extracted DNA was quantified by Qubit™ 4 fluorometer with a required concentration higher than 5ng/µL. Samples were prepared for Illumina Sequencing and sequence data was processed at Genoinseq (Cantanhede, Portugal). Sequences were then processed by the NGS analysis pipeline SILVA rRNA gene database project (SILVAngs 1.3) (Quast et al., 2013). In addition, water was characterized in terms of temperature, salinity and pH provided by the production daily monitoring.

Results & Discussion

In the sole hatchery in study, *Proteobacteria* and *Bacteroidetes* were the most abundant phyla in the diverse water compartments examined, with reported abundancies between 34-87% and 7-55%. They are followed by *Chloroflexi* (0.1-21%), *Patescibacteria* (0.1-15%) and *Planctomycetes* (1-5%). The bacterial community structure appears stable in the different sampling points belonging to the matching RAS system (Figure 1). A different observation was found when linking the three independent systems analysed, with PO being led by the genera *Leucothrix* and *Pseudoalteromonas*; the BS by *Polaribacter* and an uncultured *Cryomorphaceae*; and W2 by *Tenacibaculum* and an uncultured *Ardenticatenaceae*. The dominating genera in the OS were *Novosphingobium* and *Pseudoalteromonas*. Changes in microbiota composition appear to be connected with shifts in salinity, besides with other water quality parameters. Current work is focused on the description of the relations between the microbiota community and the environmental parameters, as well as on the relation between different microbiota groups with significance for water quality and fish welfare. We aim to add relevant data for the definition of the core microbiome diversity and structure of a healthy and beneficial community in this particular aquaculture unit, so we can determine ideal conditions for fish welfare and environmental sustainability of the production.

Acknowledgements

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A PRACTICAL SWIMMING ACTIVITY FOR THE EUROPEAN SEA BASS CULTURE (*Dicentrarchus labrax*): EFFECT ON GROWTH AND METABOLISM

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Introduction

Sustained but moderate swimming enhances growth in diverse fish species, even in early phases of culture, as we have observed in gilthead sea bream (Blasco et al., 2015). The swimming speed for optimal grow may be near optimal swimming speeds, according to Palstra and Planas (2011), where the cost of transport is lowest and the energetic efficiency highest. In sea bass yearlings, the improvement of growth through swimming activity has not yet been established. The main objective of this work focused on (1) the energy cost of sustained swimming, (2) the effect on growth and (3) on the metabolism of sea bass yearlings.

Materials and methods

Determination of a practical swimming speed for sea bass culture

Swim tests were conducted in a swim tunnel with an intermittently closed respirometer (Loligo system). Seven groups of five fish (5g mean body weight, 6 cm total length) were allowed to habituate for more than 30 minutes at a swimming speed around 0.25 BL·s^{-1} until oxygen consumption (MO_2) stabilized. Measures were taken at 0.5, 1, 1.5, 2, 3, 4, 5, … BL·s^{-1} for 15 minutes at each step, until one of the fish could no longer swim against the flo . Maximum O_2 consumption (MO_2max) was determined; the routine metabolic rate (MO_2r) was calculated from the exponential curve at zero speed, and aerobic metabolic scope (AMS) calculated as the difference.

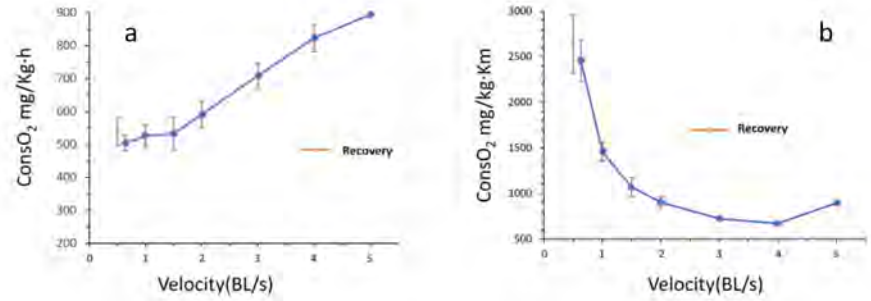

Figure 1. a) O_2 consumption rate (MO_2) and b) Cost of transport at different swimming speeds. Values are mean ± s.e.m. N=7 groups of five fingerlings.

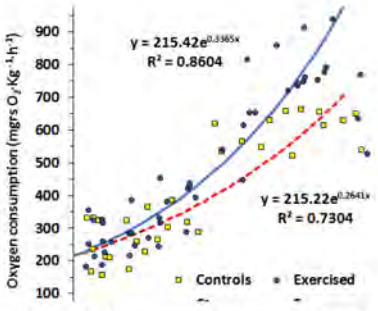

Figure 2. O_2 consumption rate (MO_2) of individual sea bass after 42 days of sustained swimming (EX) or voluntary activity (CT).

Table I. Effect of 6 weeks of sustained swimming on aerobic capacities of sea bass.

	CT (control)	EX (exercise)
MO_2 routine	215.4	215.2
MO_2 max	648.3	820.4
Aerobic scope*	432.9	605.2

aerobic capacities (Figure 2, Table I).

* MO_2 max - MO_2 routine (in mgrs O_2·Kg $^{-1}$·h^{-1}). N=6

(Continued on next page)

Swimming effects on the growth performance

Six-hundred sea bass fingerlings (3-4 g body weight) were randomly distributed into eight 200L, 60 cms diameter circular tanks (1.5 $kg \cdot m^{-3}$) and maintained during 6 weeks into a semi-closed recirculation system (22°C, 12L:12 D photoperiod). Four tanks (control group, CT) were kept on a water vertical flux, where fish movement was voluntary. Other four tanks were on a circular flux equivalent to 1.5 $BL \cdot s^{-1}$ of the initial total length (exercise group, EX). Fish were fed a commercial diet three times a day to apparent visual satiety. Feed intake and growth were recorded daily, and growth fortnightly. After 42 days, 12 fish per group were anesthetized, euthanized, and samples of white muscle were frozen in liquid N_2 and stored at -80ºC. White muscle main components (protein, lipid and glycogen) and their recycling by increasing $\delta^{13}C$ and $\delta^{15}N$ isotopic composition were analyzed, together with the genes expression and the enzyme activities of citrate synthase and cytochrome c oxidase.

Swim test response of untrained and trained fis

Six untrained and 6 trained fish were assayed as described before, and swimming performances and oxygen consumptions were compared.

Results and Discussion

Oxygen consumption increased slowly until 1.5 $BL \cdot s^{-1}$ and linearly until 4 $BL \cdot s^{-1}$. The slope change at 5 $BL \cdot s^{-1}$ shows that part of energy was supplied by anaerobic metabolism (Figure 1a). The cost of transport also changed its slope around 1.5 $BL \cdot s^{-1}$

(Figure 1b).

At the end of the experimental growth period (6 weeks), no significant differences were observed in final body weight, condition factor and growth indexes between control and swimming groups (CT, EX). Higher white muscle RNA/DNA ratio (+ 40%) and COX/CS ratio (+30%) suggested improvements of the protein synthetic capacity and of the functional mitochondria adaptation, respectively. ^{15}N-white muscle isotopic fractionation ($\Delta^{15}N = \delta^{15}N$ muscle - $\delta^{15}N$ diet) significantly decreased in swimming fish (CT: 2.0±0.04; EX: 1.7±0.06*** in ‰) suggesting protein sparing effect, as it was seen in swimming gilthead sea bream (Blasco et al., 2015). Moreover, fish swimming at 1.5 BL/s for 6 weeks significantly increased their aerobic capacities (Figure 2, Table I).

Conclusions

Sea bass fingerlings maintained at a practical swimming speed of 1.5 $BL \cdot s^{-1}$ expanded their aerobic capacity and showed white muscle metabolic changes related to potential improvements in the use of nutrients.

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THE IMPACTS OF BIOFOULING CONTROL MEASURES ON FISH HEALTH AND INFRASTRUCTURE, AND HOW TO MITIGATE THEM

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Biofouling, the unwanted growth of organisms on cage nets and other farm infrastructure, is a major challenge in aquaculture. If left untreated, it can impact fish health and welfare and affect farm integrity. To control the growth of algae, hydroids, and similar fouling organisms, nets are often coated with antifouling paint and cleaned regularly using high-pressure washers. This strategy is not only labour intense, costly, and environmentally problematic, but especially net cleaning entails risks for fish health and welfare and reduces the life-time of the applied coatings.

In multiple studies we assessed the risks of net cleaning: the release of cleaning waste particles and the abrasive treatment of the net surface. Our results show that cnidarians in the cleaning waste can damage the gills of cultured salmon, likely contributing to the dire gill health situations seen at many farm sites. An analysis of the spreading of the cleaning waste indicates that the harmful particles are not confined to the cleaned cage but can be distributed as far as to adjacent farm sites.

In addition, our experiments showed that washing of nets using high-pressure cleaners leads to abrasion of the antifouling coating, damaging as much as 30% of the coating in a single cleaning event and eventually removing almost all the coating during the grow-out season.

To address these risks and to support the sustainable growth of the aquaculture industry, novel biofouling control strategies are needed. To this end, we tested the efficacy of several coatings suggested as more environmentally friendly alternatives to today's copper coatings. However, none of the coatings based on alternative biocides or biocide-free components could compete with classic copper coatings. Thus, net cleaning remains the most important tool for non-biocidal biofouling control. Results from a study assessing novel net cleaning technologies indicates cavitation-based cleaning as an equally efficient but less abrasive alternative to high-pressure cleaning. While this technology has the potential to solve some of the challenges in aquaculture biofouling control, others remain and encourage further research in this area.

Based on the results of our latest research, current knowledge gaps will be discussed to indicate future research needs.

WHAT MAKES A FISH EGG ABLE TO BE FERTILIZED AND SUBSEQUENTLY DEVELOP INTO AN EMBRYO: NEW INSIGHTS FROM CRISPR/CAS9 GENOME EDITING IN MODEL SPECIES

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Introduction

The control of egg quality is a recurring question and a major issue in aquaculture for both traditional and new aquaculture species. Numerous studies in many different species have stressed the diversity of factors than can possibly impact the ability of the eggs to be fertilized and subsequently develop into a viable embryo. Our understanding of the mechanisms behind egg quality defects, including lack of fertilization and early embryonic failure, remains however still incomplete. In order to better understand the molecular processes that control egg quality, we have initiated a series of experiments to document the importance of various molecular factors (messenger RNAs and non-coding RNAs) for a fully fertilizable and viable egg using a genome editing approach in model fish species

Material and methods

Experiments were fully approved by INRA institutional and CREEA ethics committees and conducted using zebrafish (*Danio rerio*) or medaka (*Oryzias latipes*), two model species with short generation times and daily spawning. Candidate genes for genome editing (i.e. knock-out) were selected due to their predominant expression in the ovary in fish and, in some cases, in other vertebrates, including mammals. This strategy was applied to the following genes: *nucleoplasmin 2a* (*npm2a*), *nucleoplasmin 2b* (*npm2b*), micro RNA 202 (miR-202), *forkhead box R1*(*foxr1,* formerly known as *foxn5*), *OTU deubiquitinase with linear linkage specificity a* (*otulina*), and *solute carrier family 29, member 1a* (*slc29a1a*). Mutations (nucleotide insertions/deletions) in target genes were generated using CRISPR/Cas9 with one or several targets depending on gene size. When possible, homozygote -/- females were generated and used for reproductive phenotyping. Whenever it was not possible to transmit the mutation to the next generation, because of heavily impaired reproductive success, phenotypes were assessed in eggs produced by F0 females after checking that corresponding target transcripts were significantly knocked down

Results

In the case of *otulina* and *slc29a1a*, eggs originating from mutant zebrafish females exhibiting a knocked-down expression of target genes could not be fertilized. Similarly, mutant *npm2a* females produced eggs that were not cellularized, a phenotype resembling previously described unfertilized eggs (Cheung et al., 2018a)two npm2 (npm2a and npm2b. A similar phenotype was observed in miR-202 -/- mutant medaka females that also exhibited a dramatically reduced number of eggs per spawn and were therefore infertile or subfertile (Gay et al., 2018). In the case of *foxr1*, mutant female zebrafish produced eggs that could be fertilized but did not develop or developed abnormally and systematically died within 24h post fertilization (Cheung et al., 2018b)but the role of foxr1 in reproduction is unknown. Evolutionary history of foxr1 in vertebrates was examined and the gene was found to exist in most vertebrates, including mammals, ray-finned fish, amphibians, and sauropsids. By quantitative PCR and RNA-seq, we found that foxr1 had an ovarian-specific expression in zebrafish, a common feature of maternal-effect genes. In addition, it was demonstrated using in situ hybridization that foxr1 was a maternally-inherited transcript that was highly expressed even in early-stage oocytes and accumulated in the developing eggs during oogenesis. We also analyzed the function of foxr1 in female reproduction using a zebrafish CRISPR/cas9 knockout model. It was observed that embryos from the foxr1-deficient females had a significantly lower survival rate whereby they either failed to undergo cell division or underwent abnormal division that culminated in growth arrest at around the mid-blastula transition and early death. These mutant-derived eggs contained dramatically increased levels of p21, a cell cycle inhibitor, and reduced rictor, a component of mTOR and regulator of cell survival, which were in line with the observed growth arrest phenotype. Our study shows for the first time that foxr1 is an essential maternal-effect gene and may be required for proper cell division and survival via the p21 and mTOR pathways. These novel findings will broaden our knowledge on the functions of specific maternal factors stored in the developing egg and the underlying mechanisms that contribute to reproductive success.”,”DOI”:”10.7717/peerj.5534”,”ISSN”:”2167-8359”,”note”:”PMID: 30155373\nPMCID: PMC6109588”,”journalAbbreviation”:”PeerJ”,”language”:”eng”,”author”:[{“family”:”Cheung”, ”given”:”Caroline T.”},{“family”:”Patinote”,”given”:”Amélie”},{“family”:”Guiguen”,”given”:”Yann”},{“family”:”B

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obe","given":"Julien"}],"issued":{"date-parts":[["2018"]]}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} . Finally, *npm2b* mutant zebrafish females produced eggs that could be fertilized but developed abnormally and systematically died within 24h post fertilization (Cheung et al., 2018a)two npm2 (npm2a and npm2b, a phenotype consistent with our prior observations showing a developmental arrest at mid-blastula transition when maternally-inherited npm2b mRNA were blocked using morpholino injection in the eggs (Bouleau et al., 2014)but this maternal effect has never been demonstrated in nonmammalian species. A link between developmental competence and the abundance of npm2 maternal mRNA in the egg was previously established using a teleost fish model for egg quality. The importance of maternal npm2 mRNA for egg developmental competence remains unknown in any vertebrate species. In the present study, we aimed to characterize the contribution of npm2 maternal mRNA to early developmental success in zebrafish using a knockdown strategy. We report here the oocyte-specific expression of npm2 and maternal inheritance of npm2 mRNA in zebrafish eggs. The knockdown of the protein translated from this maternal mRNA results in developmental arrest before the onset of epiboly and subsequent embryonic death, a phenotype also observed in embryos lacking zygotic transcription. Npm2 knockdown also results in impaired transcription of the first-wave zygotic genes. Our results show that npm2 is also a maternal effect gene in a nonmammalian vertebrate species and that maternally inherited npm2 mRNA is crucial for egg developmental competence. We also show that de novo protein synthesis from npm2 maternal mRNA is critical for developmental success beyond the blastula stage and required for zygotic genome activation. Finally, our results suggest that npm2 maternal mRNA is an important molecular factor of egg quality in fish and possibly in all vertebrates.","DOI":"10.1095/biolreprod.114.119925","ISSN":"1529-7268","note":"PMID: 25009208","journalAbbreviation":"Biol. Reprod.","language":"eng","author":[{"family":"Bouleau","given":"Aurélien"},{"family":"Desvignes","given":"Thomas"},{"family":"Traverso","given":"Juan Martin"},{"family":"Nguyen","given":"Thaovi"},{"family":"Chesnel","given":"Franck"},{"family":"Fauvel","given":"Christian"},{"family":"Bobe","given":"Julien"}],"issued":{"date-parts":[["2014",8]]}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} .

Discussions and conclusions

Together, our results shed new light on the molecular processes behind fertilization and early embryonic development in fish. Here we reveal the importance of several genes (*otulina, slc29a1a, npm2a,* and *miR-202*) that are required for the production of fertilizable eggs. We also confirm the critical role of maternally-inherited *npm2b* for early embryonic development in consistency with the differential abundance of npm2b mRNA in eggs of varying quality in rainbow trout (*Oncorhynchus mykiss*) (Aegerter et al., 2005)14, and 21 days later. For all 56 collected egg batches, an egg sample was fertilized to estimate egg quality by monitoring embryonic development. Remaining eggs were used for RNA extraction and subsequent real-time PCR analysis. A significant drop of egg quality was observed when eggs were held in the body cavity for 14 or 21 days post-ovulation (dpo and report the key role played by *foxr1* in determining the ability of the eggs to support early development, once fertilized. Further investigations are in progress to understand how environmental factor and/or husbandry practices can impact egg quality through the modulation of the above studied genes.

Together, our observations indicate that the mechanisms defining egg formation involved evolutionary conserved players, not only among fish but also in vertebrates. Complementary investigations have been initiated to further study additional evolutionary conserved ovarian predominant genes, including molecular partners of nucleoplasmin. Finally, the major decrease in egg production and quality observed in KO miR-202 reported here suggests that non-coding RNA, and more specifically miRNA, could be relevant modulator of egg quality in response to environmental cues

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EFFECT OF FOUR DIFFERENT COMMERCIAL FEEDS ON GROWTH PERFORMANCE AND SURVIVAL OF EUROPEAN PERCH *Perca fluviatils* I N RECIRCULATING AQUAQUALTURE SYSTEM

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Further growing interest on aquaculture production of European perch is still limited by several bottlenecks. Economic production together with high quality and healthy meat depends essentially from artificial diets. Predicting growth rates, feed intake and energy requirements of the farmed animal in different life stages are crucial for the viability of the enterprise.

We tested four different available commercial feeds (3 mm) in a practical approach, three of them declared for percids (feed A, B, D) during grow out from 100 g to 200 g fresh weight in a three month trial in triplicate at an initial density of about 20 kg/m³ (157 fishes per treatment). Crude protein were nearly similar and ranged from 49 – 54%, crude fat was the testing variable with values from 10% (feed C and D), 15% (feed A) and 20% (feed B). Further, D was swimming feed in contrast to sinking feeds A, B and C.

At the end of the experiment we compared survival, final fresh weights, specific growth rate, Fulton's condition factor (K), coefficient of variance, filet quantity and quality (fatty acid analysis) and other relevant parameter

At the end of the experiment mean survival was highest with feed D (98.5%). Remarkable lower survival was overserved for feed A (94.1 %).

Conclusion
Under the tested conditions feed B, declared for percids, showed best results in terms of growth, survival and food quotient. Feed B contained highest value of raw fat (20 %) and was relatively expensive (€/kg feed). In contrast, not explicit for percids declared feed C revealed comparable results to feed B for growth and also good results for survival and FQ. This feed was low in raw fat (10%) and was much cheaper (€/kg feed) and could therefore recommended for economical grow out of *Perca fluviatils*.

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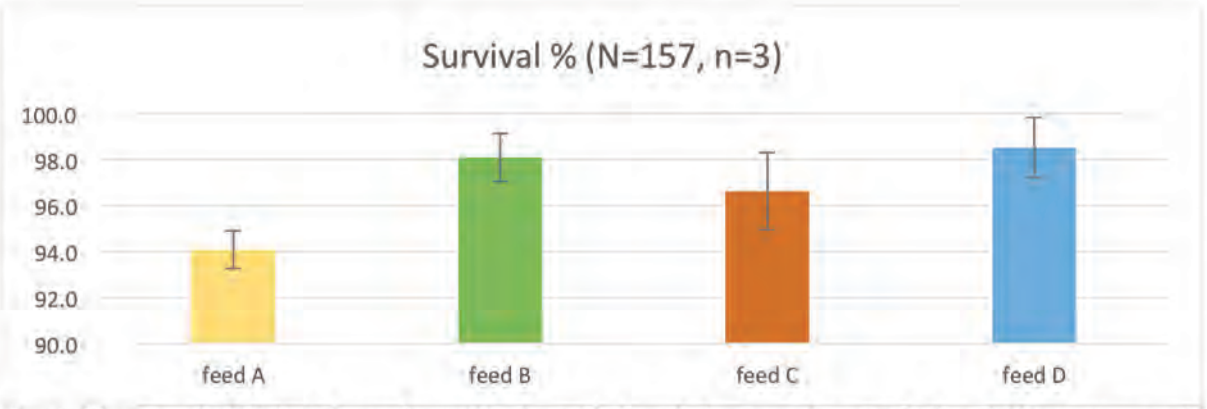

Fig. 1: Mean survival (%) of *Perca fluviatilis* after 105 days during growth out with four different commercial feeds.

Differences of food quotient (FQ) were remarkable between the tested diets at the end of the experiment ranging from 1.6 (B) to 2.7 (A) (fig 2).

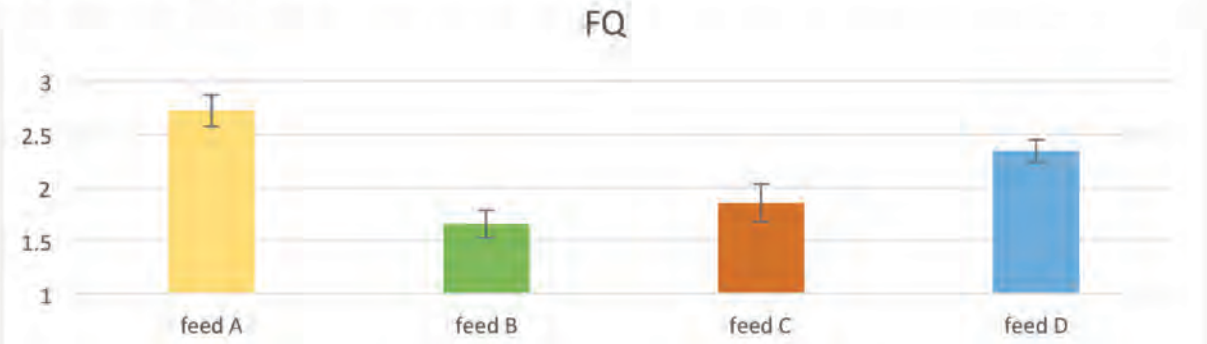

Fig. 1: Food quotient (FQ) of *Perca fluviatilis* after 105 days during growth out with four different commercial feeds.

At the end of the trial animals reached final mean body weight of 157.0 g (A), 160.1 g (D), 180.9 g (C) and 187.7 g (B) and significant differences were observed (fig. 3).

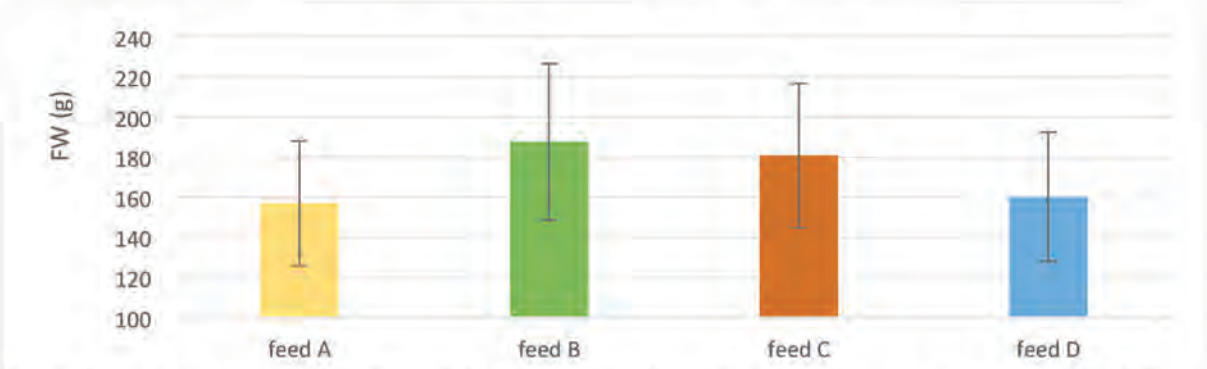

Fig. 3: Mean fresh weight (FW) (FQ) of *Perca fluviatilis* after 105 days during growth out with four different commercial feeds.

LAND-MARK BASED MORPHOMETRIC DISCRIMINATION OF NATIVE AND INTRODUCED PIKE-PERCH, *Sander lucioperca* (L.) POPULATIONS IN EURASIA

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Introduction

Since pike-perch was distributed widely in Turkish freshwater, native or introduced, the phenotypical characters might be affected by fishing pressure, different environmental conditions and feeding habits. As a first study on morphological discrimination of pike-perch population in Turkey, it is aimed in this study (i) to understand the effect of environmental conditions on pike-perch morphology and (ii) see the differences of morphometric characters in between native and introduced populations in this article.

Material Method

A total 223 S. lucioperca specimens which were collected from six geographically different regions of Anatolia and Europe were analysed (Fig. 1). All specimens were carried to the Laboratory of Fisheries Faculty of Akdeniz University and right body profile of each specimen was photographed under the day light by high resolution digital camera (D560, Nikon, Japan). 18 landmarks were identified by tpsDIG freeware Thirty-five distances between landmarks were calculated by Pythagorean. In order to avoid the effects of different specimens' size on analysis, size-dependent variation was removed in morphometric characters. For the analysis of morphometric characters, MorphoJ software was used for wireframe graphs. Normality, ANOVA, Wilks' λ, Canonical Function Analyses were done by SPSS software programme.

Results

The predicted group membership shows how many individuals are morphologically classified with which populations and 85.10 % of original grouped cases are correctly classified. In this table it can clearly be seen that the least corrected classification was in SASDL (75.00%) population because in total 12 out of 48 individuals have common morphometric characters with other populations. ETLD (94.40%) population was the most correctly classified by morphological characters in wild populations. Outside sample, DEOR showed small similarities with WALM (3.30%), SASDL (10.00%) Turkish samples. According to Wilks' λ value, it was determined that except LM1-18, LM11-13, LM01- 15, LM5-6 characters from head and second dorsal fin out of 34 investigated characters, all other 30 morphological parameters belonging to body and caudal region showed significant differences ($P<0.01$) which explained the discrimination between native and introduced population in study region. Discriminant Function Analysis showed the effect of differences of characters on population discrimination. While ETDL and NALB populations have similar body shape, when two native populations, ETDL and NALB are compared with introduced populations, it has been seen ETDL population mostly discriminate from WALM and SASDL by slightly body depth and head formation but strongly divert from SALE by head length and depth, also caudal peduncle (Fig 2.). Morphologic characters of NALB population distinguished from WALM and SASDL mostly on body depth and fin length but SALE population di fer from NABL considering the head formation**.**

Discussion

The main finding of this study is that introduced SALE population seems strongly differentiated from other introduced populations WALM, SASDL and also native populations NABL and ETDL. It was expected that European population DEOR would be classified far from Turkish native populations but results indicate that DEOR population has slightly connected with WALM and SASDL populations. Fontaine et al. (2015) found at least genetically differentiated groups in Europe and DEOR population was actually in between these two groups (Hungarian and Scandinavian). Since WALM population was introduced from Austria (Çelikkale 1990), its connection with DEOR might be understandable because of the large genetic distribution of riverine populations (Krpo-Ćetković and Stamenkovic 1996). However SALE populations should be siblings of WALM; hence, differences between these two populations seem interesting and show possible different ecological factors and feeding habits affecting morphological structure of pike-perch. In this study, it is clearly shown that two major pike perch populations from Northern Anatolia have discriminated from introduced populations (European origin). In present study it is also clearly seen that one of the two introduced pike-perch populations, Lake Eğirdir, strongly discriminated from other introduced and native populations, which shows that feeding habits, feed availability and fishing pressures have strong effects on morphology of pike-perch populations in study area.

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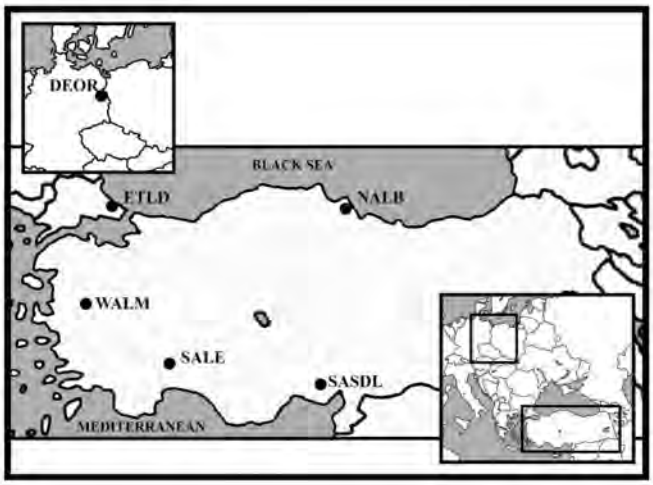

Figure 1. The map of the sampling. The symbols of pentagon indicate sampling locations. The samples referred to in the text were Lake Balık (NABL), Seyhan Dam Lake (SASL), Lake Eğirdir (SAEL), Lake Marmara (WAML), Lake Durusu (ETDL), Oder River of Deutschland (DEOR).

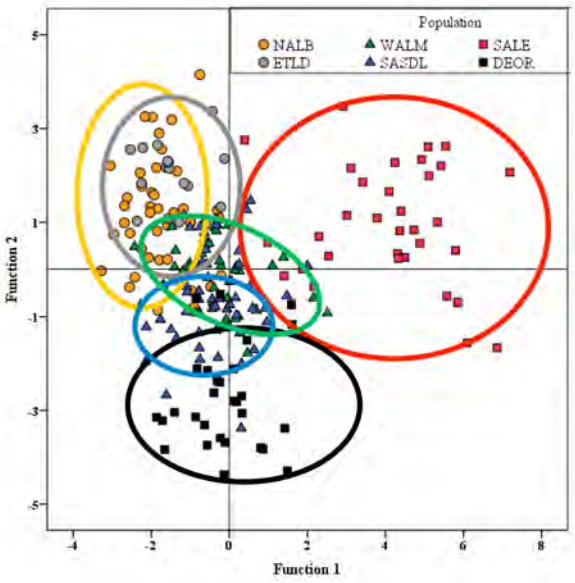

Figure 4. Canonical functions of the discriminant analysis of pike-perch populations from Turkey and Oder River (Deutschland)

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MODELLING AND DIMENSIONING AQUAPONICS AS THERMAL TREATMENT NETWORKS THAT OPTIMIZE GEOTHERMAL ENERGY USE

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Introduction

The ongoing global push for sustainability has motivated both the greenhouse horticulture and aquaculture sectors to explore the potential of geothermal energy for heating as an alternative to fossil fuels (Badiola et al., 2018; Lund and Boyd, 2016). Greenhouses and RAS experience distinct heat demand fluctuations throughout the year and therefore do not make consistent use of geothermal wells. Geothermal wells on the other hand, need to operate close to full capacity year-round to be profitable. The heat use efficiency of geothermal wells could be improved by combining both food production systems into an aquaponic-based thermal treatment network in which a RAS functions as a sink for the (low temperature) residual geothermal heat coming from the greenhouse, but also utilizes geothermal heat directly whenever greenhouse heat demand is low (Thorarinsdottir and Unnthorsson, 2018).

The EU funded GEOFOOD project aims to analyse in detail how to optimize the design and operation of such aquaponic facilities that use geothermal energy. To that end a predictive model was developed which simulates the energy balances present throughout the thermal treatment network. The model enables the user to compose a sequence of geothermal wells, greenhouses and RAS for which different scenarios can be explored by selecting several types of greenhouse, different crops and fish species as well as climates. When used as a design tool, the model is capable of dimensioning the aquaponic facilities and/or the geothermal well. On the other hand, the model can be employed to analyse the hourly heating requirements of individual components within the thermal treatment network.

Methods and materials

Geothermal aquaponics consisting of a 5 ha tomato greenhouse combined with a RAS for pike-perch has been simulated for three different locations: The Netherlands, Iceland and Slovenia. The objectives of the simulations are to dimension the geothermal well and RAS to optimize for heat use efficiency and to investigate the impact of the local climate conditions on optimal geothermal capacity and RAS size.

The model KASPRO (De Zwart, 1996) has been used to simulate greenhouse heat demand as well as flow and temperature of the residual heat coming from the greenhouse. It is a physical greenhouse climate model coupled with a virtual climate controller, consisting of modules describing the energy and mass balances present within the greenhouse.

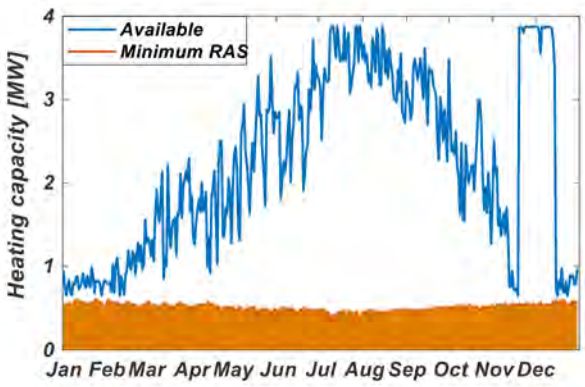

Figure 1. Heating capacity needed by the minimum size RAS (i.e. 6544m^2) and available heating capacity.

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The RAS energy model has been newly developed for the GEOFOOD project in order to predict the heat demand of RAS facilities. It is based on the energy balance for indoor aquaculture facilities as described by Timmons and Ebeling (2013). As part of the GEOFOOD project the model will be validated using on-site measurements performed at a new aquaponic facility that has been completed May 2019 in The Netherlands at the research centre of Wageningen University & Research, Greenhouse Horticulture.

Results

For a tomato greenhouse located in The Netherlands preliminary results show that geothermal heat extraction can be increased with 31% by combining it with a pike-perch RAS of 6544m^2. At this size, the RAS runs completely on residual and surplus geothermal heat, without the need of alternative energy sources during peak demands.

However, from figure 1 it can be observed that still a large part of the available heat remains unused. Simulation results also indicate that the RAS facility can be increased from 6544 m2 to 8894 m^2 (i.e. an increase of 35.9 % in fish production capacity) if only at peak demand an alternative heat source may be used, amounting to no more than 5 % of yearly RAS heat demand. In that case the simulated aquaponic system, consisting of a 5 ha tomato greenhouse and 8894 m2 pike-perch RAS, could extract 40 % more heat from the geothermal well than a 5 ha tomato greenhouse. Results for the other locations and the ongoing model validation process will be presented.

Discussion and conclusion

Integration of the newly developed RAS model and the greenhouse model KASPRO into a thermal treatment network enables assessment of geothermal energy use potential within circular food production systems, in particular aquaponics.

In the specific case of the simulated tomato greenhouse within The Netherlands, the results suggest that integrating a RAS facility contributes positively to exploitation of geothermal heating infrastructure.

Though further validation is required, the presented model is not only relevant for the current energy transition in terms of assessing geothermal potential for food production. It could also support efforts to connect established greenhouse horticulture areas with an emerging RAS sector, thereby moving towards more circular food production systems.

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DEMYSTIFYING CRITICS ABOUT HYDROGEN PEROXIDE UTILIZATION IN RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

Recirculation Aquaculture Systems (RAS) use water recycling as the base for an efficient production. Water reutilization demands cleaning techniques to remove accumulating compounds. These include mechanical filtration, biofiltration and a disinfection method. The most common disinfection methods used in RAS are UV and Ozone, both having pros and contras in terms of costs, risks, and application requirements. An alternative to these methods is hydrogen peroxide (H_2O_2), which, even been classified as a green oxidant, has not been commonly used and is hardly criticized due to acquisition, storage and secure manipulation issues. As H_2O_2 dissociates into non-toxic environmentally benign by-products including oxygen, its application offers potential savings in terms of oxygenation in RAS. Moreover, H_2O_2 is used to treat ectoparasitic illness with FDA approved H_2O_2-35% dosages between 50-1000 mg/L for 15-60 min. Minimum concentrations to which stress responses have been observed in salmonids range between 4-170 mg/L while the limits for biofilter function are around 5 mg/L [1-4].

Materials and methods

In the present study, we perform serial experiments in a RAS rearing ~65 kg European seabass (*Dicentrarchus labrax,* weight ranging from 500-800g) to test the best application possibilities for H_2O_2 into a system, with concentrations between 2.4-15.8 mg/L/h. We tested two application positions: the bypass between the protein skimmer and the sump (with higher organic burden), and the flow to the rearing tanks (low organic load). We evaluated the impact on physicochemical parameters with focus on oxygen and nitrogen species concentration and the effect of the application on bacterial community of the different compartments (assessed via total bacterial counts in CFU/ml). Dosage was applied for 4 h on a daily basis and rates of decay of H_2O_2 on the system was assessed with Quantofix Peroxide test sticks (Sigma Aldrich, from 0-25 mg/L and 0-100 mg/L) until total removal. Ozone was switched off during dosage. Smart Digital S-DDA (Grundfos, Germany) dosage pumps were used for a controlled H_2O_2 application to the system (dosing capacity between 2.4 ml/h and 7.5 L/h). Stress levels of the reared fish was assessed using cortisol and glucose as blood markers

Results

With the concentrations used, we accomplish a partial disinfection of the system without reaching levels affecting the bacterial flora of the biofiltratio units or the health of the reared fish. We found a significant reduction of the bacterial load with each of the concentration tested. The best application position was the inlet to the protein skimmer, due to the lowest impact to the bacterial flora of the filter and the reared fish. An economically relevant increase in oxygen level of the system as well as the highest disinfection levels were achieved with 15.8 mg/L/h. In this assay, an artificial oxygen depletion was simulated before applying H_2O_2 to the system, and this was recovered within the first 40 min, even during a feeding period, which normally reduces the oxygen level in the water column. After application, the rates of decay varied from 30 min to 1 h, time after which, no H_2O_2 was detected in the system.

No significant negative effects were found on fish stress markers even when cortisol (Ref: 59.8±49.5 vs. Treat: 62.2±43.5) and glucose (Ref: 131.0±34.9 vs. Treat: 155.1±45.3) levels tended to increase during the experiments using increasing H_2O_2 doses. These markers showed a high variability. During application, the number of fish showing conspicuous low cortisol levels increased.

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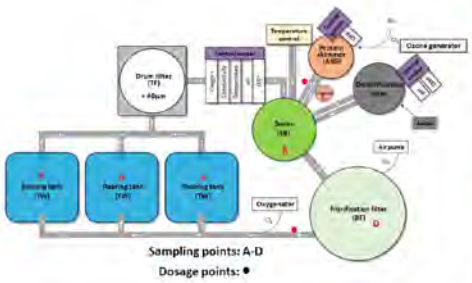

Fig.1: Schematic representation and picture a RAS to which H_2O_2 was applied. Application points: 1) bypass between protein skimmer and sump, 2) flow to the rearing tanks. Samples for control of physicochemical and microbiological parameters: A-D.

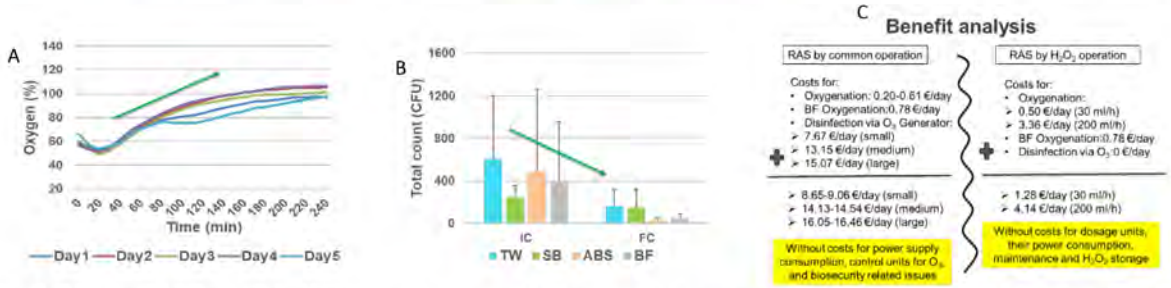

Fig.2 Effect of 15.8 mg/L/h H_2O_2 application to the oxygen concentrations (A) and bacterial burden (B) of a RAS without additional oxygenation; TW: sample from rearing tanks; SB: sample from sump; ABS: sample from the degassing compartment of the foam fractionation unit; BF: sample from the nitrification filter. C. Economic benefits of H_2O_2 application compared to common systems using Ozone disinfection on a daily basis.

Discussion and conclusion

Organic burden in the system, fish stocking density, feeding frequency and biofilter performance were all factors influencing the required H_2O_2 dosage and its rate of decay in accordance to what has been published [2, 5, 6]. In the present study, we found a relatively rapid decay of H_2O_2 remnants, supported by the organic load typically present in a RAS rearing at commercial or semi-commercial scales. We recommend to apply H_2O_2 in positions of the system with high organic burden to avoid affecting biofilter function or promoting nitrite accumulation as previously published [5]. Despite previous results showing negative stress responses of *D. labrax* to H_2O_2 application [7], we did not found critical changes on cortisol or glucose related to the treatment. These authors used smaller fish (weight 120-200 g) and doses of 50 ppm/h, which are higher than in the present study. The stress in *D. labrax* is genetically driven and highly dependent on size/age with individuals having consistent high (439.2 ± 31.1 µg/dl) or low (247 ± 85.1 µg/dl) cortisol concentrations and, as well as glucose, there is a circadian pattern for these parameters within this species [8, 9]. Glucose levels between 100-150 mg/dl have been reported in the literature for *D. labrax* [10]. With appropriate knowledge of system performance and microbiological background, H_2O_2 can be a positive complement to the disinfection spectrum of a RAS. It can be used alternating with common methods to avoid artificial selection of specific microbial groups and can have a positive impact in cases in which parasitic illness are present. Thus, as a disinfection method with a plus for its oxygenation influence, H_2O_2 can enrich the disinfection portfolio of aquaculture facilities.

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AMMONIUM REMOVAL BY ION EXCHANGER - EFFECTS ON WATER PARAMETERS, MICROBIAL COMMUNITY AND SEA BASS (*Dicentrarchus labrax*) IN A MARINE RECIRCULATING AQUACULTURE SYSTEM

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Introduction

Water treatment plays a central role in the successful operation of recirculating aquaculture systems (RAS), because high stocking densities and feeding rates rapidly impair the quality of the limited water body. In RAS, nitrogen load is typically removed by multistep biological filtration whereby the first step is ammonium oxidization to nitrite. This biological degradation is vulnerable to disturbances e.g. changing water conditions and medications of the cultivated species what can lead to an accumulation of toxic nitrogen components in the system water. Ammonium can alternatively be removed from water by using zeolite. In emergencies zeolite can decrease fish mortality by reducing dangerous ammonium concentrations. In the present study a marine RAS, stocked with Sea bass (*Dicentrarchus labrax*), was operated with the bioreactor replaced by zeolite in the RAS water. Water parameters, microbial community and reared animals were determined to evaluate the impact of an alternative ammonium removal method on RAS performance.

Material and methods

Two identical 5m^3 RAS each equipped with 3 rearing tanks, drum filte , ozone- injected protein skimmer and moving bed bioreactor were operated at constant water parameters (temperature: 17.0°C, conductivity: 50.4mS cm^{-1}, pH: 7.9, O2: 97.8%). RAS were stocked in each experiment with 9kg m^{3-1} juvenile Sea bass (102.7 ± 25.3g) and fishes were fed 8 times a day (1.02% body weight per day) during a period of 21 days. These two RAS were controlled in 3 different scenarios: 1) RAS 1 and 2 with bioreactor and ozone, 2) RAS 1 and 2 with bioreactor without ozone and 3) with zeolite and ozone (RAS 1) or without ozone (RAS 2). Nitrogen components and abiotic water parameters in the water were measured daily. At the beginning and the end of each phase bacterial count and the composition of the bacterial community were determined. At the end of each phase the blood of 12 fishes per RAS was collected and analysed for relevant parameters (cortisol, glucose, lactate, ammonium, lysozyme, haematocrit).

Results

During phase 1 and 2 ammonium and nitrite concentration in the tank waters were similar in both RAS (Figure 1). Zeolite treatment (phase 3) led to a large increase in ammonium concentration and a decrease in nitrite concentration when ozone was applied (RAS 1), while without ozone ammonium increase was much lower but nitrite was strongly elevated (RAS 2). Nitrate level in the water increased during phase 3 in both RAS but with a higher slope in the system with ozone. During phase 1 and 2 ozone treatment had minimal effect on the bacterial count in both systems. Numbers of bacterial units increased strongly during zeolite treatment, especially when ozone was not applied (Table I). Zeolith treatment had no effect on stress related blood plasma parameters of the fishes.

Discussion

Zeolite had strong effects on water parameters in both RAS. Ammonium content in the water were elevated without bio- filtration, and probably caused bacterial growth in the RAS leading to high nitrite level in RAS 2 without ozone. An additional ozone treatment could prevent the accumulation of toxic nitrite, because of the spread of the bacterial mass was reduced. Blood parameter data indicate that the zeolite treatment could be suitable to continue the normal operation of a RAS when biofiltration is affected allowing emergency situations to be managed without the need for performance-reducing emergency measures such as stopping feeding or reducing stock biomass.

EVALUATION OF A MEMBRANE-DENITRIFICATION REACTOR (MDR) IN A RECIRCULATION AQUACULTURE SYSTEM UNDER REAL CONDITIONS

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Introduction

According to the European Commission, recirculating aquaculture systems are appropriate measures to reduce the release of nutrients into the receiving waters (European commission, 2016). Developing and implementing of innovative technological solutions are suggested to achieve the objectives of the strategic guidelines for sustainable development of EU aquaculture (European commission 2013). The removal of nitrate and particles is a crucial factor not only for the environment, but also for animal welfare.

In previous investigations, a combination of denitrification and membrane cleaning was investigated in small membrane denitrification reactors, and then two semi-technical recirculation aquaculture systems (RAS) were installed for feasibility studies of the Membrane-Denitrification-Reactor (MDR) (Boley et al. 2017). In the present study, the integration of the MDR in two commercial RAS systems was investigated and evaluated.

Materials and methods

To demonstrate the functionality under real conditions, the MDR was installed in the water treatment unit of Fischzucht Rhönforelle in Marjoss, Germany (RAS1, s. Fig. 1) and thereafter at AQUA SCHWARZ GmbH in Goettingen, Germany (RAS2).

RAS2 was a smaller system only with biofilte . Two independent systems were used, one with MDR (A) and the other without MDR (B) as reference. Total fish mass increased from 39 kg/tank to 156 kg (A) resp. 164 kg (B) per tank. The fish stock was reduced several times since the biofilter capacity was insufficient. RAS1 was operated with water from a well with low hardness (0.5 mmol/L), in contrast to the water in RAS2 with a very high hardness (5 mmol/L).

For both RAS, measuring devices were installed near to the MDR for the online-measurement of different parameters (s. Fig. 2). Lab analyzes were also carried out.

Results and discussion

The denitrificati n started fast in both RAS, so that after 2 d, the nitrate was completely reduced in the effluent of the MDR (s. Fig. 2). The denitrification rates were low (1…2) mg/(L h), due to a small membrane area, compared to the large water volume inside the MDR (~600 L). The flux through the membranes reached (7…10) L/(m² h), s. Fig. 2. A special feature in RAS2 was the low consumption rate of ethanol, which could be reduced to 1.0 g ethanol/g NO_3^--N without increased nitrate concentrations in the effluent, whereas the stoichiometric consumption is 1.4 g Ethanol / g NO_3^--N. This indicates that additional organic compounds from the fish tank were used for denitrification, because here, in contrast to RAS1, a sedimentation unit was missing. This assumption was also supported by the COD concentrations in the effluent, which were significantly lower than in the influen

Therefore, the NH_4^+-N levels in the MDR effluent in RAS2 increased to 2.7 mg/L, while they remained below 0.1 mg/L in RAS1 with lower organic load. Single Nitrite peak conc. in the effluent of MDR in RAS1 were observed up to 6 mg/L. In RAS2 the NO_2^--N conc. remained low, but when the consumption rate of ethanol decreased to below 1 g Ethanol / g NO_3^--N, the NO_2^--N concentrations in the effluent of MDR increase

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Fig. 1 RAS1. Left: Fish tank; Right: MDR with measuring devices (left side)

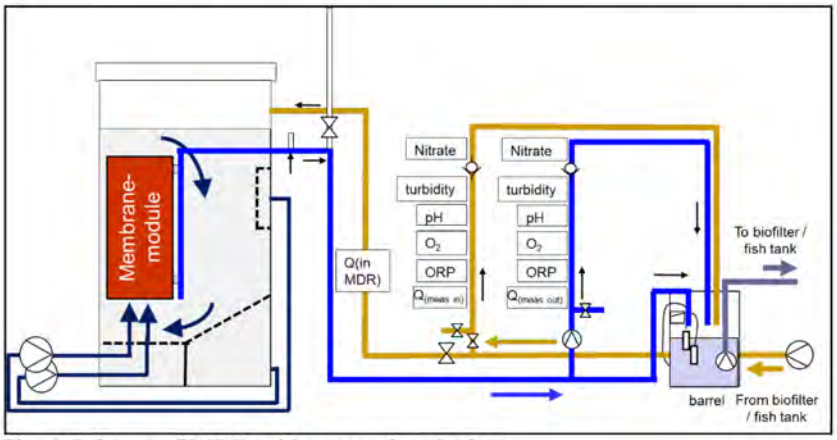

Fig. 2 Scheme of MDR with measuring devices

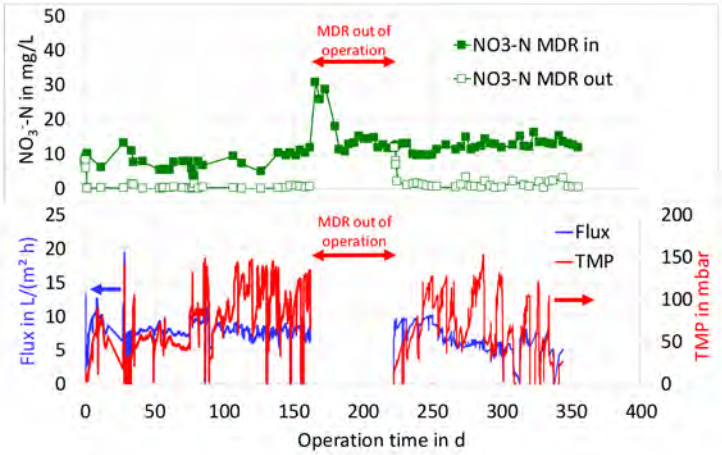

Fig. 2: RAS2: Above: Nitrate conc. as NO_3^--N (MDR in = tank A; in = influent, out = effluent of MDR. Below: flux and TMP in MDR

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A COMPARISON OF GROWTH, MORPHOLOGY, CONDITION, FILLET YIELD, DRIP LOSS AND RIGOR PROGRESSION IN TWO STRAINS OF TURBOT (*Psetta maxima)*

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Turbot (*Psetta maxima*) is a high-value whitefish that is much sought after by trade customers and consumers alike. Globally, capture production was almost 6,500 tonnes in 2017, compared to over 57,000 tonnes of farmed production in the same year (FAO, 2019). The main producers are China, the Netherlands, France and Spain, with the majority (80%) coming from China. Globally, turbot production is very small when compared to other marine species but there was rapid commercial growth between 2000 and 2010. More recently, Spanish production has increased and now accounts for more than three quarters of the European production of turbot.

While many of the major bottlenecks have been overcome, there is still some applied research to be undertaken to ensure the upward trend in production is maintained. For example, there are a number of 'strains' (or broodstock) of turbot available to turbot farmers. There is little empirical data that enables direct comparisons to objectively assess the stock performance and product quality for these 'strains'. The main traits associated with improved stock performance, from a commercial perspective, are growth performance and fillet yield, but also, from a consumer perspective, it is important to consider and examine the characteristics of the seafood products themselves including, for example; fillet quality traits, sensory attributes and shelf-life. It is imperative that stocks can be assessed for production characteristics to enable farmers to choose the best stock for their farm.

The primary aim of this experiment, as an initial study with turbot stocks, was to compare growth performance, body form and shape, general body morphometry and some selected product traits, specifically progression of rigor and drip loss, in two commonly available strains of turbot, one originating from Scotland, the other from Denmark. A selection of these results will be presented here.

GROWTH PERFORMANCE AND QUALITY TRAITS OF SIBERIAN STURGEON *A. baerii* JUVENILES FED DIETS INCLUDING *Nannochloropsis gaditana* AND *Scenedesmus almeriensis* MICROALGAE MEAL

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Introduction

The demand for aquafeed grown exponentially in the last decade and is expected to increase further in the coming years (FAO 2018). The wild fish used for the fish meal and oil, currently used in feed formulated for carnivorous species, will be used in human consumption and less available for aquaculture. Among the potential ingredients of future use in aquafeed, microalgae represent a promising matrix, as characterized by nutritional, nutraceuticals and immunostimulant properties (Camacho-Rodríguez *et al.,* 2017). However the high production cost is a limiting factor for their use. The development of a microalgae-based biorefiner , able to use some by-products and agro-industrial waste to produce biomass would enable to limit the disposal costs sustained by the companies and to lower the production costs of the microalgae. The present research was undertaken to evaluate effect on growth response and fillet quality traits of sturgeon (*A. baerii*) fed with two microalgae freeze-dried biomass *Nannochloropsis gaditana* and *Scenedesmus almeriensis* grown in Sinthetic Medium (SM) or in diluted Pig Manure (PM) and included in partial substitution of dietary fish meal and oil

Materials and methods

Four complete diets were formulated to be grossly iso-proteic and iso-lipidic. A control diet (C) was prepared using a blend of conventional animal and vegetal protein sources. The test diets coined respectively *N. gaditana* grown on Synthetic Medium (NSM), *N. gaditana* grownoin pig manure (NPM), *S. almeriensis* grown on Synthetic Medium (SSM) and *S. almeriensis* grown on Pig Manure (SPM) were prepared by replacing the 10% of protein and lipid supplied by the blend of conventional protein and lipid-rich ingredients with microalgae. All the ingredients are mixed and pelleted by a cold extrusion process (70°C). Each diet were randomly assigned to tank and tested in triplicate according to a monofactorial design. Microalgae dried biomass and diets were analized microbiologically and verified for nutritional quality. To carried out the feeding trials 240 juvenile *A. baerii* (average 12.8±0.3g each) were randomly allocated among 15 circular tanks (16 fish/tank) in RAS system under controlled rearing conditions (temperature, 19°C, DO 9.6 mg/L, artificial daylenght, 12h). Diets were offered in two daily meals with a fixed feed ratio (3% body mass) over 6 weeks and each group were weighted every week under moderate anaesthesia. At the end of the trial, survival rate (%), Final Body Weight (FBW), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Feed Intake (FI), were evaluated. Furthermore, nine fish per feed treatment were analized to determine the biometric indeces, fillet proximate composition and oxidation parameters (SOD, CAT, GPX, 8-isoprostanes). Data were subjected to ANOVA and differences tested by the Tukey's test ($P < 0.05$).

Table I Proximate composition of microalgae biomass included in the experimental diets

	Water (%)	Protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)
N. gaditana on PM	4.7	48.4	18.6	23.8	4.5
N. gaditana on SM	4.5	35.8	14.5	32.8	8.2
S. almeriensis on PM	3.4	33.8	8.4	13.7	29.1
S. almeriensis on SM	8.0	29,1	2.1	25.1	18.5

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Results

The macronutrient composition of the dried micoalgae biomass are reported in Table I.
Microbiological analysis of microalgae biomass showed no difference in TBC (Total Bacterial Count) among the different thesis (average 5.9±0.07 log CFU/g). *E. coli* were found below the detection limits of the method (< 2 log CFU/g) in *N. gaditana* grown on PM and *S. almeriensis* grown in both SM and PM, while its value was 3log CFU/g in *N.gaditana* grown on SM. Enterobacteriaceae resulted respectively 2.7 and 2.0 log CFU/g in *N. gaditana* and *S. almeriensis* grown on SM and under detection limits of the method (< 2 log CFU/g) in *N. gaditana* and *S. almeriensis* grown on PM. *Salmonella* resulted absent in all the microagae biomass. All the diets used in feeding trial resulted similar for their proximate, fatty acid composition and microbiological quality (data not reported). Dietary treatments significantly affected FBW that resulted similar in the groups C (44.2g), NSM (44.7g) e NPM (43.9g), while it was significantly lower ($P<0.05$) in the SSM (40.8g) and SPM (40.5g) groups. However, did not result in significant changes in survival rate, SGR, FCR and of the biometric index (K), nor fillet composition. Also oxidation parameters (SOD, CAT, GPX, 8-isoprostanes) of fillet were not significantly a fected by dietary treatments ($P>0.05$).

Discussion and Conclusion

Very few data are available on the use of microalgae biomass in acipenserids diet. Spirulina meal integrated with plant oils was found to be a good alternative to replace fish oil in white (*A. transmontanus*) and siberian (*A. baerii*) sturgeon diet (Palmegiano *et al.,* 2008; 2002). The data observed in this study confirm the potential use of the microalgae *N. gaditana* and *S. almeriensis* in the siberian sturgeon diet, in fact all the experimental diets tested, both based on microalgae grown on SM and on PM ensure a balanced and complete level of the nutrients, suitable for the growth of sturgeon juveniles and nutritional quality of the fillet, analogous to the control group fed with a fish meal/oil-based diet. Moreover the use of agrozootenic by-products, such as pig manure, for the growth of microalgae, appears to be a good alternative to common fertilizers, to reduce production costs.

Acknowledgments

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LIGHT-ENHANCED SHELL FORMATION IN THE FLUTED GIANT CLAM, *Tridacna squamosa*, INVOLVES LIGHT-DEPENDENT EXPRESSION OF NA^+-DEPENDENT SECONDARY ACTIVE TRANSPORTERS IN ITS CTENIDIUM

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Giant clams live in symbiosis with phototrophic zooxanthellae and strive in nutrient-deficient tropical waters where light is available. They conduct light-enhanced shell formation that involves the deposition of calcium carbonate (calcification) onto the inside surface of the shell valve following the reaction of $Ca^{2+} + HCO_3^- \Leftrightarrow CaCO_3 + H^+$. Hence, light-enhanced shell formation necessitates the increased supply of Ca^{2+} to and removal of H^+ from the extrapallial fluid, which is the site of calcification, to promote a more rapid precipitation of $CaCO_3$ as aragonite. Indeed, there is a significant increase in the pH of the extrapallial fluid in the fluted giant clam, *Tridacna squamosa*, exposed to light. H^+ could be transported from the extrapallial fluid into the shell-facing epithelial cells of the whitish inner mantle through plasma membrane Ca^{2+}-ATPase per se or as NH_4^+ through an unidentified transporter. Subsequently, the excess H^+ could be shuttled through the hemolymph to the ctenidium (gill) for excretion. The ctenidium of *T. squamosa* expresses homologs of Na^+/H^+ exchanger 3 (NHE3-like) and vacuolar-type H^+-ATPase subunit A (ATP6V1A) that have an apical localization in ctenidial epithelial cells. With illumination, the gene and protein expression levels of these two H^+ transporters increase significantl , in support of increased H^+ excretion during light-enhanced shell formation to maintain acid-base balance. In addition, the ctenidium of *T. squamosa* expresses a homolog of Na^+/Ca^{2+}-exchanger 3 (NCX3-like), the gene and protein expression levels of which are also up-regulated by light. As NCX3-like is localized to the basolateral membrane of the epithelial cells of the ctenidial filaments, there could be an increase in the transport of Ca^{2+} from the ctenidial epithelial cells to the hemolymph, and the absorbed Ca^{2+} is shuttled to the extrapallial fluid in support of light-enhanced shell formation. As both NHE3-like and NCX3-like are Na^+-dependent secondary active transporters, any increase in their activities through upregulation of expression levels would disrupt the Na^+ gradient across the plasma membrane, which must be ratified. Indeed, the protein abundance of Na^+/K^+-ATPase (NKA) α-subunit (NKAα) is upregulated in the ctenidium of *T. squamosa* during light exposure, indicating a possible increase in NKA activity to maintain the transmembrane Na^+ gradient. Interestingly, NKAα has an atypical apical localization in the ctenidial epithelial cells of *T. squamosa*, which could be related to the needs to absorb K^+ from the external seawater to maintain K^+ homeostasis due to phototrophy without food intake. Although both giant clams and scleractinian corals harbor zooxanthellae and conduct light-enhanced calcification, they have different complexities, and it is unsurprising that light-enhanced shell formation in the more advanced giant clams involves the cooperation of multiple organs. To date, many species of giant clams are either critically endangered or vulnerable. Our results provide a deeper understanding on the mechanisms of light-enhanced shell formation in giant clams, which may furnish insights into better ways to enhance their growth during mariculture for reseeding purposes.

CAN THE INOCULATION OF COMMERCIAL NITRIFYING BACTERIA IMPROVE BIOFILTERS PERFORMANCE IN THE M*acrobrachium amazonicum* LARVICULTURE?

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Introduction

The high stocking densities used in *M. amazonicum* larviculture may accumulate nitrogen toxic metabolic compounds, reaching high values (Hayd et al., 2014). An efficient biological filtration of nitrogen compounds is essential for getting satisfactory survival rates in the freshwater prawns' larviculture. Nowadays, the lack of commercial hatcheries is one of the main bottlenecks for the development of freshwater prawns' farming in Brazil. The objective of this study was comparing the performance of one artisanal model of biofilter commonly used on commercial hatcheries and a commercial one (canister filter), both inoculated with commercial nitrifying bacteria, aiming to determine the most efficient and the easiest to manage.

Materials and Methods

The study was run in the Freshwater Prawn Laboratory APTA/Pirassununga, Brazil. The treatments were Without Filtration Without Inoculation (WFWI); Without Filtration Inoculated (WFI); Canister Filter Inoculated (CFI); Dinamic Filter Inoculated (DFI), carried out in a complete randomized design with five replicates per treatment

The tested commercial nitrifying bacteria (N-Control) were previously dissolved in brackish water at salinity of 10g L^{-1} and daily added into the biofilters (treatments CFI and DFI), or directly into the WFWI treatment tanks, using the recommended doses (6mg L^{-1}), according to the producer. The larvae of *M. amazonicum* were collected from the hatching tank, counted, acclimatized, and stocked (49 larvae L^{-1}) in 20 L black rectangular plastic tanks. The water variables pH, dissolved oxygen (DO) and temperature (T) were monitored daily using an Alfakit AT-355 pH meter and an YSI Pro-20 oximeter, respectively. The salinity was kept between 10 and 11.

Water samples of all tanks were collected twice a week for determination of ammonium (NH_3), nitrite (NO_2) and nitrate (NO_3) dissolved in the water. The analyses were made by a certificated company (Aquali Assessoria e Análises Ltda). The production variables were mean weight (MW), survival (S) and days of larviculture (DL). Means were subjected to one-way ANOVA and compared by Tuckey Test ($p<0.05$). When the premises of normality or homoscedasticity, were violated, the non-parametric Dunns Multiple Comparison - Kruskal-Wallis Tests ($p = 0.05$) were applied. The software utilized for performing statistical analysis was the Toxstat, version 3.0.

Results

There were no significant differences in water variables pH, DO and T among treatments (Table I). For the nitrogen compounds, there were significant differences among the CFI and the other treatments (Table I), but no differences were detected between the treatments WFWI and WFI. According to the survival data presented in Table II, there were differences among the control (WFWI and WFI) and inoculation (CFI and DFI), due to the greater concentrations of nitrogenous compounds present in the control treatments.

Discussion and conclusion

The variables pH, DO and T ranged within the stated water parameters for hatchery of *Macrobrachium* sp according to Moraes-Valenti and Valenti (2010).

The treatment CFI was the most efficient filte , presenting the minor value to the variable nitrite. Although the survival of the treatment CFI were similar to the DFI treatment, the canister filter presented a faster assembly, easier handling and, consequently, it could means a reduction of labor costs in hatchery commercial operation. This hypothesis, however, needs to be confirmed by further economic studies

Despite the treatment WFI had presented high mortalities, we noticed that some larvae survived until the lasts larval stages. This fact indicates that the addition of commercial nitrifying bacteria directly in the larvicuture tanks, without any filtration could be a potential new hatchery system.

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Table I. Average values (± standard deviation) of pH, dissolved oxygen (DO), temperature (T), ammonium (NH_3), nitrite (NO_2) and nitrate (NO_3) for the treatments: Without Filtration Without Inoculation (WFWI); Without Filtration Inoculated (WFI); Canister Filter Inoculated (CFI) and Dynamic Filter Inoculated (DFI);

	Treatments			
Variables	**WFWI**	**WFI**	**CFI**	**DFI**
pH	7.76 ± 0.02	7.74 ± 0.02	7.76 ± 0,01	7.76 ± 0.02
DO (mg L^{-1})	4.20 ± 0.19	4.33 ± 0.27	4.25 ± 0,17	4.35 ± 0.25
T (°C)	28.81 ± 0.62	28.98 ± 0.46	29.01 ± 0.27	29.10 ± 0.62
NH_3 (mg L^{-1})	0.27 ± 0.23	0.41 ± 0.24	0.21 ± 0.06	0.28 ± 0.12
NO_2 (mg L^{-1})	0.73 ± 0.40^{ab}	1.26 ± 0.70^{a}	0.13 ± 0.03^{c}	0.36 ± 0.55^{b}
NO_3 (mg L^{-1})	1.70 ± 0.86	2.15 ± 0.74	1.19 ± 0.09	1.66 ± 1.45

Subscribed different letters in the same roll indicate significant differences (ANOVA, followed by Tukey-test, $p<0.05$).

Table II. Average values (± standard deviation) of mean weight (MW); survival (S) and days of larviculture (DL), for the treatments: Without Filtration Without Inoculation (WFWI); Without Filtration Inoculated (WFI); Canister Filter Inoculated (CFI) and Dinamic Filter Inoculated (DFI).

	Treatments			
Variables	**WFWI**	**WFI**	**CFI**	**DFI**
MW (mg)	0.94 ± 0.03	0.91 ± 0.04	0.90 ± 0.06	0.91 ± 0.04
S (%)	7 ± 2	5 ± 3	44 ± 8^{a}	46 ± 15^{a}
DL (un)	33 ± 2	31 ± 5	28 ± 4	28 ± 5

Subscribed different letters in the same roll indicate significant differences (ANOVA, followed by Tukey-test, $p<0.05$).

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PROFESSIONAL AQUACULTURE – SUPPORTING SKILLS AND CAREER DEVELOPMENT IN EUROPEAN AQUACULTURE

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Introduction
Aquaculture in Europe is diverse in its products and producers, but many parts are maturing with the involvement of major international companies and increasing technical and managerial sophistication of production systems and market chains. The industry is having to pay closer attention to recruitment and career development as well as skill development either internally or through external training and education organisations in order to compete and grow in response to emerging opportunities and challenges.

Institutional provision for aquaculture education and training in Europe
There are in the region of 100 education and training institutions in the EU and Associated States that have at least some provision for aquaculture sector training. Most formal provision is at graduate or postgraduate level although significant vocational programmes exist in countries such as Norway and France. So far there is little development of aquaculture-specific professional bodies with independent accreditation systems as exist in other sectors

Industry and private sector responses to training needs
The availability of on-the-job training within the aquaculture sector depends to a considerable degree on the economic status of the industry. The salmon industry is relatively consolidated, and individual companies have substantial internal programmes for staff training and development. There are also private companies providing specialist training especially for safety or certification compliance related issues. Online provision is still limited

EU Policy context and support actions
The European Commission supports the aquaculture sector particularly through policies and actions administered through the Directorate General for Maritime Affairs and Fisheries (DG Mare). However, to understand the wider context and support for skills and career development requires consideration of the work of the Directorate General for Employment, Social Affairs & Inclusion (DG EMPL), the Directorate General for Education, Youth, Sport and Culture (DG EAC) and the Directorate General for Research and Innovation (DG RTD). Each of these DGs have actions relevant for skills development in the aquaculture sector and work together to some extent, for instance through the Blueprint for Sectoral Cooperation on Skills. Funding provided through these DGs is supporting a range of EU projects including BlueEDU (Erasmus+ Sector Skills Alliance for cage-based aquaculture) which provided some of the analysis for this presentation.

Future opportunities
Several EU funded projects are piloting new approaches including blue careers centres (e.g MENTOR), employment of industry-based tutors (e.g CETBC), development of e-Learning courses (e.g. SEAFOODtomorrow & AQUAEXCEL[2020]) and collaborative approaches to creating and sharing learning materials (e.g. AquaCase). It is important that lessons are learned from these and incorporated into future policy and support. The long established ESCO (European Skills, Competences, Qualifications and Occupations) initiative increasingly provides a solid framework for serious strategic planning on skills and career development within the aquaculture sector. This could in the future include outreach on career opportunities; recognition and accreditation of knowledge and skills through national or independent qualification frameworks; enabling flexible and lifelong learning including through distance and work-based programmes; and greater cooperation on curriculum and learning materials for relevance and effectiveness. The industry has the greatest stake in this but need to work with the education and training sector and other stakeholders (e.g. through EATIP and its Mirror Platforms) for greater momentum and impact on industry metrics such as productivity and business growth.

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CETBC - https://stc-group.nl/en/blue-careers
EATIP - http://www.eatip.eu
ESCO - https://ec.europa.eu/esco/portal/home
MENTOR - http://mentor.cubiclemon.net/
SEAFOODtomorrow - https://seafoodtomorrow.eu/

MARENNINE, A NEW NATURAL ANTIBIOTIC AGAINST VIBRIOS IN AQUACULTURE

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We explored potential antibiotic effect of Marennine, a blue pigment produced by *Haslea ostrearia* microalgae, on the pathogenicity of *Vibrio splendidus.* These bacteria are Gram-negative marine species often considered as an eminent threat for bivalve hatcheries. Here we demonstrate that in larval rearing conditions, larva of *Mytilus edulis* and scallop *Placopecten magellanicus* showed significant mortalities exposed to high concentrations of *V. splendidus*. However, in the presence of Marennine extracts, the pathogenicity of the bacteria was suppressed. In an attempt to explore the mechanism of such effects we used Nuclear Magnetic Resonance spectroscopy to explore interactions of the pigment with intact viable *V. splendidus* at a molecular level. Our hypothesis was that Marennine interacts with the outer membrane.

Purified Marennine was exposed to ^{2}H-labelled bacteria in artifi ial sea water and in controlled conditions. NMR results showed that Marennine affects fatty acyl chain fluidit . This effect was more striking when the membrane was already fluid, during the limited nutrient conditions like the stationary phase of bacterial growth. The results suggest that Marennine is a disruptive agent of the membrane dynamics for *V. splendidus*, and that its action mechanism is compared to Polymyxins, highly regarded antibiotic family, used in aquaculture and known to interact with the outer membrane of Gram-negative bacteria.

ALTERNATIVE METHODS FOR MONITORING BENTHIC ORGANIC ENRICHMENT EFFECTS AND APPLICABILITY ACROSS A RANGE OF ENVIRONMENTAL AND AQUACULTURE CONDITIONS

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Introduction

Increasing organic matter inputs to sediments have a known effect on benthic communities due largely to oxygen depletion and consequent toxic sulfide production. Total dissolved (free) sulfide (TFS) concentrations in sediment are widely measured as a practical indicator of benthic organic enrichment effects and are used in eutrophication studies and aquaculture management frameworks in several countries as a proxy for benthic community biodiversity, including Canada. However, the need for an expanded range of performance indicators that are of use in managing effects from BOD matter effluents in soft-bottom environments is recognized to increase confidence in regulatory decisions. Previous work developed accurate and practical alternative protocols for measurement of dissolved oxygen (DO) and determination of TFS in the sediment pore-water to address issues that currently exist within the traditional ion-selective electrode (ISE) method for measuring TFS in sediment slurries (Cranford et al. 2017). The applicability of these methods, as well as spatial and temporal trends of pore-water sulfides, are being assessed across a range of environmental (seabed, water depth, hydrodynamics) and aquaculture production levels, and compared with the ISE sulfide method at multiple locations across Canada

Materials and methods

Sediment samples were collected along organic enrichment gradients using either core or grab devices at n=7 finfis and n=2 shellfish sites across Canada in 2016, 2017 and 2018. Sediment pore-water was extracted from 1 and 2 cm depth below the sediment-water interface and immediately analyzed for dissolved oxygen (DO) and TFS using the UV spectrophotometric method (UV; Cranford et al. 2017). Replicate pore-water samples were collected and fixed for later analysis by the methylene blue microplate method (MB, Wong, in preparation). Sediment plugs from the top 2 cm were also collected and analyzed for TFS using the traditional ISE method according to the current regulatory monitoring protocols for the region, in addition to samples for measurement of grain size, porosity, and organic content.

Results and discussion

The new methods (UV and MB) for measuring TFS in sediment pore-water have been validated in a range of environments and production levels, and show strong correlation with each other (Fig. 1A). Comparability of pore-water TFS with ISE TFS is more scattered and inconsistent, indicating site-specificity (Fig. 1B).

Spatial and temporal trends in pore-water DO and TFS confirm known organic enrichment patterns with respect to aquaculture inputs. Spatial variability in TFS is inherent with increasing impact across all methods used for determination. The magnitude of organic enrichment effects vary with seabed type (among other factors). Pore-water TFS measurements show a localized impact (Fig. 2A), while DO can detect impacts at a greater distance and is more sensitive to the initial stages of biochemical oxygen demand (BOD) stress on the benthos (Fig. 2B). Temporal variability in the alternative indicators are consistent with expected production level trends, and demonstrate the sensitivity of these new metrics for detection of both increasing impact levels and recovery during a fallow period (Fig. 2).

Final steps of this ongoing research will be to quantify the empirical relationships between these new alternative indicators and the benthic community biodiversity as the scientific basis for proxy use as a management tool

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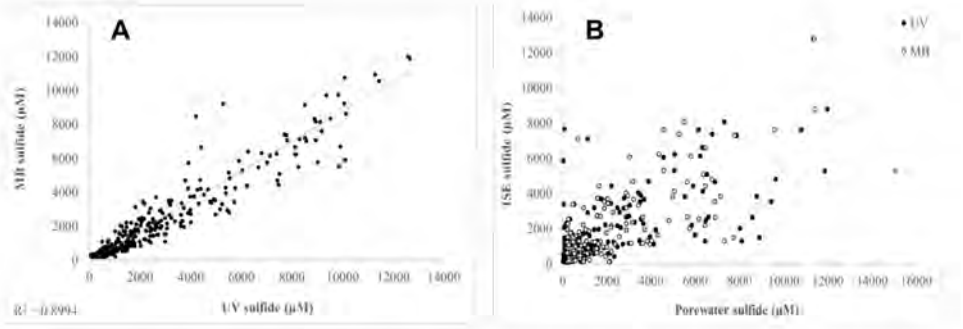

Fig. 1. (A) Relationships between MB and UV pore-water sulfide (µM) and (B) between ISE sulfide and pore-water sulfides (µM; filled circles = UV, open circles = MB).

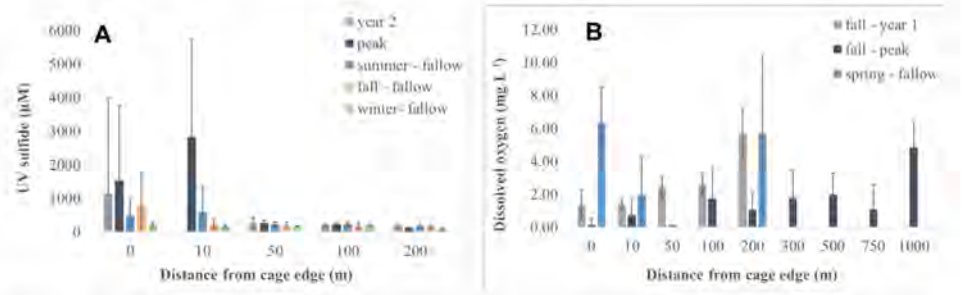

Fig. 2. (A) Mean UV pore-water sulfide (µM) and (B) mean dissolved oxygen (mg L^{-1}) measured at each station with distance from cage edge (m) during stages of a production cycle.

EFFECTS OF PLASTIC NANOPARTICLES ON DICENTRARCHUS LABRAX

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Introduction

The existence of small-sized plastic waste in the marine environment and its potential impact on aquatic life has recently become a major concern due to the large quantity of plastic debris released in coastal areas. Once in the water, large plastic debris can be fragmented, through ultraviolet photodegradation, biodegradation, and chemical degradation processes to form smaller-sized plastics, including microplastics (< 5 mm) and nanoplastics (< 100 nm) (NPs) (Koelmans et al 2015). With a decrease in size, plastic particles become more bioavailable to aquatic organisms, and potentially more hazardous. In previous studies, NPs have been reported to induce oxidative stress, immune dysfunction, teratogenic effects, and altered locomotion (Besseling et al 2014, Lu et al 2016). Furthermore, altered lipid metabolism and histopathological changes in liver (Lu et al 2016) of fish exposed to plastic particles have been reported. *Dicentrarchus labrax* (sea bass), a marine fish species and a top predator is a common and valuable aquaculture species in the Mediterranean area. This species may be exposed to NPs via water and food web and constitute a potential source and risk to human health. For these reasons, the goal of this study was to evaluate the effects of NPs on *D. labrax* at different levels of biological organization.

Material and methods

Juvenile *D. labrax* specimens (14.6 ± 2.4 cm length and 21.4 ± 6.5 g weight) were obtained from an aquaculture facility (Spain) and acclimatized for 30 days in 1000 L aquaria, with aerated ASW (salinity 30), 19 °C and natural photoperiod (14 h light: 10 h dark). Following the acclimatization period, fish were randomly distributed in the following the experimental groups: 0 mg/L (control), 0.02 mg/L, 0.2 mg/L and 2 mg/L NPs. Each experimental condition consisted of 2 tanks with 3 animals per tank, containing 20 L of experimental media. Animals were exposed to NPs for 96 h, generally following OECD guideline 203 (OECD, 1992), and kept in the conditions described for the acclimatization period (ASW, 19 °C, 14 h light: 10 h dark). At the end of the exposure, skin mucus, blood and liver were sampled and processed for further analysis. Molecular were assessed in liver and relevant biochemical markers concerning metabolism, oxidative stress and general health status were studied in plasma and skin mucus.

Results

Concerning molecular responses (Fig. 1), transcriptional levels of genes associated with lipid metabolism presented a global upregulation after exposure to NPs. Peroxisome proliferator-activated receptors', *pparα* expression was significantly increased after exposure to 2 mg/L NPs, whereas *pparγ* mRNA levels presented a significant upregulation after exposures to 0.2 mg/L and 2 mg/L NPs. The expression levels of *pparβ* and *hadh* were not significantly different from control. The mRNA levels of *nd5* were increased after exposure to NPs (in animals of all tested concentrations). Regarding biochemical responses assessed in plasma, esterase activity (EA) was significantly decreased when compared to control after exposure to 0.02 mg/L, 0.2 mg/L NPs. In skin mucus, significant differences with respect to control were found in alkaline phosphatase (ALP) levels after exposures to 0.2 mg/L and 2 mg/L NPs.

Discussion and conclusions

Overall, results showed that NPs activate transcriptional machinery in the liver of *D. labrax*, leading to increased expression of genes related to lipid metabolism. Peroxisome proliferator-activated receptors (PPARs) participate on the fatty acids signals as key regulators of lipid metabolism. The peroxisome proliferation has been proposed as a biomarker for environmental pollution assessment, as increases in the size, number and volume of peroxisomes in aquatic organisms exposed to contaminants have been documented in marine species. Alterations in mRNA levels of *ppara*, *pparγ* and *nd5* after exposure to NPs suggest an interference with the ability of fish to mobilize energy reserves. Regarding biochemical parameters, decreased EA levels were found in plasma after exposure to 0.02 and 0.2 mg/L NPs, and levels of ALP in skin mucus also decreased significantly following exposure NPs, at 0.2 and 2 mg/L. Both enzymes could play an important role in sea bass innate immunity, and their decreased levels suggests an impairment of the immune system's function following exposure to NPs.

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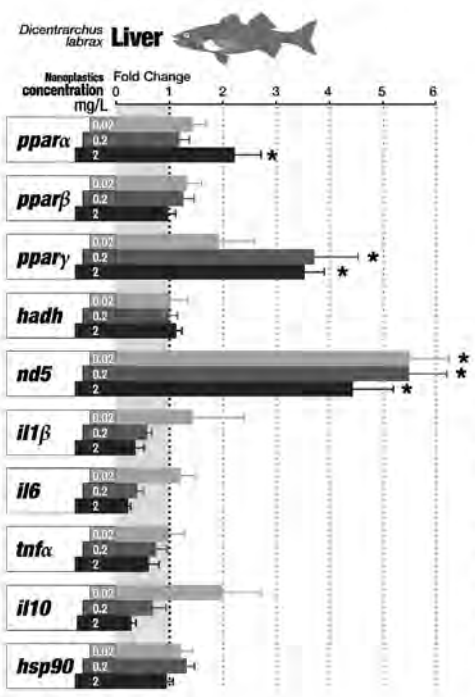

Fig. 1. Target genes mRNA levels determined in the liver of fish after 96 h exposure to polymethylmethacrylate (PMMA) nanoplastics (NPs). Statistical analysis was determined by non-parametric Kruskal-Wallis test, followed by the Dunnett test to signal significant differences from the control group ($p < 0.05$), indicated with an asterisk.

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PERACETIC ACID AND ITS ANTI-PARASITIC ACTIVITY AGAINST *Paramoeba perurans*, THE CAUSATIVE AGENT OF AMOEBIC GILL DISEASE (AGD)

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Amoebic gill disease (AGD), caused by *Paramoeba perurans*, is a major health issue for the global Atlantic salmon (*Salmo salar*) aquaculture. Even though the cases in Norway become more or less stable in the last years, AGD remains a persistent and serious gill health challenge. Hydrogen peroxide (H_2O_2) is the commonly used chemotherapeutant for AGD. However, the treatment resolution between lab-based and field-based experiments is often weak and a very high concentration of H_2O_2 is required. Peracetic acid (PAA) is being proposed as an alternative chemotherapeutant to H_2O_2. PAA is one of the most commonly used disinfectants in aquaculture because of its high treatment efficac . Earlier reports demonstrated that PAA has a far more antimicrobial effect than H_2O_2, and its effective dose against various pathogens is relatively lower than H_2O_2. In this study, we explored *in vitro* the anti-parasitic activity of PAA against *P. perurans*. We first evaluated several staining techniques for viability test to be employed in the *in vitro* exposure systems. Out of the four staining techniques (*i.e.*, MTT, Neutral Red, Resazurin, WST-1) tested, WST-1 assay was selected because of its stability and discriminatory potential. Preliminary results of the *in vitro* microplate-based exposure revealed that *P. perurans* were susceptible to PAA after a 30-min exposure at 2.4 ppm concentration. In a parallel study, we have documented that exposing salmon to PAA at similar concentration did not result to compromising health concerns. The microplate-based WST-1 assay for viability was complemented with Neutral Red-based staining, to provide a more quantifiable data of the susceptibility of *P. perurans* to PAA. PAA compromised the cell membrane of the amoeba thereby killing them, as supported by the loss of uptake of the Neutral Red dye in PAA-exposed amoeba. This observation corroborated the microplate-based assay of the killing potential of PAA against *P. perurans*. We are currently running several *in vitro* experiments to investigate how different factors (*i.e.*, dosage, temperature, starting parasite concentration and light) affects the anti-parasitic activity of PAA against *P. perurans*. Protocols will be standardised, and methods optimised continuously in the working process.

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REMOTE MONITORING OF CARDIOVASCULAR PERFORMANCE TO GAUGE STRESS RESPONSES OF RAINBOW TROUT (*Oncorhynchus mykiss*) IN AQUACULTURE

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Introduction

The welfare of farmed fish has become of increasing concern for consumers, producers, interest groups and authorities. To improve the welfare of fish, it is necessary to identify and quantify the severity of stressful farming practises early enough so that an intervention can take place before detrimental effects occur. The rapid development and miniaturization of bio-telemetry systems and bio-loggers presents a solution, as they allow us to remotely record physiological stress responses in fish over long uninterrupted periods. The remote measurement of cardiovascular variables such as heart rate and gut blood flow provide exciting avenues for determining and quantifying stress responses of fish, as these physiological parameters are affected by most behavioural responses, as well as most environmental and anthropogenic perturbations. By exploiting this technology, the aim of the present study was to investigate whether *in vivo* monitoring of heart rate and gut blood flow in freely swimming fish could be reliably used as an indicator of stress and as a tool for assessing the welfare of farmed fish during common aquaculture practises before and during harvest.

Materials and methods

20 rainbow trout (body mass: 2082±113 g) were surgically implanted with DST milli-HRT bio-loggers (*i.e.* measures heart rate and body temperature, Logger version 8, STAR-ODDI, Iceland) and 4 additional rainbow trout (body mass: 2941±166 g) were implanted with multivariate bio-loggers (*i.e.* measures heart rate, gut blood flo , activity and body temperature, Transonic EndoGear3, Transonic Systems Inc., USA). The surgically implanted individuals were then transported by well-boat and transferred to a sea cage containing ~5000 conspecifics where they remained for 16 days during which the bio-loggers were continuously recording. After these 16 days, the trout were transported by well-boat from the sea cage to the slaughterhouse. During this procedure, trout were subjected to a series of common aquaculture practices such as light crowding, severe crowding, brailing, well-boat transportation, held in a stationary well-boat, held in a holding cage by the slaughterhouse, and then finally brailing directly followed by CO_2 stunning. During this period the bio-loggers were continuously recording. Un-instrumented trout were also captured with dip nets at each of the abovementioned steps and sampled for blood via caudal puncture. Blood samples were subsequently analysed for plasma cortisol, haematocrit, haemoglobin concentration, red blood cell count, and electrolyte concentrations.

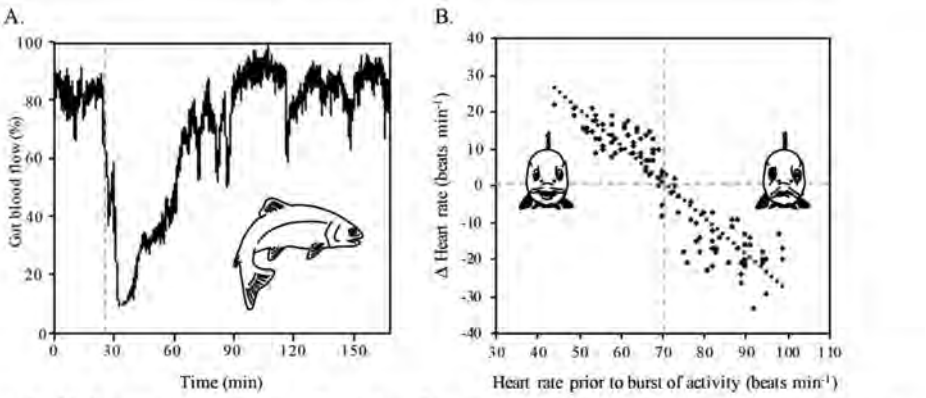

Figure 1: A) A representative trace displaying the consistent gut blood flow response to an acute stress event (dotted line). B) Chronically stressed fish display bradycardic heart rate responses in response to acute stress events or spontaneous bursts of activity, whereas relatively unstressed fish display tachycardic heart rate responses. Data from Brijs *et al.* 2018 and Brijs *et al.* submitted.

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Results

Life in the sea cage: After the initial release into the sea cage, heart rate and gut blood flow of trout were significantly elevated and it took ~7 days for heart rate and gut blood flow to stabilize. During this time, when fish experienced an acute stress event or a spontaneous burst of activity (*i.e.* during the daily approach of the feeding boat and subsequent feeding event), transient reductions in both gut blood flow and heart rate were observed. However, once fish had recovered, acute stress or spontaneous activity instead transiently increased heart rate while gut blood flow still decreased. Interestingly, a clear circadian rhythm in heart rate emerged in the recovered trout (*i.e.* average circadian fluctuation in heart rate of ~25 to 27 beats min^{-1}).

Final journey before slaughter: Stressful handling practises (indicated by elevated plasma cortisol levels) such as crowding and transportation significantly elevated heart rate whilst transiently reducing gut blood flo . In contrast, other stressful practises such as air exposure during brailing triggered significant reductions in both heart rate and gut blood flow until the trout were released back into oxygenated water, whereupon heart rate significantly increased. Repeated stress induced by multiple handling practises clearly had a cumulative and long-lasting effect, as both heart rate and gut blood flow peaked at the end of the day of transporation and remained elevated up until slaughter the following day.

Discussion and conclusion

Acute stress induced by common farming practices, as well as intense voluntary activity, consistently resulted in relatively rapid and substantial decreases in gut blood flow of farmed trout (see figure 1A). However, in contrast to reductions in gut blood flow caused by voluntary activity, recovery from stress-induced reductions involved a substantial and prolonged increase in gut blood flo . This may be necessary to repair stress-induced damages to the gastrointestinal tract. Long-term recordings of heart rate clearly demonstrated that heart rate responses to acute stress or intense activity differ depending on the physiological state of the animal (see figure 1B). Stressed fish (*i.e.* elevated mean heart rates >70 beats min^{-1}) tended to exhibit decreases in heart rate in response to acute stress or intense activity, whereas fish with mean heart rates <60 beats min^{-1} exhibited markedly increased heart rate responses. Therefore, although both parameters are useful indicators for stress in fish, researchers utilising heart rate as an indicator of stress need to be aware of the physiological and behavioural constraints that affect heart rate and thoroughly evaluate the range of heart rates normally exhibited by their model species. In conclusion, the present study demonstrates that the application of bio-loggers provides a powerful tool for the further development of management protocols aiming to pinpoint stress and improve welfare of farmed fish, which in turn may have ethical and economic benefits for the aquaculture industr .

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SPAT MORTALITY IN FARMED BLUE MUSSELS (*Mytilus edulis*) IN SCOTLAND

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Introduction

The industry for blue mussels (*Mytilus edulis* (Linnaeus, 1758)) has been a growing sector in Scotland for the past ten years, and relies heavily on the wild collection of seed (spat) for the on-growing product. There are several external risks that could impact mussel production, especially unreliable spat resources and poor water quality, pollution, bio toxins and infectious diseases (Bower et al., 1994). In recent years, problems involving spat availability and mortality have occurred impacting the mussel industry to the extent of possible closures of some businesses. One site in particular has been experiencing a severe case of spat mortality in the winter months for the past decade. Interestingly, it is only in the winter months where the mortalities have been observed and only the spat is affected, whereas older mussels from the previous season seem to remain in a healthy state. The investigation of this mortality case involves an experimental design which observes the mortality on a fortnightly basis by deploying lantern nets from the grow out lines in the affected site and in one control site.

Materials and methods

20 lantern nets, each stocked with 1000 individuals, were deployed across the affected site and 10 nets were deployed across a control site in a different body of water, which has not been experiencing any mortality, late October of 2018. The collection and counting of dead spat occurred after stocking, for a period of 11 weeks. Live and moribund samples, and eventually some survivors, were collected at each time point for analyses including histopathology, bacteriology, virology, gene expression and DNA sequencing. Temperature and salinity were monitored in the proximity of the experimental nets at both affected and control sites. Additionally, water samples for heavy metal analysis by Ion Coupled Plasma Mass Spectrometry were collected at each time point.

Results and discussion

Preliminary results confirm that spat mortality was significantl higher in the affected site compared to the control site, reaching 68.3 percent over four time points within the 11-week period. Most mortalities were observed at T1 and T2 reaching 54.9 percent (30d post stocking) after which the mortality rate then subsided at T3 (62.7%), 44d post stocking, contrasted by a 0.9 percent maximum mortality at the control site. Gene expression analysis of the spat collected during the mortality and the survivors collected at the end of the mortality period may determine whether there is a difference in response, indicating susceptibilities or compromised immunity. These results will be related to the monitored environmental parameters, histopathology examination, and heavy metals concentrations.

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EVALUATING THE EFFECTS OF FARMING REARING TOOLS ON THE QUALITY ASPECTS AND GROWTH OF THE PACIFIC OYSTER *Crassostrea gigas*

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Introduction

According to farmers and buyers, the main characteristic that defines the marketability of an oyster is the shell shape. A good oyster has a teardrop shape (thick, deep and wide) and it must be without epibionts and blisters (Doiron, 2008). Various factors are able to influence the shell shape of the oysters, among which the rearing tool adopted. The aim of this study is to evaluate the effects of the innovative Ortac farming system on the quality aspect of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) (shell shape, weight meat percentage, backward shape, epibiosis and blisters) and growth rate (weight and length). Results obtained with ortac were compared with those obtained with the traditional floating bag

Materials and methods

The experiment was conducted in a shallow lagoon located in the central-western coast of Sardinia Island (Western Mediterranean, Italy). A total of 2,400 triploid *C. gigas* spat (6±0.1g fresh weight, 40.5±0.3mm length, mean±SE) were seeded in 12 rearing tools (200 seed per tool): 6 floating bags (hereafter bag) and 6 ortacs (hereafter ortac). Oysters were reared for fourteen weeks (from September to December 2018) continuously immersed in the water during the whole experiment. At the end of the experiment, five random individuals per tool were measured for wet and dry weight of meat and shell, as well as length, width and depth. Weight and biometrics were used to calculate the shape score, an indicator of the quality shape of the oysters ((length/width)/(depth/length), Rankin et al., 2018), and the weight meat percentage of the animals (percentage of dry meat weight (g)/dry shell weight (g), Davenport and Chen, 1987). The backward shell curvature (i.e. unfavorable for holding the meat, Rankin et al., 2018) of all the animals per tool, as well as the percentage of oysters hosting epifaunal organisms (serpulids and barnacles) and the presence of blisters in the shell, were recorded. The wet weight and shell length of thirty random individuals were measured every two weeks, and mortality rate was checked in comparison to the starting number of animals. All data were analyzed by Statistica 6.1 StatSoft,Inc. (2004).

Results

The tool influenced both the quality and the growth of the animals. Oysters grown in ortac resulted in a poorer shape score, a lower weight meat percentage, a higher percentage of backward and blisters, and a lower percentage of animals free of epibionts (Tab. I). Regarding the growth rate, the bag showed a higher ($p < 0.05$) wet weight from week 8 (32.3±0.4g) to week 14 (32.4±0.4g) than in ortac (26.8±0.5 and 28.3±0.4g at week 8 and 14, respectively). The length increased significantly from week 0 to week 8, without significant d ferences between tools (Fig. 2).

Discussion and conclusions

According to our study, the rearing tool affects both the quality aspect and the growth of *C. gigas*. In the past, mortality and growth were considered the main selective breeding traits in oyster aquaculture (Mahon, 1983), while nowadays, as a niche market product, the appearance of the oyster is commercially important and shell quality is the first feature affecting buyers (Brake et al., 2003). No mortality was observed in our study, and oysters grow faster when reared in the bag (i.e. higher weight from week 8 to week 14). Better results with bag were obtained also in the quality aspect (i.e. low shape score, high weight meat percentage, low percentage of backwards and blisters), except for episiosis, since a lower shell colonization by barnacles and serpulids was obtained with ortac. Ortac is an innovative tool able to improve the growth rate and the shell quality of the oysters (Flynn, 2013) in oceanic conditions. Results obtained in our study demonstrate that, when grown in Mediterranean lagoons and by using ortac, *C. gigas* has a worse quality aspect and a lower growth performance than in bag. We hypothesize this is due to the different environmental conditions of Mediterranean Sea in comparison to the oceans, mainly the lower water movement (i.e. tides, currents and waves). The adoption of more accurate husbandry practices and/or adjusting the ortac farming system to Mediterranean features could improve the efficiency of the tool, in terms of quality aspect and growth.

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Tab. I: quality aspects of *Crassostrea gigas* grown in ortac and bag (mean ± SE, n=6).

	ORTAC	BAG	*p-value*
Shape Score	6±0.2	5.3±0.1	**0.037374**
Weight meat percentage	6.3±0.2%	8.5±0.2%	**0.003948**
Backwards	33.3±2.1%	19.5±1.7%	**0.003948**
Blisters	18.3±3.1%	2.4±1.5%	**0.003948**
Epibiosis	87±2%	95±1%	**0.015152**

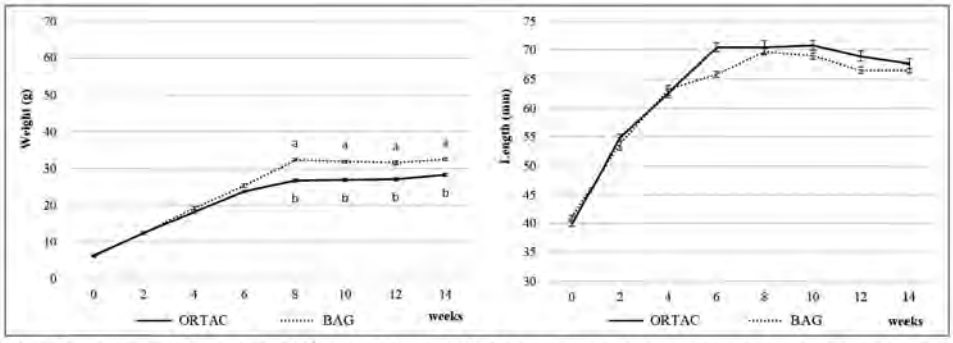

Fig. 1: wet weight (g) and length (mm) of *Crassostrea gigas* grown in ortac and bag (mean±SE, n=6). Superscripts indicate significant differences between tools.

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A PILOT SCALE HATCHERY OF SHELLFISH IN SARDINIA: A REGIONAL DEVELOPMENTAL STRATEGY WITH RESEARCH AND TECHNOLOGICAL OPPORTUNITIES

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Introduction

In Europe, the demand for shellfish products is constantly expanding and the Mediterranean Sea could offer potential locations for expansion. In Italy, Sardinia records the greatest national productivity of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793), about 60% of total production accounted to just five oyster farmers operating in small coastal lagoons (FAO, 2019). Since Sardinia has many areas covered by shallow coastal lagoons that could represent potential sites for oyster farming, the Sardinia Region funded the OstrInnova project. The general objectives of the project were to increase the oyster production, by identifying new potential farming sites and assessing the productivity by implementing a bio-energetic growth model. One of the main outcomes of the project was a pilot scale bivalve's hatchery at the International Marine Centre - IMC facilities (Oristano, Italy). This was designed for research and technology transfer on the production of shellfish. The hatchery, together with the knowledge and skills required for its management, represents an after-OstrInnova asset, available for the development of shellfish production and research in Sardinia.

Materials and methods

In the OstrInnova project, shellfish producers, universities and research centers from Sardinia and UK were involved. An expert in shellfish production and research was involved for the hatchery design, this was made taking into account the location and the existing facilities. OstrInnova was launched on April 2016, and from the beginning of the project the IMC staff was trained on shellfish production techniques. During the training period, a 2-months stage in a leading research institute (Cawthron Institute, New Zealand) on shellfish research and production was done by a member of the IMC staff, aiming to improve the expertise in bivalve's hatchery protocols. The pilot scale hatchery, built during summer-autumn 2018, allows research at different scales for each developmental stage of shellfish species and is useful for investigating on the reproduction and rearing of diploid and triploid oysters.

Results

The pilot scale hatchery is composed by four units, phytoplankton production, broodstock conditioning, larval rearing and post-larval stages. The phytoplankton production unit is provided with nine polyethylene photobioreactors of 200-300 l capacity each one. The photobioreactor are equipped with a lighting systems consisting of 40W cool-white fluorescent tubes. The system is supplied with CO_2 and equipped with a blower and a micro filtration system for the air. In order to facilitate the distribution of phytoplankton and to minimize potential contamination from the outside, a system of pipes and a volumetric pump were set up to connect the photobioreactors to the different hatchery units. The broodstock unit includes six 150 l slope bottom customized tanks and a spawning table equipped with a chiller/heater unit. This design allows the maintenance of six separate broodstock tanks, with a maximum capacity of 300 adults. The larval rearing unit includes two separate systems, one static and one flow-through. The first system consists of nine cylindrical tanks with conical bottom, with a capacity of 180 l each. The second one is composed by twenty 5 l polymethyl-methacrylate transparent cylindrical containers that works as a flow-through system. The post-larval stages unit is composed with i) a settlement unit (3 tanks), ii) a micronursery made of 20 upwellers, and iii) a nursery tank operating either in downwelling or upwelling. For biosafety reasons, each system is equipped with a filtration system (sand or cartridge filter), UV sterilizer, chiller/heater and protein skimmer.

Discussion and conclusions

Sardinia is the highest oyster producer in Italy, and this production is completely focused on triploids Pacific oyster *C. gigas*. Due to the absence of oyster hatcheries in Sardinia, and that in Italy exists only an oyster hatchery located in Ferrara which produces just diploid oysters for local farmers, the total amount of *C. gigas* spat seeded in the region comes from French hatcheries. Taking into account the current and potential shellfish production in Sardinia, the government has started to invest on the improvement and development of this industry. In this line, the pilot scale hatchery built at the IMC laboratories has not the purpose to produce *C. gigas* for farmers but to improve the scientific research about reproduction

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and rearing of triploid individuals, increase knowledge and skills, and develop and transfer the required technology to the productive sector. This strategy is aimed not only to *C. gigas* but also to other shellfish species of interest, such as *Ostrea edulis*. On the basis of the information obtained during the OstrInnova project, the future production of shellfish in Sardinia is focused on the rearing of the European flat oyster *O. edulis* as an alternative species to the Pacific oyster. For this reason, and considering that along the Sardinian coasts exist well-established natural populations of *O. edulis*, one of IMC's research lines on shellfish production will be focused on the reproduction and rearing of this species. Research on *O. edulis* will have a local impact and can contribute to the global production of the species and to the restocking efforts invested in other areas. Shellfish production in Sardinia is a regional development strategy that can contribute to local and global challenges.

Acknowledgements

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PRODUCTION POTENTIAL OF BLUE MUSSEL (*MytiluS* SPP.) FARMING ALONG THE SALINITY GRADIENT OF THE GERMAN BALTIC SEA

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Introduction
Aquaculture production is globally increasing (80 mio t in 2016) but production in Europe contributes to only 3% while Asia dominates with 85% (EEA 2018). For shellfish aquaculture (mainly *Mytilus* spp.) production in Europe even decreased from 190,000 t in 2000 to 155,000 t in 2016. To support blue growth in Europe, the EU Blue Growth Strategy (COM(2012) 494/F1, EC 2012) was developed, including the expansion into areas, with sub-optimal growing conditions for *Mytilus* spp. as the Baltic Sea (Beyer et al., 2017). Mussel cultivation is an extensive aquaculture without additional feeding, avoiding an input of extra of nutrients. The blue mussel is distributed in most parts of the Baltic Sea and juvenile mussels (spat) settle naturally if collector material is provided. By its filter feeding behaviour, it removes particles and binds nutrients in biomass. When harvested, it can then support nutrient removal in that waterbody (Petersen et al., 2014)Denmark where biological and economic parameters related to nutrient removal was monitored throughout a full production cycle (1yr. The limiting factor here is the decreasing and highly variable salinity, which hampers growth of *Mytilus* spp. To further investigate the potential of blue mussel farming and nutrient extraction in the Baltic Sea, where tradition is scare, several studies have been conducted or are ongoing in many member states. We focused on the southern Baltic Sea, where along the German coast a salinity gradient from 1 to 24 psu is covered. We compare growth rates of four mussel farm trials in German coastal waters to estimate production potential and nutrient removal depending on salinity. Furthermore, a Dynamic Energy Budget (DEB, Maar et al., 2015) model based on environmental parameter (salinity, temperature, chlorophyll a concentrations) is verified with measured growth data to predict mussel growth under varying conditions in di ferent areas.

Material and methods
Test mussel farms in the longline system were set up in Kiel, Nienhagen, Greifswald Bay (GWB) and Wieker Bay (WB) at salinities ranging from 7 to 17 psu and growth data of the first 12 to 16 months collected. Results were entered into the growth model (DEB) combined with environmental monitoring data and growth potential at twelve different locations along the German Coast estimated covering a wide salinity range. Based on modelling results, potential usages of the biomass yield after 6 and 18 months and the economic feasibility was investigated.

Results and discussion
Kiel Bay and Nienhagen show fast growth, nearly comparable with North Sea mussels (Walter and De Leeuw, 2007)with 11 000 to 64 000 individuals per meter collector. The shell length of suspended mussels increased in their first summer at an average of 1.2 mm per week. Between the end of August until the end of September a mean of 2 to 9 kg mussels per meter equalling 4500 to 20 300 individuals per meter were harvested. Relayed on bottom cul- tures the mussel seed continued to grow and could be marketed as consumption mussels after their second summer. Kurzfassung Seit 2001 ist die Langleinenkultur von Miesmuscheln (Mytilus edulis. Nonetheless, modelling predicts far slower growth in Nienhagen and hereby highlights the dependency on environmental parameters such as temperature and chlorophyll followed by annual divergences in mussel growth. A continuous yield is important for economic feasibility as well as fast growth and a market size of min. 5 cm after 18 months which can only be reached in areas of a salinity of 11 psu or more. If aiming for small size mussels, a compensation fee is necessary for the removal of nutrients to make mussel farming economically attractive in regions of low salinity.

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ASSESMENT OF ENVIRONMENTAL SAFETY OF A COMBINATION OF *Melissa officinalis* AND MAGNESIUM ON SENSITIVE SPECICES

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Introduction

Regrouping, loading and transport are common sources of stress in farm. This is also true for fish. Well-boat transport and loading are very stressful for fish such as salmon (Delfosse, 2017; Iversen *et al.*, 1998). Composed of *Melissa officinalis* and soluble magnesium, a natural product is used to reduce aggressiveness and to improve animal welfare. A previous study set up in fishes shown a decrease of stress in fish at a recommended dosage of 0.25ml/l. (Labalett *et al* 2018).

The aim of the present study is an assessment of environmental safety of this solution by studying biological biomarker. The toxicity of this product was evaluated using bioassay. The mortality of two copepods (*Daphnia Magna* and *Tisbe Longicornis*) and the growth rate inhibition of marine micro-algae (*Selenastrum Capricornutum* and *Dunaliella tertiolecta*) were analyzed at different dosages in order to evaluate the eventual toxicity of this natural product.

Material and method

The methods used to measure the livability or growth population inhibition were different. Growth population inhibition test was done on 50 ml with a density of 10,000 cells by ml, the culture of *S. capricornutum* and *D. tertiolecta* was continuously exposed to light and mixed twice a day. Three replications and six different dilutions were applied, the initial solution containing sea water and nutrients for the right development of the organism. The dosages applied were 0; 0.625; 1.25; 2.5; 5; 10 ml/l of *Melissa officinalis* combined with magnesium (Durelax® Liquid, Norfeed, France), the micro-algae were exposed to each dilution. The cellular density was measured 48h after the start of the trial.

The objective of the second test was to measured the incidence of the natural solution on two copepods, *D.magna* and *T.longicornis* livability. Juveniles copepods were used in this trial. *Daphnia Magna* was used after 24 hours of clutching. *T.longicornis* were chosen according to their size (125-250 µm). Four replications by treatment and seven different dilutions were done: 0, 0.25; 0.5; 1; 2;4;8 ml/l of water. The mortality was measured after a time exposure of 96 hours.

Results and discussion

No mortality on *D.magna* or *T.longicornis* was observed showing the safety of the product even at doses 32 times the recommended dosage.

There was no growth inhibition concerning *D.tertiolecta* even at doses 40 times the recommended dosage. However, we observed a significant speed growth reduction of *S.capricornutum* for dosages from 10 times the recommended dosage compared to the control.

At the recommended dosage 0.25ml/l and up to 10 times the recommended dosage there is no incidences on sensitives biomarker species . This combination of *Melissa officinalis* and magnesium could show great potential of use for aquaculture

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Labalette et al. 2018 Water supplementation of a mixture of Melissa extract (*Melissa officinalis*) and magnesium to reduce physiological stress reactions of salmon (*Salmo salar*)

GREENHOUSE GAS ASSESSMENT OF ATLANTIC SALMON (*Salmo salar*) AQUACULTURE ON CERTIFIED (ASC) FARMS

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Introduction

With many capture fisheries already being fished above sustainable limits (Froese et al., 2018), the only way to produce more fish, is through aquaculture (World Bank, 2013). However, in view of food production having a major environmental impact, e.g. to climate change through greenhouse gas (GHG) emissions (Steinfeld et al., 2006), assessing the impact of the production chain is crucial to ensure a sustainable growth of the sector by understanding how to limit or prevent environmental impacts. Globally operating certification schemes, such as the Aquaculture Stewardship Council (ASC) set limits on these impacts for participating farms. The ASC was established in 2010 in order to provide certification for farmed fish based on standards of best practice defined through multi-stakeholder dialogues. Because climate change is a global problem and the biggest of the environmental factors to be addressed, the ASC promotes efficient and sustainable energy use and requires their farms to monitor energy use on the farm sites and feed use (Aquaculture Stewardship Council, 2019).

The ASC currently has nine certification standards available, of which salmon is the most important one in Europe in terms of production volumes and value (European Commission, 2017). At present, ASC has 27% of global salmon farming under certificati n, which is about 40% of all ASC-certified farms (Roebuck and Wristen, 2018). The ASC strives to lower GHG emissions from these certified farms by lowering the energy use. Therefore, the aim is to investigate and evaluate the environmental impact in terms of GHG emissions from energy use and feed at ASC-certified salmon farms sites.

Materials and methods

For this study GHG analysis will be performed using data available from ASC-certified farm sites to evaluate energy use and associated GHG emissions (CO_2-equivalents (CO_2e)) and GHG emissions resulting from feed from grow-out sites of Atlantic salmon production. GHG will be assessed using one tonne of live-weight salmon as functional unit for the assessment, limited to the grow-out site of salmon, as data from smolt production will not be used and chain of custody is not available. This data will be compared as far as possible to secondary data from non-certified farms obtained from literature resources. Comparisons within certified data will made in regard to companies and countr .

Data submitted from a total of 176 salmon farming sites contain quantity and type of energy used on each farm site per production cycle and the calculated GHG emissions in CO_2e thereof. Moreover, farms also provided data on GHG emissions from feed (CO_2e) calculated by themselves multiplying the specific GHG emission factors per kilo of feed provided by the feed manufacturer by the amount of feed used per production cycle on the farm site. Since data for GHG emission related to energy use are required to be submitted per year, conversions will be applied in order to have the same time period for all indicators when needed. Data could be accessed through the database of ASC, being submitted for each farm site in order to obtain ASC certification

As there is no uniform format for data submission in place, differences in detail of the received data were present. Therefore, submitted data was classified in terms of detailedness and completeness as ‘poor’, ‘medium’ or ‘good’. Classification ‘poor’ was given for farm sites that just provided the resulting values for each indicator without further detailing or when data was missing. Whereas, ‘good’ meant a detailed report including all necessary background information and sources. And ‘medium’ covered all the basic required data points but certain factors were left out. Poor quality was excluded from further analysis, leaving 89 farm sites for assessment. As farms are allowed to use different sources for the calculation of emission factors, difference in these will be investigated and GHG emissions will be recalculated with one standardized value for all of them.

An overall comparison of GHG emissions from energy use as well as feed of all farm sites will give an indication of the ranges of the farms under certification

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Results / Discussion

The results of this study will be used as a first step to evaluate the current practice of ASC-certified farms on GHG emissions. This might help to alter the requirements on these indicators to further drive reduced energy use and GHG emissions or to help in the improvement of data collection.

This abstract will be adapted as soon as the results are available.

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EFFECTS OF DIFFERENT FEEDING FREQUENCIES ON GROWTH, FEED UTILIZATION, PLASMA BIOCHEMISTRY AND DIGESTIVE CONDITIONS OF GILTHEAD SEA BREAM (*Sparus aurata*) FED WITH DIFFERENT FISH MEAL AND FISH OIL DIETARY LEVELS

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Introduction

The optimization of feeding management is essential to improve the key performance indicators such as growth rates, feed efficienc , mortality and quality traits of the Mediterranean aquaculture. Optimal feeding frequencies under farming condition may contribute to maximise feed utilization reducing wastes and production costs. Several effects of the feeding frequency on performances have been observed in different fish species mainly depending on the fish size and on the interactions between feeding frequencies and type of administered diets (Yúfera et al., 2014; Enes et al., 2015; Guo et al., 2015; Zeytin et al., 2016). This study was undertaken in order to assess the effects of different feeding frequencies on growth, feed digestibility, somatometric indexes, fish plasma biochemistry, and digestive condition of gilthead sea bream fed different fishmeal and fish oil dietary levels with the final aim to provide guidelines designed for the daily management of feeding practices under farming condition.

Materials and methods

Two isoproteic and isoenergetic experimental extruded diets with different FM and FO dietary level (FM/FO 30/15 and 10/3, A and B, respectively) were formulated using practical aquafeed ingredients. Each diet was randomly assigned to fish groups of 60 individuals (initial weight: 88.3 ± 2.4g) each submitted to a different feeding frequency (1, 2, 3 meals day^{-1}) in triplicate: (1) a daily meal at 8.30am, (2) two meals a day at 08.30 and 16.00 and (3) three daily meals at 08.30, 12.30 and 16.00 respectively, over 109 days. All the fish received the same total daily feeding ration that was determined based on the feed ingested by the group 1 which was fed close to satiation (90-95% of the satiation level). At the end of trial growth, feed utilization, feed digestibility, digestive enzyme activity, gut histology and plasma biochemistry were measured. Data were analysed by a two-way ANOVA followed by a Tukey's multiple comparison test.

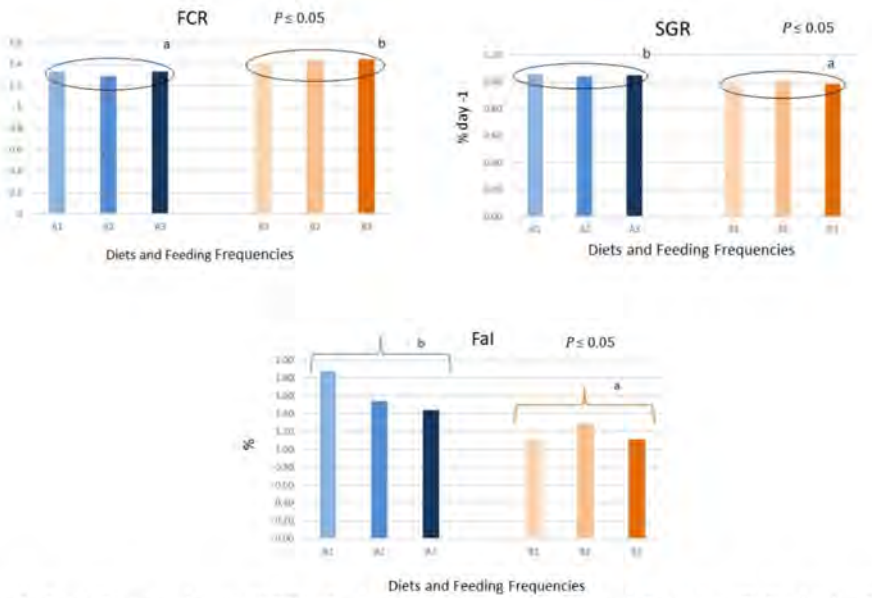

Fig.1. Specific growth rate (SGR), feed conversion rate (FCR) and fat index (FaI) obtained at the end of the trial under different feeding frequencies (1, 2, 3 meals) and fed high (A) and low (B) fishmeal and fish oil dietary level. Different superscript letters a, b denote significant differences among diets.

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Results

At the end of the trial, no significant differences ($p > 0.05$) due to the different feeding patterns were observed in terms of final body weight, specific growth rate (SGR), voluntary feed intake (VFI), feed conversion rate (FCR) and no significant differences were found in the viscerosomatic index (VSI), hepatosomatic index (HSI), condition factor (CF) and fat index (FaI) values between different feeding frequencies. SGR and FaI values were slightly higher in diet A compared to diet B while FCR showed lower values (Figure1).

Discussion and Conclusion

The results showed that it is possible to obtain an equivalent growth performance and feed utilization regardless of the number of the meals daily administered under both high and low FM and FO dietary level. No effect on fat deposition was determined by feeding frequencies even if a tendency toward lower values were observed in the 3 meals group fed high FMFO level. Based on the present results frequent feeding do not support an increase in feed utilization during the on-growing of sea bream and this could be a useful indication to plan feeding activity at farm level which maximize growth and costs of feeding procedures.

Acknowledgements

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DEVELOPMENT OF A BIOECONOMIC MODEL TO VALUE ECOSYSTEM SERVICES IN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEMS

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Seafood production through aquaculture is in a unique position to contribute to healthy and sustainable diets and help to tackle the rising burden of chronic noncommunicable diseases and malnutrition in the UK, if environmental sustainability challenges and barriers to consumption are adequately addressed. Diversifying production, especially towards species of higher environmental sustainability, such as seaweed, mussels and sea urchins, and in particular through Integrated Multi-Trophic Aquaculture (IMTA), could serve a significant purpose towards bioremediation, while also allowing for product diversification and increased public acceptability of the industry. However, continued challenges to the commercial implementation of IMTA have hindered investment.

The Diverseafood project addresses barriers to the diversification of aquaculture systems in the UK, notably the development of economically attractive business models, a suitable regulatory framework and the consumer market of IMTA produced seafood, while evaluating the contribution of IMTA to the nutritional value of aquaculture-produced seafood and to the environmental sustainability of the sector. One key challenge is that current IMTA systems do not consider the economic value of the bio-mitigation services provided by extractive species or the value they might represent in terms of social licence to operate. This work will identify the value of provisioning, regulating and socio-cultural ecosystem services associated with IMTA by developing the first bioeconomic model for IMTA to value diversification and nutrient removal and analysing the effect of IMTA practices on the social licence for companies to operate.

This research will evaluate the impact of a transition from salmon monocultures to IMTA systems on the value of provisioning, regulating and socio-cultural ecosystem services. The project will develop a bioeconomic model of growth and harvest based on existing IMTA optimisation models of multispecies productivity and studies that examine the economics of IMTA. Data requirements include farm layout, investment and operating costs, sale prices, cultivation (species, harvest weight, culture period, mortality), environmental drivers (water temperature, salinity, total particulate matter, etc.) and culture practice (stock density, feed applied, etc.) and will be sourced from previous experiments and existing literature.

The bioeconomic model will couple an economic value function with the dynamics of a conventional monoculture salmon farm and then add in co-cultured groups (bivalves, seaweeds). The main difference between the monoculture and the IMTA system formulations is that these versions will include extra terms for the revenue and costs from the addition of co-culture groups (impact on provisioning services), as well as extra bio-mitigation terms in the equation of motion (impact on regulating services). Thus, it will consider two elements that can potentially add to economic welfare using diversified systems, (i) increased profitability and reduced risk through diversification, and (ii) nutrient remova

Several interesting aspects of the management of an IMTA system operation in contrast to monoculture will be analysed. We will begin by introducing a fixed site area for the operations, which amounts to imposing an additional constraint in the optimization problem. This expanded model will be used to consider the current regulatory regime in Scotland that involves the use DEPOMOD/AUTODEPOMOD models by the Scottish Environment Protection Agency (SEPA) (Ecasa Toolbox 2007) to regulate aquaculture discharges. The case of a nutrient tax will then be considered, showing how this leads to different management and welfare implications and, finall , extend the analysis to consider the often-discussed notion of a “nutrient credit”. The model will provide a useful quantitative tool for the aquaculture industry, regulatory bodies, and the scientific community to understand how diversified aquaculture systems can support an environmentally sustainable and economically profitable expansion of aquaculture. The recognition of both provisioning and regulating ecosystem services and their association with a system of nutrient trading credits, which still has to be put in place at a national or international level, can represent financial incentive tools to encourage producers to contemplate alternative diversified aquaculture systems as viable options to their current practices

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With few ecological-economic assessments of IMTA vs. monoculture done to date (e.g. Shi et al. 2013, Nobre 2010), the development of a bioeconomic model will help create financial and regulatory incentives for governments and the industry to jointly invest in IMTA systems and for companies to improve operating practices. It will also be used to support the analysis on establishing and implementing a scheme for the payment of credits or other incentives for bio-mitigating services.

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Acknowledgments

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TOWARDS IMPROVED COMMUNICATION IN AQUACULTURE: EXPLORING CONSUMERS' PERCEPTIONS AND ATTITUDES

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Public perception is considered one of the limiting factors to the expansion of aquaculture, as it translates not only in seafood purchasing and consumption attitudes, but also on the shaping of public support for the industry and social licence to operate. However, to build social approval it is essential to understand perception and attitudes and their underlying factors, and use this to improve how businesses, third sector and regulators operate and engage with the public.

Though several studies have focused on analysing consumers' knowledge and consumption habits, the stated preference for aquaculture or wild-caught seafood products, the relevance of key topics (e.g. welfare), trends in media coverage in European countries; literature remains scarce on the determinants of public perception of aquaculture and associated attitudes, as well as contrary or supporting arguments, with studies on social licence to operate in aquaculture just recently starting to populate the literature. There is also limited evidence that informing the public by itself is an effective strategy to raise acceptability or to increase consumption and there is high uncertainty on what should be communicated to the public or communities and how this engagement should occur. The role of others, such as corporate social responsibility or certification, in shaping the image of aquaculture also remains poorly explored.

A systematic literature review was conducted on perception and attitudes towards aquaculture at European level and used to inform subsequent focus groups with public and communities and stakeholder interviews exploring communication in aquaculture. Focus groups were designed to investigate seafood purchasing and consumption behaviour, as well as public perception and attitudes to aquaculture production and products; with a total of 6 focus conducted (6-8 participants each) with different groups of UK consumers (low-frequency, high-frequency), non-consumers (general, vegan) and communities living in proximity of operating aquaculture facilities (older members, younger community members). A series of in-depth interviews were also be conducted with different UK stakeholders (producers, processors, retailers, third sector and regulators; 3-4 per group) to identify perceived public perception issues, communication challenges and pathways for improved engagement. This research aims to support the development of best communication practices, improve public engagement with the aquaculture sector and sustainable seafood production and consumption.

Acknowledgments
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ANTIMICROBIAL ACTIVITY OF *Nannochloropsis* spp. AGAINST FISH PATHOGENS

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Introduction

Microalgae are the primary sources of many bioactive compounds like proteins, carbohydrates, lipids, vitamins and enzymes. It is considered a promising organism for industrial and biotechnological applications because of its ability to accumulate high levels of polyunsaturated fatty acids (Durmaz, 2006). Microalge are used in food additive for human or animal nutrition, pharmaceutical industry, organic fertilizer, biofuel production, drug therapy and antimicrobial, antifungal agent for human pathogens (Spolaore, 2006; El-Sheekh et al., 2006; Dussault et al., 2016).

One of the most important bacterial diseases that cause economic losses in aquaculture is rainbow trout fry syndrome caused by *Flavobacterium psychrophilum*, affecting hatchery reared rainbow trout fry and fingerlings. Synthetic antibiotics and vaccine are used in treatment and cure however, accumulation and resistance to these drugs by aquatic-environment are increasing problems. Therefore, finding alternative agents for pathogen control are crucial for Turkish aquaculture sector which has around 240 thousand tons production capacity cultured.

Materials and Methods

Walne media was used to culture *Nannochloropsis* spp. in 1 lt glass bottles were ventilated and incubated at 26 °C, in daylight, for 9-15 days (Rippka et al., 1988). *F. psychrophilum* were grown according to Austin&Austin, (2007). Ethanol and methanol solvents were used as a extraction. Disk diffusion method was used to determine the algal antimicrobial effect on *F. psychrophilum*. At the end of the incubation period at 22-25°C for 24 hours, the presence of antimicrobial activity was determined according to the inhibition zones formed around the discs.

Results

In this study, the antimicrobial activity of *Nannochloropsis* extracts were tested for *F. psychrophilum* that was found to be sensitive to both ethanol and methanol. The antimicrobial activity of the methanol extract formed against *F. psychrophilum* was 15.0±0.4 mm, while the ethanol extract had a lower zone diameter and was measured to be 9.0±0.5 mm. As a result, it was observed that *Nannochloropsis* spp. showed quite high antimicrobial activity against *F. psychrophilum* (Figure 1).

Discussion and Conclusion

F. psychrophilum have shown antimicrobial activity positively as the inhibition zones were 9-15 mm respectively. Higher antimicrobial activity was observed in the extracts obtained with the methanol extract. It has been observed that the extracts obtained from sea and fresh microalgae have antimicrobial activity on human pathogens (Özdemir et al., 2004; Abo State et al., 2015). However, a few studies have examined the antimicrobial activity of some algae against some fish pathogens. Bhuvaneswari et al. (2013) studied the antimicrobial effect at the molecular level of the cyanobacteria *A. platensis* against *A. hydrophila* and *E. tarda*. In the light of these results, *N. oculata* extracts should be performed and used fish disease prevention studies and/or feeding trials as an alternative environmental friendly, low costs chemotropic reagent for aquaculture that will open new horizons for green and sustainable aquaculture.

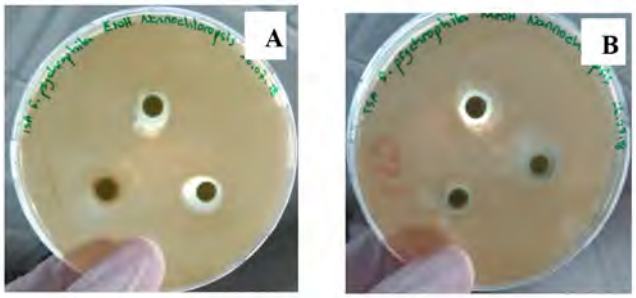

Figure 1. Zones show antibacterial effects of *Nannochloropsis spp.* extracts on **A)** *F. psychrophilum* (ethanol) **B)** *F. psychrophilum* (methanol).

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QUANTIFYING THE CONSEQUENCES OF DELOUSING ON FISH WELFARE IN SALMON FARMING

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The ability to delouse salmon cages efficiently when sea-lice counts exceed legal limits is critical, as uncontrolled outbreaks of sea-lice may lead to impaired fish welfare and health and have severe consequences for wild salmonids in the environment near the farm (Bjørn et al., 2001, Igboeli et al., 2014). Today, the most common delousing methods are non-medicinal treatments such as freshwater, thermal or mechanical delousing (Lekang et al., 2016), while chemical methods such as immersing the fish in closed/semi-closed volumes containing anti-louse chemicals have been abandoned since sea-lice populations have undergone a survival-driven genetic selection for resistance against these substances (Aaen et al., 2015). All the current non-medicinal treatments consist of the following two steps: **crowding** the fish at higher than normal densities, and **pumping** the fish from the cage, through a barge or ship that contains the delousing treatment system, and back into a different section of the cage or into a new cage (Føre et al., 2018b). **Both phases are extremely dangerous for the fish well-being. Crowding** may subject the fish to lowered welfare and lead to detrimental health and increased mortality since crowding at very high concentrations may induce mechanical damage, and increased stress levels to the fish (Erikson et al., 2016). **Pumping and delousing** expose the fish to mechanical stress due to low pressure, turbulent flo , confined space, thermal shocks, collisions with pipe walls and metal walls and presence of rotating equipment such as impeller pumps or brushes (Svendsen et al., 2017).

Although delousing is the riskiest fish treatment operation in the production cycle for farmed salmon, **there exist no knowledge-based and objective methods** for handling these challenges and the present industry practice in parasite management is founded in **experience-based reasoning, and manual monitoring and operation** (Føre et al., 2018a). This practice typically entails that operators first observe the fish via direct visual observation or underwater cameras and then interpret this information primarily based on their subjective experience, yielding a subjective perception of the current lice level and state and condition of the fish. These interpretations are then used as a foundation for making decisions concerning crowding and pumping, making the delousing process prone to **huge variations in fish mortality and stress**, both between operation crews and delousing units (Lekang et al., 2016).

Inspired by the core principles of **Precision Livestock Farming** (Berckmans, 2004), researchers at SINTEF Ocean have coined the **Precision Fish Farming** concept whose aim is to apply control-engineering principles to fish production, thereby improving the farmer's ability to monitor, control and document biological processes in fish farms, including the fundamental **delousing process** (Føre et al., 2018a). In relation to salmon delousing and within the Precision Fish Farming framework, the SINTEF Ocean scientist have been **investigating the consequences that crowding, pumping and delousing have on fish welfare** since non-medicinal delousing treatments were introduced by the salmon farming industry in Norway. Among several activities, the projects called **CROWDGUARD** and **KVALISYS** are the most relevant. The **CROWDGUARD project** (RCN project no 282371) develops technology for quantificat on and documentation of fish welfare related states during **crowding** focusing on sensor platform, instrumentation and communication, operational testing and data collection, data processing, analysis and validation. The goal of CROWDGUARD is to take control over the crowding situation and the biomass density to ensure that the fish is not exposed to excessive physical loads.

The **KVALISYS project** (FHF project no 901397) aims at quantifying the mechanical loads inflicted by **pumping and delousing** equipment to Atlantic salmon by employing a new generation of passive data loggers – "sensor fish". The scope of KVALISYS is to establish a standard methodology and an accepted baseline to compare different pumping and mechanical delousing systems. This may yield concrete guidelines and suggestions on how to design pumps for fish as well as delousing units.

The two projects, CROWDGUARD and KVALISYS address specific aspects of delousing operations, either by making some quantities observable and measurable (i.e. mechanical loads, stress level or biomass density), either by improving single machinery components such as pumps, or by applying feedback control exclusively to one single phase of the overall process. The hereby presentation will show the latest activities and results within the **CROWDGUARD** and **KVALISYS** projects.

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SHELFISH FARMING IN OPEN SEA WITH ADVANCED CONCRETE RAFTS

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Introduction

Approximately 85% of the mussel aquaculture around the world is suspended and off-bottom culture (McKindsey et al. (2011)), including among the techniques the floating raft system, the used in Spain to produce 40% of the EU mussel. Since its origins in Galicia to our days, the rafts have been built with eucalyptus wood. This imply a short lifetime, the need to use paints for protection and a high carbon footprint. In 2015 the company RDC developed a 540 m^2 precast raft made of Ultra-High-Performance Fiber-Reinforced-Concrete (UHC), an extremely compact and durable composite (this project received funding from the EU's Horizon 2020 research and innovation programme under grant agreement No 738777, SELMUS, SME Instr. Phase 2). Four years after the idea, there are more than 5.000 m^2 under operation and proving high resiliency and very low maintenance costs.

Considering the UHC raft performance, a new design is under development to enable it to cover other needs (project OpenMode-863562, co-funded by the European Maritime & Fisheries Fund (EMFF)). This new generation is adapted for open waters, connectable and sensor-equipped, allowing intensive harvesting in new areas, adapting the modules to the farm needs and facilitating their transport and assembling. The project will test these capacities with eight pilots floated in five countries across three EU sea basins

The problem of the traditional mussel rafts

Wooden rafts for mussel farming are used in Spain since 1900 due to their reduced purchase cost, lightness and flexibilit . The intensive system is feasible due to the upwelling-downwelling dynamics on the continental shelf (Figueiras et al. (2002)). The structure can carry up to 500 ropes and produces nearly 60 tones/year. However, its use has three type of disadvantages: 1) Economic: It has low durability (≈12 years) and needs periodic investment in maintenance which undermines the sector's profitabilit . 2) Industrial: each raft is built manually through a high-risk job in the inter-tidal zone using hammers. Replacing the damaged elements is a slow work. 3) Environmental: it implies intense deforestation. The degradation of the wood and the products used to protect it cause water pollution.

The 540m^2 UHC raft

The modular raft designed in 2015 was made of UHC (Utility Model ES1147609U), an innovative material which permeability is nearly 1,000 times lower than concrete, is five times more flexible than steel (elastic modulus) and its strength is six times higher than for wood. With the same area than the traditional raft (20x27 m), it is formed by three frames where six steel floaters are connected, and 10 secondary beams perpendicular to the first. Its lifetime is expected to exceed 30 years. The first prototype was precast in 2016 for the Technology Center AZTI. In October 2019 there are already ten units harvesting mussel and oyster in both basins of the Spanish coast. The continuous monitoring systems and cameras are proving the robustness and resiliency of the rafts, which are already demanded by the Galician farmers because they have proven to minimize the operating expenses.

The product is aligned with the EU Blue Growth strategy, as: 1) it boosts the shellfish aquaculture competitiveness. 2) It improves maritime spatial planning and integrated maritime surveillance concentrating the farms on controlled and visible modules. To reach a similar production some regions use a tangle of kilometers of submerged ropes (long-line method) and plastic elements.

The OpenMode project: Towards a global and sustainable impact in shellfish farmin

The 540m^2 UHC raft is useful in semi-opened waters with very high production capacities, and the special transport required for the precast elements is complex and expensive. The project OpenMode aims to build on the progress achieved up to now adapting the UHC rafts for open waters. For this, they need to be: 1) Modular: The length of its beams will be 12 m to be exportable in 40' containers, so that an EU-produced hi-tech solution can be competitive in any country. The smaller size will also reduce the stresses under intense swell. The simple packing and assembling facilitates its reuse. 2) Connectable: Elements can be connected onshore or offshore to form larger structures (12x24 m, 12x36 m…), with a type of connection that depends on the swell and application. The module configuration will facilitate the adaptation to other BG sectors. 3) Sensor-equipped: A remote system will send weather, marine, water and structural parameters to support the decision taking and minimize costs, risks and environmental impacts of visiting the platforms.

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The project will float eight 140 m^2 pilots before 2021. Four of them to test the connection systems in the Bay of Biscay (Atlantic basin), one in the North Sea with the support of the Technical University of Denmark to scale-up the compensation policies, two in the Dalmatian coast to increase the productivity and face their problems with the predatory fishes, and one in Malta to explore the possibilities to combine the structure with solar energy. There are specific tasks dedicated to BIM, maritime safety, Life Cycle Assessment, User Experience, trainings and optimization of harvesting through Big Data analytics.

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MARINE SPATIAL PLANNING FOR THE DEVELOPMENT OF SUSTAINABLE MARINE AQUACULTURE IN ITALY – A GIS ANALYSIS FOR THE DEFINITION OF CONSTRAINTS MAP AND IDENTIFICATION OF AZAs

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Introduction

The lack of suitable sites for aquaculture is one of the factors limiting the development and growth of European aquaculture (COM 229/2013). The national Multiannual Strategic Plans for Aquaculture prepared by Member States (MS) in 2014 (Reg. 1380/2013/EU) foresee the commitment in improving spatial planning to identify marine areas to be allocated to aquaculture by 2020. In 2014 entered into force the Directive 2014/89/EU establishing a EU framework for Maritime Spatial Planning (MSP) for a sustainable coexistence of uses and, where applicable, the appropriate apportionment of relevant uses in the maritime space. The MSP directive includes aquaculture among the innovative economic sectors to be considered to promote the Blue Growth and the sustainable development of maritime sectors. MS are responsible and competent for designing and set up maritime spatial plans, which identify the spatial and temporal distribution of relevant existing and future activities and uses in their marine waters. Italy implemented the MSP directive with a national decree in 2016 and the following guidelines of the Ministry of Infrastructures and Transport in 2017. The objective of this study is to develop aquaculture suitability maps in Italian seas to facilitate the allocation by regional governance of marine areas for aquaculture development and for further detailed local studies for site selection for shellfish and fish farming activities in coastal and offshore areas.

Materials and methods

Primary tools used for spatial planning are the Geographic Information System software, which allow the management and analysis of geo-referred datasets related to multiple kinds of information, such as infrastructural, social and environmental, returning readily interpretable, highly visual maps (Malczewski, 2010). The IT-AQUA GIS was build using the ESRI ArcGis 10.3 Geographic Information System and the data have been mostly sourced from many Open GIS Consortium Web Feature Services (OGC WFS) Geoportals. The scientific literature on geospatial analysis used for the identification of Allocated Zones for Aquaculture (AZAs) was reviewed (Aguilar-Manjarrez et al, 2017) and relevant data layers, at national scale, representing potential constrains to aquaculture development were selected. The principles and criteria for the identificatio of the categories of constraints and interactions, the list of constraints and the relative "respect zone" have been discussed in two round meetings within the Italian Aquaculture Platform (ITAQUA, Ministry of Agriculture). The most relevant stakeholders involved were Regional and local Competent Authorities, Central Authorities (Ministries of Environment, Health, Research, Economic Development), Producer's Associations, aquaculture companies, cooperatives and scientific experts, who validated the process for the identification o AZAs in the Italian Regions.

Environmental data, as Natura 2000 network sites, Marine Protected Areas (MPAs), natural benches of bivalves, have been sourced from ISPRA repository, from the National Geoportal of Ministry of the Environment and from Regional Resolutions. Infrastructural data as pipelines and submarine cables, military polygons, wrecks and hydrocarbon exploration, extraction areas, etc., have been sourced from the General Management of Environmental Safety and Mining and Energy Activities (DGS UNMIG) and from the Hydrographic Institute of the Italian Navy. General interaction as sea routes, boating, sport fishing, tourist settlements, etc., have been sourced from the Opendata of the Italian Public Administration (dati.Gov.it) and from Ordinances and Decrees of the Port Authorities. Aquaculture licensed sites have been sourced from the repository of the State maritime property register, and further validated and updated.

Results

Data was harmonised according to the Infrastructure for Spatial Information in the European Community (INSPIRE) directive and then compiled into a geodatabase which comprised, 12 environmental, 13 infrastructural and 9 other human interaction geospatial layers (constraints). A constraint - free area layer was then calculated and identified using a simple boolean overlay of the identifi d constraints. Where appropriate, a buffer (Respect Zones) was added to the constraint layers.

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The IT-AQUA GIS geodatabase, containing all the layers of constraints, has been then used as background dataset to perform a case study in the Latium Region, where the process of identification of zones have been started following the Guidelines for the identification of AZAs drafted by ISPRA to facilitate the planning and management of of marine waters for aquaculture use at regional level and the integration of aquaculture activities within the Regional plans for MSP by 2020.

Discussion and conclusion

The purposes of the identificati n and allocation of zones suitable for aquaculture, through the establishment of AZAs and within the wider MSP process, are multiple and encompass, among others, the minimization of multi–use conflicts, the reduction of environmental impacts, the optimization of production cycles, the streamlining of the licensing procedures, the increasing of confidence of investors. Furthermore, the involvement of a number of stakeholders in the decision making process strives to include different coastal users in the identification of the AZAs increasing the social acceptance of aquaculture in the region (FAO, 2010).

Acknowledgments

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SULFUR AMINO ACID REQUIREMENTS AND INTERACTIONS IN JUVENILE YELLOWTAIL KINGFISH (*Seriola lalandi*)

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Introduction

Sulphur-containing amino acids (SAA), which include methionine and cysteine, allow normal growth and functioning of fish. Methionine is an essential amino acid and cannot be synthesized *de novo* in sufficient quantities to meet metabolic requirements. In fish, cysteine has the potential to spare 33 to 60% of methionine requirement (Abidi and Khan, 2011; Harding et al., 1977; Moon and Gatlin, 1991). The relatedness, interactions and sparing effects of SAA imply that quantifying the requirement of a species for one essential SAA must be done within the context of other SAA present in the diet. The objective of this study was to quantify the dietary methionine requirement and sparing effect of cysteine in juvenile Yellowtail Kingfish (YTK)

Materials and methods

YTK (mean weight 52.6g) were fed one of ten diets, containing five incremental levels of methionine, ranging from 7.9 to 25.2 kg^{-1}, crossed with two levels of dietary cysteine (i.e. 5.5 or 13.9g kg^{-1}). All diets were isonitrogenous (≈600g crude protein kg^{-1}), isoenergetic (≈22MJ gross energy kg^{-1}), and prepared using practical raw ingredients. YTK were held at 22°C for 8 weeks and fed twice daily to satiation.

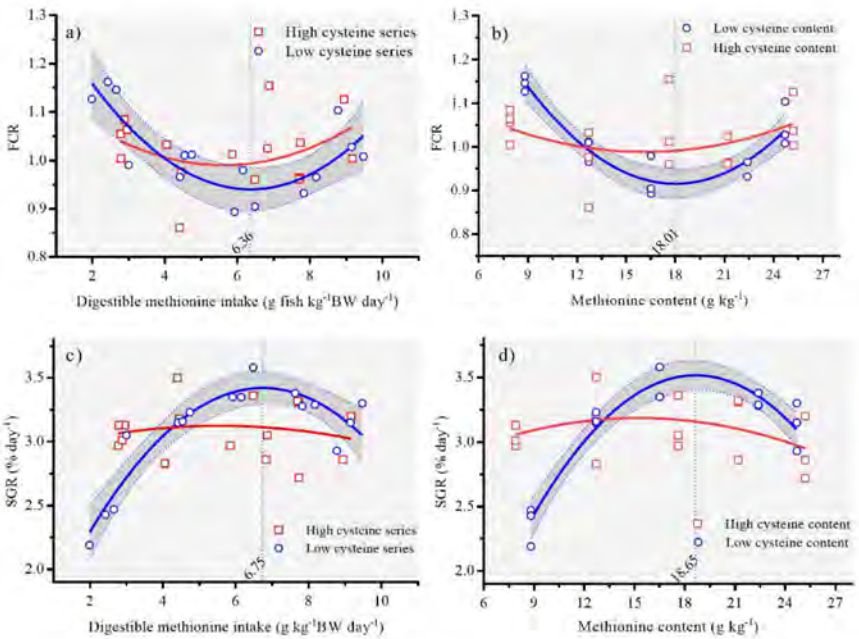

Fig. 1. FCR response relative to digestible methionine intake (a) and dietary methionine content (b); SGR response relative to digestible methionine intake (c) and dietary methionine content (d). Regression models for fish fed diets low (blue) and high (red) in cysteine. Grey bands indicate the 95% confidence interval for low cysteine series. Dotted, vertical lines indicate the optimal methionine intake (a,c) and dietary specification (b,d) for YTK fed diets containing low level series of cysteine.

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Results

Applied models indicate that the maximum growth and feeding efficiency of YTK occurs at a digestible methionine intake of 6.75 and 6.36g kgBW^{-1} day^{-1} respectively, at an average digestible cysteine intake of 1.73g kgBW^{-1} day^{-1} (Figure 1). Furthermore, the continuing increase in methionine intake caused a reduction in SGR and a worsening in FCR. YTK fed the diets containing the higher level of cysteine, recorded high variability in both SGR and FCR and no specific methionine requirement could be established. Generally, YTK fed the diet higher in cysteine performed more poorly relative to YTK fed the diet lower in cysteine.

Discussion and conclusion

The dietary crude level of total sulphur amino acids (TSAA; methionine+cysteine) is approximately 24.3g kg^{-1} diet which equates to a daily digestible intake of TSAA of 8.4g kgBW^{-1} d^{-1}. Based on the SGR response of YTK, cysteine can spare at least 48.2% of the dietary methionine requirement in YTK, considering molecular weight and digestibility. The decline of SGR in YTK past the optimum methionine intake, indicates a maximum methionine threshold, possibly induced through the buildup of excess methionine derivates via transamination or transulfuration pathways. The results suggest the dietary specification of methionine, currently relied on in commercial aquafeeds for YTK, may be inadequate. This study provides new data on the SAA requirements of juvenile YTK and will facilitate the formulation of better diets for this species.

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DETERMINATION OF CARRYING CAPACITY IN INLAND WATERS AND VARIOUS MODELS

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INTRODUCTION

The amount of inland water aquaculture, their environmental impacts and potential must be revealed for the sustainability of aquaculture and the efficient use of water resources. However, development of inland aquaculture sector is under the responsibility of researchers, public institutions, non-governmental organizations and cultivators working in this field by ensuring the sustainable use of the water body without limiting other uses. Use of models to estimate the environmental impact of inland water aquaculture or, in other words, determination of the carrying capacity of inland waters should be considered as the first stage for the development of accountable sustainable fisher . In all of the fish farming methods, fish feces and wastes from inedible feed accumulate in the environment (Beveridge, 1984). Effects of fish farming on aquatic environment depend on the method and application of aquaculture, the feed quality, the stock amount and the hydrography of the area for cultivation (Wu, 1995). Carrying capacity is a scientific term that tolerates a harmful effect of accumulated concentration of a substance on an ecosystem in a given aquatic environment.

While one portion of solid organic material containing mainly carbon and nitrogen entering the receiving water body from the fish screens (approximately 15%) are remained in the water column as suspended solid, some of them are consumed by fishes outside the cage. In addition, an important portion of them are accumulated in the sediment and causes organic enrichment of benthic system, and significant changes in benthic macrofauna and sediment chemistry (Ackefors and Enell, 1990).

Beveridge (1984) model and MOM system (Modeling-Ongrowing fish farm monitoring) are used as the determinants of the carrying capacity.

MODELLER

BEVERIDGE MODEL

Various models can show us that how much fish can be grown by phosphorus entering the ecosystem from feeds and other sources and without deteriorating the environment in dam lakes carrying out intensive fish farming (Beveridge, 1984)

MOM

There is a need for systems where modeling and monitoring programs and environmental quality standards are existed together in order to prevent excessive use of the aquaculture area and to make the use of resources sustainable. The monitoring program consists of three types of research (A, B and C). A-research type is based on simple measurement of the sedimentation rate of organic material on the ground of cage companies; B-research type is performed in the local active zone, which includes three groups of parameters. In the C-research type, the focus is on the structure of benthic communities in the central and local active zones (Hansen et al. 2001). The parameters used include biological (macrofauna existence), chemical (pH, redox potential) and sensory (gas discharge, color, odor, viscosity, deposit thickness) parameters. Mom model is shown below according to Hansen et al. (2001).

DISCUSSION AND CONCLUSIONS

It is very important to calculate the carrying capacity in order to ensure sustainable aquaculture and reduce the pollution caused by the aquaculture. Knowing how much fish you can grow before starting inland water aquaculture is important to ensure making production here for many years, and also the capacity estimates for aquaculture should be made. In addition to existing models with carrying capacity of aquaculture, it is possible to develop new models and obtain more effective and wide-ranging parameters. Future studies should be designed so as to obtain instantaneously the past, current and future values of the carrying capacity of aquaculture.

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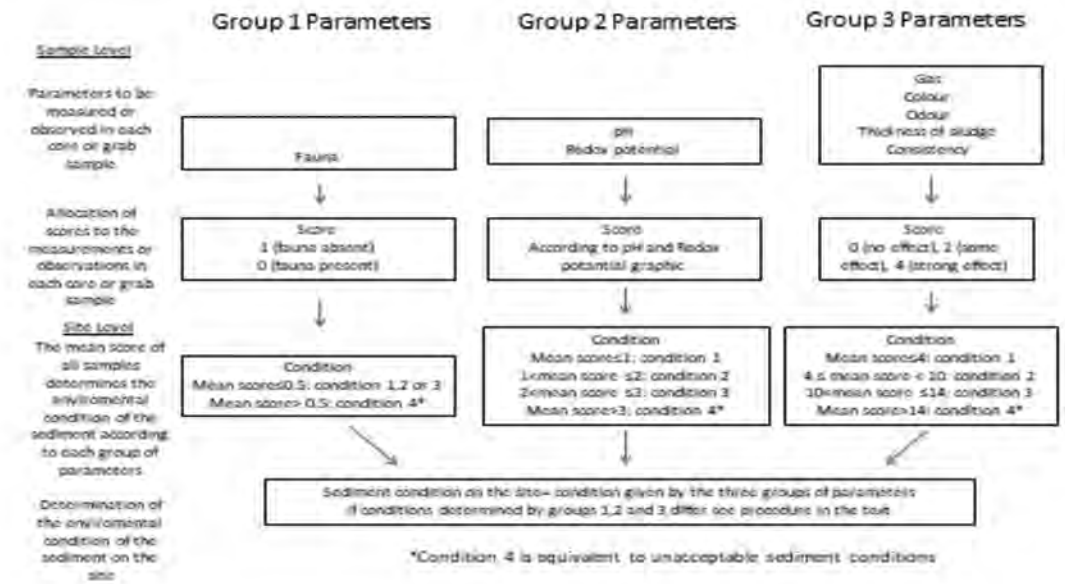
Values	Values	Units
FCR		
Phosphorus in feed		Kg P/tonnes feed
FCR* Phosphorus in feed		Kg P/tonnes fish
Phosphorus in fish		kg P/tonnes fish
Çevreye salınan fosfor		
P(enviroment)=P(feed)-P(fish)		kg P/tonnes production
Mean depth		Meter
Flushing rate		Annual
R(fish) (sediment in phosphorus)		
Area		m^2
Phosphorus level after production		mg/m^3
Phosphorus level before production		mg/m^3
Changes in phosphorus level		mg/m^3
Accumulation from fish		mg/m^2/year
= Delta P*Z*p/(1-R)		g/m^2/year
Accumulation from fish* area		g/year
Total allowable production		
[Accumulation from fish* area]/ releasing phosphorus to enviroment		tonnes/yeat



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DETERMINANTS OF FISHERY AND AQUACULTURE PRODUCTS CONSUMPTION AT HOME IN THE EU28

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Introduction
The European Union is the world's largest market for fishery and aquaculture products in nominal terms. Given the importance, the European Commission aims to balance distinct stakeholders in the Common Fisheries Policy. For this reason, a better understanding of the EU internal market is needed, and a survey of 27732 EU residents was carried out to analyse the fishery and aquaculture EU market (European Union, 2018). The data provide the base for the estimation of two ordered probit models and the estimation of the marginal effects for different consumption frequencies. The models aim to determine the main determinants of fishery and aquaculture products consumption at home for the EU residents. The results of the survey show that home is the most common place to consume this kind of products, with around 70% of people in the EU28 consume fishery and aquaculture products at home at least once a month (European Union, 2018). To our best knowledge, in the current literature, there is not a similar approach that considers such an extensive sample which is representative of the EU28. The results provide important insights for different stakeholders such as policy makers, nutritionists, fishery and aquaculture associations and marketing manage .

Materials and methods
The database used for the estimation of the models was obtained from the Special Eurobarometer 2018 (European Union, 2018), which includes questions that analyse the internal market for fishery and aquaculture products of the EU. For the present study, we focus on the responses for the frequency of consumption at home for fishery and aquaculture products (dependent variable) and correlate them with variables related to attitudes of respondents, socioeconomical and demographic factors (independent variables). Two ordered probit models were estimated, one of them including the standard deviations of the independent variables involved (heteroscedastic model). The marginal effects for the different consumption patterns at home were estimated for both models. The methodology was selected considering that the ordered probit models are an appropriate analytical framework when survey responses are ordinal (Kumar et al., 2008; Thong and Solgaard, 2017).

Results
Preliminary results indicate that the largest marginal effects on home consumption frequency depends on how clear and easy to understand is the information accompanying the fishery or aquaculture products. In fact, understanding clearly or to some extent that information would increase by 40% and 27% respectively the probability to consume products at least once a week.

Other important aspect that determine home fish consumption frequency is the attitudes regarding the main reasons to buy or eat fishery and aquaculture products. Particularly, it was found that considering important that they are healthy, taste good and less expensive than other food, would increase the probability to consume them at least once a week by 20%, 18% and 18% respectively; while other reasons such as being easy or quick to prepare, quick and easy to digest, would increase the probability by around 11% each one.

It was also found that consumers who have a clear preference for wild products, tend to consume the products more frequently. Similarly, older generation of residents, especially those older than 45 years are more eager to consume fishery and aquaculture products more frequently at home. On the other hand, the attitude toward the importance of the cost of the product was found to be not significant in general

Discussion and conclusions
The results provide valuable information for producers, retailers and authorities Particularly, they give important insights to establish common policies for the EU28 countries, by using a generous database that enhances the credibility of the results. Results indicate that there is a great possibility of increasing the consumption of fishery and aquaculture products at home in EU28 by just providing clearer and easier information to understand about the products, specially, keeping in mind, that around 25% of respondents from the Eurobarometer survey indicated that the information accompanying fishery

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and aquaculture products is not clear or easy to understand (European Union, 2018). This strategy is also supported by several studies in the literature, that conclude that giving quality information to consumers, enhances the product attributes (Bronnmann & Hoffmann, 2018; Kumar, 2018). Following this, additional studies are required to understand how the information can be provided in a clearer and easier way, and which is the information needed by consumers, in order to develop a common policy regarding the minimum requirements of the information accompanying the products, so in a way it can be standardized.

Regarding to the importance of certain attitudes than increase fish consumption frequency at home, producers need to focus on alternatives than enhance the healthiness and the taste of the products; while also provide products that are easy and quick to prepare, which are currently not as common in the market as for other food categories. Moreover, the fact that those who have a clear preference for wild products have a higher probability of frequency of consumption, is a clear sign to aquaculture producers and authorities, alerting them about the need to keep looking for strategies that offset the negative image that aquaculture products currently have. Similarly, given that younger people have a lower frequency of consumption at home in comparison to older people, is important to identify how through some marketing strategies such as product differentiation or online shopping, this can change.

Finally, it is important to notice that since the attitude towards the importance of the cost is not significant for fish consumption frequency at home, producers should risk on looking for higher quality products that might be more attractive to consumers despite their higher costs. However, prior to this, studies that evaluate the willingness to pay for these new products should be performed to understand their feasibility, since consumers who consider that one of the most important reasons to buy these products is that is less expensive than other food, tend to have a bigger consumption frequency.

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IDENTIFICATION OF EPITOPES TARGETED BY IMMUNE RESPONSE AGAINST SALMON PANCREAS DISEASE VIRUS IN NORWEGIAN SALMON AQUACULTURE

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Background Pancreatic Disease (PD) is one of the globally important viral pathogens in salmonid aquaculture. Sick fish have extensive pancreatic damage and inflammation of the heart and skeletal muscles, which can cause major financial losses due to poor growth and reduced slaughter quality. PD is caused by a virus called Salmonid alphavirus (SAV) or PD virus. In Norway, there are two of six known subtypes of SAV (SAV2 and SAV3) (Hjeltnes, Bang et al. 2018). Vaccination has already proven to be an important tool and the humoral immune response is a major factor helping fish to survive SAV infection (Mérour and Brémont 2014). Commercial vaccines against PD are available, while the efficacy of vaccine has been debated (Hjeltnes, Bang et al. 2018).

Object E2 protein is an envelope protein of SAV, which supposed to be the carrier of neutralizing epitopes and represents a promising target for subunit vaccine against SAV. In our previous study, we demonstrated humoral responses against E2 protein in farmed salmon post natural SAV infection (Cao, Stene et al. 2017). The aim of this study is to further identify linear B-cell epitopes recognized by these anti-E2 antibodies and map to their structural localization.

Method Atlantic salmon reared in sea cage culture at the north western part of Norway was used in this study. Twenty salmon plasma samples were identified as serologic positive against E2 protein (provide by Dr. Tøndervik, SINTEF Norway) and against SAV3 virus (provide by Dr. Aas-Eng, Pharmaq AS) (Cao, Stene et al. 2017). The overlapping peptides (10 mers with an offset of 3) from Mimotopes were designed for screening linear antibody epitopes using an enzyme-linked immunosorbent assay (ELISA) (Hammarlund, Lewis et al. 2005). A western blot (WB) assay was applied for the verification of specific binding. To identify the structure of E2 protein and epitope on the molecular, the in silico tools special for designing peptide-based subunit vaccines and immunotherapeutics against various pathogen-causing diseases were applied (Dhanda, Usmani et al. 2016).

Results The serologic positive plasma were examined for the fine specificity of the antibody responses. Several linear epitope recognized by the plasma antibody were identified and spontaneous responses most frequently recognized two specific E2 epitope, namely E2127-136 and E2214-223 , which is in accordance with the results calculated with the B cell epitope prediction program BepiPred-2 (Jespersen, Peters et al. 2017). Using BepiPre-2, we calculated the location of linear B-cell epitopes and demonstrated that the sequence DGTRH in E2127-136 and KSADSA in E2214-223 both have random coil-structure, which represent the potential epitope regions (Li, Liu et al. 2013). Furthermore, both sequence have relative surface accessibility as show in the structure model produce by SWISS-MODEL program (Waterhouse, Bertoni et al. 2018) (Figure 1)

Conclusion In this study, we identified and characterized two linear epitope motifs in SAV virus E2 protein. These results further our understanding of the epitope distribution of SAV E2, and provides critical knowledge that will aid in the understanding of SAV infection and immunity, vaccine design, and pre-clinical efficacy studies. Results from this study provide also information for the development of peptide-based immunodiagnostic tests to detect SAV in serum.

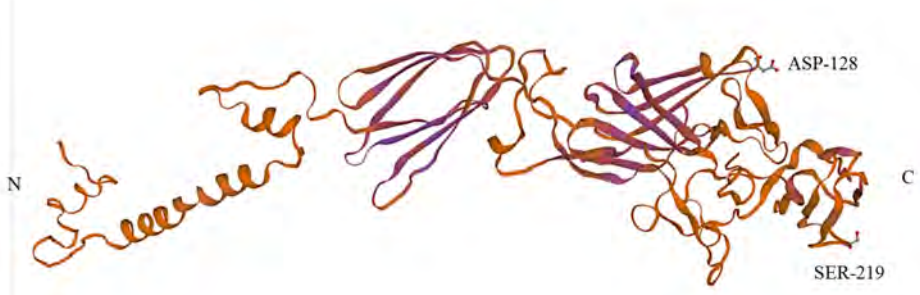

Fig. 1: SWISS-MODEL: homology modelling of E2 protein structures and complexes. ASP: Aspartic acid; SER: Serine

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RESTORATIVE AQUACULTURE WITH OFFSHORE STRUCTURES

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Introduction
Flexible floating reefs, pictured at right, support a new off-shore aquaculture ecosystem where the essential inputs are sunlight, and ammonia or nitrate, and phosphate. This new ecosystem supports a free-range fishery more sustainably and at lower cost than traditional ocean fish pens.

Existing wild fisheries production should increase due to increased habitat and food suppl .

OceanForesters assembled a team that received funding from the U.S. Department of Energy Advanced Research Projects Agency-Energy (ARPA-E) through the University of Southern Mississippi to design these aquaculture systems that could eventually produce inexpensive biofuels.

Nutrient Recycling
In ocean forestry, nutrient conversions, per the figure at right, combine with photosynthesis to add carbon to inorganic nutrients creating food for animals. As a result, the flexible artificial reef can have an optimally balanced suite of products including macro-algae, shellfish, finfish and other seafood. Operation using recycled inorganic nutrients and sunlight is much less expensive and more easily scaled than when using fishmeal.

Chambers, et al. (2014) observed that the macroalgae and shellfish absorbed more nutrients than the fish emitted, thus cleaning up excess nutrients in the Piscataqua River.

Economic Analysis
Because our natural-like ecosystem produces its own food for free-range fish, it eliminates the need for fishmeal to feed penned fish. A rough comparison of economics is that our expensive structure may cost $1000 per ton of seafood but it could save as much as $2000 for fishmeal per ton of fish.

We are also working with CleanCarbon Energy to develop a new hydrothermal liquefaction system that could eventually produce biocrude oil for as low as $70 per barrel, because income from our seafood sales offsets the cost of a structure designed to submerge to the seafloor to survive a Category 5 tropical storm

Application to Jamaica
Roughly 80 square kilometers of seafloor off Alligator Pond (west of Kingston) could ultimately support 1,000 hectares (fifty 20-ha reefs) of OceanForesters' hurricane-surviving floating flexible reefs. Restorative aquaculture of conch on fifty reefs could sustainably produce over 10,000 tons of conch per year.

The total of all production from the fifty reefs (conch, lobster, crab, oyster, sea cucumber, sea urchin, kingfish, jack, mackerel, whiting, bonito, and other free-range finfish may be as high as 150,000 tons per year. If the average export value is $2.5/kg, the total value of exports could be over $300 million per year.

High reef productivity can start with commercial fertilizers. Sustainable operation requires recycling inorganic nutrients from Jamaica's municipal wastewater. Pasteurized nutrients from people are brought to the reef for recycling. Recycling of people wastes also addresses other United Nations Sustainable Development Goals, creates more local jobs, and ensures local control and availability of the essential fish-growing nutrients. Ocean forests will also clean up excess ocean pollution from offshore runoff, thus improving water quality and increasing tourism.

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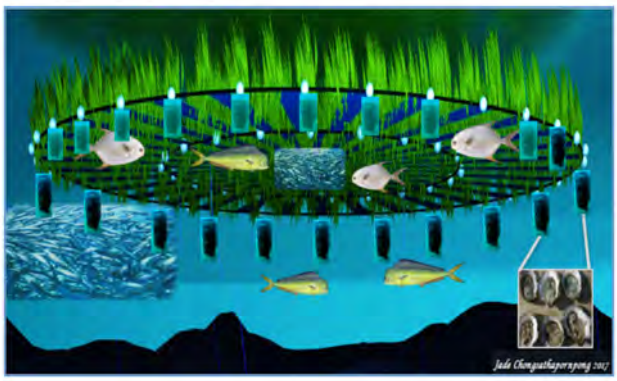

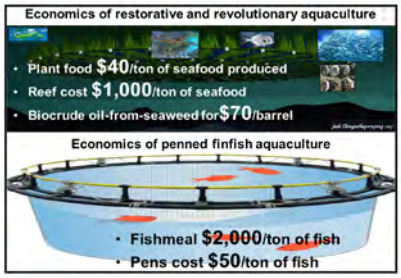

Benefits

Floating reefs, like natural ones, produce abundant shellfish and finfish put local fishers and seafood processors back to work, remediate ocean pollution, and increase ocean biodiversity through natural nutrient recycling. The system uses the natural recycling of nutrients (see diagram to the right) in which shellfish eat plankton and other organic nutrients while their wastes are food for the seaweed, which in turn provides food and habitat for finfish which are naturally attracted to the reef.

Benefits include: increasing food supplies, cleaning up dead zones, reducing ocean acidification, and eventually producing enough biofuels to replace all fossil fuels, while capturing sufficient C_2 to reverse climate change.

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CIRCULATING MICRORNA IN PLASMA AND OVARIAN FLUID AS POTENTIAL NON INVASIVE BIOMARKERS OF NUTRITION AND REPRODUCTION OF FEMALE RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

MicroRNAs (miRNAs) are small noncoding RNAs (~22 nt) that hold important post-transcriptional regulatory roles in most biological processes. The most striking characteristic of miRNAs is their high stability in extracellular spaces including biological fluids, which allows their utilization as noninvasive biomarkers. Several reports have highlighted the potential of circulating miRNA (c-miRNA) as predictive and diagnostic markers of biological process related to nutrition, reproduction or pathology (Turchinovich et al, 2012).

MicroRNAs have been less studied in fish than in mammalian species but knowledge in this area is continuously growing, especially in rainbow trout (Onchorynchus mykiss), which represents a key sector of aquaculture production. MiRNAs seem also to play important roles in the development and physiological processes in fish (Wienholds et al, 2005; Bizuayehu et al, 2012). A recent study reveals that miRNA are present in the blood of rainbow trout and are subjected to postprandial regulations in blood as in other tissues. As significant correlations were found between circulating miRNAs and expression of genes in liver, the authors suggested that circulating miRNAs could be good indicators of metabolic pathways in rainbow trout (Zhu et al, 2018).

In this context, the aim of the present study was to perform an extensive profiling of wide plasma and ovarian fluid miRNAs in female rainbow trout and assess the impact of moderate (i.e. 20%) feeding restriction, during oogenesis process, on c-miRNAs, in order to evaluate the potential of c-miRNAs to be used as non-invasive biomarkers of metabolic and physiological status of the fish during the reproduction cycle

Materials & methods

For that purpose, two feeding strategies were implemented during the last five months before the reproduction. Females were either fed (1) ad libitum or (2) 80% of the ad libitum (restriction). During the trial, two artificial photoperiods were applied to trigger reproduction during summer and plasma sampling was carried out at different times during oogenesis process. Ovarian fluid was sampled during spawning period.
To investigate the abundance of circulating miRNAs, small RNA libraries were generated and sequenced using Illumina small RNA deep sequencing. Validation by RT-qPCR was carried out.

Results & discussion

More than 500 microRNAs were successfully detected in the plasma and ovarian fluid of female rainbow trout. Some miRNAs were exclusively detected in the ovarian fluid whereas others were only found in the plasma. Circulating miRNAs only found in ovarian fluid showed significant different abundance according to feeding level (ex: mir 202, mir2928…). Inversely, some c-miRNAs specific of the plasma have seen their abundance significantly changed during the oogenesis or depending of feeding level (ex: miR 206, miR122, miR10…).

Conclusion

To conclude, the present study demonstrates for the first time that trout plasma and ovarian fluid contain large amounts of miRNAs. Some of them are significantly affected by oogenesis stage and/or feeding rate suggesting that c-miRNAs could be considered as promising non-invasive biomarkers of nutrition and reproduction for further application in the research or aquaculture areas.

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IMPACT OF BOTTOM SUBSTRATE TYPE ON THE RESUSPENSION OF ATLANTIC SALMON (*Salmo salar*) FAECAL MATERIAL

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Introduction

Faecal material discharged from open ocean salmon fish farms generates a direct impact to the bottom dwelling communities, modifying both population structure and diversity via organic enrichment. Therefore, accurate modelling of such particles is critical for the planning and licensing of fish farming locations in Norway. To attain such accuracy, particle transport models should include relevant small-scale processes that influence particle movement, such as particle resuspension and re-entrainment into the water column. Approaches to simulate such phenomena tend to overlook the role of bottom substrate on the onset of particle resuspension, assuming the same value of critical shear stress to be applicable for all bottom types even when there is evidence against it in the body of knowledge (e.g. Law et al. 2016).

We performed controlled lab experiments using tubular flow raceways to determine near-bed flow conditions necessary to resuspend fish faeces over the most common bottom types in existing aquaculture locations in Norway. These novel parameters will help to expand existing modelling tools for the Norwegian salmon fish farming industr .

Materials and methods

Faecal pellets were collected from a research pen holding near 2 kg individuals of Atlantic Salmon in Masfjorden (Norway). A total of 42 experiments were performed, with faecal pellets being placed in a tubular raceway equipped with a propeller to induce step-wise increases in mean-flow velocity and a bee-hive panel to generate quasi-homogeneous turbulence in the system. Different bottom substrates were placed in the flume according to a randomized experimental design. A Nortek Vectrino ADV was used to record 3D velocity components near the bed while two (2) downward looking GoPro Hero 5 cameras were used to record the experiments. Mean flow conditions for faeces resuspension were extracted from the synchronized videos and velocity records, while a second moment statistic method based on the turbulent kinetic energy was used to derive bottom shear stresses and shear velocities. Influence of substrate and the time the faecal material was let to sit in the flume (particle age) was analysed using parametric ANOVA.

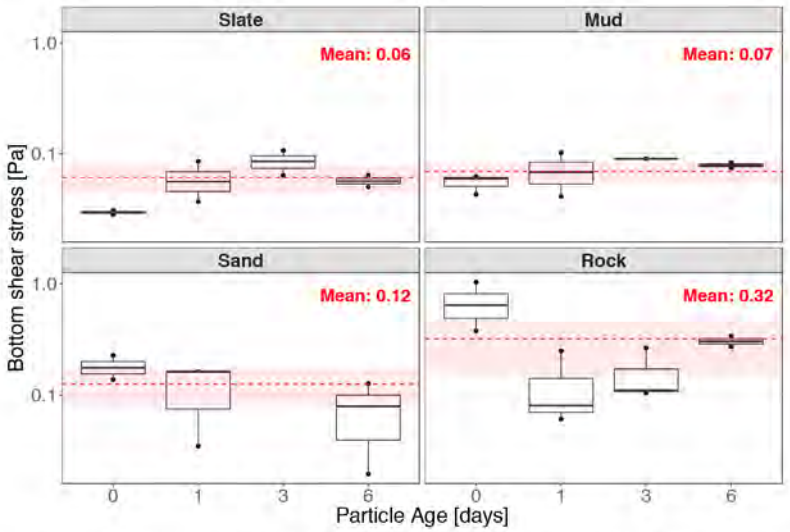

Fig. 1. Critical shear stresses required to resuspend Atlantic Salmon faecal pellets over different bottom substrates

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Results

Tests indicated there is a significant effect of the substrate type on the shear stress required to onset the resuspension of faecal material once it has been deposited on the sea bottom (Fig. 1). Faeces sitting on smooth regular surfaces such as muds or rock slates were resuspended when exposed to fairly low bottom shear stresses, in the order of 0.06-0.07 Pa. Slightly higher values of shear stress for the muddy substrates can be associated to the build-up of bedforms between sequential tests and interactions between the cohesive material and the organic particle.

Particles laying on sandy bottoms required a higher level of shear in order to be resuspended from the bottom when compared to the smooth substrates group. Post-Hoc analyses showed no significant difference between sandy substrates and smooth surfaces, but a strong difference between the aforementioned groups and exclusively rocky substrates (i.e. pebbles, boulders) can be observed. Particles sitting on rocky substrates, which are typical for some fiord systems in the Norwegian coast, are mobilized until they fall into fractures and gaps between rocks, once trapped they need to be exposed to very high shear to leave the cracks and be re-entrained into the bulk of the flo .

Discussion and conclusions

Our results indicate there is a clear effect of substrate type on the bottom shear stress required to resuspend faecal material from salmon holding aquaculture pens once they have reached the seafloo . These results validate previous evidence by Law et. al (2016) who observed differential shear requisites according to surface type using a Gust Microcosm Erosion Chamber, but who still required further testing in larger scale flumes which could reproduce field conditions more accurately. As a natural continuation of this work, the differential critical shear stresses obtained will be implemented in a particle tracking model and compared to the constant-value approach introduced by Cromey et al. (2002) and currently used in most fish farm particle resuspension modules. By validating these upcoming results against available field data in different fish farm locations in Norway, we will be able to assess the required level of model complexity for future aquaculture-related particle dispersion studies.

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THE COMBINATION OF POULTRY AND ALGAE OILS AS REPLACERS OF FISH OIL IN DIETS FOR GILTHEAD SEA BREAM (*Sparus aurata*) FINGERLINGS

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Introduction
Sustainability of marine aquaculture is nowadays dependent on finding alternative cost-effective lipid sources that can replace fish oil. Potential alternatives should provide high energy lipid but also meet fish n-3 LC-PUFA requirements. While poultry oil (PO) is considered a good non-protein energy source, some algae oils, particularly from microalgae, can provide high n-3 LC-PUFA contents. However, algae oils are frequently expensive and with limited availability. Therefore, the objective of this study was to evaluate the combination of PO with two different microalgae oils as replacers of FO in diets for gilthead seabream (*Sparus aurata*) fingerlings, under two different dietary fish meal (FM) levels and a cost-effective approach.

Materials and methods
Seven isoproteic and isolipid experimental diets (50% CP and 17.5 % CL) were formulated under two different fish meal (FM) contents (15 and 7.5%). A positive control was formulated with 15% FM and 5% FO. For each dietary FM content, FO was replaced by a combination of poultry oil and one of two microalgae oils that were named as algae oil (POALG) or DHA oil (PODHA). A negative control replacing FO by exclusively PO was also formulated for each dietary FM content. Experimental diets were assigned to triplicate groups of gilthead fingerlings (IBW 2.5±0.01g) allocated in 250 l tanks, at a stock density of 0.55 kg m^{-3}. Fish were fed manually 3 times a day, 6 days per week for 74 days. Temperature along the trial was 23.1±0.3ºC and dissolved oxygen was maintained above 6.0 mg l^{-1}. Fish were individually weighed and measured at day 1, 30, 45, 60 and 74 of the feeding trial. Besides, at the end of the trial, whole-body samples were collected for biochemical analysis. A one-way ANOVA was applied to analyze differences ($p<0.05$) in the different lipid sources with the same dietary FM content, while t-Student test was applied to detect differences ($p<0.05$) between each lipid source but with different % FM.

Results
Results showed that fish fed POALG and PODHA presented similar growth performance than fish fed FO, but better growth than those fed on only PO, for both dietary FM contents. Besides, fish fed 7.5% FM diets presented lower growth than fish fed their homologous 15% FM diet, except for fish fed PO whose growth was similar regardless the content of FM in the diet. Furthermore, fish fed the most extreme diet (7.5% FM and PO) presented the highest mortality during the trial. Fish fed 15% FM and POALG showed similar FCR and LER than fish fed FO, but higher than fish fed PODHA or PO. Besides, whole-body fatty acid composition showed that fish fed POALG or PODHA showed EPA+DHA contents than fish fed PO. Contrary to growth performance, whole-body FA was not influenced by the dietary content of FM, with fish fed either 15 or 7.5% presenting similar FA composition.

Discussion and conclusions
The results of the present study indicate that both combinations of PO with the two microalgae oils tested were efficient total replacers of FO at both dietary FM contents. However, the substitution levels used were insufficient to eliminate the effect of the decrease from 15% to 7.5% FM in the POALG and PODHA diets on fingerlings growth and feed utilization, even when fatty acid composition was similar between the diets and fish composition after the feeding period. These results suggest that some other factors independently from dietary fatty acid composition could be affecting growth performance and feed utilization of fish fed 7.5% FM diets.

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IMPAQT – AN INTELLEGIENT MANAGEMENT SYSTEM FOR INTEGRATED MULTI-TROPHIC AQUACULTURE

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Introduction

European aquaculture is met with many challenges in the face of increasing demand for its products. Global estimates forecast that aquaculture will supply over 60% of the fish intended for direct human consumption by 2030, beyond this aquaculture will dominate global fish supply (World Bank, 2013). With fish stocks approaching maximum sustainable yields, aquaculture production must increase in order to meet the demands for the 70% more protein required by the predicted world population by 2050 (Anon. 2017). In Europe the demand supply gap for fish derived protein continues to increase. The EU is the world's largest importer of fisheries and aquaculture products, importing 70 % of the EU consumption (EUMOFA, 2018). Aquaculture is one of the pillars of the EU's Blue Growth strategy and its development can contribute to the Europe 2020 strategy (Anon, 2013). The FAO's Blue Growth Initiative, also focuses on innovation, integration and the adoption of a multi-sectoral approach, to maximize ecosystem services while providing social and economic benefits.

Integrated Multi Trophic Aquaculture (IMTA), is acknowledged as a promising solution for sustainable development of aquaculture. The concept of IMTA is to farm species of different trophic levels, complementary to each other, so that the wastes and by-products of one species become the feed, fertiliser and energy source for another. As yet, IMTA is not widely adapted at a commercial level. It has been only tested at a very small scale in Europe and the management of large-scale areas remains challenging. Culture of extractive species with fed species in the same aquaculture sites is encouraged, and this practice is shown to remove waste materials from fed species and lower the nutrient load in the water (FAO,2018).

Methodology

IMPAQT aims to promote the eco-intensification of aquaculture by demonstrating the eco-efficiency and minimization of environmental impacts, enabling socio-economic benefits and ecosystem services, and promoting the transition towards a circular economy business model. IMPAQT will develop and deploy novel sensors and data sources, together with smart systems required for long-term autonomous monitoring in the field. IMPAQT also aims to provide an advanced IMTA model for users, which yields spatially explicit information on how the different farm components interact with the environment on the scale of an ecosystem and that can be used for planning decisions by both farmers and regulators.

Results

This integrated management system, operating at the scale of an IMTA farm and comprising analysis and decision support functionalities, will be enable enhanced operational decisions for animal welfare, production optimization, environmental protection and food-quality assessment.

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PROBIOTIC *P. acidilactici* MA18/5M ENHANCES SHRIMP GROWTH AND ANTIOXYDANT DEFENCES: BENEFICIAL EFFECTS AND POSSIBLE PHYSIOLOGICAL MECHANISMS

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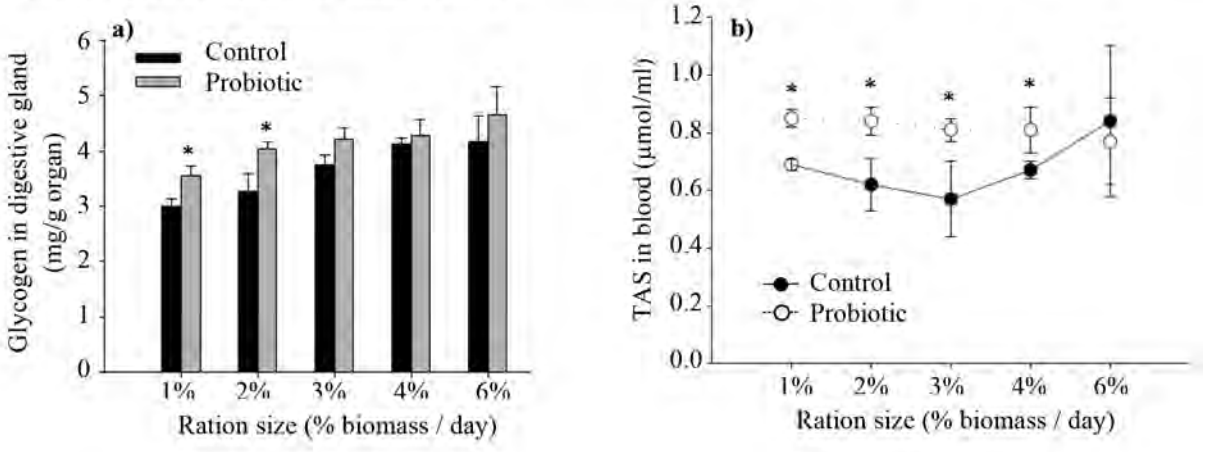
High metabolic activity, increased environmental stress and infectious challenges are few conditions known to increase the intracellular production of reactive oxygen species (ROS) hence the need for higher antioxidant defense to protect cellular and animal integrity. Accordingly, the beneficial effects of in-feed antioxidants to sustain animal health and performance start to be recognized across the livestock industry. There is less notoriety about the potential link between (dietary) carbohydrates metabolism and the antioxidant system, particularly as a mechanism by which probiotics can positively influence the animal s oxidative status.

A controlled feed-intake trial documented the positive influence of the well know probiotic *P. acidilactici* MA18/5M on the growth, carbohydrate digestibility, glycemia and antioxidant status of peneid shrimp (**Fig**). The positive effect of probiotic on the shrimp antioxidant status was further confirmed under semi-commercial conditions as well as when exposed to a controlled vibriosis challenge where enhanced survival was also documented. As supported by knowledge in other species, it is hypothesized that enhanced carbohydrate utilization as a result of *P. acidilactici* would decrease cellular oxidative stress level via the direct ability of glucose to scavenge OH-radicals or by fueling the pentose-phosphate pathway generating NADPH. NADPH is indeed a key player against oxidative stress and free radicals propagation in particular.

Together, this shades light on a scarcely explored mechanisms by which the probiotic *P. acidilactici* MA18/5M can enhance shrimp health status and growth via its nutritional benefits.

Fig: Positive effect of *P. acidilactici* MA18/5M on **a)** carbohydrate utilization and **b)** blood Total Antioxidant Status (TAS)



SEA URCHIN RECRUITMENT: THE EFFECT OF DIATOM BASED BIOFILMS ON *Paracentrotus lividus* COMPETENT LARVAE

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Introduction

In Europe, sea urchin *Paracentrotus lividus* aquaculture still faces certain constraints which have to be solved for a sustainable and cost-effective industry. Settlement, metamorphosis and post-metamorphosis survival rates are still not high enough to produce juveniles in hatcheries at an industrial-scale (Carbonara et al., 2018). It seems that the most successful metamorphosis-inducing signals are microbial biofilms (Hadfield and Paul, 2001). Recent studies have shown the metamorphosis induction effect of benthic diatoms on *P. lividus* (Rial et al., 2018; Zupo et al., 2018) and the potential to regulate developmental transition in sea urchins of neurotransmitters as histamine (Swanson et al., 2012) and Gamma-Aminobutyric Acid (GABA) (Rahman and Uehara, 2001). The main objective of the present study was to compare the effect of these different inducing cues on the metamorphosis of *P. lividus* and to identify those that could be of easy and cheap application.

Materials and Methods

Sea urchins competent larvae were exposed to the following treatments: *Nitzschia laevis* biofilm (NL), *Halamphora coffeaeformis* biofilm (HC), Mix biofilm of both *N. laevis* and *H. coffeaeformis* (MIX), Natural biofilm sampled from refining oyster ponds (NATURAL), Broken oyster shells 10 g (SHELL), Histamine 10^{-6} M (HIS), GABA 10^{-3} M (GABA), GABA 10^{-3} M + Mix biofilm of both *N. laevis* and *H. coffeaeformis* (GABA+MIX) and a negative control of filtered seawater (FSW). All experimental treatments were carried out in four 80 mm diameter Pyrex® Crystallizing Dishes filled with 100ml of filtered seawate .

Every 24h metamorphosis rate was recorded in each treatment. The first four treatments providing a metamorphosis rate of more than 90% were transferred into tanks filled with aerated filtered seawater in order to assess survival of early juveniles. At 10 days post-metamorphosis (DPM), survival was assessed by counting living juveniles on each treatment.

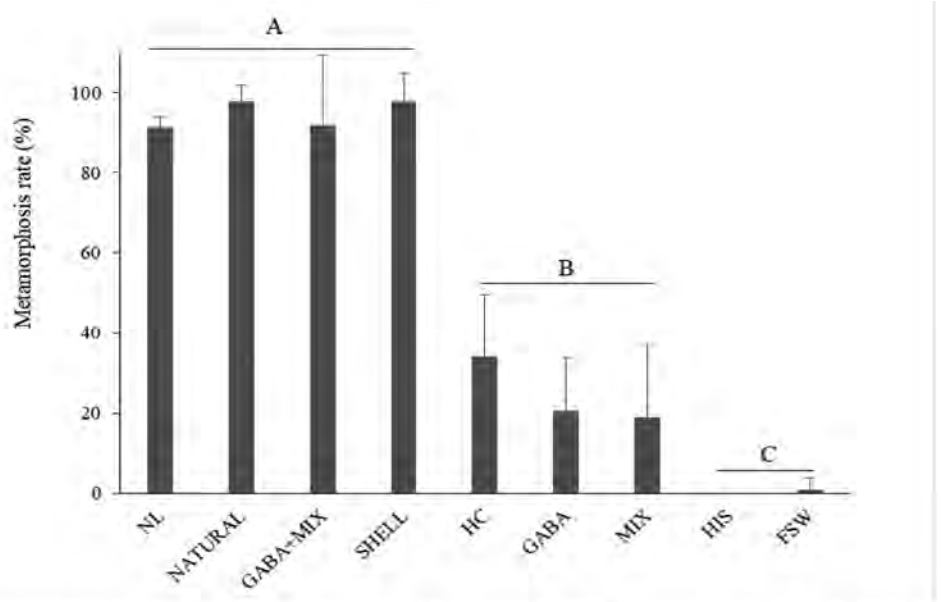

Figure 1 *Metamorphosis rate (%) of P. lividus larvae after 72h exposure to the different treatments. NL = N. laevis, NATURAL = natural biofilm, GABA+MIX = GABA+N. laevis+H. coffeaeformis, SHELL = broken oyster shells, HC = H. coffeaeformis, MIX = N. laevis+H. coffeaeformis, HIS = histamine, FSW = filtered seawater. Data are expressed as mean ± sd.*

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Statistical analysis of results were carried out using SigmaStat® 9.0 software. One-way ANOVA tests or Kruskal-Wallis tests were realized. Student-Newman-Keuls *a posteriori* multiple comparisons tests were carried out when significant differences ($P < 0.05$) were observed between treatments.

Results

After 72h of experiment, we can divide the treatments in three groups according to their effects on metamorphosis rate: group A - NL, NATURAL, GABA+MIX and SHELL, group B – HC, GABA and MIX, and group C - HIS and FSW. The sea urchins larvae in group A showed significantly higher metamorphosis rates than in groups B and C ($P < 0.001$). Significant differences were also found between larvae in groups B and C ($P < 0.05$). Between treatments in the same group there was no statistical difference (Figure 1). Sea urchins of the group A were those transferred to the tanks for survival assessment. Survival 10 DPM was higher than 60% for the four treatments. No difference was found between them.

Discussion and Conclusion

In this study we demonstrated that the transition from planktonic larvae to benthic juvenile could be promoted through diatom-based biofilms. As no difference was found between survivals in the NL, NATURAL, SHELL and GABA+MIX treatments, these represent efficient metamorphosis inducers for *P. lividus* larviculture. However, in order to promote practical and easy solutions for farmers, this study suggests to use preferably *N. laevis* biofilms or the treatments coming from the oyster ponds (i.e. natural biofilms or oyster shells). Monospecific diatom biofilms, as *N. laevis,* provide advantages for the industry: their growing is simple all around the year and they allow an easier quality control in terms of growth rates and nutritional profile in an industrial production cycle. In another way, using natural biofilms and oyster shells from the oyster ponds could represent a practical and sustainable source of metamorphosis inducing cue for oyster farmers.

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A cINTEGRATED MULTI-TROPHIC AQUACULTURE ASSAY: BIOREMEDIATION POTENTIAL OF *Palmaria palmata* ASSOCIATED TO REARED OYSTERS AND SEA URCHINS

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Introduction

Integrated Multi-Trophic Aquaculture (IMTA) still faces considerable controversy over its advantages in the ecological, social and economic levels (Troell et al., 2009)”container-title”:”Aquaculture”,”page”:”1-9”,”volume”:”297”,”issue”:”1”, ”source”:”ScienceDirect”,”abstract”:”The marine aquaculture sector is growing rapidly. Offshore aquaculture installations have been drawing increasing attention from researchers, industry and policy makers as a promising opportunity for large-scale expansion of the aquaculture industry. Simultaneously, there has also been increased interest in both land-based and nearshore aquaculture systems which combine fed aquaculture species (e.g. finfish This study is concerned by the question of the sustainable aquaculture technical feasibility and searches to provide an alternative source of seaweeds to sea-urchin farmers in order to promote a French oyster aquaculture diversification.

The specific objective of the experiment is to determine *Palmaria palmata* growth rate and yield when cultivated in an IMTA system with effluents from oysters *Crassostrea gigas* and sea urchins *Paracentrotus lividus*. This assay is still in course.

Materials and Methods

Seven to ten cm long thalli of *P. palmata* are maintained by vegetative propagation in air-agitated tumble in triplicate tanks and stocked to a total biomass of 2g/L (Chow et al., 2001). Algae tanks are submerged in a thermoregulation pond in order to maintain temperature at 13°C. They are exposed to 120 µmol.photons m^{-2} s^{-1}. Growth of algae is being measured every week (weight and surface gain) and expressed in yield (g DW m^{-2} d^{-1}) according DeBoer and Ryther (1977) and in specific growth rate (% d^{-1}) according D’Elia and DeBoer (1978)and showed transient rates of NH4+ accumulation which did not greatly exceed the capacity to incorporate N in steady-state growth. NH4+ was preferred over NO3−even in plants preconditioned on NO3−as the sole N. source, NO3− uptake was suppressed at 5µM [NH4+], but simultaneous uptake occurred at unsurpressed rates at lower concentrations. Potential for N accumulation was greater via NH4+uptake than via NO3−uptake. Changing capacity for NH4+ uptake with N content appears to be a mechanism whereby excessive

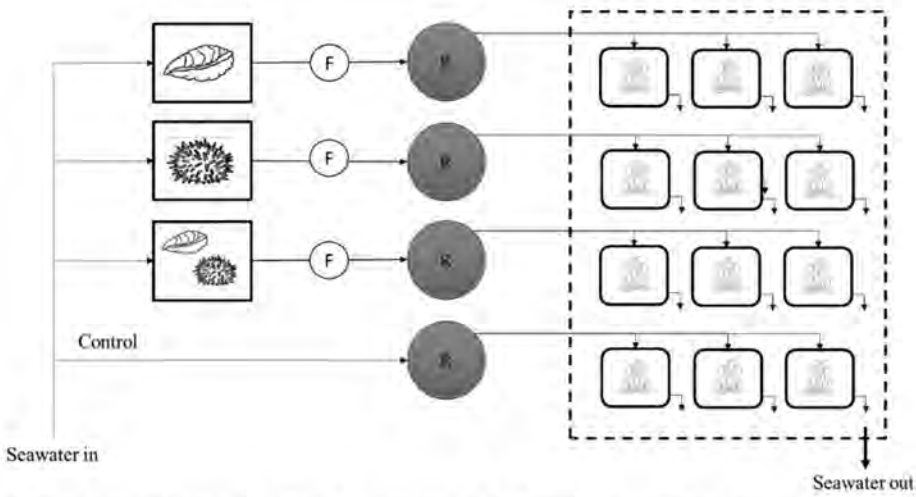

Figure 1 Experimental design. F = filter; R = reserve tanks. Dropped lines design the thermoregulation pond.

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accumulation of N was avoided by N-.satiated plants but a large accumulation was possible for N-depleted plants.”,” DOI”:”10.1111/j.1529-8817.1978.tb00297.x”,”ISSN”:”1529-8817”,”language”:”en”,”author”:[{“family”:”D’Elia”,”given”:”Christopher F.”},{“family”:”DeBoer”,”given”:”James A.”}],”issued”:{“date-parts”:[[“1978”,9,1]]}}],”schema”:”https://github.com/citation-style-language/schema/raw/master/csl-citation.json”} . Nitrogen uptake efficiency (NUE) and nitrogen uptake rate (NUR) are also measured weekly. Excess algae resulting from growth is removed every week and initial density restarted.

Oyster culture contain individuals at a density of 43 kg/m^3 in tanks filled with filtered sea water (FSW). They are fed with 2 x 10^9 to 3 x 10^9 cells per day of cultures of *Dunaliella tertiolecta* or *Skeletonema* sp. The sea urchin culture contain individuals at a density of 23 kg/m^3 in tanks filled with FSW and they are fed *ad libitum* with *P. palmata* or *Ulva* sp. collected from natural populations. Linear and specific growth rates of the animals are assessed every week.

Oysters, sea urchins and algae are reared in an open through-flow system (Figure 1). Sea water effluent from animals is filtered, accumulated in reserve tanks and passed at a constant flow (30% per day) on to the tanks with algae. Additionally triplicate algae control tanks without animal effluent are used in order to study the growth of algae without providing nutrients.

Results

First results show a macroalgae mean growth of 30% and a mean NUE of 95%. However, several problems have induced a high variability within results and a bias that must be taken into account: a development of microalgae and epiphytes in the algae tanks and a cross contamination between them. Moreover, four weeks after the start of the experiment, a high mortality rate (20-40%) has been observed in the sea urchin culture due to a low water exchange rate. For these raisons, some adjustments have been made on the experimental design in order to reinitialize the acquisition of data. This assay is still in course.

Discussion and Conclusion

Land-based IMTA systems have already been demonstrated to be technically feasible (Troell et al., 2009). However, in Europe, most of the IMTA systems are based on fish farms with fishes as main link. Only few studies based IMTAs on invertebrates, seaweeds or microalgae (Jacquemin et al., 2018). Due to an anecdotal fish production in our region (non-traditional activity, poor image of farmed fish by consumers), the present work has proposed an innovative study of an IMTA focused on fed invertebrates and seaweeds as extractive species. It seems that ammonium-nitrogen concentrations produced by the invertebrates are high enough to allowing macroalgae growth. However, higher water exchange rates have to be tested in order to improve sea urchin survival without compromising the ammonium-nitrogen concentration available for algae.

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USE OF SPIRULINA *Arthrospira platensis* FOR DAIRY BYPRODUCTS TREATMENT: GROWTH AND QUALITY TRAITS

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Introduction

Some byproducts of the dairy industry, such as exhausted whey and buttermilk, represent a problem both from an economic point of view, as the dairies have important disposal costs, and from an environmental point of view, due to their high microbial and COD content. The traditional treatment technologies are particularly expensive and of hard management. In this respect a possible solution may be given by microalgae, as a sustainable biotechnology able to assimilate the nutrients present in the dairy byproducts, which permits not only to reduce the COD load in the byproducts, but also to obtain biomass that can be valorized in various sectors of commercial interest (nutraceuticals, pharmaceuticals, cosmetics, food and feed supplements, bioplastics and phytobiostimulants).

Materials and methods

A cyanobacterial strain of *Arthrospira platensis* (21.99), purchased from the Culture Collection of Algae at Göttingen University (Germany), was grown in semi-batch cultures in 100-liter column photobioreactors. The culture broth was composed in exhausted whey (EW) diluted with Zarrouk medium (Zarrouk, 1966), commonly used as standard medium (SM) for *A. platensis*, in ratio 1:4. The exhausted whey came from a big dairy factory located in the Po Valley (Italy). Simultaneously a control trial in which *A. platensis* was cultured on SM as such without addition of any byproduct was carried out for comparison. The trials, conducted in duplicate, lasted 25 days in controlled conditions, at temperatures of about 25-27° C, a light intensity of 30 $\mu E\ m^{-2}\ s^{-1}$ and with a Hydraulic retention time (HRT) of 12 days.

The parameters measured were dry biomass, total nitrogen (N_{tot}), nitric nitrogen ($N\text{-}NO_3$), orthophosphate-phosphorus ($P\text{-}PO_4^{3-}$) and Chemical oxygen demand (COD).

A. platensis biomass was regularly harvested, freeze-dried, analyzed microbiologically and characterized for macronutrient and fatty acid composition.

Results

The nitrogen removal with *A. platensis* grown on medium supplemented with EW was absolutely efficacious, as $N\text{-}NO_3^-$ concentration was almost completely uptaken after few days, also thanks to the presence of a relevant microbial community that competed with *A. platensis* for the consumption of nitrogen. Nevertheless, inorganic nitrogen for growth of *A. platensis* was always available, as the microorganisms themselves were able to convert the fraction of organic nitrogen present in the whey into $N\text{-}NO_3^-$, resulting in a highest N_{tot} removal of 84%. This phenomenon also permitted a COD reduction of 96%. With regard to harvested biomass, the average value obtained was 0.85 g/l dw, compared with control that reached an average value of 0.67 g/l dw. The macronutrient composition of the dried *A. platensis* biomass, control and test, is reported in Tab I.

The fatty acid analysis showed a high difference in n-3 composition between control (traces) and test (29.72 g/100 g dw) ($p>0.05$). Microbiological analysis showed no difference in TBC (Total bacteria Count) between test and control (average: 5 ± 0.07 log CFU/g). Enterobacteriacea and *E. coli* were found below the detection limits of the method (< 2 log CFU/g). *Salmonella* and Sulfite-reducin *Clostridia* spores resulted absent.

Tab I: macronutrient composition.

	Water (%)	Protein (%)	Lipid (%)	Carbohydrate (%)	Ash (%)
A. platensis EW	11.30	35.91	2.59	5	34.02
A. platensis CW	8.05	47.43	5.26	9.7	24.80

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Discussion and conclusion

A. platensis proved to grow slightly better in experimental medium than in the control, thanks to presence of microbial community naturally present in the whey and with which it integrated in a symbiotic equilibrium: Cyanobacteria (as well as other microalgal strains) produce through photosynthesis oxygen, that can be exploited by microbes that provide CO_2 and nutrients that improve photoautotrophic growth of microalgae (Subashchandrabose et al. 2011). The most important result of this work is the reduction of COD in the mixture whey-SM by over 90%, until values that are compatible with the law-limits concerning emissions of wastewater into the sewerage system (0.5 g O_2/l). The microbiological analysis and biochemical composition show that the microalgal biomass grown on exhausted whey has excellent nutritional values, potentially useful as feed in aquaculture. Further studies should be carried on, in other types of PBRs, in order to verify the feasibility and reliability of these systems in producing *A. platensis*.

Acknowledgements

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THE EFFECTS OF DIETARY CREATINE ON MUSCLE QUALITY AND ALLERGEN PARVALBUMIN MODULATION OF *Dicentrarchus labrax*

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Introduction

Food allergies represent a serious public health problem worldwide and fish, being one of the highly consumed foods worldwide, represents a group that limits more people in their food choices, essentially among the young population. Parvalbumin, which is a stable white muscle protein involved in the processes of contraction and relaxation of fish muscle, have been identified as the main responsible proteins of allergic reactions by the consume of fish, including farmed fish. Creatine is used parallelly as a molecule to enhance bioenergetics by increasing efficiency of Ca2+ uptake and to modulate the expression of parvalbumin. Therefore, the supplementation of fish diets with creatine aimed at reducing the expression of fish allergenicity factors and increase flesh quality, is of higher interest and beneficial in terms of food safety and human health. The present study was conducted to produce a low allergenic fish species using enriched diets diets for Atlantic seabass (*Dicentrarchus labrax*) supplemented with different percentages of creatine (0%, 2%, 5% and 8%). Its effects on muscle quality, physiological stress, proteome and parvalbumin modulation were analyzed.

Methodology

Triplicate groups of 20 seabass (initial body weight: 186,17 ± 0.83g) were randomly distributed in 500-L tanks under standard rearing conditions. Fish were hand-fed ad libitum twice a day during 91 days, with four agglomerated diets formulated at different concentrations regarding creatine concentration (CTRL; Crea2%; Crea5%; Crea8%). Fish were then euthanized and sampled for i) growth performance evaluation, ii) nutritional status by body composition analysis and iii) stress levels. Comparative proteomics was used to look into changes in muscle tissues at protein level and to assess the effects of fish dietary creatine on alle gen parvalbumin modulation.

Results

After 12 weeks of trial, growth performance was similar between diets. Hepatic visceral indicators, body proximate composition and metabolites fingerprinting were not affected by the creatine dietary levels. Nevertheless, higher cortisol levels were found in fish fed with higher dietary creatine concentrations. Muscle proteome is differently expressed in fish fed with 2% dietary creatine.

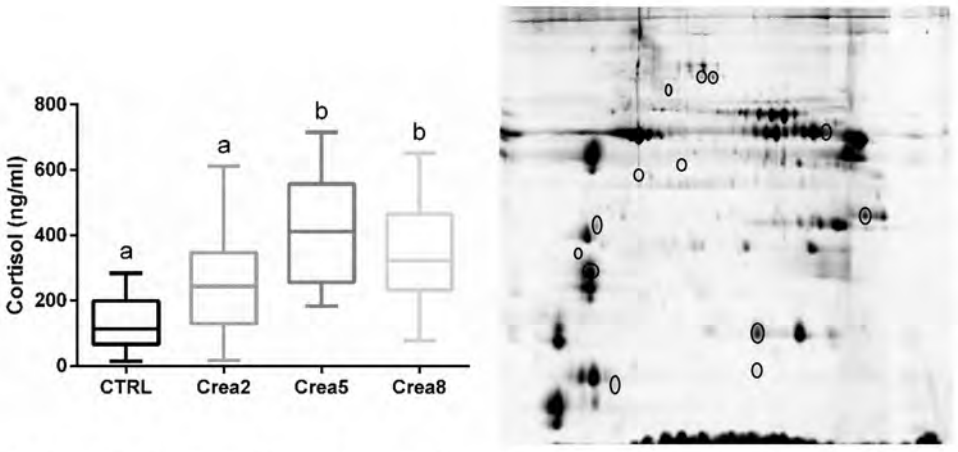

Fig. 1. Left) Fig. 1. Cortisol (nM) concentration in plasma of *D. labrax*. Values are means (n=18) and error bars represent standard error of the mean (SEM). Different letters represent significant differences (one-way ANOVA followed by post-Hoc Tukey, p<0.05); Right) 2D gel of *D. labrax* muscle on a 12.5% polyacrylamide gel and a pI range from 3-7.

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Discussion and Conclusion

Our findings suggest that the dietary supplementation of creatine do not constitute a valuable nutritional approach towards either productivity parameters or lowering allergenicity of fish, but could, however, impact its quality. Nevertheless, proteomic technology demonstrates to be valuable for the dietary nutrients/compounds standardization limits, which don´t disturb the biological processes in fish. Such findings can impact fish safety and the aquafeeds industry, to making aquaculture more resilient and competitive.

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IN VITRO PROTEIN HYDROLYSIS OF NOVEL AQUAFEEDS INGREDIENTS BY THE DIGESTIVE ENZYMES OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Interest in identifying novel sustainable ingredients in aquafeeds such as insect and microalgae biomass meals increased in recent years. A number of experiments have evaluated their potential as dietary protein sources in the diet of several fish species, however, to our knowledge, little research has been carried out on the way digestive enzymes of fish hydrolyze their protein and contribute to their protein digestibility (Tibbetts *et* al. 2017, Vizcaíno et al., 2019). Digestibility of proteins is obviously a basic factor to establish the nutritional value of a protein-rich ingredient in particular for carnivorous fish species. Nevertheless, determination of *in vivo* digestibility requires long time frames, specialized facilities, high labor costs and ethical control. In this regards, *in vitro* assays can be used as preliminary alternative tests in order to evaluate the quality of protein-rich ingredients for aquafeeds, based on the extent of protein degradation and the amount of free amino acids released by proteolytic enzymes extracts obtained from a given fish species (izcaíno *et al*., 2019).

The present study was aimed at evaluating the *in vitro* degradability of the protein fraction of novel ingredients such as microalgae dried biomass (*Tetraselmis suecica, Arthrospira platensis*) and insect meals (*Hermetia illucens, Tenebrio molitor*) compared to that of a fish meal as a reference, using rainbow trout enzyme extracts

Materials and methods

The reference fish meal as well as all test ingredients were obtained from commercial farms. Crude digestive enzyme extracts were obtained from fifteen rainbow trout (1,0 kg average body weight) reared at the facilities of the University of Udine and fed for one month a commercial diet not including the novel ingredients. Fish were euthanized 6 h after the last meal using an overdose of anesthetic according to EU Directive 2010/63/EU. Total alkaline protease activity was determined using 5g l^{-1} casein in 50 mM trisHCl buffer (pH 9.0) as substrate (Alarcón *et al.*, 1998). The *in vitro* protein hydrolysis of the different ingredients was carried out according to the methods described by Alarcón *et al.* (2001) using five jacketed reaction vessels connected to a circulating water bath in order to maintain a constant temperature (37°C) and continuous agitation. For each assay, a known amount of each ingredient, providing 160mg crude protein, was suspended in 50mM Tris HCl buffer pH 9.0. After 15min stirring, the addition of a previously calculated volume of rainbow trout digestive enzymatic extract comprising 3040U of total alkaline proteolytic activity started the enzymatic reaction. A control assay with fishmeal as reference protein source was carried out. Protein degradation was monitored at different times by electrophoretic techniques using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to obtain a quantitative coefficient of protein degradation (CPD) (Vizcaíno *et al*., 2019). In addition, total amino acids released by digestive enzymes were also quantified using L-leucine as substrate (Church *et al*., 1998).

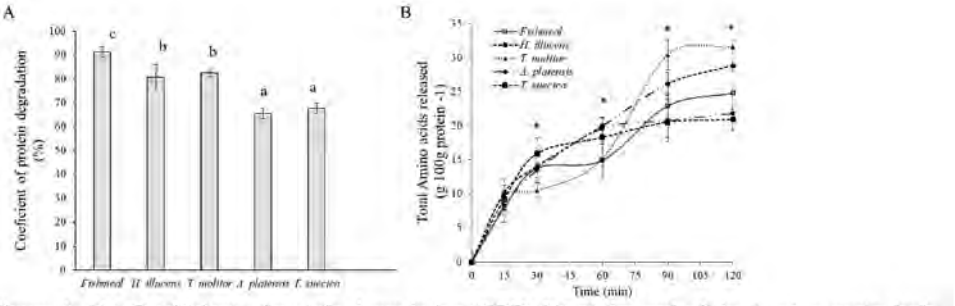

Figure 1. (A) Coefficient of protein degradation (CPD, %) at the end of the *in vitro* proteolysis. Different letters symbolize significant differences among ingredients tested ($p<0,05$). (B) Concentration of free amino acids released (g 100 g $protein^{-1}$) during the *in vitro* proteolysis. Asterisks symbolize statistically significant differences among ingredients for each sampling time ($P < 0.05$).

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Results and discussion

Overall, the results obtained have shown an efficient protein hydrolysis of the different tested protein sources. Densitometric analysis revealed that more than 60% of proteins or polypeptides of raw ingredients visualized in the polyacrylamide gels were hydrolysed by rainbow trout digestive proteases. The CPD values (Figure 1A) were higher in Fish meal (90%) and just slightly but significantly lower in *T. molitor* and *H. illucens meals* (*nealy 80%*), while in case of the microalgae meals, they were similarly lower and around 60%, a fact demonstrating high proteolysis capability by rainbow trout digestive proteases.

In turn, the quantification of free amino acids released by digestive enzymes can be used to estimate the bioavailability of dietary protein. At the end of the *in vitro* assay, fish digestive proteases were able to release from 20% to 31.6% of total amino acids contained in the protein of the different raw sources (Figure 1B). The amount of free amino acids released after 120 min of the hydrolysis of insect meals (*T. molitor* 31.6g 100g protein^{-1} and *H. illucens* meals 28.9g 100g protein^{-1}) are both significant y higher ($p<0.05$) than fish meal (24.8g 100g protein$^{-1)}$, instead microalgae dried biomass (*T. suecica* 20.9g 100g protein^{-1} and *A. platensis* 21.7g 100g protein^{-1}) are significantly lower ($p<0.05$) than the value obtained from the degradation of the ingredient used as control. Probably this is caused by the presence of the wall that limit the availability of the protein to be degraded.

Conclusion

Insect meals exhibit slightly lower coefficient of protein degradation and higher amount of free amino acids released than fish meal. Overall, the extent of protein hydrolysis and the concentration of free amino acids released reveal that the microalgae and insect biomasses tested here can be used as potential protein ingredients in practical diets for rainbow trout. Despite this, further research to improve understanding of the factors that determine microalgae protein bioavailability and the biological effect on fish

Acknowledgments

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AN AUTONOMOUS UNDERWATER VEHICLE FOR AUTOMATED INSPECTION OF AQUACULTURE NET PEN CAGES

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Introduction

An Autonomous Underwater Vehicle (AUV) is presented, as a useful tool with advanced capabilities that can be used to automatically inspect the net structure of aquaculture net pen-cages for possible dysfunctionalities, such as net holes of various shapes and sizes. For the acquisition of underwater image data and the inspection of the installation, a methodology to efficiently navigate the AUV under real operational conditions has been implemented. The navigation procedures and the required data collection are performed without the intervention of a human user, using real-time processing and assessments. The automated fish net consistency inspection is based on video data recorded from the entire net pen-cage area and is performed off-line.

The system

The AUV is based on the BlueRobotics' BlueROV2, the technical details of which are available from the manufacturer. Existing open-source navigation and guidance software have been enhanced with underwater capabilities, while the AUV is considered as IoT-enabled in the sense that all relevant communication and data-exchange (e.g. images and video, sensor data etc.) are performed through Internet Protocol (IP) networks. To cope with lack of GPS or similar systems underwater, computer vision techniques that facilitate correct positioning within the cage are utilized. The AUV monitoring system comprises of three subsystems: a) AUV, consisting of the vehicle, its sensors and the internal processing/control unit, b) Off-line Processing and Communications, and c) Energy Provision and Management, containing the energy sources and the charging docking station.

Operation

For the location and navigation of the AUV inside the fish cage, the proposed solution involves a combination of inertia and magnetic sensors with a real-time optical recognition system enhanced with photogrammetry fundamentals applied to reference targets attached to the net at known depths and bearings. To achieve the required communication speeds with the land base, the AUV docks to a surface-based station that has direct link to the internet. Through this link, the time-tagged and position-tagged hi-res video is uploaded to a surface-based server for further image processing and fish-cage net dysfunctionality detection. The same docking station is used for the inductive charging of the AUV batteries.

One of the main aspects of the monitoring is net inspection assessments, mainly focused on the detection of possible problems existing at the underwater net infrastructure, like holes or 'broken' patterned structure that may appear on the nets. Since AUV provides video data of the net, appropriate advanced image processing techniques are used for the calculation and extraction of all the parameters that can accurately describe the subjects under consideration and alert properly.

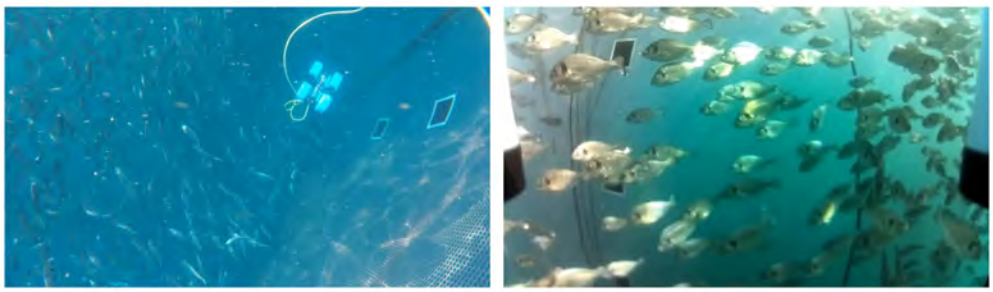

Fig. 1. AUV trials in aquaculture net-pen cages at the HCMR pilot farm

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Trials and Results

The AUV has been successfully tested in a real aquaculture environment, within a squared section fish-cage (6m × 6m × 8m). Test-missions and trials were conducted in various lighting conditions and depths, with and without fishes in the cages and under different conditions.

Acknowledgement

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EFFECT OF A DIGESTION ENHANCER IN DIETS WITH PARTIAL FISHMEAL SUBSTITUTION BY PLANT PROTEINS ON GROWTH PARAMETERS, BODY COMPOSITION AND DIGESTIBILITY IN EUROPEAN SEABASS D*icentrarchus labrax*

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Introduction

Over the last decade, sources of vegetable proteins (VM) and oils (VO) have been increasingly used to replace fishmeal (FM) and fish oil (FO) in feed for marine finfis (Jobling, 2016). The vegetable proteins as substitutes of fish meal present various challenges, due to the quality and the protein concentration from plant sources that are generally lower than FM, and the lower palatability of most plant protein (PP) meals (Drew *et al*., 2007). The present study aimed to evaluate the effect of a digestion enhancer, algae sulphated polysaccharides (MSP®LIPID) on diets with partial FM substitution by PP on growth parameters, body composition, and digestibility in European seabass. Attention has been focused on seaweeds as a rich source of bioactive compounds and their polysaccharides as antioxidants to prevent oxidative damage in living organisms (Hassan *et al*., 2011). The algae sulphated polysaccharides activate the enterocytes and hepatocytes and this initiates several mechanisms (Qi *et al*., 2012). First, the bile acid secretion is increased. The release of the bile by the gall bladder allows the digestive organs to exclude rejected toxins, thus improving the detoxification of the liver, blood, and kidneys. The stimulation of bile secretion and excretion leads to obtain smaller lipid micelles formation which improves the efficiency of the lipolytic enzymes (Hassan *et al*., 2011). Also as the enterocytes and hepatocytes are activated in a way that some enzyme are stimulated these two actions help to improve digestive system and nutriment assimilation (Qi & Sheng, 2015).

Materials and methods

Three isoproteitic, isolipidic and isoenergetic diets were fed to 3 groups of European seabass in triplicate. Groups A were fed the control diet containing 18% fish meal, group B fed a diet in which fish meal was substituted at 33% by PP and group C in which a digestion enhancer (MSP®LIPID) was added in the fish meal substituted diet (Table I). Three hundred and sixty fish (14.4±0.06g) were randomly distributed in nine 500 l tanks, at 19 °C, and fed three times a day, 7d a week, until satiation for 95d initially with 2 mm pellets and after xx g bodyweight 3 mm pellets. The growth parameters and the apparent digestibility coefficients (ADC) were calculated according to the following formulas:

Feed Conversion Ratio (FCR)=feed intake/weight gain

Specific growth rate (SGR) (%day 1) = 100x(ln final body weight−ln initial body weight)/days

Daily Growth Index (DGI)=[(final weigh $^{1/3}$ − initial weight$^{1/3}$)/days]×100

Average daily gain (ADG)=Weight gain(g)/days

Hepatosomatic index (HSI)=100×weight of the liver/whole body weight

Viscerosomatic index (VSI)=100×weight of digestive tract/whole body weight

ADC=100–100x[(marker in the diet/marker in faeces)x(nutrient or energy in faeces/nutrient or energy in the diet)].

Results

By the end of the growth trial, no significant differences were observed for weight gain and SGR among sea bass fed diets A, B and C. All groups had excellent growth rates (SGR above 1.1%/day) relative to water conditions. In addition there were no significant differences in feed conversion ratio between groups, which had a value of 1.3 - 1.4. Replacement of fish meal by plant protein ingredients did not affect DFI which was at 1.1-1.2% bw/day. The whole-body proximate composition was representative of the size of fish tested and did not reveal differences between treatments (Table II). The use of the digestion enhancer proved to be beneficial as it improved the protein ADC significantl , and increased numerically all other digestibility values, however without statistical significance (able III).

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Table I. Feed ingredients and proximate composition of the dietary treatments

	Diet A	Diet B	Diet C
Raw materials%			
Fish meal 999 LT	18,0	12,0	12,0
Wheat flour	13,6	12,6	12,6
SPC	10,0	10,0	10,0
Fish oil	8,0	8,0	8,0
Hipro Soya (48%)	10,0	16,0	16,0
Wheat gluten	3,5	6,4	6,4
Rapeseedmeal	8,0	5,6	5,6
Rapeseed oil	4,3	4,8	4,8
Corn gluten	8,0	8,0	8,0
Poultry meat meal	8,0	8,0	8,0
Haemoglobin meal	7,0	7,0	7,0
Lmethrnin	0,5	0,5	0,2
Mono Cal Phosph	0,0	0,1	0,1
Premix	1,00	1,00	1,00
Dipentene	0,00	0,00	0,50
Total%	100,0	100,000	100,0
Composition%			
Protein	47,0	47,0	47,0
Fat	16,0	16,0	16,0
Ash	7,0	6,4	6,4
Fiber	2,38	2,2	2,2
Moisture	7,0	7,0	7,0
NFE	20,7	21,4	21,4
Starch	10,6	10,4	10,4
Total %	100,0	100,0	100,0

Table II. Growth performance and whole-body composition of *Dicentrarchus labrax*

	Diet A	Diet B	Diet C
Initial body weight (g)	14.4±0.5	14.4±0.3	14.5±0.4
Final body weight (g)	47.2±1.1	44.5±3.5	46.9±1.6
FCR	1.4±0.1	1.4±0.2	1.3±0.0
SGR	1.2±0.0	1.1±0.0	1.2±0.0
ADG	8.3±1.2	8.2±1.5	8.5±0.6
DFI	1.1±0.1	1.2±0.0	1.1±0.0
HSI	1.8±0.1	1.6±0.1	1.6±0.1
VSI	9.2±0.6	9.7±0.6	10.0±0.7
Whole body composition (% Dry Weight)			
Dry matter	37.3±0.7	36.1±1.0	35.9±1.6
Ash	9.9±0.8	9.6±1.5	8.8±0.7
Protein	49.4±3.4	48.8±3.8	49.0±2.7
Lipids	38.7±2.7	40.4±2.7	39.6±2.2

Table III. Apparent digestibility coefficients (ADCs, %) of nutrients and energy of *Dicentrarchus labrax*

	Diet A	Diet B	Diet C
ADC protein	90.7±1.3 [a]	89.8±2.0 [ab]	93.6±0.9 [b]
ADC fat	87.9±2.1	88.9±2.9	91.2±1.0
ADC organic matter	83.0±1.4	83.2±1.5	85.9±1.4
ADC dry matter	75.4±2.4	76.3±2.2	79.1±1.8
ADC energy	72.0±5.5	74.7±4.5	77.9±1.3

n=3, mean±standar error, different letters suggest statistically significant difference

Discussion and conclusion

Although commercial diets for sea bass contain high levels of FM, it has already been shown that young sea bass can accept dietary protein-based diets almost devoid of FM without affecting the growth and use of feed (Kaushik *et al*., 2004). The results of this work support these results and show that a 33.3% substitution of FM in a 18% fishmeal diet -during a 95 day trial- is possible without affecting the growth performance and body composition of the seabass, the whole-body composition and feed digestibility, with zero mortality rates (Kaushik *et al*., 2004; Tibaldi *et al*., 2006). Under the present experimental conditions the inclusion of PP also did not affect feed intake. The addition of algae sulphated polysaccharides showed a tenedency of a beneficial effect as far as protein digestibility is concerned. Algae polysaccharides may have an impact on a number of biological processes such as reducing oxidative stress and provide antihyperlipidemic benefits by increasing the activities of antioxidant enzymes and limiting lipid peroxidation (Hassan *et al*., 2011). Furthermore, they exert therapeutic effects on metabolic diseases (Qi & Sheng, 2015).

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INTEGRATED CULTIVATION OF EUROPEAN SEA BASS *Dicentrarchus labrax* AND *Ulva* sp. IN RECIRCULATING AQUACULTURE SYSTEMS WITH TWO DIFFERENT DIETS

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Introduction

The rapid increase of intensive aquaculture has resulted in the accumulation of nutrients which can disturb the sustainability of aquatic ecosystems, degrade the water quality and cause eutrophication. One potential way is the increased use of land-based recirculating aquaculture systems (RAS). Such systems allow more effective control of culture conditions, and through filtratio , minimize many negative impacts on the surrounding environment (Martins et al., 2010). Integrated Multi-Trophic Aquaculture (IMTA) gives a new prospective to the preservation of the environment. Through the combination of different species that interact and use each other's by products as a source of nutrients, IMTA can contribute to the bioremediation of seawater and provide economic and environmental sustainability (Granada et al., 2009). *Ulva* has been successfully integrated into mid- to large-scale animal mariculture systems, showing high growth rate (Troell et al., 2003; Neori et al., 2004). A good potential for profitability has been estimated for integrated sea bream - *Ulva* farms (Neori et al., 2004). In this study, European sea bass (*Dicentrarchus labrax*) and *Ulva* sp. were cultured in indoor RAS with biofilm filtration in an effort to optimize growth performance and quality of both species. Two different trials were performed where dietary fish oil was replaced by vegetable oils (rapeseed and palm oil) at the first trial, and at the second, pellets containing sardine oil were used for the fish nutrition. The cultivation of sea bass and *Ulva* in IMTA-RAS is a case study of the project IMTA-EFFECT (Integrated MultiTrophic Aquaculture for EFFiciency and Environmental ConservaTion) in the frame of EU ERA-NET project, aiming to evaluate IMTA-RAS performance using alternative diets to finfis and to contribute to food web modelling through fatty acids characterisation.

Materials and Methods

In each trial, two identical RAS units with separate biofilters (bacteria compartments) and similar fish biomass were used. In one unit (*Ulva*-Ras), water compartments and sea bass (fish compartments) were grown in separate tanks (3 tanks per species) in order that the water from upper tanks to insert into the fish tanks and thereafter to flow in a lower biofilter (bacteria compartment). The waste water treated by the biofilm pumped into the water tanks. Rearing conditions were similar in both RAS units in terms of water flow rate, salinity, oxygen, photoperiod and light intensity. Fifty fish were placed in each tank. Fish were fed extruded pellets at apparent satiation twice a day (except week end). Dietary fish oil was replaced by a mixture of rapeseed oil and palm oil (1:1) at the first trial, whereas pellets containing sardine oil were used at the second diet. Sea bass were reared for 12 weeks. Body weight (W) and length (L) (standard and total) were measured at the beginning and the end of each trial. FCR, SGR and Condition Factor ($K=W*L^{-3}*100$) were calculated for each trial. *Ulva* was collected from the Saronic gulf, sorted and cleaned with seawater and weighted after drying in open air. Unattached thali were transferred to the upper level tanks of *Ulva*-Ras and were kept suspended by air diffusers situated at the bottom of the tanks. *Ulva* was kept at densities of 0,5-1 kg $m^{-2.}$ S. *Ulva* was harvested weekly (2nd trial) or biweekly (1st trial) and replaced. Samples were collected at the beginning and the end of the growing period of each species for chemical composition and fatty acid analyses.

Results

In both trials Body Weight Increase (BWI), Specific Growth Rate (SGR), Feed Conversion Rate (FCR) and mortality did not show significant differences between Control and *Ulva*-RAS. Sea bass in *Ulva* containing units (UF) showed a higher feed intake during the 1st trial, while lower in the 2nd trial compared to control fish (CF). Condition Factor (K) was higher in *Ulva*-RAS in both trials. Sea bass chemical composition showed significant differences, between fish UF and CF in both trials regardless feed intake. In both trials, moisture was significantly ($P<0,05$) higher in CF than in UF groups, whereas ash and lipid were significantly higher in UF than in CF groups in both trials. Protein content was significantly higher in UF than in CF groups only at the 1st trial. Fatty acid profile analysis of sea bass showed significant differences in the content (% total lipids) between UF and CF groups. After single or bi-week, culture in indoor RAS *Ulva* showed an increase in Nitrogen and Phosphate content.

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Conclusions

Ulva cultured in indoor RAS did not increase its biomass, however chemical composition analysis, shows improved quality, due to the decreased carbohydrates and the higher protein content. Fish in *Ulva*-RAS show an increased Condition Factor (K), higher lipid (% wet weight) and increased EPA & DHA (g/100g) content compared to control. High lipid content in cultured fish is often associated to physiological disorders (e.g dietary EPA and DHA deficiency) or increased feed intake. On the other hand, it may be an indicator of a good physiological condition. For fish in Ulva-RAS, feed consumption increased when fed VO-diet and decreased with FO-diet compared to control fish. However, increased lipid content was observed in both diets, regardless of dietary EPA and DHA content or feed intake. Although *Ulva* was not a dietary component in this study, the results support the previously reported hypothesis that supplementation of fish diet with algae is associated with activation of fish lipid metabolism and good physiological status (Nakagawa et al., 1987). More research is required to reveal the effective substances due to *Ulva* presence in fish culture. Nevertheless, taking into consideration the results of the chemical composition, we can assume that the co-culture of sea bass and *Ulva* could result in a higher nutritional value for both species.

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NUTRIENTS INVOLVED IN DIGESTION AND TRANSPORT OF LIPID ACROSS THE INTESTINAL MUCOSA OF ATLANTIC SALMON
PART 2: LIPID MALABSORPTION IN SIX, NORWEGIAN ATLANTIC SALMON (*Salmo salaris* L) FARMS

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Optimal gut health is a key component in improving feed utilization, for better fish growth and health, reduced production costs and for minimizing the environmental impact of Atlantic salmon industrial production. There is a dearth of knowledge on gut health of farmed fish stock. This is being addressed in the GutMatters project, funded by the Norwegian Seafood Research Fund (FHF), in a national survey to investigate the prevalence of gut health disorders and their incidence during a production cycle in sea farmed Atlantic salmon.

In the survey that ran from October 2017 to November 2018, 6 sea farming sites along the Norwegian coast were monitored starting from smolt transfer to harvest fish size (>4 kg) (sampling 3). At each of 3 samplings per site, fish weight and length; plasma; gross pathology observations of the fish, intestinal mucosa and liver; intestinal, liver, and other samples for histology, qPCR, microbiota, metabolomics, and digestive enzyme activity were analyzed from 20 fish. Additionally, site physicochemical data, fish genetics, population records, feeding regimes, growth and health history data were also collected. Results from the preliminary gut health assessment from gross pathology and intestinal histopathology will be presented.

Histopathology revealed enterocyte steatosis, indicating a lipid transport disorder, in the pyloric caeca and occasionally the mid intestine was a noticeable finding whose prevalence and severity appeared to increase in warmer periods of the yea .

Inflammatory changes, resembling the salmonid soybean-meal-induced distal intestinal enteritis, were also observed in some fish from all participating sites. A trend of a general increase in occurrence and severity of the enteritis over time was noted for most of the sites.

In conclusion, the gut health of some fish from participating farms was hampered by enterocyte steatosis and enteritis. More survey results and follow-up plans for the GutMatters project will be presented.

COMPARISON OF EXPRESSION LEVEL OF GLUCOCORTICOID RECEPTOR (GCR) GENE IN ORIENTAL WEATHERFISH (*Misgurnus anguillicaudatus*) ACCORDING TO REARING DENSITY

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Introduction

The oriental weatherfish (*Misgurnus anguillicaudatus)* is considered as popular food with high level of nutrition value and high medical function in Asian countries for a long time. It is anticipated that commercial farming for *M. anguillicaudatus* will come into the spotlight and increase requirement of research in aquaculture field in Korea, though it has a problem which occurred mass mortality at early stage without any special reason. It is assumed that stress due to rearing density is one of the reasons. Cortisol and Glucocorticoid receptor (GCR) are relative with level of stress in fish, and inversely proportional when stress factor activated them. Stress tolerance and effect can be determined by the GCR gene level (Wendelaar Bonga, 1997). The purpose of this study is to confirm quantitatively difference with expression level of GCR gene in *M. anguillicaudatus* depending on different rearing density.

Materials and methods

We produced fingerlings of *M. anguillicaudatus* by artificial reproduction in 10L plastic tank. We made 3 groups of different rearing density (3,000; 6,000; 15,000 fish/ ^{2}). We provided live feeds (rotifer, Artemia) or commercial feed, and other environments were suitable for fish. We sampled 3 individual in each tanks (n=3) at 0, 5, 10, 12 days after hatch out(0d, 5d, 10d, 12d). mRNA was extracted from each sample by Trizol method, and synthesized cDNA for quantitative Real-time PCR (RT-PCR). RT-PCR was performed using SYBR green PCR Master Mix (Life technologies, USA) and GCR gene-specific primer set. The PCR were performed as follows: 10min at 95°C; 40 cycles of 15sec at 95°C, 1min at 60°C. The data from RT-PCR (C_T value) was analyzed by $2^{-\Delta\Delta CT}$ method, and statistical analysis was performed using the SPSS Version 10 (SPSS Inc., USA).

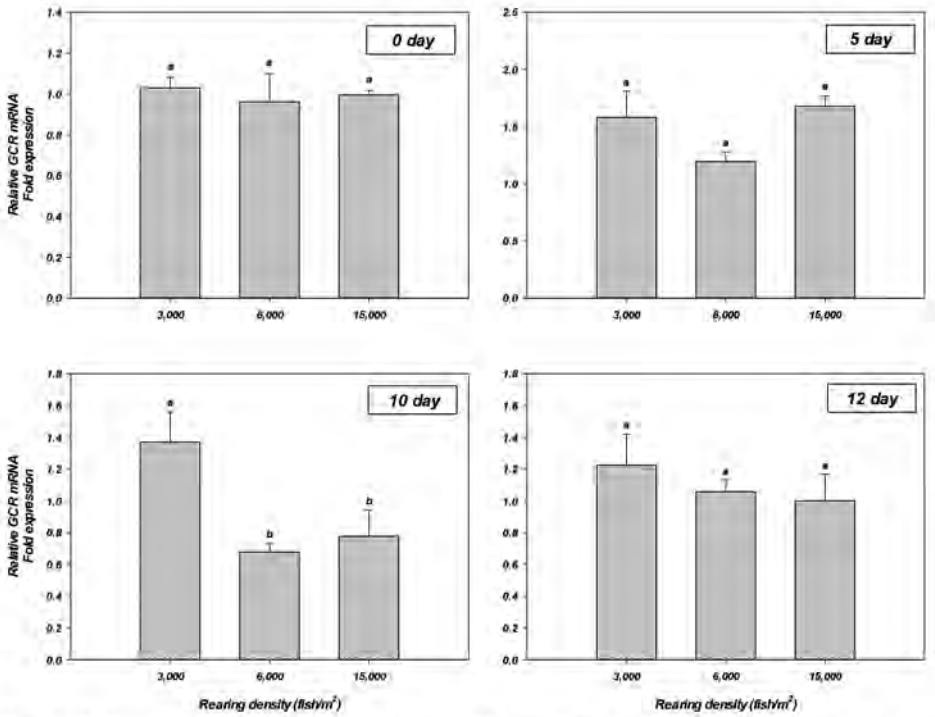

Fig. 1. Relative fold expression of *M. anguillicaudatus* GCR gene according to rearing density. The letters upper the bar within each group marks significant differences by duncan's multiple range test (P<0.05).

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Results

The Expression of GCR gene of 10d sample decreased at 6,000 group, and it was significantly difference at 6,000/15,000 group (Fig. 1) ($P<0.05$). Samples of 0d, 5d was not significant y different ($P>0.05$). In the comparison according to the rearing density, 6,000 group was significantly di ferent expression level of GCR gene at 10d.

Discussion and conclusion

We demonstrated that the relationship between stress of fish and rearing density in this study. We produced fingerlings of *M. anguillicaudatus* in different density, and determined expression level of GCR gene from individual samples using Real-time PCR. Expression of GCR gene was same until 5d after hatching in all group, than significantly decreased at 10d in 6,000/15,000 group (Fig. 1). As a result about rearing density, 6,000/15,000 group was significantly decreased at 10d, but 3,000 group was not. This result demonstrated that rearing density over 3,000 fish/ ^{2} affect the fish stress in artificial reproduction of *M. anguillicaudatus*. It was similar to the previous study that stress in fish affect by high rearing density (Witeska et al., 2005). Occurring stress in fish for many factor (environments, pathogen, etc.), Secreted cortisol combines with GCR, and cortisol-GCR complex react to stressful situation (Park et al., 2011). Previous studies reported that down-regulation of GCR expression content with elevated cortisol concentrations (Park et al., 2011; Terova et al., 2005). As a result, we confirmed that stress due to rearing density may cause the mortality of fish, and established the adequate rearing density of *M. anguillicaudatus* for aquaculture.

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THE ENVIRONMENTAL EFFECTS OF WATER MOVEMENT ON THE ABALONE MARINE AQUACULTURE AREA

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Introduction

The marine aquaculture cage systems use more intensive energy in the limited area than natural ecosystems to increase the productivity. In particular, it is known to change the marine environment around farms (aquaculture areas) due to the large and dense facilities and intensive food supply (and excretion). In this study, we investigate the change of characteristics of seawater movement according to the increase of facilities and discuss the effects on the aquaculture environment in terms of geochemical process.

Materials and methods

To analyze the variability of the water movement, geochemical process and environmental characteristics around the abalone marine aquaculture area, seasonal hydrographic survey were carried out and time series datasets of hydrodynamic were collected in the study area simultaneously.

Results

According to the preliminary results from hydrodynamic data, the seawater movement in the study area was characterized by tide and tidal current with a semi-diurnal cycle. However, the distortion of tidal current velocity along water depth was observed in the vicinity of abalone aquaculture cage. The minimum and maximum velocity of tidal current were measured at the surface and middle layer respectively. The change of the vertical structure of current velocity seems to be related to the increased abalone aquaculture facilities (cages). Eventually, the increased current velocity at the middle layer would

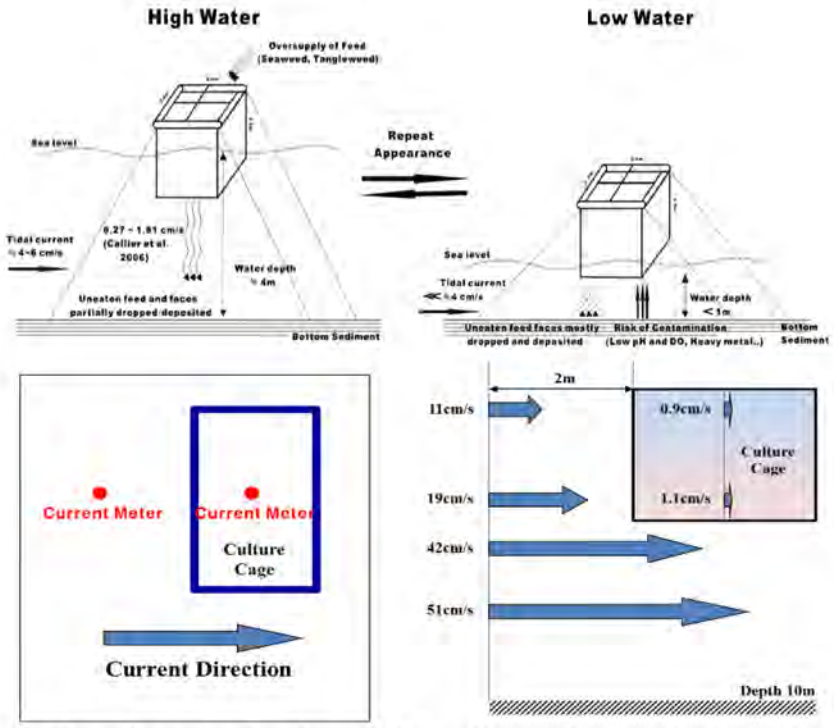

Fig. 1. The schematic drawing of the sedimentation mechanism of organic matters, the deployment of current meter and the vertical structure of tidal current velocity in the vicinity of abalone aquaculture cage.

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influence the vertical velocity shear increase at the bottom layer. The periodical variation and high concentration of the suspended particulate matter at the bottom layer and the inside of the abalone aquaculture facilities can be explained by the increased shear velocity at the bottom layer. The decreased current velocity at the surface layer by the dense and massive abalone aquaculture facilities can also be related to the main cause of environmental degradation in the study area due to the restriction of water-mass exchange. The results of the water quality parameters (dissolved oxygen, chemical oxygen demand, nutrients, suspended particulate matter, etc.) analysis indicated that the changes of environmental condition are closely related to the current velocity.

Discussion and conclusion

From the preliminary results, the extent of the environmental impact on the abalone aquaculture area seems to be highly correlated to the change of seawater movement as well as geochemical processes such as the amount of organic matter and the concentration of nutrients. Further research for the interaction of the hydrodynamic and geochemical processes including the variation of the sea surface temperature is essential to understand the environmental effects on the abalone aquaculture area.

NEED OF FEED ENZYMES FOR EFFICIENT RECIRCULTATION AQUACULTURE (RAS) SYSTEMS: A PROTEASE PERSPECTIVE

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Introduction

Frequent disease outbreaks and unpredictable weather conditions are significant threats to intensive farming systems. Farmers are seeking more control over the operations to ensure better return on investment and as well as to reduce the ecological footprint.

Recirculating aquaculture or RAS systems are therefore becoming increasingly popular. They provide better control culture environment and improved waste management.

Efficiency of a RAS system largely depends on its' nutritional wastes removal capacity. Better waste management primarily depends on reducing the waste output and then, efficient removal of the wastes.

In this paper, we summarized the findings from several studies where use of a specific protease improved nutrient utilization thus reducing the waste output to the culture environment.

Methods

We summarized the findings from several commercial studies conducted on farms or in controlled laboratory conditions worldwide. The meta-analyses were mainly focused on protein-efficiency ratio (PER), nitrogen digestibility, and nitrogenous waste-output.

Results

Marked improvement in protein efficiency ratio (PER) was observed when a specific dietary protease complex was supplemented to the diets compared to the same diet without supplementation in studies with tilapia, yellow catfish, snakehead and Chinese mitten crab shown (Fig. 1).

Apparent nitrogen digestibility also showed significant improvement in both fish (rainbow trout and Atlantic salmon) and crustacean (Pacific white shrimp)

Findings of a laboratory trial with yellow catfish have shown a linear decrease in hourly ammonia excretion with graded level of the specific protease (Fig 2

COMPUTER VISION BASED FISH INDIVIDUAL IDENTIFICATION AS AN ALTERNATIVE TO FISH TAGGING

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Introduction

The optimization of the fish production in intensive aquaculture can lead to the increase of the production with the respect to the fish welfare. The new technologies enable to develop the Precision Fish Farming (PFF) (Fore et al. 2017) concept whose aim is to apply control-engineering principles to fish production, thereby improving the farmer's ability to monitor, control and document biological processes in fish farms. Animals identification is known concept in the precision agriculture used for livestock farming, stock identification or monitoring of endangered species (Bendik, 2013). The same concept can be adopted in precision fish farming.

There are many different ways for individual fish identification. The widespread and popular methods of fish identification are tagging and marking (PIT, RFID or VIE tags). Most of the commonly used methods are invasive and can have adverse effects on fishes, increasing the risk of sequelae or mortality, mainly for small and sensitive fish as most of the stream fish species.

Non-invasive fish identification based on the fish appearance is cheap, less stressful for the fish and accurate. Human based identification of individual fish of the same species using the skin pattern was proved by Hirsch (Hirsch 2015). There are several different patterns which can be observed on the fish body (eye pattern, scale pattern, body texture). The aim of this paper is to demonstrate the usability of visible patterns for individual fish identification from the point of view of identification accuracy and pattern stability for long term identificatio

Materials and methods

The results of three different studies are reported here to demonstrate the ability of computer vision for individual fish identification

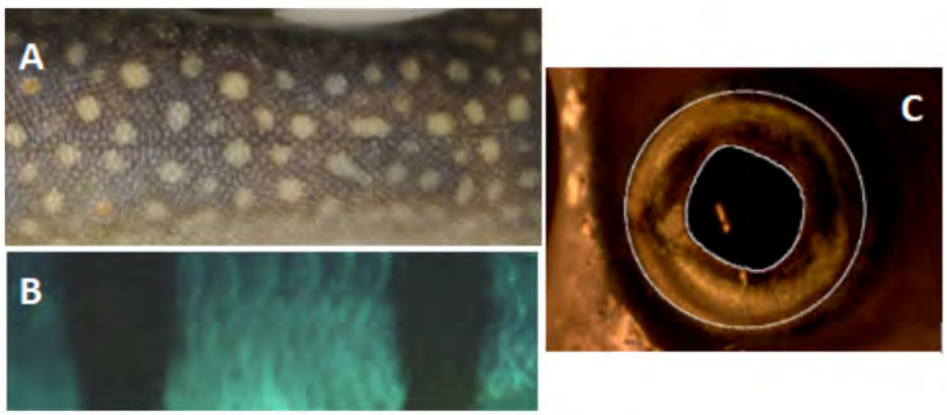

Figure 1. Example of visible pattern used for fish individual identification for different fish species: A – dot pattern of tail part of rainbow trout, B – stripe pattern of middle body part of Sumatra barb, C – iris pattern of salmon

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Rainbow trout identification – The aim of the study was to test image based individual identification for the group of 32 rainbow trout. The image database contains 3 datasets representing three data collections of images of the same fish for 3 weeks. One image of each fish out of the water was taken for each session. The fish was automatically detected and the region of interest (ROI), containing unique dot pattern, was determined, Fig1-A. The texture descriptor histogram of oriented gradients (HOG) (Dalal 2005) was used to create the feature vector for identification. The identification was performed for all combinations of the data collection sessions.

Atlantic salmon identification – The image database of 330 fish eyes was collected. From 4-8 images per fish taken for each individual in one session. The iris images were then collected for 30 tagged fish in next 3 sessions (2 months in between the sessions). The inner and outer boundary of iris was automatically detected, Fig 1-C. The image rotation was compensated, and fish iris code based on 1D-Log-Gabor filters was calculated. The power of the iris pattern was tested on the task of identification 330 individuals.

Sumatra barb identification – The 25 fish individuals were used for fish identification within one data collection and between 2 data collections (14 days between data collection). Three images of the lateral view of same fish under water were collected using unified background (green colour). The fish was automatically detected in the image and the central parts containing middle part of the body with two stripes was used as ROI. The identification was tested between data collections and within data collections.

Results

Rainbow trout identification: All 32 fish were correctly identified between all three sessions which corresponds to the 100% recognition accuracy.

Atlantic salmon identification: The equal error rate for the 330 individual fish was 0.84% which correspond to the high accuracy of individual identific tion. The equal error rate for identification between four data collections varies from 10 to 43%.

Sumatra barb identification: The classification accuracy of the fish within the same data collection was 100% (all fish were correctly identified). The classification accuracy between the data collections was 88% (3 individuals were identified incorrectly).

Conclusion

Individual fish identification is one of the keystones of the emerging concept of Precision Fish Farming. This study showed the usability of the visible fish patterns (iris, body pattern) for individual identification using computer vision. The identificati n was tested on three species representing different visible patterns. The identification power and pattern stability for long term identifica ion was studied. High accuracy of the identification was reached for all used patterns with different stability of the patterns for long term identification.

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COMPARATIVE STUDY OF CAROTENOIDS IN SHRIMP AND SOFT-SHELL CRAB SAMPLES: ELECTROANALYTICAL APPROACH

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Introduction

In the aquaculture industry, the quality of products is essential to obtain good market price, so maintaining their excellent nutritive value throughout the industrial processing is vital. Carotenoids are compounds responsible for the natural colour of different products which is one of the main criteria to consumers (García-Chavarría and Lara-Flores, 2013). They have great antioxidative activity and astaxanthin is considered as one of the strongest antioxidants and most distributed carotenoid in the aquaculture industry. Among the 750 reported carotenoids found in nature, more than 250 are of marine origin (Maoka, 2011). Until now, carotenoids were mostly analyzed using chromatographic and spectrophotometric methods (Maoka, 2011), which require time-consuming preparation of the sample and high cost of the reagents and analysis. Electrochemical methods could provide a cost-effective and fast-screening alternative. Therefore, in this study, we investigated the electrochemical behaviour of astaxanthin using voltammetry of immobilized microdroplets and its application to determine the carotenoid concentration in shrimp and soft-shell crab samples that are common in the human diet.

Materials and methods

All experiments were performed in the dark by placing aluminium foil around the experimental cell. The electrochemical technique square-wave voltammetry (SWV) was performed in two electrolyte solutions, 0.1mol dm^{-3} $HClO_4$ and 0.1mol dm^{-3} KNO_3 on paraffin-impregnated graphite rod (PIGE) which was mechanically cleaned before each experiment. Voltammetry of immobilized microdroplets (Scholz et al., 2000) was employed using a potential step increment of 2mV and a square-wave amplitude of 50mV. The frequency varied from 10Hz to 1000Hz. Extraction methods according to Michelon et al. (2012) were preceded for analysis of carotenoids in real samples with minor modifications. Spectrophotometric analysis was performed for determination of total carotenoid according to the method by Wellburn (1994).

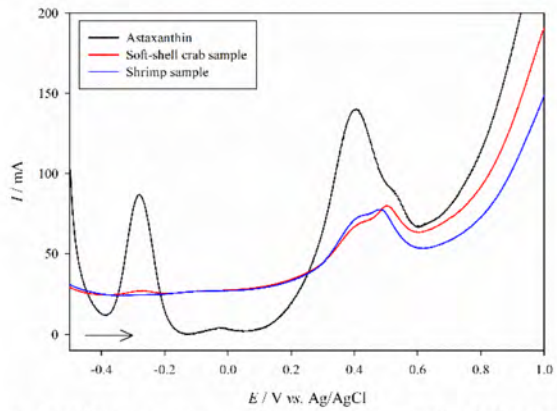

Fig. 1. Square-wave voltammograms of astaxanthin and of the extracts from shrimp and soft-shell crab samples in the form of microdroplets (5µl). The frequency is 100Hz, pulse amplitude is 50mV and the step potential is 2mV.

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Results

Voltammetric response of astaxanthin microdroplets consists of four oxidation peaks at potentials E_{P1} = -0.276V, E_{P2} = -0.032V, E_{P3} = 0.529V and E_{P4} = 1.026V when measured in the acidic electrolyte, while increasing pH-value has drastically influenced net peak potentials i.e. responses were shifted toward more negative values. The accuracy of the method is expressed as a recovery for peak P1 and the obtained value was 121.70%. The relative standard deviation for maximum astaxanthin concentration in the form of a droplet expressed for the current of peak P1 was 3.69% and for potential was 0.413%. The limits of detection and quantification were 69.6μmol dm^{-3} and 211.0μmol dm^{-3}. Electrochemically determined concentrations of the astaxanthin in shrimp and soft-shell crab samples were 33.11μg g^{-1} and 52.03μg g^{-1}, while total carotenoid content was determined using spectrophotometry at the λ_{max} = 480nm.

Discussion and conclusion

The optimized voltammetric technique has been developed for the characterization of carotenoids in shrimp and soft-shell crab samples. Electrochemical oxidation mechanism of astaxanthin was further explained using voltammetry of immobilized microdroplets and serves as a "fingerprint" for its determination in real samples. Low standard deviations imply non-contaminated surface of the working electrode and high repeatability in the identification of this oxidation peak. For both shrimp and soft-shell crab samples, 20 times higher values were obtained using the spectrophotometric method when compared to the electrochemical method. This can be explained with non-selectivity of the spectrophotometric method, i.e. carotenoids have similar absorption maxima and they contribute to the total carotenoid content in the samples. In contrast, voltammetric responses for sample extracts are in an agreement with the voltammetric response of astaxanthin (figure 1). Therefore, only astaxanthin is quantified in samples using voltammetry and total carotenoids present in the samples were spectrophotometrically measured. Voltammetric technique has shown to be a more selective method.

The applied electrochemical method allowed quantification of a specific carotenoid in shrimp and soft-shell crab samples and could possibly be applied in industrial processes for measuring carotenoid content in newly developed products.

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THE INFLUENCE OF GROWING TANK SIZE ON PADDLEFISH *Polyodon spathula (WALBAUM, 1792*)

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Introduction

The various diversity of shapes and sizes of the current fish species is due to the intercondi- tional reciprocal bonds between them and the biotic and abiotic environment. The different conditions that the aquatic environment offers to fish have led to the appearance of various fish forms (Stancioiu, 2006). The capacity of adaptability of fish to different environmental conditions is taken as a consequence that determines the great diversity of body shapes. Functions of the locomotor system of the fish contribute, as a first fact , to the formation of the shape of the body.

Materials and methods

Research regarding the behavior and growth performance under the influence of the growing tanks size on *Polyodon spathula* were carried out at the pilot research station of the Faculty of Food Science and Engineering, „Dunărea de Jos” University of Galati, România. The experiment took place in a Recirculating Aquaculture system (RAS), provided with four growing tanks, two with the the construction dimensions of 1.40×1.40×0.60m (V1-Basin 1 and Basin 2) and the other two units with the dimensions of 0.50×0.50×0.45m (V2-Basin 3 and Basin 4). Each growing unit was populated with the same stocking density of 1.2 kg m^{-3} (figure 1)

The establishment of the stocking densities was made based on biometrics measuremts, such as: total length (TL-cm)- from the top of the rostrum to the top of the upper lobe of the caudal; the length of the rostrum (LR-cm)- from the top of the rostrum to the eye, and weight (W-g). So, in the presented context, the purpose of this research was to study if the growth performance and behaviour of this species is influenced by the tank size

Results

There are few information in the literature regarding the influence of the growing tank size on behavior and growth performance of fish. Kirschbaum, F., (2006) reported no significant difference regarding the growth performance in a similar study performed on *Acipenser sturio* fish in growth tanks with a volume of 11.6m^3 and 6.8m^3. In this study, the behaviour of *Polyodon spathula* was evaluated, and it was observed that, after two weeks the fish from the V2 variant swim „stuck” by the sidewalks of the tanks over a time interval of 10-15 minutes, or even more. This reaction is a response to the stress caused by the lack of space, and led to the frontal rotation of the rostrum and its damage, but also to the partial or total depigmentation of the fish.

Regarding the mean weight, in the variant V1, fish almost double their weight, while in the variant V2, average weight decreased from 13.79 ± 2.0g/fish to 5.82 ± 0.17g/fish. In the basin B4 there was a slight increase of individual weight from 13.72±1.9 g/fish to 15.56±4.47 g/fish

Regarding the total length of poliodon juvenile it was observed an increase with 31% in variant V1 compared to the basin from the variant V2 where there was an increase of 7.96% in B3 and, 14.02% respectively in B4.

Table I. Average of the biometric measurements at the begining and in the end of the experiment

Growing tank		Mean weight (g)		Total length (cm)		Rostrum length (cm)	
		Initial	Final	Initial	Final	Initial	Final
V1	B1	13.67±3.64	23.39±5.6	16.36±1.78	21.31±1.78	5.56±0.6	7.46±0.53
	B2	13.67±3.8	24±3.95	16.43±1.8	21.83±1.23	5.54±0.7	7.8±0.47
V2	B3	13.79±2	5.82±0.17	16.58±0.9	17.9±2.21	5.56±0.45	6.1±0.8
	B4	13.72±1.9	15.56±4.47	16.4±0.9	18.67±1.85	5.49±0.2	6.48±0.74

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The correlation between the TL (cm) and LR (cm) highlights that the fish from the V2 variant registered a short period of growth. At the end of the experiment, the mean of the LR registered in the V1 variant a value of 7.46±0.53cm in B1, and 7.8±0.47cm in B2, while in V2 the growth of the juvenile in the TL and in the LR did not have the same increase as in variant V1 (table 1).

Discussions and conclusion

The results obtained in this experiment highlights the fact that growing tanks of small dimenision have a negative effect on the growth performance of paddlefish. Limiting the swimming space for this fish is a major stressor factor that inhibits the retention capacity of nutrients from the administated feed. The specific characteristics of the polyiodone for feeding by active and passive filtration, as well as those related to the plasticity of the paddlefish feeding mode, to consume the feed deposited on the bottom of the growing tanks, were affected by the limited surface of the basins. In variant V2, due to the small distance between the opposite walls of the tanks (50 cm), the filtration capacity of paddlefish was shortly (2-3 seconds) in comparation with the filtering activity of the fish from th V1 variant, which lasted for 1 to 3 minutes.

In conclusion, *Polyodon spathula* demonstrated a good technological plasticity by subjecting it to different environmental conditions. However, in extreme cases, the growth of the paddlefish in growth tanks with a reduced surface result in a decrease of the growth performance and survival.

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PREDICTING LONGTERM EFFECTS OF SMALL CHANGES IN AQUAFEEDS PERFORMANCE USING A DYNAMIC METABOLIC MODEL

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Introduction

The aquaculture industry has been pushed towards a significant reduction in the use of fish meal and fish oil, replacing it with alternative protein and oil sources, often of plant origin, driven by economic and environmental pressures. The introduction of plant- based ingredients in feed formulations can affect fish performance through effects of on nutrient digestibility, but these effects are normally considered small as such feed formulations are well well-balanced in nutrients. Still, such small effects, may bring result in considerable impacts when accumulated over time. Moreover, such plant-based feed formulations can also have major impact on nitrogen and phosphorus outputs to the environment. It is therefore of the utmost importance to assess the effect that these changes in feed formulations can may have on fish performance, feed costs, consumer-value, and the environmental impact of aquaculture.

A metabolic, or nutrient-based, model (FEEDNETICS™, Silva and Soares, 2018) was here used to predict the long-term interactive effects of alternative feed formulations, resulting in small changes in nutrient digestibility, on growth performance, feed conversion, and release of nitrogen and phosphorus to the environment.

Materials and methods

The main data (inputs) needed to run the metabolic model are proximal and amino acid composition of the feed and apparent digestibility coefficien s (ADC's) for protein, fat and phosphorus, along with a feeding table and a temperature profile. We have generated two virtual trials using FEEDNETICS™ to generate predictions on the effect of: a) inclusion of the enzyme phytase in feed as to render phosphorus in phytic acid more available (digestible) to Atlantic salmon, against a scenario where the lower phosphorus digestibility was compensated by adding inorganic phosphorus (Mono-Calcium Phosphate, MCP) to the feed, a common practice in the industry; and b) the inclusion of a probiotic that improves protein digestibility in gilthead seabream.

Results

FEEDNETICS™ predicts that salmon fed the Phytase supplemented feed will have a better performance and lead to a considerably lower total phosphorus waste production, compared to the MCP supplemented diet. In fact, the prediction suggests a 6.2% higher fish weight after one year (Fig. 1), and 35% lower total phosphorus waste (Fig. 2). Moreover, FCR is predicted to be 2.5% lower for the Phytase supplemented feed.

Discussion

This study confirms that modelling approaches are useful to study the effect of very small changes in feed formulations on fish performance and environmental impact of aquaculture. A small change (1%) in protein digestibility, along with an improvement in phosphorus bioavailability brought by introduction of an enzyme, lead to small but economically significant changes in growth and FCR in Atlantic salmon, together with a very significant reduction in phosphorus waste production.

Therefore, we trust modelling tools are able to generate realistic predictions of concrete scenarios, including the interactions between feeding level, feed formulation and water temperature, thus being useful tools to optimize the efficiency of feed formulations and feeding regimes, while reducing environmental impacts in fish farming

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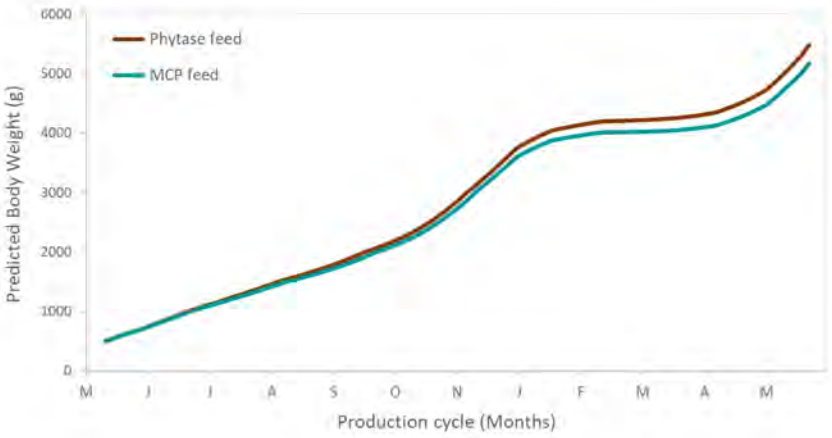

Fig. 1. FEEDNETICS™ predictions for growth in body weight in Atlantic salmon feed the phytase and MCP supplemented feeds over a period of one year.

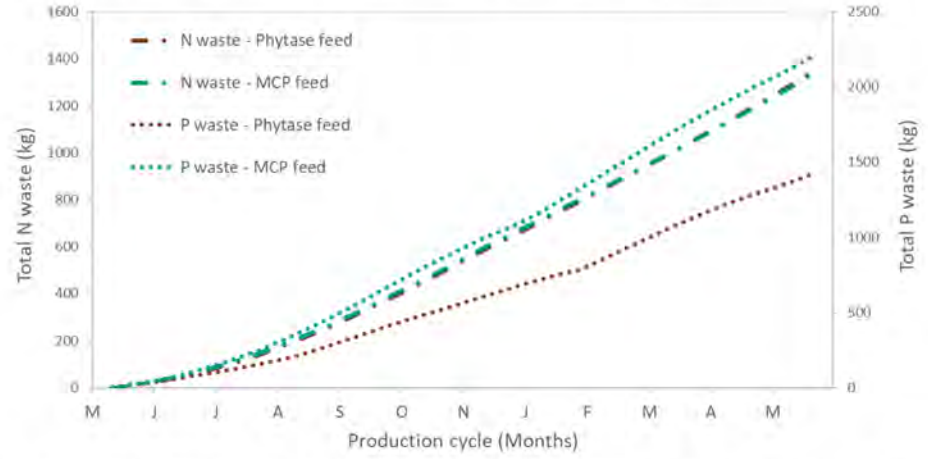

Fig. 2. FEEDNETICS™ predictions for total phosphorus and nitrogen waste production in Atlantic salmon fed the phytase and MCP supplemented feeds over a period of one year.

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Acknowledgements

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SPERMATOPHORE PRODUCTION AND SPERM QUALITY OF THE RIVER PRAWN *Macrobrachium americanum* (Bate, 1869) FED WITH DIFFERENT DIETS

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The effects of different diets on spermatophore production and sperm quality were investigated in the river prawn *Macrobrachium americanum*. Male river prawns were cultured during 244 days fed with three diets: fresh food (50% jumbo squid meat, and 50% Pacific sardine muscle), commercial pellets (Camaronina 35 Purina®), and a 50:50 mix of both diets. Spermatophore production was recorded on average every 24 days as the percentage of spermatophores produced per extraction per diet, their weight and biochemical composition. Sperm quality was measured as the total number of spermatozoa and the proportion of live/dead sperm, and normal/abnormal sperm morphology. Mean biochemical composition of *M. americanum* spermatophores was 36.3% proteins, 25.8% carbohydrates, and 4.6% lipids. Weight of spermatophores and sperm counts were not significantly different among diets, neither as a function of the male initial total length ($p > 0.05$). Male river prawn reproductive exhaustion was observed by the decline of the spermatophore production, weight of the spermatophore, and the number of spermatozoa per spermatophore with an increasing proportion of dead and abnormal sperm throughout the experiment (Figure 1). The recommended period of maintenance in captivity for male broodstock is below 115 days, after which male showed evidence of reproductive exhaustion. It is recommended to feed broodstock males of *M. americanum* with commercial pellets, because they are cheaper and easier to use than fresh food, ensuring a good spermatophore production and sperm quality

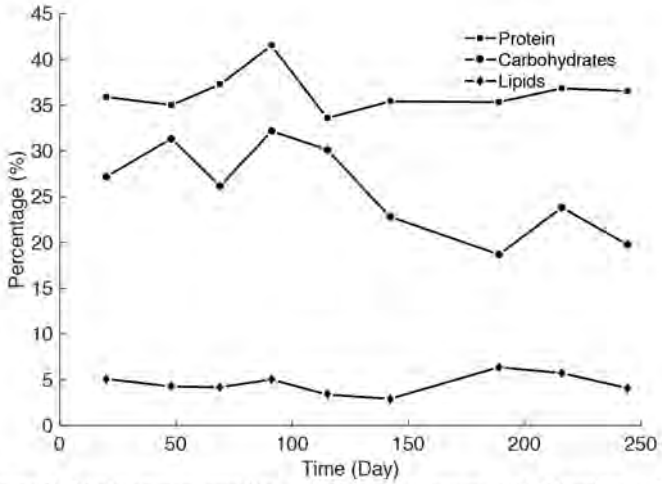

Figure 1. Male sperm quality of *M. americanum*. (A) Number of spermatozoa per spermatophore, (B) Average percentage of abnormal sperm, and (C) Proportion of dead sperm per spermatophore recorded throughout the experiment per diet.

ANCHOVY AND GIANT SQUID HYDROLYSATES CAN ENHANCE GROWTH AND THE IMMUNE RESPONSE OF EUROPEAN SEABASS (*dicentrarchus labrax*) FED VEGETABLE BASED-DIETS

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Introduction

Vegetable protein sources are considered sustainable alternatives to fish meal, but they can present anti-nutritional factors, amino acids imbalances and low palatability with concomitant negative effects on fish growth. Marine protein hydrolysates were recently shown to stimulate fish growth and immunity (Gisbert et al., 2018), but different sources and production methods of hydrolysates may cause distinct responses in fish (Martínez-Alvarez et al., 2015). The aim of the present study was to evaluate if the inclusion of marine protein hydrolysates at 3% in a plant-based diet can result in similar growth and immune status of juvenile European sea bass, *Dicentrachus labrax,* compared to a fish meal-based diet

Material and methods

Four hydrolysates developed by Austral S.A.A. obtained from anchovy (ANC) or giant squid (GS), using either a single protease (P1, endopeptidase activity, and P2, endo - and exopeptidase activities) or a protease mix (MIX) were added to a plant protein-based diet at 3%, resulting in four experimental diets: ANC-P1, ANC-P2, GS-P2 and GS-MIX. A plant protein-based diet, without hydrolysates, was used as a negative control, NC, and a fish meal-based diet as a positive control, PC. Diets were fed to triplicate groups of European sea bass juvenile (initial body weight 7.0 ± 2.0 g) allocated to triplicate tanks, and fed the experimental diets for 60 days. At the end of the trial, growth performance, nutrient utilisation, whole body composition and fish immune status were evaluated. The bactericidal and bacteriostatic activities of each hydrolysate were also assessed *in vitro*.

Results

All the hydrolysates showed bactericidal and bacteriostatic activities against some bacterial fish pathogens belonging to the *Vibrionaceae* family, but when the GS was hydrolysed by a single peptide (P2) resulted in a significantly lower killing ability against *Vibrio harveyi* compared to all other hydrolysates. The experimental diets were well accepted by fish that more than tripled their initial body weight (BW). The final BW and specific growth rate of fish fed diets ANC-P1, ANC-P2 and GS-MIX were similar to those fed the PC, and significantly higher than those fed the NC. Growth performance of fish fed GS-P2 did not differ from those fed the NC. The feed conversion ratio (FCR) was low in all dietary treatments (1.0-1.1) and did not differ among diets. Likewise, final whole-body composition, nutrient retention and gain remained similar in all diets.

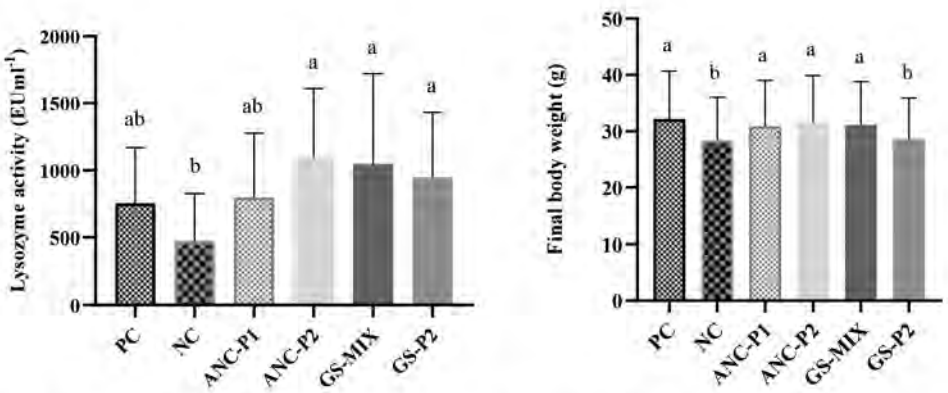

Fig. 1. Final body weight and innate immune status (lysozyme activity) of *D. labrax* after feeding the experimental diets for 60 days.

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Total blood cell numbers, haematocrit and haemoglobin concentration were not affected by the experimental diets. Fish fed GS-P2 had a mean corpuscular volume comparable to those fed the NC, but significantly lower than all other diets. Moreover, the ANC-P2, GS-MIX and GS-P2 diets led to a significantly higher plasma lysozyme activity ($P = 0.002$) compared to the NC.

Discussion and conclusions

The present results showed that the inclusion of 3% ANC or GS hydrolysates in a plant protein-based diet was effective in promoting European sea bass growth performance and innate immune status, resulting in values comparable to those obtained by using a fish meal-based diet. However, the hydrolysis process directly affects the benefits of ANC and GS hydrolysates on fish growth and immunity. For instance, the use of a single protease (P2) with both endo- and exopeptidase activities in GS translated in a decreased growth and bactericidal activity against *V. harveyi* compared to the other treatments. These results showed that the application of combined enzymes in the hydrolysis process had an important role in the production of bioactive GS hydrolysates, although that was not observed for ANC hydrolysates. The differential effects of hydrolysates on fish growth and immunity are most likely related to the mechanisms of action of food-borne antimicrobial and growth stimulating peptides (Martinez et al., 2015), suggesting a decreased content of these bioactive compounds in GS-P2 hydrolysate. In fact, the latter presented a lower proportion of high molecular weight peptides (> 6 500 Da) that might act as growth stimulators (Ho et al., 2014).

It can be concluded that hydrolysates obtained from anchovy, mainly those hydrolysed by a single protease with endo-and exopeptidase activity (P2), or from giant squid hydrolysed by a protease mix (MIX) were the most effective in promoting European sea bass growth and innate immunity. Further studies could provide more insight about the influence of distinct hydrolysis methods and hydrolysate sources on the bioactivity of hydrolysates added to fish diets

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PLANT SALE PRICE AND PRODUCTION PROFILE AS TOOLS TO IDENTIFY PLANTS WITH POTENTIAL FOR AQUAPONICS SYSTEM IN URBAN AREAS, CASE OF STUDY: JABOTICABAL, BRAZIL

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Introduction

The economic performance and suitability of aquaponics farms has contradicting views (Greenfeld et al., 2018). One point that has been identified as a key factor for the economic success of aquaponics entrepreneurs is the choice of the cultured aquatic and plant species (Bosma et al., 2017; Engle, 2015; Tokunaga et al., 2015). Furthermore, the plant production has been recognized as the main source of income in aquaponics (Adler et al., 2000; Bailey and Ferrarezi, 2017; Love et al., 2015; Quagrainie et al., 2018). To provide the highest return to the farmers, some of the variables to consider when selecting the plant species are the productivity ($kg.m^{-2}$), crop density ($plants.m^{-2}$), production period (weeks) and unit value or sale price (Bailey and Ferrarezi, 2017). Usually leafy vegetables are most cultured in aquaponics systems (Love et al., 2015; Yep and Zheng, 2019), mainly because the combination of the variables $\$.m^{-2}$.week provides a common point for comparison among crops (Bailey and Ferrarezi, 2017). Even when some crops have a higher sale price, a short culture period could be more profitable or attractive. The regional characterization of plant production systems is also a valuable information source for producers as well as for the creation of public policy (Batalha et al., 2010; Hoffman et al., 1987; Mattei, 2014). In Brazil, vegetable production comes mostly from family-owned operations with a little commercialization and small supply chain, supported mainly for a strong relationship between the confidence of the buyer and the availability of production (Celestrino et al., 2017). Given that relationship, the monitoring of vegetable sale prices brings certainty and benefits to the consumers and sellers since this increases the confidence of a fair trade. This work aimed to prospect vegetables of economic interest that have the potential to be produced in the aquaponics system, based on a variety of prices and technical production data collected from local markets and farms in Jaboticabal, Brazil.

Material and Methods

Located in São Paulo State, Brazil, the municipality of Jaboticabal has an estimated population of 76,196 (IBGE 2016). A survey focused on retailers and urban plant producers was carried out in November of 2018. Six retailers were selected (one supermarket chain, two individual supermarkets, two plant retailers and one farmers market) to collect the prices of 42 varieties of herbs, fruits and vegetables for six months, from November 2018 to April 2019. Nominal prices were collected and transformed to real prices using the General Price Index (IGP- DI by its initials in Portuguese) of Getúlio Vargas Foundation (FGV) by using May 2019 as the base year and afterwards they were converted to US dollars (US$1 = R$3.92). Given the real sale price for each plant variety, the real average price was calculated and with the average of the six months, the plants were grouped into five categories. To categorize the plants, the highest and lowest price were identified and five equidistant categories between those values were established. Regarding the urban plant producers, to access the farm production, data were collected with a questionnaire. For each farm were collected: the cultured plant, production area, monthly productivity, average production cost, production cycle duration, number of months of the year in which the production can be done, and frequency of occurrence for the vegetable in the farms. Data were standardized and afterwards a cluster analysis was performed.

Results

The prices of 42 brands of plants (herbs, vegetables and fruits) were collected. A total of 25 plant varieties were identified as feasible to be produced in aquaponics. The plant prices were from US$0.66 $packet^{-1}$ for watercress, to US$2.57 $packet^{-1}$ for "sul"strawberry. However, the majority of the plant prices (66.7%) presented a price lower than US$1.42 $packet^{-1}$ (Table I)

According to the data obtained from urban farms, 15 vegetable species are being produced in the urban zone of Jaboticabal, classified into four groups. Group 1 consisted of four vegetables that have larger productivity (average 2.46 packets. $month^{-1}$) and short growing cycle (46 days). Group 2 comprised of vegetables cultured in a small area in the farms (average 25.25m^2) and produced throughout the year (except for coriander which is produced only for ten months), some of the

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vegetables of this group showed high productivity, such as Oakleaf lettuce (average 3.17 packets m^{-2}) and spinach (average 2.83 packets m^{-2}). The iceberg lettuce stands out because it has the largest cultured area in the farms (average 610m^2), it is produced throughout the year and thus, it is separate from all the other vegetables in the group 3. In the group 4, the vegetables have high culture area in the farms (average 401.56 m^2), the production can be done throughout the year, production cost less than US$0.19 per packet and the productivity is low (average 1.39 packet m^{-2}) (Table 2).

Discussion and Conclusion

From the 15 vegetable species that are being produced in the urban area of Jaboticabal, spinach has the highest average sale price (average US$1.45 $packet^{-1}$), while the others show average prices between US$0.66 $packet^{-1}$ and US$1.42 $packet^{-1}$. The production of these vegetables in the region shows that they are adapted to the climate conditions, there are production input suppliers and a market that could be advantageous by showing the potential of these vegetables for use in the aquaponics system. However, the low price of some of the plants is a point to be considered and could be unfeasible in an aquaponic farm. Plants of high value that are commercialized but are not produced in local farms have production potential in aquaponics systems given that the existing demand. These factors are important when considering that the aquaponics system requires high investment for construction and maintenance of the system. The plants with high potential for aquaponics in urban Jaboticabal are those in groups 3 and 4, which have prices over US$1.43 $packet^{-1}$ and over the average, and a good market.

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ARGININE IMMUNONUTRITION IN FISH: A DOUBLE-EDGED SWORD?

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Introduction

The concept of maintaining animal health through the best possible nutrition is well accepted in modern animal farming and functional additives appear to be good candidates to improve health and survival. Amino acids (AA) in particular have known roles in the improvement of the immune response to infection and recent evidence indicates that several immune mechanisms are influenced by their availability in fish (Conceição et al., 2012; Herrera et al., 2019). Still, the potential use of dietary AA supplementation for animal health management is not fully developed. This is particularly important for the aquaculture industry, where few therapeutic possibilities are available against infectious episodes.

Arginine in particular appears to have important roles regarding immune modulation since it is required for macrophage responses and lymphocyte development. For instance, dietary arginine supplementation has been shown to enhance the innate immune system of the Senegalese sole (Solea senegalensis) (Costas et al., 2011). Here, it is intended to give a comparative overview of our recent research using arginine surplus as a tool to improve the health status of farmed fish. Therefore, two independent studies with the same experimental set up aimed to assess whether an increased availability of dietary arginine can improve the immune status of European seabass (Dicentrarchus labrax) and gilthead seabream (Sparus aurata).

Materials & methods

European seabass and gilthead seabream juveniles (8.4 ± 0.4 and 23.1 ± 0.4 g, respectively) were fed 3 experimental diets. A control (CTRL) diet was formulated to meet the indispensable amino acids profile established for both species. Based on this formulation, two other diets were supplemented with DL-Arginine at two different levels (i.e. 0.5 % and 1 % of feed, ARG1 and ARG2, respectively). Fish were fed these diets in triplicates for 2 or 4 weeks until visual satiation under controlled conditions. Blood samples were withdrawn from the caudal vessel at the end of each feeding period for the evaluation of the haematological profile. Blood smears were prepared for differential cell counting. Blood was finally centrifuged and plasma samples were collected and stored at -80 °C until assayed. Head-kidney tissue was also collected for the assessment of immune-related gene expression and kept at -80 °C until processed.

Results

Seabass trial
Fish showed a time-dependent decrease in peripheral lymphocyte numbers regardless of dietary treatment, whereas plasma peroxidase values dropped in time in seabass fed ARG1 and ARG2 and was lower at 4 weeks in fish fed ARG1 than in fish fed CTRL. At the head-kidney level, a slight modulation of the immune status was observed, with some genes pointing to an immune-suppressive state, such as the decrease in matrix metalloproteinase 9 in fish fed ARG2 compared to those fed CTRL.

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Seabream trial
The bactericidal activity increased in fish fed the highest supplementation level after 4 weeks. A tendency to increase monocytes relative proportion of peripheral blood leucocytes was observed in fish fed the ARG2 diet after 2 weeks of feeding, compared to their counterparts fed the lower supplementation level. Peripheral monocyte numbers also correlated positively with plasma nitric oxide levels, which showed an increasing trend in a dose-dependent manner. At the head-kidney level, the mRNA expression of the colony stimulating factor 1 receptor tended to be up-regulated at the final sampling point regardless of dietary treatments.

Discussion

As in other vertebrates, a balanced diet is a key factor for an adequate performance including immune response and disease resistance. However, the present study showed opposite effects regarding the fish immune status. Although the observed inhibition of cellular and humoral defences in seabass fed arginine supplemented diets seemed to be somehow faint, data suggested that arginine supplementation could compromise at some extent the seabass immune response. In contrast, results from the seabream trial suggested that dietary supplementation with arginine may have an immunostimulatory effect after 4 weeks of feeding.

Although more health-related biomarkers are being processed for both trials, data point to opposite effects in terms of arginine immune-modulatory effects depending on the species. Having this in mind, further studies should be developed to unravel this double-edge sword effect regarding arginine surplus in diets for these important species for Mediterranean aquaculture.

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EFFECTS OF SHORT-TERM FEEDING MICRO- AND MACRO ALGAE SUPPLEMENTED DIETS IN INNATE IMMUNITY AND OXIDATIVE STATUS OF *Sparus aurata* JUVENILES

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Introduction

An activated immune system has specific nutrient requirements and increases the competition between nutrients available for maintenance purposes, functioning of the immune system and for body protein deposition in growing animals. In this context, algae have gained a lot of attention as a source of biomass and biomolecules for feed purposes, sine these specialties contain bioactive compounds, or phytochemicals, that may benefit health beyond the role of basic nutrition (Wells et al., 2017). For instance, microalgae exploited at the industrial scale are of increasing interest as a source of valuable compounds such as extracellular polysaccharides, phycobiliproteins and long chain PUFAs (Gaignard et al., 2019). However, microalgae biomass is hardly exploited commercially as aquafeed components, primarily due to their unavailability in large volumes and high price of the marketed products. Therefore, research efforts should be directed to explore the potential of low levels of dietary algae biomass inclusion in animal health.

This study aimed to evaluate the effects of short-term feeding with marine macro and microalgae biomasses on gilthead seabream (*Sparus aurata*) health status.

Materials and methods

Six isonitrogenous (45% protein) and isolipidic (18% fat) diets were formulated. Diet A consisted of a commercial based diet and served as control whereas five other diets consisted of diet A with a 2% inclusion of different algae: Diet B (*Tetrasselmis striata*); Diet C (*Phaeodactylum sp.*); Diet D (*Nannochloropsis sp.*); Diet E (*Gracilaria gracilis*); Diet F (*Ulva rigida*). Diets were randomly assigned to triplicate groups of 110 fish/tank (IBW: 11.7 ± 1.0 g) and fed to satiation twice a day. Fish were maintained in a recirculated seawater system (temperature 22.4 °C; salinity 35.2 ‰; photoperiod: 12 h light: 12 h dark). After 1, 2 and 4 weeks of feeding, 4 fish/tank were sampled for blood, plasma and tissues collection (i.e. head-kidney, liver and gut) for further analyses according to Machado et al. (2015).

Moreover, 15 fish/treatment were also subjected to an inflamm tory insult by intraperitoneally injecting heat inactivated *Photobacterium damselae piscicida* following 1 and 2 weeks of feeding and sampled at 4 and 24 h post-injection. Blood, plasma and liver and gut tissues were collected.

Results and discussion

Total red blood and white blood cells remained unchanged throughout the feeding trial. Plasma bactericidal and anti-protease activities decreased over time while IgM levels followed an opposite trend, increasing from week 1 to the end of the trial. Since fish were not challenged, results showed changes over time, most likely related to fish normal growth and development. At the end of week 1, fish subjected to the inflammatory insult showed a decrease in plasma bactericidal activity at 4 and 24 h when fed diets C and F, respectively. Plasma anti-protease activity also decreased after 4 h post-injection in fish fed diet F. After fish stimulation, preliminary results suggest a possible immune-modulatory effect of dietary treatments after one week of feeding.

Further analyses are currently on-going to obtain a more accurate picture of fish immune response and oxidative status attributable to the different dietary treatments.

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CRYOPRESERVATION OF FISH EMBRYOS: THE USE OF MELATONIN AS AN INHIBITOR OF THE APOPTOTIC PROCESS

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Introduction

Despite concerted efforts the cryopreservation of fish embryos has remained a challenge, the development of this technique would significantly contribute to the genetic conservation of endangered species and lineages of interest, facilitating the exchange of genetic material between stocks, as well as enable the year-roundproduction of fish species including those with seasonal reproductive cycles (Digmayer, 2013). The current difficulties in cryopreservation of fish embryos are due tolack of ideal cryoprotectants and necessary advances in related technique to overcome the challenges of relatively large fish embryo size; complex compartmentalization with different properties and permeabilities that hamper the flow of cryoprotectants and water; presence of fat-rich yolk sac and associated susceptibility to intracellular ice formation (Zhang and Rawson, 1995; Lahnsteiner, 2008). Although, recent advances in vitrification techniques are showing promise, the formation of reactive oxygen species (ROS) and apoptotic events triggered during the process are posing considerable hindrance. In this context, the main objective of this study was to determine the use of melatonin hormone, an important inhibitor of ROS formation, in the vitrification of zebra fish embryos *Danio rerio*).

Materials and methods

Four treatments were performed: Control group (non-cryopreserved embryos, without melatonin); Treatment 1 (cryopreserved embryos, without melatonin); Treatment 2 (cryopreserved embryos + 10^{-6}M of melatonin) and Treatment 3 (cryopreserved embryos + 10^{-3}M of melatonin). The vitrification solution as described by Keivanloo and Sudagar (2013) was used as a basis and adapted following toxicity tests. The embryos used were between developmental stage of 22 to 24 somites and had their chorions removed. After cryopreserving embryos for seven days, 30 embryos per treatment were thawed and incubated to evaluate the possible hatching rate. In parallel (immediately post thaw) four embryos per treatment to quantify the relative expression of keygenes involved in apoptotic processes (*Bcl-2* - Anti-apoptotic; *Bax/Caspase-3* - Pro-apoptotic); ten embryos per treatment for quantification of ROS (molecular probe 2′,7′-dichlorofluorescin diacetate); ten embryos per treatment for the study of DNA fragmentation (TUNEL method); and six embryos per treatment for the study of cryo-lesions by scanning microscopy; totaling 240 embryos were analyzed. For the parameters of hatching rate, quantification of ROS and DNA fragmentation, a factorial analysis (Shapiro-Wilk at 5% of significance) was performed, and the significant differences were evaluated by the Tukey test at 5% significance, using the SAS statistical analysis program. For the relative quantification of gene expression, the method $^{-\Delta CT}$ was used.

Results

The control group had an average survival rate of 92.30 ± 4.90%, on the other hand, 100% of the vitrified embryos entered apoptosis after thawing. In these embryos, the most observed cryo-lesions were: vitelynic synchicial layer (VSL) with irregular and/or ruptured forms, invaginations and wrinkling or perforations in the epidermal layer of the embryos. Regarding the relative expression of the genes, *Bax* and *Bcl-2* presented lowerlevels of expression for the control groupof embryos, without cryopreservation. Among the cryopreserved treatments, the inclusion of 10^{-6}M of melatonin improved the results obtained ($p<0.05$) both for the anti-apoptotic (*Bcl-2*) and for the pro-apoptotic (*Bax/Caspase-3*) genes. The analysis of ROS formation showed that the control treatment, that were not cryopreserved, had a lower percentage of affected surface area of the embryo (3.80 ± 0.40%) when compared to Treatments 1 (one) (25.59 ± 8.80%), 2 (34.45 ± 7.90%) and 3 (26.64 ± 12.47%), most of the observed fluorescence was located in the region of the VSL. The cryopreserved embryos with the addition of melatonin, treatments 2 (53.93 ± 18.50%) and 3 (45.41 ± 10.20%) showed a significant reduction in DNA fragmentation($p<0.05$) compared to treatment 1 (one) (cryopreserved/without Melatonin - 79.74 ± 1.00%), and were statistically compare to the control group (47.47 ± 10.82%).

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Discussion and conclusion

It was clear that the vitrification solution used was not able to adequately protect all the regions of the embryos, especially the VSL as was also observed by Ninhaus-Silveira (2006), who identified this structure as one of the main obstacles to the successful cryopreservation of fish embryos, because it has extremely sensitive to cryoinjury compared to the rest of the embryonic tissues. Based on favorable expression of the apoptotic genes in embryos cryopreserved with melatonin (10^{-6}M) infused cryoprotectants, it can be inferred that melatonin may be involved in a process of supra regulation that affects negatively the cells. Felici et al. (1999) obtained similar results in fetal primordial germ cells of mice. The increased formation of ROS in the cryopreserved embryos evidences once again the inefficacy of the vitrification solution. As for the analysis of DNA fragmentation, the significant reduction in the presence of fragmented DNA in cryopreserved treatments with melatonin (Treatments 2 and 3), being comparable to the non-cryopreserved embryos of the control group, can be attributed to theuse of this hormone. In general, the inclusion of 10^{-6}M of the melatonin hormone in the vitrification solution of zebrafish embryos significantly improved the response to the post-thaw apoptotic process, especially the expression of the genes involved in apoptotic process and DNA fragmentation. These results will contribute to the future development of an appropriate protocol for the vitrification of fish embryo

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TOWARDS A REVISED CLASSIFICTION SCHEME DESCRIBING BENTHIC IMPACTS OF MARINE AQUACULTURE BASED ON SEDIMENT GEOCHEMISTRY

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Introduction

The vertical flux of organic matter (waste feed and faeces) at finfis and shellfish aquaculture farms has a known effect on benthic habitat and microbial and faunal communities. Organic matter deposition stimulates benthic aerobic metabolism and anoxic conditions are created in surficial sediments if oxygen consumption exceeds resupply across the sediment-water interface. Under anoxic conditions, sulfate reducing bacteria continue to decompose the organic matter and produce hydrogen sulfide. H_2S and ionization products (HS^- and S^{2-}), collectively referred to as total free sulfides (TFS), are toxic to benthic organisms. Organic enrichment impacts at marine aquaculture farms in Canada, the US, New Zealand and elsewhere are assessed, at least in part, by monitoring TFS concentrations in sediment and comparison of these data against a classification scheme that indicates benthic impacts (Hargrave et al., 2008). However, recent research has shown that the regulatory TFS protocol (ion-selective electrode; ISE), on which the classification scheme was derived, provides biased results owing to the inclusion of nontoxic mineral sulfides and/or oxidation and volatilization of TFS prior to analysis (Brown et al, 2011; Cranford et al. 2017). A new classification scheme will be presented that is based on TFS data obtained with a more reliable protocol that is analytically robust and practical for rapidly measuring TFS concentrations in the field.

Materials and methods

Surficial sediment samples were collected at different distances (0 to 1000 m) from four Atlantic salmon, one trout and one mussel farm in Canada, and five chinook salmon farms in New Zealand. Sampling in Canada employed a core sampler and a grab sampler was used in New Zealand to collect multiple sediment samples at each location. Macrofauna samples (92 cm^2 surface area) were screened through a 0.5 mm mesh and preserved in formalin. Pore-water was extracted for analysis of TFS concentrations using RhizoCera® samplers connected to a syringe. RhizoCera were inserted through the side of core samples at 1 and 2 cm depth below the sediment/water interface and directly into the surface of grab samples to a depth of 2 cm (Fig. 1). Approximately 1 mL of pore-water was extracted under gentle suction to fill and flush the inside of the RhizoCera tubes. 100 µL of water was removed from inside each RhizoCera with a syringe for immediate TFS_{UV} analysis onboard the boat according to the ultraviolet spectro-photometric method (Cranford et al. 2017). In Canada, additional subsampling was conducted for analysis of grain size, dissolved oxygen, total organic content, and TFS based on the standard ISE protocol (TFS_{ISE}). The New Zealand samples were also analysed for TFS_{ISE} and redox potential.

Results and discussion

The elimination of particulates and prevention of pore-water exposure to the atmosphere resolved any possibility for mineral sulfide contamination or loss of TFS through volatilization or oxidation. Comparison of TFS_{UV} and TFS_{ISE} results from New Zealand show the magnitude of errors caused by the known issues with the ISE method (Fig. 2a; also see Cranford et al. 2017). This is supported by comparing redox potential and TFS data (Fig. 2b). Sulfide reduction is associated with negative redox values (Brown et al, 2011) and this is consistent with the TFS_{UV} results. However, the TFS_{ISE} method often gave high TFS values under positive redox conditions; indicating contamination with iron sulfides and pyrite as previously suggested (Brown et al. 2011). The benthic classification scheme based on the ISE approach will exhibit a similar bias. At the time of abstract preparation, the infauna taxonomy was nearing completion. These data will be analysed to determine relationships between organic enrichment indicators (macrofauna diversity, TFS_{UV} concentration, organic content, redox, etc.) for each aquaculture setting and bottom type towards revising the organic enrichment classification scheme.

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Fig. 1. Extraction of pore-water from grab and core samples using RhizoCera/syringe samplers. Dissolved oxygen (right) and redox (left) probes are also shown.

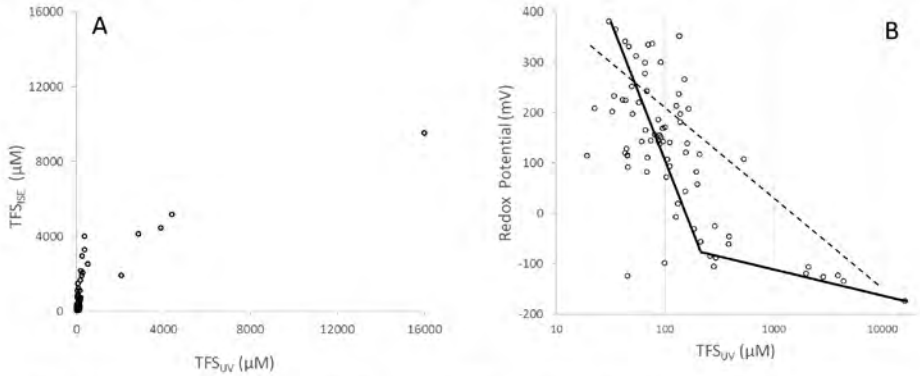

Fig. 2. Relationships between TFS_{ISE} and TFS_{UV} (A) and redox and TFS_{UV} (B) at New Zealand farms. The broken line is the regression between redox and TFS_{ISE}.

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THE OPPORTUNITIES OF PRODUCING FRUITY VEGETABLES IN AN AQUAPONIC SYSTEM ON COMMERCIAL SCALE

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Introduction

Vegetable production in aquaponic systems on a commercial scale is a challenge, but can be achieved whenever optimal growing conditions for both fish and vegetables are realised. This means fish waste water cannot be used as such but water parameters as pH, nutrient content and total EC have to be adjusted to the demand of the plant. These ideal growing conditions can only be obtained in a decoupled recirculating aquaculture system (dRAS). The past years, experience was gained with the culture of tomato, cucumber and sweet pepper in a decoupled aquaponic system. The aim of the research was to determine the sodium tolerance of these vegetable crops and so the potential of growing these vegetables in an aquaponic system. Sodium can be a problem in an aquaponic production system as it is essential for fish but not for plants. In a recirculating hydroponic growing system, used for vegetable production, the accumulation of sodium in the nutrient solution can have a negative effect on yield and quality. Therefor the choice of fish in the system is very important and preference should be given to a vegetarian fed fish as feed and therefor waste water from these fishes have a low sodium content.

Material and methods

A 400 square meter greenhouse for fruity vegetable cultivation is connected to a fish stable, this way forming an aquaponic system. In the greenhouse 11 rows of plants are grown in a hydroponic production system. The two outer rows are buffer rows, the nine inner rows are each individually connected to one of the nine fish tanks in the fish stable, forming 9 individual aquaponic systems. Each fish tank has its own drum filter and biofilte . While rinsing the drum filte , waste water is produced which is used for the preparation of the nutrient solution for the vegetable plants in the decoupled aquaponic system. Before using this water for the cultivation of vegetables, the water parameters are optimized, meaning the pH is adjusted to a correct value for vegetables and the electrical conductivity (EC) is increased by adding the necessary nutrients. After using this nutrient solution for fertigation of the vegetables, the drain water is collected for each row individually and recirculated. There is no backflow from the plant production to the fish. The fish used in this aquaponic system is Omega perch (*Therapon barcoo*), sourced from the neighboring fish farm Aqua4C. Omega perch is a fish that can be produced on a vegetarian diet, meaning the sodium content of the feed can be kept on a low level, avoiding the postulated negative effects of sodium on the cultivation of vegetables.

For each trial, the same three objects were included: one object where the nutrient solution was prepared with rain water (common practice), a second object where waste water from fish production was used to prepare the nutrient solution and a third object where extra sodium was added to the nutrient solution prepared with rain water, to test the sodium tolerance of the crop. During the cultivation period several parameters were intensively monitored. Yield and quality of the fruits were registered every harvest and plant growth, stem diameter and fruit dimension were measured every two weeks. Next to that, every 2 weeks a water analysis was conducted on fish waste wate , drain water and nutrient solution.

Results

Results of the do’s and don’ts in commercially growing tomato, sweet pepper and cucumber in aquaponics will be communicated during the conference as the trials are still in progress.

MICROALGAE DYNAMICS IN A CLOSED FARMING SYSTEM IN THE SEA FOR ATLANTIC SALMON

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Introduction

The global production of farmed Atlantic salmon exceeds two million tons, Norway being the main contributor (FAO, 2016). Currently, most of this production occurs in net-pens in coastal areas where environmental concerns, such as sea lice, diseases and escapes of farmed fish are hindering increased production. Prolonging the time fish spend in closed-containment systems (CCS) before they are transferred to open sea cages is a potential solution which could help resolve the mentioned sustainability issues. In recent years, besides keeping fish longer in land-based facilities the use of CCS in the sea has become increasingly popular in Norway. These facilities commonly pump in deep water to avoid surface layers where sea lice are the most abundant. In traditional net-pens the exchange rate of water is generally high as water is moved by currents through the farming site, in CCS there are normally only one or a few water/waste outlets hence the water exchange rate is lower. This increases the risk of accumulation of waste and pathogenic microorganisms in CCS, such as harmful microalgae. However, taking in deep water could also reduce the risk of harmful microalgae as they may be more abundant in surface waters. In open net-pen culture it is known that blooms of harmful algae (HABs) can lead to significant fish losses (Berdalet et al. 2016; Landsberg et al. 2005), however very little is known about the microalgae communities in CCS pumping in deep water and the potential risk for HABs in these systems. To understand more about microalgae (MA) dynamics in CCS, data from two monitoring programs at a CCS site in south-western Norway were analysed.

Materials and methods

The CCS prototype involved in the monitoring program is situated in the Hardangerfjord in south-western Norway. The water is taken in from 26m depth, to avoid the sea lice belt, and the total volume of the CCS is 21 000m^3. The data analyzed are from two monitoring programs from the time period 2017- 2019. The programs typically started when smolts were transferred to the CCS from a land-based smolt farm normally in October or November and ended when fish were transferred from the CCS to an open net-pen site in April. Samples for MA analysis were collected every other week in Oct., Nov. and in Feb. to April, in the winter (Dec. and Jan.) samples were collected monthly. Samples were collected with a standard water sampler from 3m depth in the CCS prototype and from 3m depth upstream of the prototype (reference station). 25 ml water samples were fixed with Lugol iodine on site and sent to the lab where they were analysed following the Utermöhl technique. Samples were settled for 24h in composite sedimentation chambers and the organisms were counted to the species level when possible using an inverted microscope.

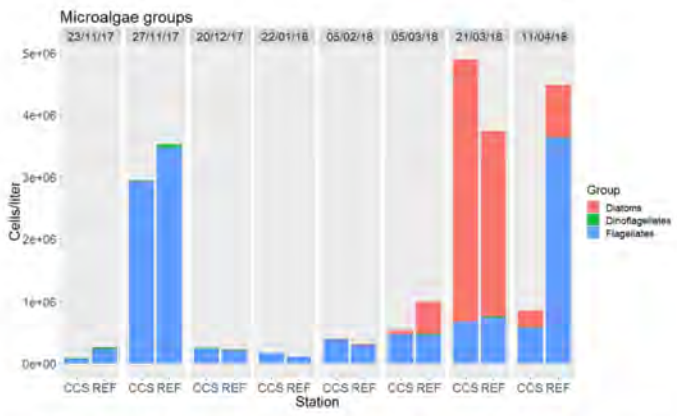

Fig 1. Total number of cells (cells/liter) of the principal microalgae groups (Diatoms- in red, Dinoflagellates- in green, Flagellates- in blue) in the closed containment system (CCS) and at a reference station (REF) during monitoring program 1 (Nov 2017-April 2018).

(Continued on next page)

Results and discussion

Monitoring program 2 (Oct. 2018-May 2019) is currently still running hence these results will be presented at the conference. A comparison between monitoring program 1 and 2 will also be made at the conference. Following are the preliminary results from monitoring program 1 (Nov. 2017 until April 2018). In general, the number of MA cells and the total biomass were low in the period Nov. to Feb. (max 10^6 cells/liter; biomass 30µgC/liter) both in the CCS and at the reference station (REF). The spring bloom of diatoms started slightly earlier at the REF (fig. 1), peaking the 23th of March, when a similar bloom was observed in the CCS. In April 2018 the number of MA cells and the total biomass was lower in the CCS compared to the REF. Overall, the composition of MA by taxonomic group (diatoms, dinoflagellates and flagellates) was similar between the CCS and the REF at most sample points, with flagellates dominating throughout the monitoring period except in March when diatoms were more abundant (fig. 1). *Skeletonema spp*. was the dominating diatom in both the CCS and the reference station sample in the March bloom. *Skeletonema* spp. has long silica spines that protrude outward from the cell, that can irritate and potentially damage the gills of fish. There has been at least one case were species of *Skeletonema* have been associated with fish mortality (Kent et al. 1995). At what concentration certain diatom species become gill irritants is very difficult to conclude from the available scientific literature. This is because most of the studies are not based on experiments, but on field observations where one has little control over other potential factors that may have contributed. However, we believe it is unlikely the concentrations observed in the monitoring program had a negative effect on the fish since Skeletonema spp is a very common species that regularly occurs in high numbers during the spring bloom in Norway.

Heterosigma akashiwo, *Dichtyocha speculum* and *Prymnesiales* spp. were detected at several sampling points in the monitoring program. These species are known to be toxic to fish (Davidson et al. 2011; Henriksen et al. 1993; Johnsen et al. 2010; Moestrup 1994). However, these were mainly observed at the reference station and in very low concentrations.

In conclusion the results from monitoring program 1 indicate that overall the number of MA cells, the total MA biomass and the number of toxic MA may be lower in CCS compared to the surface waters that fish in a traditional net-pen are exposed to. These results will be interesting to compare to the results from the ongoing monitoring program 2.

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EFFECT OF STARVATION AND RE-FEEDING WITH DIFFERENT DIETARY PROTEIN LEVEL ON SOME HEMATOLOGICAL PARAMETERS OF JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss,* WALBAUM, 1792)

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Introduction

Both in the wild environment and in the aquaculture, fish may be starved when, due to a variety of reasons, such as the occurrence of diseases or changes in the environmental conditions. In aquaculture, compensatory growth can be seen as a management tool to reduce the cost of fish feeding (Guzel et al., 2011) or as a measure to improve water quality (Turano et al., 2008) by controlling the production of residues resulting from overfeeding the fish (Davis et al., 2011). During starvation periods, fish may face various physiological responses to cover their metabolic needs. Rainbow trout is a carnivorous fish, and the necessity of crude protein from diets is between 40 to 50 %, making the cost of feeding very high. In this context, in order to reduce these costs, starvation followed by subsequent feeding of fish, without negatively affecting their growth performance and physiological state can be used as a management tool. In his context, the aim of this experiment was to analyze the effect of applying of cyclical short periods of starvation (2 days and 4 days) on the hematological profile of rainbow trout

Materials and methods

Experimental design. This experiment lasted for 46 days and was carried out in the facility of the University "Dunărea de Jos" from Galați, Faculty of Food Science and Engineering, Department of Food Science, Food Engineering, Biotechnologies and Aquaculture, România. 228 trout (initial weight 111.93±15.76g) were randomly distributed in twelve rearing units (water volume, 132L) of a recirculating aquaculture system. Six treatments with duplicate were assigned, as follows: two control groups, feed daily, ad libitum, with commercial pellets containing 41% crude protein (D41) and 50% crude protein (D50); two groups starved for 2 days (D2) and then fed with commercial pellets with 41% crude protein (D2/41), respectively 50% crude protein (D2/50) and two groups starved for 4 days (D4) and then fed with commercial pellets with 41% crude protein (D4/41), respectively 50% crude protein (D4/50). At the beginning of the experiment the fish from the fasted groups (D2/41; D4/41; D2/50 and D4/50) were deprived of food for two and four days, respectively. After these days, the starvation groups were fed until their food consumption did not differ from the food consumption of the control's groups more than 10% (calculated as the average of three previous consecutive feeding days). This feeding regime was previously demonstrated to elicit super-compensation of growth in the case of *Lepomis cyanellus*×*Lepomis macrochirus* (Hayward et al., 1997).

Blood sampling and biochemical analysis. In order to evaluate the physiological state of the fish, at the end the experiment ten fish were randomly selected and anaesthetized with 2-pnenoxiethanol and 1 ml of blood was collected by caudal vein punction. Red blood cells (RBCx10^6/μL) were determined by dilution with Vulpian diluting solution and counted in a haemocytometer Neubauer. Haemoglobin (Hb, g dL^{-1}) was determined by the cyanometahaemoglobin colorimetric method using Drabkin reagent and then read at spectrophotometer Spectrocord Analytikjena, at a 540-nm wavelength. The hematocrit (PCV, %) was determined in duplicate using heparinized capillary tubes centrifuged 5 minutes at 12000 rotations $minute^{-1}$ at Hettich Hematocrit 210 Centrifuge. The mean corpuscular volume (MCV, μm^3), the mean corpuscular hemoglobin (MCH, pg) and the mean corpuscular hemoglobin concentration (MCHC, g dL^{-1}) were calculated according to Ghergariu et al., 1985.

Table I. The hematological profile of rainbow trout at the end of the experimental period

Exp. Variants	*Hematologixcal parameters (X±SD)*					
	PCV (%)	Hb (g/dl)	RBC x10^6/μl	MCV (μm^3)	MCH (pg)	MCHC (g/dl)
D_{41}	38±0.63	9.48±0.22	1.06±0.04	358.84±14.63	89.60±4.93	24.96±0.39
$D_{2/41}$	38.17±0.41	8.74±0.42	0.98±0.05	392.13±21.55	89.72±4.81	22.90±1.16
$D_{4/41}$	38.33±1.37	8.35±0.29	0.90±0.05	425.92±18.50	92.83±5.17	21.80±0.83
D_{50}	37.17±0.75	9.53±0.36	1.09±0.02	342.63±10.25	87.85±4.37	25.64±1.02
$D_{2/50}$	37.67±0.52	9.35±0.23	0.99±0.04	380.19±12.33	94.41±3.15	24.84±0.79
$D_{4/50}$	37.83±0.41	9.23±0.25	0.88±0.11	436.82±15.48	106.76±15.62	24.40±0.62

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Results

The effect of application of cyclical short periods of starvation (2 days and 4 days) and re-feeding with different protein level for rainbow trout is presented in Table I.

Starvation and re-feeding with different dietary protein level had no significant ($p>0.05$) effect on some hematological parameters including PCV, MCV, MCH, while Hb, RBC and MCHC registered significant differences ($p<0.05$). Significantly higher ($p<0.05$) concentration of hemoglobin was observed in the case of fish fed with higher protein content, while the increasing of the starvation period led to a significant decrease of the hemoglobin concentration. Furthermore, starvation and subsequent feeding led to a significant decrease of the erythrocyte number with the increasing of the starvation period. Generally, the decrease of the number of erythrocytes in the period of starvation is associated with the reduction of oxygen by the control of hematopoiesis, and implicitly the decrease of the metabolic activity.

Discussions and conclusion

From the overall analysis of hematological parameters, it can be concluded that the change of the hematological values occurred as a reaction of adaptation of the fish organism to technological conditions. Thus, the significant reduction in the number of erythrocytes and the amount of hemoglobin associated with starvation has implicitly led to a significant increase in mean red blood cell volume (MCV) and decreases in mean hemoglobin (MCHC), which means the occurrence of hemoconcentration, which may be a consequence of water loss in the body during the period of starvation (Weinberg et al., 1972).

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TESTIS DEVELOPMENT AND ULTRASTRUCTURAL FEATURES OF SPERMATOGENESIS IN BURBOT *Lota lota*

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Introduction

Generally, spermatogenesis of teleost varies depending on species and inhabited environments. The burbot *Lota lota* is the only freshwater member of the cod family, Gadidae, living in cold waters (Scott and Crossman, 1973; McPhail, 1997). Almost no report is available on gonad development and ultrastructure of spermatogenesis in burbot. Therefore, aim of the current study was to investigate testis structure and ultrastructure of germ cells during spermatogenesis using transmission electron microscopy.

Materials and methods

The testes were cut into small fragments and fixed for 2 h in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and stored at 4° C. Thereafter, the material was post-fixed for 1–2 h in 1% osmium tetroxide in the same buffer, dehydrated in a graded ethanol series and subsequently transferred to ascending concentrations of acetone. Ultrathin sections were cut with ultramicrotome, stained with uranyl acetate and counterstained with lead citrate for later examination using a JEOL Ltd. transmission electron microscope.

Results

The testis of the burbot was tubular and the germ cells were arranged in cysts or clusters within the seminiferous lobules. The differentiation of spermiogenesis stage (spermatids) in this species was characterized by flagellum development, nuclear rotation, nuclear fossa formation, and excess cytoplasm elimination. Spermatozoon ultrastructure revealed that it is anacrosomal teleostean type with round head consisting oval heterogeneously electron dense nucleus. The distal centriole and proximal centriole constituting the centriolar complex were oriented incompletely perpendicular to each other.

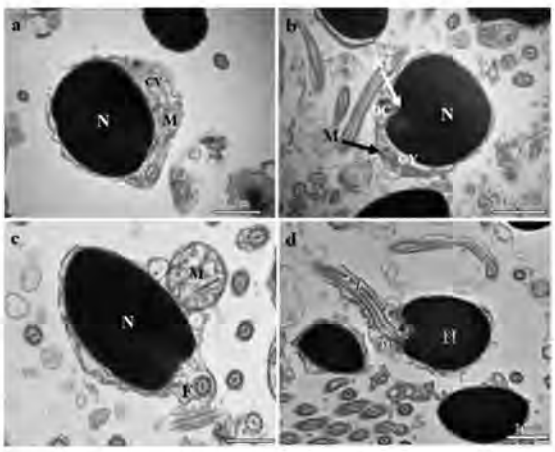

Fig 1. (a) Transmission electron micrograph (TEM) of newly formed spermatids in *Lota lota* showing regular distributed electron-dense nucleus; (b) TEM of spermatid at the second stage of development displaying distal centriole and nuclear fossa (white arrow); (c) The third stage of spermatid development and large mitochondria showing flagellum formation; (d) TEM of mature spermatozoon showing joint centriolar complex (proximal and distal centrioles) in the nuclear fossa (black asterisk) and fully developed flagellum. CY, cytoplasm; DC, distal centriole; F; flagellum; M, mitochondria; N, nucleus; Nf, Nuclear fossa.

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Discussion and conclusion

The burbot gonad is tubular anastomosing type with an unrestricted distribution of spermatogonia. This pattern of gonad is in line with identified structure in zebrafish *Danio rerio* (Rupik et al., 2011). The stages of spermiogenesis in burbot are similar to streaked prochilod *Prochilodus lineatus* (De Melo Dias et al., 2017). However, spermiogenesis has shown to proceed to four stages in zebrafish (Rupik et al., 2011).The results of this study provide an opportunity for better understanding of the structure of testis and characterization of the spermatogenesis in burbot.

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FLESH QUALITY OF ATLANTIC SALMON SMOLTS REARED AT DIFFERENT TEMPERATURES AND PHOTOPERIODS

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Introduction

Flesh quality is a complex set of characters involving factors such as texture, chemical composition, color and fat content (Fauconneau et al. 1995). The impact of temperature and light on these mechanisms depends on the affected life stages, as reviewed by Rowlerson and Veggetti (2001). The effect of season may overshadow endogenous rhythms and affect quality (Roth et al., 2005). The aim of this study was to study the combined effect of two photoperiod regimes, continuous light (LL) and simulated natural photoperiod (LDN, Tromsø, North-Norway) at low temperatures (4, 6 and 9°C) on flesh quality and textural properties in Atlantic salmon smolts.

Material and methods

Juvenile (initial mean weight 96.0 g ± 3.1 SEM) Atlantic salmon were reared at six different combinations of temperatures and photoperiods. The experimental groups are abbreviated as: 4LDN, 4LL, 6LDN, 6LL, 9LDN and 9LL. The experiment was carried out in the period from 16 October 2013 to 17 March 2014. At arrival at Bergen High Technology Centre the salmon (95 fish in each tank) were distributed among twelve 1 m^2 (400 l) tanks with each experimental group in replicate. At termination of the trial (17 March 2014) the fish were slaughtered and flesh samples taken to investigate quality and textural properties in the different experimental groups. The chemical analysis included flesh gaping, muscle pH, water content of fillet, water holding capacity (WHC), and texture properties (hardness and breaking force). Possible interactive effects of temperature and photoperiod on flesh quality in Atlantic salmon post-smolts was studied.

Results

Final weight in the six experimental groups varied between 174 and 345 g. The 4LL group was significantly larger compared to the 4LDN group from January onwards (Fig. 1A) and displayed 30% higher overall growth rates, whereas no growth enhancing effect of LL was seen at 6 and 9°C. As a result, an overall interaction effect of photoperiod and temperature on growth rate was found. Softer texture was seen in the fast growing groups. Hardness decreased with increasing temperature, size and growth whereas no effect of photoperiod was found. Accordingly, there was an overall significant linear relationship between fillet hardness and individual growth (Fig. 1B

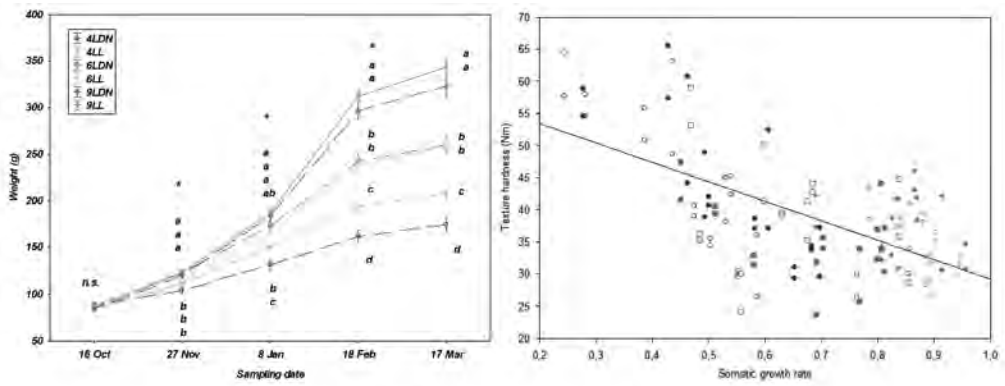

Fig. 1. (A) Mean weight (g) and (B) texture hardness (60% compression) of juvenile Atlantic salmon reared at three temperatures (4, 6 and 9°C) and two light regimes (LL = continuous light, LDN = simulated natural photoperiod for Tromsø, Norway). Letters indicate significant difference between treatments on sampling date. *denotes significant interaction between photoperiod and temperature. Open symbol = LDN. closed symbol = LL. Blue = 4°C and circle symbol, green = 6°C and square symbol and red = 9°C and diamond symbol.

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Discussion and conclusion

Results on textural properties in the present study, measured as breaking and hardness, suggest that changes in quality was effected by growth properties and temperature, but photoperiod played only a minor role. Previous studies on other teleost species show the shear forces of the muscles increases in periods with low growth (Hagen et al., 2007). This could help to explain the overall relationship between textural hardness and somatic growth rate seen in the present study (Fig. 1B).

In line with previous studies (Imsland et al., 1995; Jonassen et al., 2000) an interactive effect of photoperiod and temperature on somatic growth was found in this study. Fish exposed to low temperature and continuous light regime (4LL) had a significantly higher growth (30 % gain in overall SGR) than the 4LDN group. These findings demonstrate that the growth promoting effect of continuous light can be stronger at low temperature compared to near optimum temperature.

We conclude that quality in salmon muscle is dependent on growth, where temperature has the major impact, whereas photoperiod only has minor effect on flesh quality and textural properties. The present findings indicate that slaughter of salmon should be avoided in periods of high growth.

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MOLECULAR AND PHYLOGENETIC ANALYSIS OF *Dactylogyrus* SPP. PARASITES IN CULTIVATED SILVER CARP (*Hypophthalmichthys molitrix*) AND BIG HEAD CARP (*Hypophthalmichthys nobilis*) IN IRAN

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Introduction: *Dactylogyrus* is one of the most common external parasites on the gills of Cyprind fish. These parasites are highly host specific and many species only have a specific host. Since there are reports of silver carp specific *Dactylogyrus* species isolated from big head carp and vice versa, the molecular investigation of Dactylogyrids have done in these two fish species.

Material and Methods: 81 silver carp and 82 big head carp were caught from 10 fish farms in Guilan province, North of Iran, and after preparing wet mounts of body surface *Dactylogyrus* parasaites divided and fixed by glycerin jelly. In order to morphometric assessments on captured images, Image J soft ware were used for 7 point to point distances. Drawing of parasites was done by drawing tube and then compared by identification keys and parasites identified For molecular investigation the genomic DNA was extracted from one parasite specimen and 28S rDNA region of *Dactylogyrus* specimens were amplified by related primers in PCR (Plaisance *et al*., 2005). Purified fragments of PCR products were sequenced from both forward and reverse sites of each PCR product. Sequences of the parasites were aligned using the clustal W software and then manually adjusted to perform the phylogenetic analysis. The phylogenetic tree was built using Mega 6.0 by the UPGMA method. The pairwise genetic distances also calculated by BioEdit software.

Results: Sequences were deposited in GenBank with accession numbers MG825611 and MG825765 respectively for *Dactylogyrus hypophthalmichthys* and *Dactylogyrus suchengtaii* isolated from *Hypophthalmichthy smolitrix*, and also MH023397 and MH023399 respectively for *Dactylogyrus aristichthys* and *Dactylogyrus nobilis* isolated of *Hypophthalmichthy nobilis.* The phylogenetic tree (Fig 1.) shows the genetic affinity of isolated parasites from these two fish. The pairwise genetic distances (Fig 2.) also shows the isolated *Dactylogyrus* species of these two fish are very closely related.

Discussion and Conclusions: Probably this genetic relationships between *Dactylogyrus* specimens isolated from silver carp and big head carp can be considered as one of the main reasons for the success of transgenic *Dactylogyrus* species between these two fish and the failure of this transfer to common carp and gibel carp, as mentioned in studies by Molnar et al. (1984). Referring to this phylogenetic tree and the results of other studies (Musselius, 1968; Jalali and barzegar, 2004; Molnar et al., 2005), it seems that occasional silver carp parasites may appear in big head carp and vice versa. Most likely the racial impurity of silver carp and big head carp in Iran is the reason for hosting of each other parasites.

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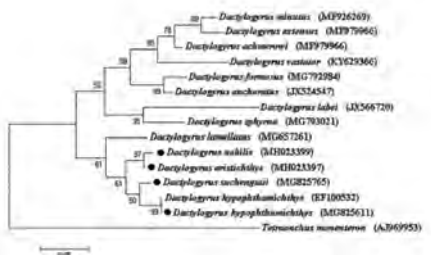

Figure 1. Phylogenetic tree constructed by the UPGMA method

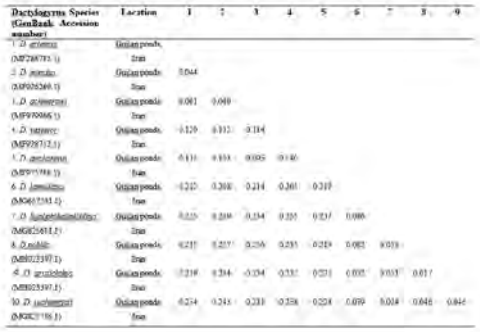

Figure 2. Pairwise genetic distances

IMPUTATION AND GENOMIC PREDICTION ACCURACIES FOR SEA LICE RESISTANCE IN ATLANTIC SALMON

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Introduction

Genomic selection (GS) can increase the rate of genetic gain in aquaculture breeding. Unfortunately, the cost of GS is high because a large number of individuals need to be genotyped in aquaculture breeding schemes. However, cost can be reduced by genotyping them with a lower-density single nucleotide polymorphism (SNP) panel and impute them to a higher-density panels (Goddard, 2008; Habier et al., 2009). In this study, we quantified the potential of imputation from low marker density for cost effective genomic selection in Atlantic salmon breeding.

Materials and Methods

A dataset from Mowi breeding population was obtained from a sea lice challenge trial. Genotypes and phenotypes from 4564 individuals were available. From each family, parents were genotyped with ~ 53K SNP and the genotypes of the offsprings were strategically reduced from 53K to 0.5K SNP. Three strategies were used to reduce the marker density: 1) proximity to the center of SNP windows (EQ), 2) highest allele frequency in a SNP window (MAF) and 3) based on maximum R-squared of a marker with all markers in the window (R2). In addition, some combinations of the above approaches (i.e. EQMAF, R2MAF and EQR2MAF) were also tested. For the combination strategies, weights were placed on individual strategies. The genotypes of the offsprings were imputed from 0.5K to ~53K SNPs and imputation accuracy was calculated as a correlation between true and imputed genotypes (Calus et al., 2014). Genomic breeding values (GEBV) were predicted using true and imputed genotypes. From each family, the phenotypes of 15% of the sibs were masked (i.e. validation individuals) and accuracies of prediction were calculated as correlations between GEBV and the masked phenotypes. The correlations were weighted by the inverse of the square root of heritability.

Results and Discussion

The imputation accuracies for the different marker selection strategies were between 0.63 - 0.65 when no parents were genotyped and between 0.80 - 0.81 when only one of the parents (either dam or sire) was genotyped (Table 1). When both parents were genotyped, the imputation accuracies were between 0.88 - 0.90 (Table 1). There was no difference in imputation accuracy between genotyping only dams and only sires. It was also observed that there is slight difference in imputation accuracies among marker selection strategies. The results also showed that there no difference in imputation accuracy between male and female offsprings.

Genomic prediction accuracy was 0.78 ± 0.1 when using true genotypes compared up to 0.76 ± 0.11 when using imputed genotypes. Among the marker selection strategies, EQR2MAF gave a slightly higher prediction accuracy (Table 1). Similar trends were also observed for three other disease traits and two weight related traits. The results showed that imputation can be leveraged to minimize genotyping cost without losing too much prediction accuracy. It also noted that both parents needed to be genotyped to get a reasonable imputation accuracy.

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TABLE 1. Imputation and genomic prediction accuracies for Sea lice resistance in A. salmon

Marker selection strategy	Imputation accuracy			Prediction accuracy[1]
	No parents	One parent	Two parents	
Non-imputed	-	-	-	0.78±0.10
MAF	0.65±0.10	0.81±0.04	0.90±0.02	0.74±0.09
EQMAF	0.65±0.09	0.81±0.04	0.89±0.02	0.71±0.10
EQR2MAF	0.63±0.08	0.80±0.04	0.88±0.02	0.76±0.11
R2MAF	0.64±0.08	0.81±0.03	0.89±0.03	0.72±0.09

[1]For the imputed genotypes, offsprings with both parents are used for the genomic prediction of breeding values.

GENOMIC PREDICTION AND GENOME-WIDE ASSOCIATION STUDIES FOR FEMALE REPRODUCTION TRAITS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Rainbow trout is a worldwide cultured salmonid species with numerous breeding programs implemented over the last 50 years in closed populations. Inbreeding is therefore expected to be at moderate to high levels in broodstock populations (D'Ambrosio et al. 2019). Previous works showed significant inbreeding depression effects in female body weight at spawning and spawn weight (Kincaid 1983) as well as in egg number and spawning age of females (Su et al. 1996). Therefore it is important to evaluate the performance for those female reproduction traits and their genetic architecture in current broodstocks.

The aims of the study were to estimate genetic parameters, to identify quantitative trait loci (QTL) and to assess the efficiency of genomic selection (GS) compared to pedigree-based selection (PS) for female reproduction traits in rainbow trout. Six traits were studied: ready-to-spawn female body weight (FW), spawning date (SD), egg numbers in the spawn adjusted for FW (EN), spawn weight adjusted for FW (SW), egg weight (EW) and egg diameter (ED). As far as we know, it is the first report on G AS and efficiency of selection for fish reproduction traits

Materials and methods

1,346 fish were phenotyped (Table I) and 15,265 individuals were recorded in the pedigree (10 generations). The 1,346 fish and their parents (87 dams and 72 sires) were genotyped with the Axiom™ Trout Genotyping array. After quality control, 29,799 SNP were retained for the analysis.

AIREMLf90 program was used for variance component estimation (Misztal et al. 2002) and POSTGSf90 program for GWAS (Aguilar et al. 2014). A 1-Mb portion of the genome was declared as a QTL when this 1 Mb-window explained at least 1% of the genetic variance of the trait.

The (genomic) estimated breeding values (G)EBV were estimated by pedigree-based BLUP and GBLUP linear mixed models using BLUPf90 package (Misztal et al. 2002). To assess the GS and PS efficienc , 40 replicates of Monte-Carlo 'leave-one-group-out' Cross Validation tests were performed considering 1,078 individuals and 269 individuals in the training and validation sets, respectively. Accuracy of selection was derived as the mean over the 40 replicates of the correlation between (G)EBV of individuals in the validation set and their phenotypes corrected for the fixed effects depending on the traits (cohorts, spawning date and over mature egg) divided by the square root of trait heritability.

Results

Genetic architecture. All heritability estimates were medium (Table I). At least 1 QTL was detected for any trait, but no QTL explained over 2.5% of the genetic variance. Therefore the genetic architecture of female reproduction traits appeared to be highly polygenic. For SD, 4 QTLs were detected (on Omy5, Omy9, Omy11 and Omy26), each of them explaining only 1.0 to 1.5% of the genetic variance. For FW, a single QTL was detected on Omy23 and explained 2.4% of the genetic variance. This QTL has not been yet reported in the literature for rainbow trout growth traits. Unique QTLs were also detected for EN and SW, explaining 1.3% on Omy8 and 1.0% on Omy2, respectively. For EW and ED respectively, 2 and 3 QTLs explained between 1.0 to 2.5%; the same 2 QTLs were detected on Omy 1 and Omy8 with the last one showing the largest effect for both EW and ED (Fig1.)

Accuracy of selection. Genomic EBV were more accurate than pedigree EBV for all traits. The gain in accuracy varied from 16% (FW) to 37% (EN) (fig.2)

Conclusion

Female reproduction traits are polygenic with intermediate heritability values. Only 2 QTLs over the 12 identified across the 6 traits studied explained over 2% of the genetic variance. These results suggest that gene-assisted selection will be useless. However, genomic selection based on a reference population of only a thousand individuals related to candidates may improve the efficiency of selection from 16 to 37% depending on female reproduction traits

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Traits	N	Mean	Raw variance	Phenotypic variance	h^2(se)
Female spawning body weight (FW, g)	1,345	2,090	200,433	118,777	$0.32^{(0.06)}$
Spawning date (SD, divided in 5 weeks)	1,346	2.57	2.38	2.08	$0.28^{(0.06)}$
Egg number in spawn (EN)	1,345	4,801	1,771,891	1,746,357	$0.33^{(0.06)}$
Spawn weight (SW, g)	1,345	190	3,150	2,591	$0.43^{(0.06)}$
Egg weight (EW, mg)	1,344	40	40.0	35.8	$0.27^{(0.05)}$
Egg diameter (ED, mm)	1,344	0.40	0.035	0.032	$0.46^{(0.07)}$

Table I: Summary statistics and heritability of female reproduction traits in rainbow trout

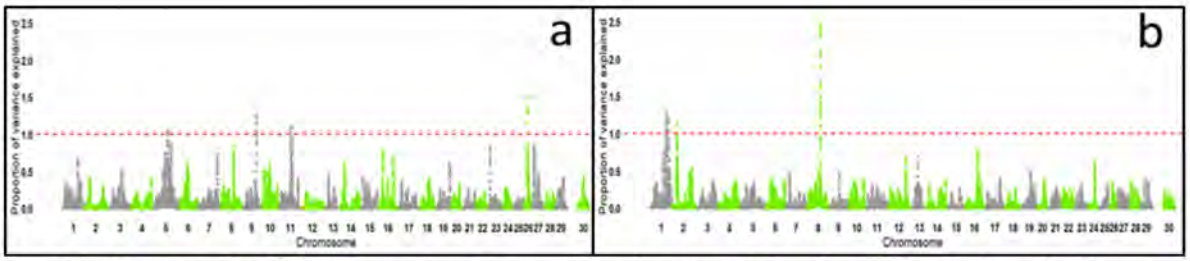

Fig. 1 Manhattan plot of the proportion of genetic variance explained by 1Mb-window of adjacent SNPs for spawning date (a) and egg diameter (b). The red line represents 1% of variance genetic.

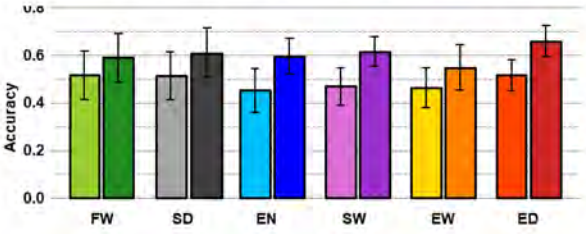

Fig. 2 Mean and standard deviation of accuracy of BLUP EBV (light colors) and GEBV (dark colors) for the six traits: FW, SD, EN, SW, EW and ED

Acknowledgement

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EVALUATING THE FEASIBILITY OF INCOPORATING MEMBRANE BIOLOGICAL REACTORS WITHIN RAS: EFECTS ON WATER USE, WATER QUALITY, AND RAINBOW TROUT (*Oncorhynchus mykiss*) PERFORMANCE

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Introduction

The membrane biological reactor (MBR) is a proven wastewater treatment technology that is used in municipal and industrial applications (Hai and Yamamoto, 2011). MBRs balance denitrification and nitrification within an activated sludge system. Fine-pore (≤ 0.2 µm) membranes within the MBR produce a filtered permeate that excludes solids, fine particles, bacteria, viruses, and other water quality constituents (Hai and Yamamoto, 2011). Onsite research indicates that MBRs can effectively treat concentrated wastewater from aquaculture facilities (Sharrer et al., 2007; 2010). The low-solids filtrate produced by MBRs also appears to be well-suited for return to recirculation aquaculture systems (RAS) which could result in further water savings, reduced discharge volumes, and water treatment advantages.

Materials and methods

A 4-month study was carried out to evaluate the feasibility of incorporating MBRs with the water recycle loop of experimental-scale (9.5 m^3) RAS while culturing rainbow trout. Triplicate RAS with and without MBRs were used for the trial. Control RAS were operated with standard flushing rates known to support acceptable rainbow trout performance and welfare. Three single-vessel MBRs with flat plate membranes (Alfa Laval, Sweden) received, processed, and retained RAS backwash water that otherwise would be removed, and a head-pressure-driven permeate was returned to RAS, creating a nearly closed loop system. Equal numbers of rainbow trout (103 ± 1 g) were stocked into each RAS prior to the study. Water use, water quality, and fish performance were assessed

Results

Integrating MBRs with RAS to reclaim backwash wastewater resulted in significantly reduced water usage, i.e., six and a half times less than RAS operated with traditional flushing rates. Mean daily water exchange rates for RAS with and without MBRs was 1.2 ± 0.4 and 7.8 ± 0.5 % of the RAS volume, respectively. A range of water quality concentrations were significantly greater ($P < 0.05$) in the culture water of RAS with MBRs including: heterotrophic bacteria counts, nitrate-nitrogen (NO_3-N), total ammonia nitrogen, total phosphorous, and true color, as well as dissolved calcium, copper, magnesium, and sulfur. Alkalinity and ultraviolet transmittance levels were significantly lower in RAS with MBRs.

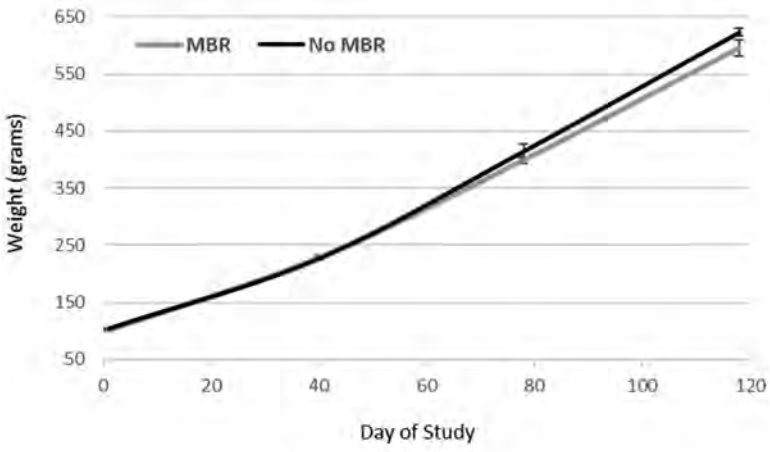

Fig. 1. Rainbow trout growth (mean weight ± standard error) in RAS with and without membrane biological reactors during the 4-month study.

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Infrequent addition of sugar enhanced denitrification, resulting in decreased NO_3-N and increased alkalinity in the MBR permeate. Water chemistry differences in MBR-integrated RAS did not affect ($P > 0.05$) rainbow trout growth performance (Fig. 1). At the end of the trial, the mean weight of rainbow trout cultured in RAS with and without MBRs was 595 ± 14 and 623 ± 6 g, respectively. Deficiencies in MBR function were observed including less than optimal MBR permeate production rates.

Discussion and conclusions

Results from this preliminary trial indicate that integrating MBRs within the water treatment loop of RAS is feasible for rainbow trout production. The substantial water savings resulting from MBR integration could have important implications for the developing RAS industry. RAS already reduce the water requirement for fish production; however, large, commercial-scale facilities still have a significant water requirement. We estimate that a 1,000 MT salmonid RAS farm could require ≥ 3,000 m^3 of daily makeup water. However, new RAS farms are being constructed at larger scale (up to ≥ 30 MT) which will demand even greater water volumes. The water savings provided by MBRs could offset this expanded water requirement, which would broaden the scope for facility site selection and potentially allow location of commercial RAS farms near major seafood markets. More research is needed to optimize MBR integration within RAS including faster permeate production rates, enhanced denitrification efficienc , and improved RAS water quality. Use of ozone in MBR-integrated RAS would likely produce significant water quality improvements (Davidson et al., 2011). Future research that seeks to optimize MBR integration with RAS is planned, including the combined use of ozone.

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EFFECTS OF HEAT-KILLED *Lactobacillus plantarum* (HK L-137) ON THE HEALTH STATUS AND GROWTH-RELATED GENE EXPRESSION OF GENETICALLY IMPROVED FARMED TILAPIA

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Introduction

Genetically improved farmed tilapia (GIFT), an enhanced strain of Nile tilapia (*Oreochromis niloticus*), exhibit better growth and survival rates than routinely available strains of tilapia. Currently, intensive culture systems are common for tilapia culture and often cause stressful conditions that reduce fish growth and wellbeing. The use of functional feed additives as bio-friendly agents is a sustainable way to improve cultured fish performance, and the use of functional ingredients with immune modulatory properties has become more prominent across the animal feed industry.

Heat-killed *Lactobacillus plantarum* (HK L-137) can positively impact the performance and wellbeing of aquatic animals, as reported in several studies.

Thus, the present study investigated the potential value of HK L-137 as a dietary probiotic for GIFT.

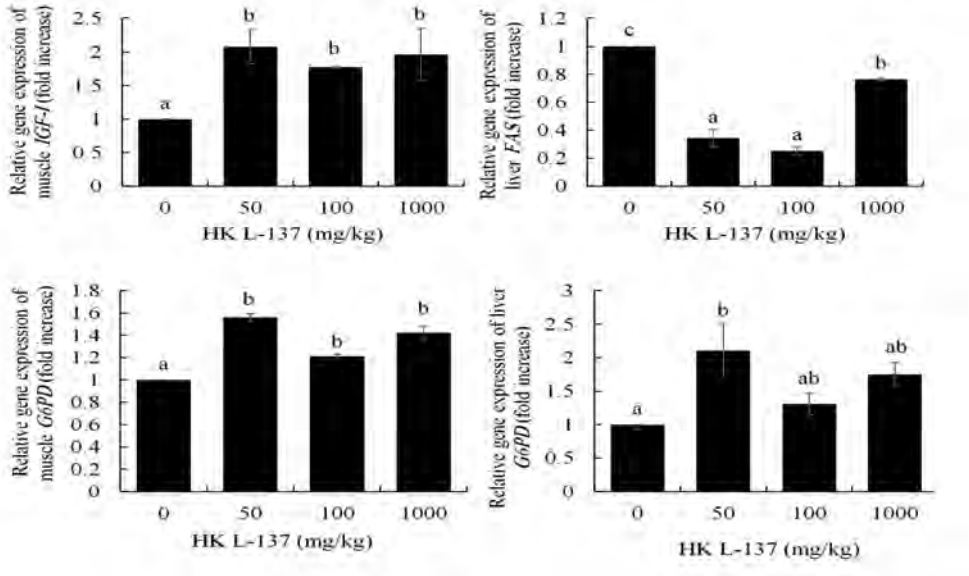
Materials and methods

For 12 weeks, fish were fed a control diet (HKL0) or a diet supplemented with HK L-137 at a concentration of 50 (HKL50), 100 (HKL100) or 1000 (HKL1000) mg kg^{-1} feed.

Table 1. Growth performance and nutrient utilization in GIFT tilapia*

	Test diets			
Item	HKL0	HKL50	HKL100	HKL1000
Initial body weight (g)	16±0.04	16±0.03	16±0.03	15.97±0.04
Final body weight (g)	$36.16±0.9^{a}$	$41.2±1.3^{b}$	$45.2±0.7^{c}$	$42.2±0.93^{b}$
Weight gain (%)	$126.3±5.4^{a}$	$158.3±8.3^{b}$	$183.4±4.3^{c}$	$167.4±5.83^{b}$
Specific growth rate (% BW/ day)	$1.82±0.1^{a}$	$2.1±0.1^{b}$	$2.3±0.04^{c}$	$2.04±0.05^{b}$
Feed efficiency ratio	$0.54±0.02^{a}$	$0.61±0.03^{ab}$	$0.73±0.02^{c}$	$0.66±0.02^{b}$
Survival	$91.1±2.2^{a}$	$97.8±2.2^{b}$	$100±0.00^{b}$	$98.9±1.1^{b}$
Condition factor (%)	1.82±0.1	1.83±0.03	1.84±0.06	1.84±0.04
Hepatosomatic index (%)	2.24±0.2	2.39±0.13	2.09±0.34	2.24±0.17
Viscera somatic index (%)	2.9±0.1	2.87±0.13	2.6±0.03	2.74±0.1

*Values expressed as means ± SE (n = 3). Different superscript letters indicate significant differences for each pairwise comparison between treatments.



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Results

At the final sampling, the HKL100 group had significantly ($P < 0.05$) increased performance parameters (FBW, WG, SGR and FER) compared to the control group, while the HKL50 and HKL1000 groups showed weaker improvements. Mucosal thickness and villus length were significantly ($P < 0.05$) increased in the HKL50 and HKL100 groups in the anterior, middle and posterior intestine, but muscle thickness was significantly ($P < 0.05$) improved only in the anterior and middle intestine. Amylase, lipase and protease activity was significantly ($P < 0.05$) increased in fish fed 50 or 100 mg HK L-137 per kg diet compared to control fish. Significant modulation of blood haematocrit, haemoglobin levels, and RBC and WBC counts ($P < 0.05$) occurred in fish fed HK L-137, while total cholesterol and GPT were decreased by HK L-137. Furthermore, antioxidative enzyme (SOD and CAT) activity were significantly ($P < 0.05$) higher in the HKL100 group than in the control group, while MDA levels were lower. Furthermore, fish fed HK L-137 showed enhanced total serum protein and IgM levels. Interestingly, qRT-PCR revealed significant ($P < 0.05$) upregulation of the growth-related gene IGF-I and the glucose regulation gene G6PD but downregulation of the fatty acid synthase (FAS) gene in all HKL groups compared to the control group.

Conclusion

Thus, we conclude that the use of HK L-137 is an efficient strategy to achieve economically feasible and sustainable tilapia production.

COMPARATIVE GONAD HISTOLOGY AND SEMEN QUALITY OF NORMAL (XY) AND NEO-MALES (XX) OF ATLANTIC SALMON (*Salmo salar*)

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Introduction

In Australia the commercial propagation of Atlantic salmon relies heavily on crossing homogametic brood stock i.e. normal females (XX) and neo-males (XX) which results in the production of 100% female offspring. It is necessary because in most salmonids' females have extremely favourable production traits compared to males (Inanan and Yilmaz, 2018). Typically, however, neo-males gametes can only be extracted after sacrifice and dissection, which often results in inconsistencies in quality and quantity of semen used for fertilization, compromising the seed production efficiencies in commercial hatcheries (Parodi et al. 2015; Vikingstad et al. 2016). The objective of the present study was to comparatively evaluate the gonad histology and semen quality of normal (genotype XY) and neo-males (genotype XX) with a long-term view to improve hatchery production of all-female progeny of the Atlantic salmon.

Materials and methods

Four normal (XY) and seven neo-males (XX), distinguished by their ability to- or lack of expressing semen respectively, from the same brood stock, were comparatively evaluated. The sperm collection from both genotypes was made by testis maceration. The Left lobe (from each fish) was fixed in buffered formalin for histological analysis, while the right lobe used for extracting semen by maceration. Prior to sperm extraction, key measurements of fish (body weight and length) and their testis (weight, length, width and depth) were taken and gonadosomatic index computed. Semen qualitative and quantitative analyses (Motility by CASA, duration of motility, viability of spermatozoa, sperm concentration and structural abnormalities) as well as gonad histology (Hematoxylin/Eosin staining and *in situ* cell death detection assay by Tunel) were carried out. All quantitative data followed the normal distribution (Shapiro-Wilk test 5%) and the statistical differences were determined by analysis of variance and mean test (Tukey 5%), using the statistical software R.

Results

Differences were found in body size between the genotypes, with neo-males being on average six times heavier and 42% larger than the normal males. Similarly, their gonads were four times heavier, almost double in length and thickness compared to normal males. Morphometric observations revealed that neo-male gonads are irregularly shaped, have poorly formed seminiferous ducts and lower gonadosomatic index ($P<0.05$).

The CASA analyses showed higher percentage ($P<0.05$) of semen motility for normal (80.69 ± 24.00%) than neo-males (57.2 ± 36.50%). Similarly, the duration of semen motility was also significantly longer for XY (99.31 ± 28.03 sec) compared to the XX (66.84 ± 23.83 sec) males. Significant differences were also found for curvilinear velocity, where the normal males (190.25 ± 56.50 $\mu m/s^{-1}$) had mean values higher ($P<0.05$) than neo-males (155.27 ± 48 $\mu m/s^{-1}$). The average of the remaining semen motility parameters measured i.e. linearity, progression, straight-line velocity, path velocity, wobble and beat cross frequency did not show statistical differences ($P>0.05$). No statistical differences ($P>0.05$) were observed for viability of spermatozoa, sperm concentration and structural abnormalities between normal and neo-males.

Histological analysis performed by H&E staining revealed the existence of consistent differences between the genotypes. A larger area of interstitial tissue, bigger size of Sertoli cells, greater number of Sertoli cell cysts (consisted of more than two cells in a cluster) were found in neo-males ($P<0.05$). Interestingly, the Tunel assay showed that most cells including hypertrophic Sertoli cells of neo-males predominantly remained non-florescent, suggesting that they are live and active.

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Discussion and conclusion

The results of this research further highlight the disparity in quality of neo-male semen of Atlantic salmon when compared to the normal males that are routinely encountered by hatchery managers, particularly that these are not just restricted to the harvest method. The key bottleneck is an inability of neo-males to express semen freely, likely contributed by poorly developed seminiferous ducts, irregular shape of the testes and the presence of hypertrophied and cyst forming Sertoli cells. Similar observations have been made in rainbow trout (*O. mykiss*) by Kowalski et al. (2011), suggesting this is a shared bottleneck in salmonid aquaculture, perhaps also applies to other cultured species where reliance on neo-males is a norm. This typically, requires maceration of testis for collection of semen, a relatively complex process that can contaminate the semen samples with impurities (such as blood) and results in lowers parameters of semen quality (Parodi et al. 2015; Vikingstad et al. 2016). Despite the standardization of sperm collection method in this study, neo-males had relatively poor semen quality. Such poor quality of neo-male sperm has been attributed to physiological/genetic obstacles to final maturation for these animals (Kinnberg et al., 2000a). Collectively, these two factors i.e. method of collecting semen and problems related to final maturation, along with the individual maturity variations make it difficult to refine and develop standard and reliable hatchery protocols that consistently yield high fertilization rates for neo-males. Conceivably this has flow on to nursery and grow-out performances and ultimately to economic efficiency of the farming operations. Nonetheless, the study implies that the neo-males may be amenable to physiological priming, which along with improved brood stock management practices may lead to production of semen, without the need for euthanasia. The benefits of such an ability i.e. without compromising the advantage of unambiguously selecting the XX-male brood stock, will improve hatchery efficienc es, including repeat use of neo-male broods. Results and inferences of this study should serve as foundation for future investigation aimed at delineating the causes that preclude expression of semen by neo-males as well as in optimizing general hatchery practices.

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STUDY OF GENOMIC VARIATION WITH WHOLE GENOME SEQUENCING IN PIKEPERCH *Sander lucioperca*

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Introduction

Pikeperch (*Sander lucioperca*) is a freshwater and brackish fish species natively distributed in Europe and Asia. It is a valuable fish due to its growing consumer demand and its high potential for inland aquaculture. In order to improve the production and profitability of fish in the aquaculture industry, the use of domesticated stocks and breeding programs is inevitable (Gjedrem et al., 2012). Although the impact of several environmental factors has been studied on productive and reproductive pikeperch traits (e.g. Mattila and Koskela, 2018; Olin et al, 2018**)**, to our knowledge, to date no attempt was made to study the influence of genetics. However, for managing genetic diversity and for sustainable breeding, the relationship between genetic and phenotypic variation has to be elucidated. To this end, the study of genomic variation is required. The most common form of genetic variation is the single nucleotide polymorphism (SNP). The identification of SNPs allows its use as markers for the analysis of the genetic structure of populations, for the construction of genetic maps and to help locate genes associated to traits of interest. The objective of this study is to identify SNPs based on a large-scale experiment in a pikeperch breed and to investigate the genomic variation using information of parents and offspring.

Material and methods

Seven full-sib families of pikeperch were generated by a fish production facility (State Research Institute for Agriculture and Fisheries in Hohen Wangelin, Mecklenburg-Western Pomerania, Germany). The progeny obtained from each family were transferred into a common tank and sampled after reaching fingerling stage. Genomic DNA from a total of 394 individuals, including broodstock, was extracted from blood or flash-frozen caudal fin for whole genome sequencing with Illumina (NovaSeq 6000). SNPs were detected using the Haplotypecaller tool of the Genome Analysis Toolkit v4.0 (GATK) pipeline for DNA-Seq. Identifie SNPs are further processed with the genome association analysis toolset PLINK. To study genomic variation, SNPs are filtered by call rate, minor allele frequency and Hardy-Weinberg equilibrium. After filtering, a set of high-quality SNPs are selected for parentage testing (ParentSearch; Boerner, 2017). Then progeny haplotypes are phased from SNP genotypes and blocks of linkage disequilibrium are computed for elucidating regions of particularly high or low recombination activity. Furthermore, runs of homozygosity are investigated to study the extent of inbreeding, which is an important measure of genetic diversity (Curik et al., 2014).

Results

[The computing process is currently taking place.]

Discussion and conclusion

The analysis of the genomic variation is a necessary step for understanding the relationship between genotype and phenotype. This study revealed an extensive panel of high-quality SNPs that can be further employed in genomic selection accelerating the establishment of breeding programs and therefore a more efficient aquaculture industr .

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ARE GENETICS AND GENOMICS HELPFUL TO IMPROVE FEED EFFICIENCY IN NILE TILAPIA *Oreochromis niloticus*?

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Introduction

Improving feed efficiency in aquaculture is crucial to develop a more sustainable aquaculture. Feeds represent 30 to 70% of the total production costs, and have major environmental and social impacts (de Verdal et al, 2017).

Until now, the contribution of genetic improvement to this goal has been hampered by the lack of efficient phenotyping methods and of accurate genetic parameters of feed conversion rate (FCR) in fish. Different methods have been used to measure individual FCR in fish, but all of them have pros and cons, i.e. the loss of social interactions between fish, the low repeatability of measurements, or the time needed to measure the phenotype.

Indvidual FCR is a difficult trait to measure. Using genomic selection would help to reduce the phenotyping effort by phenotyping fish that are not the candidates to selection (training population). These phenotypes are needed to establish a genomic prediction equation for the selection candidates, thus potentially reducing the phenotyping effort with a higher accuracy. The aim of the present study was to 1/ estimate the genetic parameters of performance and feed efficiency traits and 2/ assess the potential of genomic selection for feed efficie cy. The GIFT strain, selected for growth for more than 15 generations, of Nile tilapia *Oreochromis niloticus*, was used for this purpose.

Material and methods

Video assessment of feed intake (FI) on individual fish reared in groups was used to estimate the genetic parameters of growth traits (body weight gain, BWG), FI, FCR and relative FI ($FI.BW^{-1}$) on 1,000 fish from 40 pedigreed full-sib families of Nile tilapia. Juvenile fish (22.4 g) were evaluated during 13 consecutive meals over 7 days (de Verdal et al., 2018). Each phenotyped individual was genotyped using DarT-seq methodology. A total of 4930 SNPs were used after quality control. Heritability was estimated with a pedigree-based or a genomic approach. Five-fold cross validation was apllied using different methodologies: pedigree-BLUP (pBLUP), genomic BLUP (gBLUP), and single-step gBLUP (ss-gBLUP) and twoBayesian methods (Lasso and BayesC-π) to estimate the reliability of EBVs.

The BLUPF90 family of programs (Misztal et al, 2002) was used for all analyses except for the Bayesian methods where GS3 (Legarra et al, 2010) was used.

Results

As expected, heritability estimates revealed a genetic control for feed efficiency traits in tilapia. Pedigree- and genomic-based heritabilities were rather different for all traits (Table 1). Estimates were lower with genomic data for FI and $FI.BW^{-1}$ but higher for BWG and FCR.

EBVs estimated with different methodologies (pBLUP, gBLUP, ss-gBLUP, Lasso and BayesC-) were compared to observed phenotypes for all traits. Results for FCR and FI corrected for fixed effects are shown in Figure 1. Globally, all correlations were close to the expected accuracy, whatever the trait. The EBVs of FI were the most accurately predicted, with 95 to 100% of the expected accuracy, for pBLUP and Lasso, respectively.

Discussion and conclusions

Using this specific methodology to measure individual FI and estimate FCR, it is clear that genetic improvement of feed efficiency is possible in tilapia. These traits have moderate to high heritabilities. Genetic parameters estimates were not similar using the classical pedigree-based method and the genomic-based method, which can be partly explained by some intra-family effects, not visible using the pedigree-based estimations. The heritability estimates for FCR are relatively close

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Trait	Pedigree h^2	Genomic h^2
BWG	0.27 ± 0.08	0.37 ±0.06
FI	0.45 ±0.09	0.17 ±0.05
FI.BW^{-1}	0.31 ±0.001	0.19 ±0.06
FCR	0.32 ±0.11	0.59 ±0.06

Table 1 – Estimation of pedigree and genomic heritabilities ± S.E.

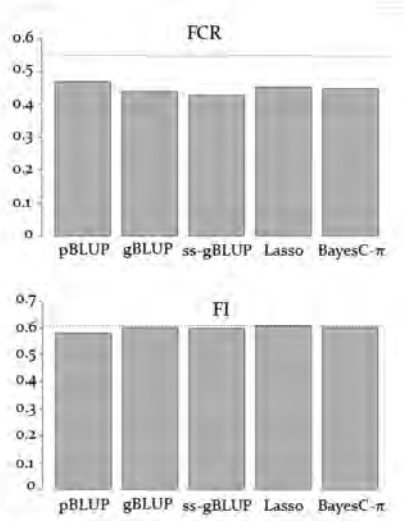

Figure 1 – Correlations between predicted EBVs for fish in the validation data in a given model (predicted from the training data) and the recorded FCR and FI. The dotted line represent the expected accuracy estimated using the equation of Daetwyler et al (2010).

to what was found by Besson et al (2019) in European sea bass, who estimated heritabilities of 0.25 and 0.47 for FCR (log transformed) using pedigree and genomic data, respectively. Regarding FI traits, the heritability drop could be explained by the relatively low number of SNPs used in the analyses, not all the QTLs being tracked by the SNPs.

We showed that accurate predictions of EBVs can be performed using different methodologies. According to the considered trait, the best methodology was not the same but accuracy differences between methodologies were rather small (less than 10%), and all methods were close to the expected accuracy. More genotypes and phenotypes might help increase the reliability of predictions and as a consequence, increase the impact of genomic selection on feed efficiency traits. Finally, although the heritability of FCR using genomic data was much higher than using pedigree data, the accurecy was very similar. As a consequence, using this number of SNPs and this measurement methodology, the clear advantage of using genomic data is reducing the phenotyping efforts, which can be useful knowing the complexity to measure FCR in fish

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EXPRESSION PROFILES OF MIR-462 AND 734 IN ZEBRAFISH LARVAE UPON *Aeromonas hydrophila* AND *Edwardsiella piscicida* EXPOSURE

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MicroRNAs (miRNAs) constitute a group of small non-coding RNAs (~22 nucleotides) and act as post-transcriptional regulators of target mRNA. The miRNA can be described as "multivalent" by its ability to target several genes, thereby regulating the expression of several proteins. Apart from miRNAs functions in mammals, teleost miRNAs also have been involved in the development and various physiological processes including growth, development, immune responses, etc. Most miRNAs are highly conserved within the vertebrates but miR-462 has only been described in teleost fishes according to miRBase. In addition, miR-734 is also specific to teleost and has not been reported in higher vertebrates. Therefore, understanding the expression of miR-462 and 734 is important to understand the immune functional role of those specific miRNAs.

We first focused on the constitutive expression of miR-462 and 734 in different tissues (liver, gill, spleen, gut, kidney, brain and muscle) of adult zebrafish (*Danio rerio*). The ubiquitous expression of miR-462 and 734 was detected in all tested tissues, with the highest levels in gills (23.9-fold) and brain (42.1-fold), respectively. Furthermore, we investigated the immune responses of these miRNAs in larvae exposed to pathogenic bacteria *Aeromonas hydrophila* and *Edwardsiella piscicida* at 3, 24 and 48 hours post exposure (hpe). Based on the qRT-PCR results, upregulation patterns of miR-462 and 734 were identified in larvae, upon exposure to *A. hydrophila* (2.4×10^8 CFU/mL) and *E. piscicida* (5.0×10^{10} CFU/mL). The findings of this study imply that miR-462 and 734 could ubiquitously express in a variety of tissues in adult zebrafish and these could be infection inducible miRNAs in zebrafish larvae upon *A. hydrophila* and *E. piscicida* exposure.

QTLs LINKED TO SURVIVAL AND GROWTH OF RAINBOW TROUT FED A 100% PLANT-BASED DIET IN RAINBOW TROUT SINCE THE FIRST MEAL

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Introduction

To ensure aquaculture development, composition of aquafeed has changed a lot in the past years. Indeed, classical aquafeed contained high proportion of fish oil and fish meal while these ingredients produced by fisheries are limited by quotas. Use of plant-based diets is a widely adopted option because the availability of plant products allows covering the needs for aquafeeds. For Rainbow trout, substitution rates up to 80 % have no impact on growth and survival. However higher substitution rates, especially total substitution, decrease survival in early stages and growth. Previous works with Rainbow trout showed that selection is highly efficient for survival and growth when trouts are fed a 100 % plant-based diet since the first meal (Callet et al., 2017). The present work aimed to characterize the genetic basis of survival and growth in a commercial population of Rainbow trout fed a 100% plant-based diet from the first meal onwards

Material and methods

20 dams and 10 sires from a selected line of the French breeder, Viviers de Sarrance, were crossed according to a full factorial mating to produce 3000 fish. Fish were fed, since their first meal, a 100% plant-based diet (without any fish oil and any fish meal) manufactured by INRA-NuMeA. Dead fish were collected each day. At 3.5 months, a mink entered and killed or ate a large part of the fish. After 4 months, the 549 surviving fish were weighed, measured and a piece of fin was collected. The 592 fish dead before the 7th week were also considered in this study. The 592 dead fish and the 549 surviving fish were genotyped with the Axiom® Trout Genotyping Array, containing 57k SNPs. After quality control, 28 310 SNPs were retained for the analysis. For the 2 traits, survival at 7th week and weight at 4 months, a GBLUP model was considered to estimate the effects of all SNPs simultaneously. AIREMLf90 program was used for variance component estimation (Misztal et al. 2002) and POSTGSf90 program for GWAS (Aguilar et al. 2014). A 1-Mb portion of the genome was declared as a QTL when this 1 Mb-window explained at least 1% of the genetic variance of the trait.

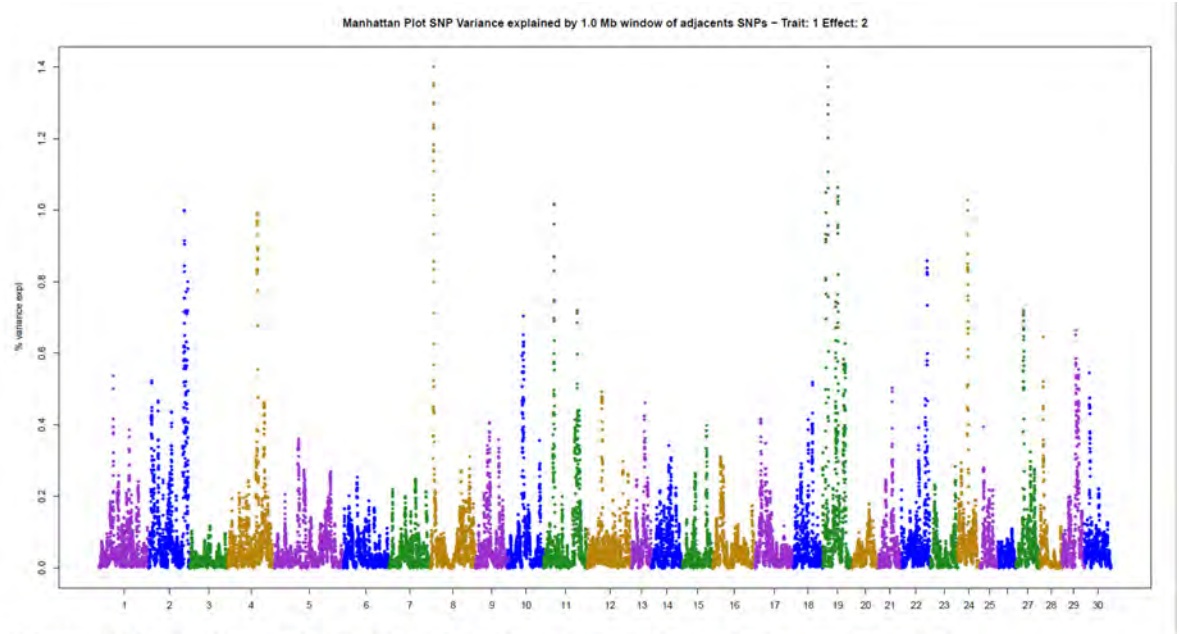

Figure 1-Manhattan plot of the proportion of genetic variance explained by 1Mb-window of adjacent SNPs for survival.

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Results and discussion

Mean weight of the 549 surviving fish was 16.73 ± 6.88 g and their mean length was 111.9 ± 18.37 mm at the end of the experiment. Four fish were discarded because of inconsistency between their weight and length

Genomic heritability was estimated at 0.38 ± 0.07 for body weight and 0.31 ± 0.05 for survival. Genetic correlation between body weight and body length was 0.96 ± 0.01 so that only body weight was further considered.

As shown in Figure 1, five QTLs were detected for survival on Omy2, Omy8, Omy11, Omy 19 and Omy24. All of them explained only between 1 and 1.4% of the genetic variance.

For body weight, four QTLs were detected on Omy3, Omy5, Omy16 and Omy18. The last QTL explained over 2% of the genetic variance.

Globally, these results confirmed the polygenic inheritance of survival and growth traits with a 100% plant-based diet. A more precise design (considering more animals and a higher-density chip) should be considered in the future to fine map the QTLs as well as to accurately estimate the QTL effects. A signature of selection on the 5th generation of the INRA selected line (Callet et al., 2017) is under analysis.

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EVALUATION OF DIETARY INCLUSION OF INSECT MEAL AND POULTRY BY-PRODUCT MEAL IN COMBINATION TO PLANT PROTEIN-RICH INGREDIENTS ON STRESS RESPONSE AND NUTRITIONAL STATUS OF RAINBOW TROUT *Oncorhynchus mykiss*

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Introduction

Innovation of aquaculture feeds is progressing fast. Reducing feed costs and improving feed efficiency represents a major challenge for economic and environmental sustainability of aquaculture. Plant ingredients have been extensively investigated for their stable production and cost-effectiveness, however their use involves some nutritional and environmental drawbacks (Baruah et al., 2017). In this scenario, insects and animal by-products are regarded as attractive ingredients to replace fish meal in alternative or in combination to plant ingredients (Belghit et al., 2019; Lu et al. 2015), however their effects on fish physiology and welfare have still been little investigated (Elia et al., 2018).

The aim of this study was to evaluate stress response and nutritional status of rainbow trout fed new formulated diet containing insect meal from *Hermetia illucens* larvae and poultry by-product meal as partial substitutes of plant meal, by analyzing blood chemistry parameters.

Materials and Methods

Diets and feeding trial - Six extruded isoproteic (42% DM) and isolipidic (24% DM) diets were formulated: **CF -** a fish based diet with 90:10 and 80:20 ratios between fish and a vegetable mixture derived proteins and lipid, respectively; **CV -** a vegetable mixture based diet with inverted ratios respect to CF diet (10:90 and 20:80 respectively); two insect meal based diets (**IM30**-**IM60**) and two poultry by-product meal based diets (**PBM30**-**PBM60)** formulated by replacing 30% and 60% of vegetable mixture derived proteins with the alternative ingredients and by maintaining constant the 20:80 ratio of crude lipid from fish sources and vegetable/alternative animal sources.

The feeding trial was carried out at Experimental Aquaculture Centre of the Foundation Edmund Mach (Italy). Juveniles rainbow trout (54.2±1.45g) were reared in 1600 l triplicate flow-through tanks at 13°C for 13 weeks

Sampling and analysis - 15 fish for each tested diet were sampled at the end of the trial after 24 h of fasting. Blood was withdrawn from the caudal vein of anaesthetized fish (100mg/l MS 222; Pharmaq, Italy). After centrifugation, serum was collected and stored at -80°C for analysis of cortisol, metabolites and enzymes according to Di Marco et al. (2017). Biometric measurements and autoptical analyses were also performed. Kruskall wallis test and post-hoc multiple comparisons were performed to evaluate the effect of dietary treatment on blood chemistry parameters.

Results

Dietary treatment did not significantly affect fish growth (One way ANOVA ns). The hepatosomatic index were significantly higher in CF compared to the other groups ($p<0.001$). Diet composition significantly influenced the levels of glucose, total proteins, albumin, cholesterol, creatinine and transaminase (Kruskall wallis test $p<0.01$). Glucose level was significantly lower ($p<0.001$) in CV compared to CF group and similar to IM60 and PBM60 groups (Fig.1). Total protein content was significantly lower in all groups except CV ($p<0.05$), compared to CF group. Conversely, albumin concentration was significantly higher in all groups, except PBM30 group, respect to CF ($p<0.05$). Cholesterol and creatinine concentration were significan ly lower in all groups than in CF group ($p<0.001$). AST and ALT values were significant lower in IM60 group compared to PBM60 and PBM30 groups respectively ($p<0.05$).

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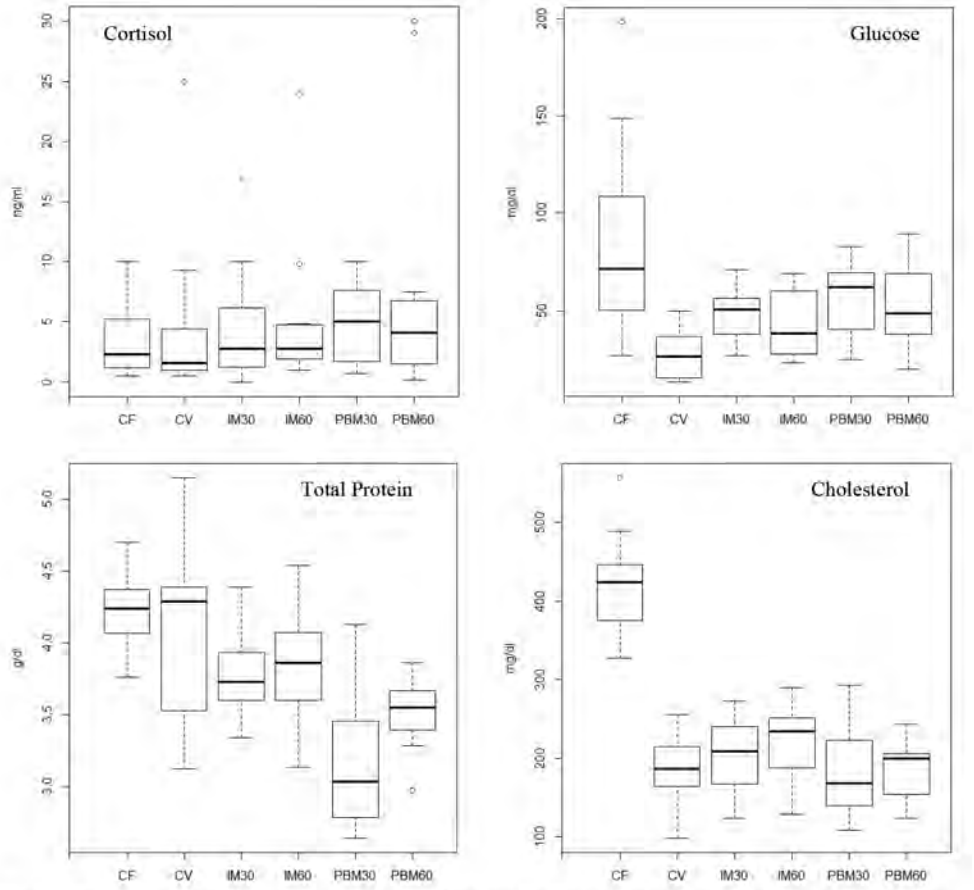

Fig.1. Cortisol, glucose, total protein and cholesterol levels in rainbow trout fed experimental diets.

Discussion and conclusion

All formulated diets sustain the fish growth without differences in relation to type of ingredients and levels of inclusion. Cortisol and metabolites levels measured in all groups are within the normal range reported for the species, suggesting no fish stress and a good nutritional status (Manera and Britti, 2006; Pinedo-Gil. et al. 2019). A comprehensive evaluation of blood lipid and protein content indicates that fish fed IM diets have a slightly higher metabolic profile compared to fish fed PBM diets and also a minor liver lipid accumulation as results from hepatosomatic index and liver histological analysis.

This study suggests that IM and PBM meals are promising alternative sources of protein for rainbow trout diet. Long-term feeding trials at commercial farms are needed for further investigations on fish physiology and welfare over the production cycle.

Acknowledgments

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USE OF STINGRAYS OF THE GENUS *Potamotrygon* (GARMAN, 1877) (POTAMOTRYGONIDAE, MYLIOBATIFORMES), FOR ELABORATION OF NEW PRODUCTS AND EVALUATION OF THE POTENTIAL PRESERVATIVE OF SPICE (*Eryngium foetidum* L.)

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Introduction

The change in eating habits causes consumers to seek products with greater quality and convenience to their taste, so the demand for new processed foods from fish has increased. According to Gonçaves (2011) the importance of processing and elaborating products based on fish, be in what concerns: food safety; quality; insertion of species that are not consumed in the market; adding value to unattractive fish; and encouraging the consumption of these fish. Therefore, as a way of adding commercial value to streaks, allowing an increase in its consumption, and also to verify the use of regional spices capable of increasing the shelf life of elaborated products based on fish, it was a concern to direct efforts for the development of this research. Therefore, the present work had as objective to elaborate small cake from stingray (*Potamotrygon sp*.) And to evaluate the anti-rancid potential of the spice *Eryngium foetidum L*., used as additive in the elaborated product.

Materials and methods

The stingray (*Potamotrygon sp.)* and spices (*Eryngium foetidum* L.) were purchased in the state of Amapá, Eastern Amazonia, Brazil. For the preparation of the small cakes, the stingrays were processed, cooked for about 40 minutes and then the protein material (MP) was separated from the skin and cartilage. The small cakes were made according to Sary et al. (2017), with adaptations, where they were divided into four groups: negative control (GCN), positive control (GCP), with spice powder (GPE) and another with spice extract (GEE). The prepared product was stored and frozen in a freezer at -20ºC. For the oxidative rancidity analysis (TBARS) in the small cakes, the methodologies described by Yagi (1976) were used. Moisture, lipid and ash determinations were also performed according to the methodologies proposed by Official Methods Of Analysis (AOAC, 1990), carbohydrate and caloric value as described in the literature. The analyzes of centesimal composition were made by groups of triplicate.

Results

The small cakes made from MP from *Potamotrygon sp*, were shown to be easy to prepare, the results obtained for the centesimal characterization are described in table 01, for fish in nature and for the elaborated product, with averages and standard deviations, for each parameter, through the quantitative analyzes of the samples.

The results for the evaluation of oxidative rancidity are shown in figure 1, for analyzes performed after 01 day and 07 days, divided into groups, in which they are expressed in mmol of products produced (malonaldehyde) from lipoperoxidation, per liter (mmol / L).

Discussion and conclusion

The elaborated product presented with smaller amounts for the nutritional constituents and calorific value when compared to the fish in nature, this can be explained due to the previous cooking process. According to Pedrosa and Cozzolino (2001) in the cooking process, there is an expressive loss in the nutritional composition present in the fish, thus evidencing what was found in these analyzes.

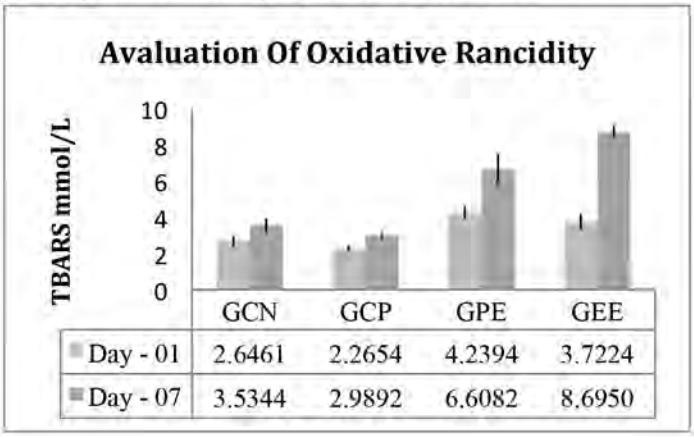
On the evaluation of oxidative rancidity (Figure 01), comparing the others with the control groups, it can be inferred that the spice used as an increment to increase the shelf life of the processed product is possibly inducing and accelerating the process of oxidative rancidity instead of rezudi it, thus causing the rapid damage of the product elaborated, even this being stored in place and the appropriate temperature. The result for the evaluation of oxidative rancidity is worrisome because according to Cluton (1997) and Ferrari (1998) the ingestion of substances produced from the lipoperoxidation process promotes irritation of the intestinal mucosa and tends to cause diarrhea, hepatic degeneration and even death of cells.

(Continued on next page)

Table 1 - Centesimal characterization of stingray of the genus *Potamotrygon*, in nature and the product elaborated from this raw material, both acquired through donations, in the state of Amapá, Eastern Amazon.

Parameter	Product	
	In nature	Elaborate
Humidity (g/100g)	61,80±3,26	79,38±0,60
Mineral waste (g/100g)	02,30±0,29	01,10±0,01
Total lipids (g/100g)	04,11±0,04	1,16±0,11
Total proteins (g/100g)	19,42±1,30	16,52±1,71
Carbohydrates (g/100g)	11,75±1,19	01,55±1,50
Calorific value (Kcal/100g)	164,84±1,87	95,62±9,59

Figure 1 - Results of oxidative rancidity evaluation, for analyzes after 01 day and 7 days, in which the Thiobarbituric Acid Reacted Substances (TBARS), expressed in mmol/L, were quantified in small cakes made from stingrays (*Potamotrygon sp.*), divided into four groups: GCN; GCP; CPE; e GEE.



Thus, it was possible to conclude that the product is easy to prepare and prepare, and can be replicated without many costs, since products have been used that can be easily found in markets and fairs. Results of centesimal composition for *Potamotrygon sp.* where, therefore, these data may serve as a subsidy in nutritional diets for humans, thus allowing an adequate dietary use of the said species.

Regarding oxidative rancidity analyzes, the use of *Eryngium foetidum L.* as a spice in the process of elaboration of fish products should be avoided, in which they will be stored for a long period of time, since this spice may possibly accelerate the oxidation process in the food. There is a warning about the use of spices in the preparation and production of fish-based foods, without previous study of their oxidizing activity. More studies will be carried out, aiming to certify and elucidate the oxidant activity processes of the spice used in this work.

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COMBINATIONS OF BROWN SEAWEEDS AS FEED ADDITIVES IMPROVED THERMAL SHOCK RESISTANCE OF *Litopenaeus vannamei* POST-LARVAE

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Introduction

Brown seaweeds have important nutritional and functional properties. These seaweeds show higher antioxidant activity than red and green seaweeds, and have a diverse content of bioactive compounds, such as polysaccharides, terpenes, phenols and polyphenols, minerals, polyunsaturated fatty acids, vitamins, and carotenoids (Balboa et al., 2013; Gómez-Gil et al., 2000). The present work aimed to assess the effect of different combinations of the brown seaweeds *Sargassum filipendula* and *Undaria pinnatifida* as feed additives on growth parameters and thermal shock resistance of Pacific white shrimp post-larvae reared in biofloc system, during nursery phase.

Materials and methods

The experiment was performed at the Marine Shrimp Laboratory (LCM, *Laboratório de Camarões Marinhos*), of the Federal University of Santa Catarina (UFSC, *Universidade Federal de Santa Catarina*, Brazil), using *Litopenaeus vannamei* post-larvae at the stage of 20 days post-hatchery (PL20, ± 0.006 g). PLs20 were stocked in 400 L tanks at 1500 PLs m^{-3}, and the rearing was managed as a superintensive biofloc system for seven weeks (until they reach 1 g), under four treatments: PLs fed diets containing different ratios 0.5%:2%, 0.5%:4%, 1%:2% and 1%:4% of the dry biomass of *S. filipendula* (S):*U. pinnatifida* (U), respectively, and control diet (without addition), all in triplicate. During the experiment, animals were fed six times day^{-1} and the water quality parameters were monitored (temperature, pH and dissolved daily, and total ammonia, nitrite and nitrate once a week). Following the feeding trial, we determined the growth parameters, and subjected the PLs to thermal shock (28.6 °C to 13.5 °C for 1h, and then back to 28 °C).

Results

The water quality parameters remained at acceptable levels for shrimp nursery, as suggested by Van Wyk and Scarpa (1999), and did not differ among the treatments. Growth parameters also did not show any significant differences among the treatments.

On the other hand, PLs fed diets containing the 1S:2U ratio showed higher survival after thermal shock (Table 1.).

Table 1. Survival rate (%) of *Litopenaeus vannamei* post-larvae fed diets containing 0.5%:2%, 0.5%:4%, 1%:2% and 1%:4% of the dry biomass of *S. filipendula* (S):*U. pinnatifida* (U), and the control diet with no supplementation, after 24 hours post-thermal shock .

Treatments	Survival rate (%)
Control	50.0^{a*}
0.5S:2U	44.5^{a}
0.5S:4U	46.7^{a}
1S:2U	73.3^{b}
1S:4U	55.6^{a}

*ANOVA one-way, followed by Tukey test (p=0.0001)

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Discussion and conclusion

Many studies have shown that brown seaweeds can affect the resistance of different organisms to thermal stress (Kandasamy et al., 2011, 2014; Schleder et al., 2016). In previous reports, shrimp fed *S. filipendula* did show higher survival after thermal shock (around 97% compared to 43% in the control group), while increasing levels of *U. pinnatifida* had negative effects on thermal shock resistance, with the higher level (4%) causing 100% mortality. The combination of both brown seaweeds in the diets (0.5S:1U, 0.5S:2U and 0.5S:4U) avoided the negative effect of *U. pinnatifida* on the resistance of shrimp juveniles to thermal shock, but the survival remained similar to the control group (around 30%) (Schleder et al., 2016, 2017). Interestingly, in the present work, the combination 1S:2U improved significantly the resistance of shrimp post-larvae to thermal shock.

In conclusion, the combination of *S. filipendula* and *U. pinnatifida* in the diet can improve PLs resistance to acute thermal stress without affecting their growth performance. These results are particularly important for shrimp industry, since after nursery phase shrimp are transferred to grow-out tanks in the shrimp farms, where they face several environmental stresses, such as thermal stress.

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BACTERIAL FISH DISEASE MONITORING OF RAINBOW TROUT IN TURKEY: 3 YEARS OBSERVATION OF AEGEAN REGION

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Introduction

The fish farming industry in Aegean Region is mainly based on rainbow trout (*Oncorhynchus mykiss*) in Turkey and the production rates have steadily grown every year (TUIK, 2019). It is important to determine new and emerging bacterial fish diseases due to their substantial economic and environmental impacts. Some of the most known diseases were reported over the years such as Lactococcosis (Çağırgan and Tanrıkul, 1995), Vibriosis (Tanrıkul, 2007) and Yersiniosis (Çağırgan ve Yüreklitürk, 1991) in rainbow trout from this region. However, it is substantial to track disease threats and their effects to the area along with containment and eradication of fish disease. For these purposes, 3 years of observation was conducted in the most productive area for rainbow trout farming in Turkey.

Materials and methods

Bacterial fish pathogens were isolated from 10 different rainbow trout farms in Aegean Region of Turkey during 3-year research. The commercial size diseased fish (250-350g) were detected in ponds and moribund fish were transferred to Izmir Katip Celebi University Faculty of Fisheries Fish Disease and Biotechnology Laboratory. Clinical and pathological symptoms were detected after full necropsy. The primer isolation of pathogens was conducted on both TSA (Tryptic Soy Agar, Merck) and proper selective medium based on presumptive identification. The initial identification of pathogens was conducted with using API identification products (BioMérieux, France) then further characterization and identification were performed with amplifying the 16S rRNA gene of the strains. The antibiotic susceptibility of the strains was determined by Kirby-Bauer disk diffusion susceptibility test (Hudzicki, 2009) and considered due to The Standards of Clinical and Laboratory Standarts Institute (CLSI) agains 20 different antimicrobial agents (Oxoid, UK). The Minimum Inhibitory Concentration (MIC) values of the pathogens were also determined with E-test strip (BioMérieux, France) according to EUCAST and Austin and Austin (2007).

Results

The current status of the bacterial fish pathogens was determined during 3-year field research from 10 different rainbow trout farms and the profile of the bacterial fish pathogens are shown in Table I. *Lactococcus garvieae* is the most abundant pathogen with 37.8% ratio among others which is followed by *Yersinia ruckeri* with 32.4%. *Pseudomonas fluorescens* and *Hafnia alvei* are the least common cause of disease and determined only 3 and 2 times during 3 years, respectively. *L.garvieae* strains were not susceptible to 20 different antibacterial disks which were used in the study and many of them were resistant to antibiotics. Also, all pathogens have different MIC parameters to different antibiotics according to the E-test results.

Table I. Isolated bacterial fish pathogens during this research

Pathogen	Number of identified strains	Isolation percentage (%)
A.hydrophila	5	6.8
L.garvieae	28	37.8
P.fluorescens	3	4.1
V.anguillarum	12	16.2
Y.ruckeri	24	32.4
H.alvei	2	2.7
TOTAL	74	100,0

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Discussion and conclusion

In the present study, the main cause of bacterial disease of rainbow trout is determined as *L.garvieae*. It was recorded from all the rainbow trout farms during spring and summer months and observed chronic effects of Lactococcosis during the winter period. Generally, the pathogen does not exist in hatcheries, but if the sanitary conditions were not provided properly, it was isolated from even 3g fry with clinical symptoms. Furthermore, the implication being that the vaccines are not effective if fish become infected in the hatchery. The antibiogram and E-test results show that, the pathogen develop resistance to different antibiotics which is well supported by several research (Kubilay et al., 2005; Raissy and Ansari, 2011; Raissy and Moumeni, 2016). Vibriosis usually observed in fish farms which are located in high altitude, besides it was determined only in the winter season from other farms. Yersiniosis was not seen in temperatures below 12 °C and observed above 13 °C from almost every rainbow trout farm. On the other hand, *Hafnia alvei* were mostly confused with *Yersinia ruckeri* based on API 20E identificatio results (Popovic et al., 2007). In this study, *H.alvei* was isolated from 2 different rainbow trout farms which had high stocking density and low water conditions and confirmed with molecular identification. *Vagococcus salmoninarum* was reported in rainbow trout broodstocks from the same region before (Tanrıkul et al., 2014), but it was not observed during 3 years of field research from commercial size fish in this study. It is important to determine the disease profile of a region to detect a proper treatment strategy and prevent antibiotic resistance over time. This study highlights the profile of bacterial diseases in the most productive area for rainbow trout in Turkey.

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ESCAPED FARM SALMON AFFECT THE GENETICS OF WILD SALMON (*Salmo salar*) POPULATIONS ALL ALONG THE NORWEGIAN COAST

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Introduction

The Norwegian Institute for Nature Research and Institute of Marine Research have classified 225 wild Atlantic salmon populations according to the level of genetic introgression from escaped farmed salmon (Diserud et al. 2019). The assignment given by the Norwegian Ministry of Climate and Environment is to classify wild Atlantic salmon populations in terms of "Genetic Integrity" according to the "National Quality Norm for Wild Salmon (*Salmo salar*)".

Material and methods

Genetic introgression from escaped farm salmon has been quantified for more than 40 000 salmon (Diserud et al. 2019). These analyses include only salmon that hatched in the wild, so escaped farm salmon were excluded from the data set, identified by differing growth patterns in their scales. Only populations with a sample of at least 20 individuals from no earlier than year 2000 were included among the final 225 populations.

The genetic introgression is quantified using molecular genetic markers (Karlsson et al. 2011, 2014, 2016; Glover et al. 2013). The genetic integrity of the wild salmon populations is distributed among the four quality classes defined by the Quality Norm:

Green Status very good or good: No genetic introgression observed
Yellow Status moderate: Weak genetic introgression indicated
Orange Status poor: Evidence of moderate genetic changes
Red Status very poor: Evidence of large genetic changes

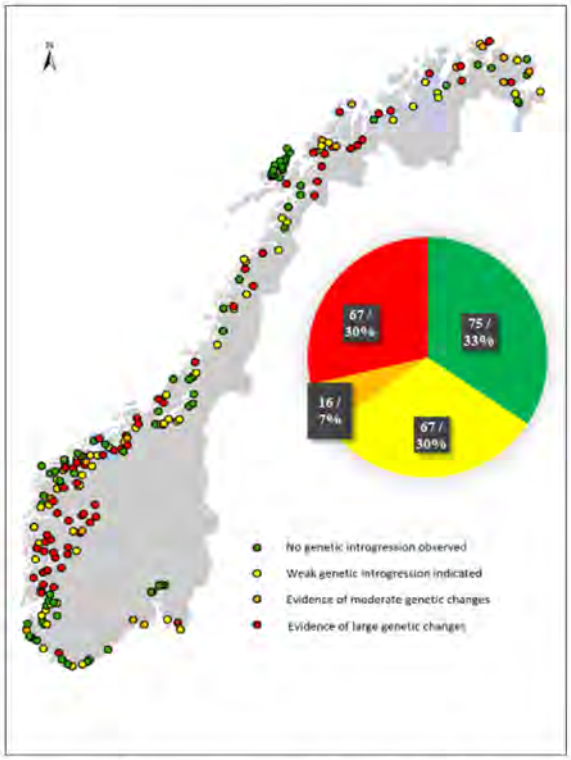

Fig. 1. Genetic introgression of escaped farm salmon into 225 Norwegian wild Atlantic salmon populations.

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Results

No genetic introgression was observed in 75 wild salmon populations (33%), weak genetic introgression was indicated in 67 populations (30%), evidence of moderate genetic changes was found in 16 populations (7%), while evidence of large genetic changes was found in 67 populations (30%) (Diserud et al. 2019). The genetic status was classified as very good or good in a third of the populations, as moderate in another third, and as poor or very poor in the last third of the populations (figure 1). The populations with poor or very poor genetic status are more common in western Norway and in Troms county in northern Norway, while the south-eastern parts of Norway have more populations with very good or good genetic status.

Discussion and conclusion

Genetic introgression has been analysed for half of the Norwegian salmon populations, representing more than 94% of the total spawning stock in Norway. The level of introgression in a wild population is significantly associated with the average proportion of escaped farm salmon in the river over the last 25 years (Diserud et al. 2019; Glover et al. 2019). Our approach allows quantification of genetic introgression from the individual level to populations, regions and the national level, and in rivers with and without a historical baseline. The highest average levels of genetic introgression were found in the regions of Norway with the highest aquaculture activity. A large proportion of the variance in the level of introgression could not be explained by proportions of escaped farm salmon though, river properties like water discharge and wild population characteristics like population size or density will also affect the genetic introgression.

To protect the genetic integrity of wild Atlantic salmon populations, only low levels of introgression from escaped farm salmon can be allowed into wild populations. Further introgression is likely, unless substantial reduction of escaped farm salmon in the wild, or sterilization of farm salmon, can be achieved. This is a unique work; no other country has a similar documentation of genetic introgression from escaped farm salmon into wild salmon populations.

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STRATEGIES FOR WATER PREPARATION IN A BIOFLOC SYSTEM: EVALUATION OF A CHEMOAUTOTROPHIC, HETEROTROPHIC AND MATURE SYSTEM FOR PACIFIC WHITE SHRIMP REARING

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Introduction

Biofloc technology (BFT) has advantages over traditional shrimp farming systems, such as improved space utilization, reduced water uses and reduced effluent discharge (Bossier and Ekasari, 2017). However, one of the main questions about BFT, is how to achieve a stable system faster, aiming to prevent spikes of nitrogen compounds such as ammonia and nitrite. In BFT the nitrogen compounds can be recycled basically by two pathways: heterotrophic bacteria and chemoautotrophic bacteria (Ebeling et al., 2006). Heterotrophic bacteria require organic carbon sources such as sugar, dextrose, molasses, or other sources as energy, in addition to requiring a (C/N) ratio higher than 10:1. Chemoautotrophic bacteria have slower growth rates and use inorganic carbon instead (Ebeling et al., 2006). When comparing the performance of chemoautotrophic and heterotrophic bacteria to fix 1 g of ammonia in the BFT system, produce a very low bacterial biomass (0.2 g) compared to heterotrophic (8.07 g) (Ebeling et al., 2006). However, the chemoautotrophic bacteria are slow growing, taking about 20 days to appear on the system. The use of chemoautotrophic bacteria in the beginning of BFT systems could then be an interesting strategy in order to avoid the formation of excess sludge and peaks of nitrogen compounds as seen at the beginning of an organic fertilization period. Thus, the objective of this work was to assess different water preparation strategies (heterotrophic, chemoautotrophic and mature) on BFT system for the Pacific white shrimp (*Litopenaeus vannamei*) rearing.

Material and Methods

Pacific white shrimp (*Litopenaeus vannamei*) from the lignage HB16 (Aquatec, Rio Grande do Norte), free of pathogens that require notification of the International Organization of Epizootics, with 3,45g were used on the trial. The experiment evaluated shrimp rearing in different biofloc systems: heterotrophic, chemoautotrophic and mature biofloc. The experiment was randomized with three treatments of four replicates each. It consisted of twelve fibe glass tanks with a volume of 300L of water (0.58 m^2 bottom area), with fou artificial substrates and stocked with 350 shrimp m^{-3}. Shrimp feed (Guabi Potimar 1.6mm – 35% crude protein) was added to the tanks four times a day. For the heterotrophic system, three days before stocking of shrimp, water was fertilized with feed and molasses at carbon/nitrogen ratio (C:N) of 15:1 in order to reach 200 mg L^{-1} of total suspended solids (TSS) at the beginning of the rearing period. During the experiment, the same C:N ratio was maintained. When TAN surpassed 1 mg·L^{-1}, additional carbohydrate (molasses) was added to the system at a ratio of 20g of carbohydrate per each gram of TAN. For the chemoautotrophic system, 30 days before stocking shrimp, water was fertilized with 1 mg L^{-1} of sodium nitrite $NaNO_2$ and 1 mg L^{-1} of ammonium chloride NH_4Cl per day in a bioreactor tank with 400L. The bioreactor tank was equipped with 16 artificial substrates (needlona®), heaters, aeration system and no light. After thirty days, the water of the four experimental tanks was prepared to receive shrimp by adding 100L of water from the bioreactor tank and 200L of seawater. For the mature biofloc system, the experimental units were prepared to receive shrimp by adding 90L (30%) of water from shrimp in the biofloc system (matrix tank) and 210L of seawater. Dissolved oxygen and temperature were monitored twice a day. TAN, nitrite, alkalinity, salinity and pH were monitored three times per week. Nitrate, orthophosphate, TSS, volatile and fixed solids were analyzed once a week. TAN concentration of nitrite, nitrate and orthophosphate was analyzed using the colorimetric method according to the procedure described by Strickland and Parsons (1972). After 35 days, shrimp performance (final weight, weekly growth rate, survival, feed conversion ratio and yield) was evaluated .

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Table 1. Shrimp performance of the Pacific white shrimp *L. vannamei* using biofloc with heterotrophic, chemoautotrophic and mature biofloc systems for 35 days stocked at a density of 350 shrimp m^{-3}.

Parameters	Heterotrophic	Mature	Chemoautotrophic
Mean final weight (g)	9.85±0.87[a]	9.81±0.47[a]	9.42±0.83[a]
Growth rate (g $week^{-1}$)	1.28±0.70[a]	1.27±0.44[a]	1.19±0.41[a]
Final biomass (Kg)	942.87±120.21[a]	961.70±51.98[a]	938.87±54.08[a]
Survival (%)	90.9±6.24[a]	93.3±2.57[a]	91.6±3.59[a]
Feed conversion ratio	1.48±0.34[a]	1.64±0.24[a]	1.29±0.04[a]
Yield (Kg m^{-3})	3.14±0.40[a]	3.20±0.17[a]	3.12±0.18[a]

Means ± SD. The different letters indicate significant difference ($p<0.05$) between treatments in the same line by Tukey test.

Results and Discussion

The pH, alkalinity, nitrite and SS were different among treatments in that variables were higher in heterotrophic treatment tanks compared to mature and chemoautotrophic treatment tanks. No significant differences were observed in salinity, nitrate, VSS and FSS concentration among the treatments. Total ammonia and nitrite were higher in heterotrophic treatments tanks. Orthophosphate was lower in chemoautotrophic treatment tanks compared to heterotrophic and mature treatment tanks. This study demonstrated that the stimulation of the nitrification on the water can maintain the nitrogen compounds on the water (ammonia and nitrite) in low levels, avoiding peaks, without the addition of organic carbon sources. No significant differences were observed in the average weight, growth rate, final biomass, survival, feed conversion ratio and yield among treatments (Table 4). Therefore, the growth performance of shrimp was not affected by the different water preparation evaluated.

Conclusion

Mature and chemoautotrophic system controls better the nitrogen compounds on the water, without the use of extra carbon source. Shrimp performance in all systems (mature, chemoautotrophic and heterotrophic) is not affected.

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ASSESSMENT OF SUSTAINABLE AQUAFEED FORMULATIONS USING TWO APPROACHES: GROWTH TRIAL AND A DYNAMIC NUTRIENT-BASED MATHEMATICAL MODEL

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Introduction

The current drive for aquaculture sustainability has pushed feed formulators for the design of novel aquafeeds, where alternative ingredients (e.g. insect products, by-products of fisheries and animal production, and algae-based products) have been prefered in relation to those commonly used up to date (e.g. fish meal, fish oil, soybean meal) due sustainability issues (Oswald et al., 2019). A challenge associated with this transition is ensuring that novel feeds provide all required nutrients, without affecting, and when possible improving, fish farming performance (feed conversion, fish composition, and environmental impacts).

Thus, a nutrient-based model (FEEDNETICS, Silva and Soares, 2018) was applied *a priori* of a nutrition trial with Gilthead seabream (*Sparus aurata*) performed in the framework of the H2020 GAIN project, in order to have a preliminary assessment of the performance of three novel aquafeeds formulations against a traditional diet. The objective of this study was to compare these results with the ones that will be obtained by the nutrition trial with the same feed formulations, in order to evaluate to what extent this model can be used as a decision-support tool in the formulation of novel aquafeeds.

Methods

Four different diets where tested in both the nutritional trial and the virtual trials performed using FEEDNETICS model (calibrated for Gilthead seabream): 1) a control diet containing traditional soy products (CONTROL); 2) a diet rich in processed animal proteins (PAP); 3) a diet with alternative ingredients without the inclusion of PAP (NPAP); and 4) a diet containing a mixture of alternative ingredients (MIXED).This nutritional trial was performed with four replicates, each one with 55 fish with an initial weight of 30g. Each treatment followed a single-dietary regime (i.e. diets abovementioned), under a temperature of 23º±C, during 9 weeks. The results of virtual and nutritional trials were compared, in terms of growth, FCR, fish DHA+E A contents, and total nitrogen excretion (g N/kg biomass gain).

Results

Results after running the model revels that the average body weight tends to be greater in fish fed with MIXED diet and the lower body weight was predicted as being in fish fed with PAP diet (Figure 1). This is consistent with outcomes observed in other parameters such as the lower FCR and N waste predicted for fish fed with MIXED diet (Table 1). On the other hand, percentage of DHA seems to be greater in all formulated diets when compared to CONTROL. However, EPA percentage was higher in CONTROL diets (Table 1).

Discussion and Conclusions

The model suggests that some of the formulated diets for this experiment bring different results in fish performance, nitrogen waste and lipids composition. The model has also shown adaptability in running predictions for diets with different formulations for specific temperatures in gilthead seabream. The results of the virtual vs. actual nutritional trial will be compared, and divergencies will be discussed taken into account differences in digestibility and feed intake of the diets. Results suggest that the use of a modelling tool such as FEEDNETICS can help on the screening of formulated diets before *in vivo* experiments and decrease the number of fish used in aquaculture trials, reducing costs and increasing speed of development of novel aquafeed formulations.

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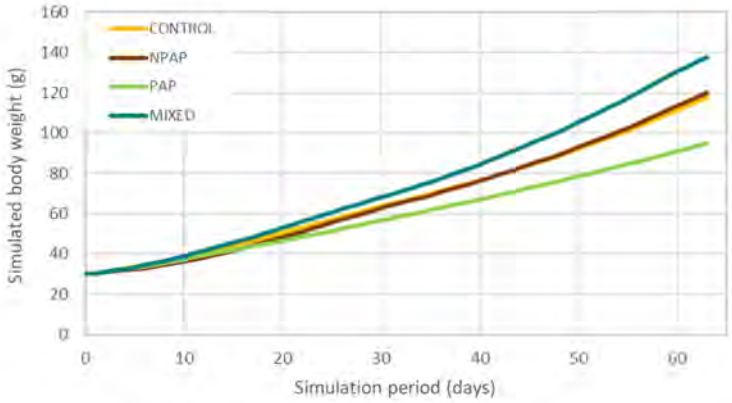

Figure 1: Final simulated body weight run by the model.

Table 1: Food Convertions Factor (FCR), total nitrogen waste and fatty acid percentage (DHA and EPA).

	CONTROL	NPAP	PAP	MIXED
FCR	1.12	1.09	1.38	0.98
Total N waste *(g N/kg of biomass gain)*	44.19	44.58	62.09	37.05
DHA *(% of total FA)*	5.45	6.17	6.51	6.37
EPA *(% of total FA)*	6.12	3.13	3.25	2.99

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LEVELS OF VITAMIN A, D AND K IN DIETS HIGH IN PLANT BASED FEEDSTUFFS FOR GILTHEAD SEA BREAM (*Sparus aurata*) FINGERLINGS

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Introduction

Substitution of fish based ingredients may alter the nutritional profile of the feeds, including the vitamins contents, ultimately leading to unbalanced vitamin supply (Hansen et al., 2015). Requirements for several vitamins have been established for species such as carps and salmonids (NRC, 2011), but adequate levels for gilthead sea bream are yet unknown for most vitamins.

Vitamin A is directly involved in preserving the epithelium, cell differentiation and reproduction, and is also required for regeneration of rhodopsin, essential for vision (Halver and Hardy, 2002; NRC, 2011). It also intervenes in chondrocytes development, hence playing a role in skeletogenesis (Lall and Lewis-McCrea, 2007). Therefore, low levels of vitamin A supplementation may cause poor growth and abnormal bone development amongst other symptoms (Halver and Hardy, 2002; NRC, 2011). Besides, in gilthead sea bream excess vitamin A led to changes in bone homeostasis and structure (Fernández et al., 2012).

Vitamin D is mainly involved in Ca homeostasis by regulating Ca uptake and liberation from bone intervening in bone remodelling (Halver and Hardy, 2002; NRC, 2011; Boglione et al., 2013). Fish are unable to synthesise vitamin D and so require absorbing it directly from the diet (Lock et al., 2010). Once it is absorbed deposition takes place mainly in liver, intestine, kidney, spleen, gills, skin and muscle (Lock et al., 2010). Bone mineralization has been used as a biomarker for vitamin D optimum levels (Fleming et al., 2005).

Vitamin K is involved in blood clotting, and inhibiting bone resorption (Halver and Hardy, 2002; Lall and Lewis-McCrea, 2007; NRC, 2011). Deficiency curses with increased blood clotting times, anaemia, haemorrhages and skeletal anomalies (Halver and Hardy, 2002; Roy and Lall, 2007). Skeletal anomalies and bone mineralization have been described as biomarkers for vitamin K requirements (Roy and Lall, 2007).

The essentiality of these vitamins for gilthead sea bream nutrition is evident, thus the aim of these three studies was to evaluate the effect of dietary vitamin A, D and K supplementation in gilthead sea bream growth, productive parameters and health status when fed diets low in FM-FO.

Material and Methods

Three parallel trials were conducted based on a similar plant-based diet (FM 10% and FO 6%) containing five increasing supplementation levels for the different vitamins. Feeds from the vitamin A trial were supplemented with 0.38, 0.75, 1.50, 3.00 and 6.00 mg kg^{-1} retinyl acetate; those from the vitamin D trial contained 0.054, 0.057, 0.060, 0.066 and 0.078 mg kg^{-1} cholecalciferol; while the vitamin K trial included 0.0, 4.9, 9.8, 19.6 and 39.2 mg kg^{-1} menadione. Gilthead sea bream fingerlings, weighing 20.4 ± 1.3 g were distributed into 45 tanks (15 per trial) in triplicate groups per diet and fed until apparent satiation thrice daily for 70 days. Water temperature, oxygen and feed intake were monitored daily. Growth and productive parameters were monitored along the trial and samples for X-ray analyses, bone molecular markers, histology and proximal composition were taken.

Results

At the end of the feeding trials, fish had doubled their weight independent of the diet, and no mortalities were registered. Fish fed vitamin A presented an increase in growth when fed up to 1.50 mg kg^{-1} retinyl acetate. At the end of the trial skeletal anomalies were predominantly found in the anterior region including cranium and pre-haemal vertebrae, especially due to the high prevalence of pre-haemal lordosis. However, the prevalence of pre-haemal lordosis was not directly related to the dietary inclusion of retinyl acetate. Only maxillary anomalies seemed to follow a quadratic regression which appeared to be reduced with increasing levels of dietary retinyl acetate.

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Vitamin D supplementation did not produce a significant effect on growth regardless of the different levels. The prevalence of total severe skeletal anomalies, anomalous maxillary and caudal vertebrae seemed to decrease until 0.06mg kg $^{-1}$ cholecalciferol supplementation, while higher supplementation translated in an increase in the prevalence of anomalies.

Only fish fed 19.6 mg kg^{-1} menadione presented an increased growth. The prevalence of severe skeletal anomalies and abnormal caudal vertebrae decreased with increasing dietary menadione up to 19.6 mg kg $^{-1}$ supplementation.

Discussion and conclusions

Overall, the results on growth, analysis of skeleton anomalies and molecular markers denoted the importance of these vitamins on bone development of gilthead sea bream fed diets containing low levels of fish ingredients

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EFFECTS OF DIFFERENT LEVELS OF VITAMINS B1, B9 AND B12 IN DIETS LOW IN FISHMEAL ON GILTHEAD SEA BREAM (*Sparus aurata*) FINGERLINGS

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Introduction

The current trend in aquaculture production is to shift from the traditional fish to plant based ingredients due to the rising prices and to the competence with other industries. However, the profile of vitamins in the plant based diets tend to be different to traditional fish based feeds, thus requiring vitamin supplementation in order to cover fish requirements. Vitamins are essential micronutrients which cannot be synthetized by the fish, and must be supplied through the diet in order to improve growth and health status. Amongst these B-vitamins are a group of water-soluble vitamins, that include thiamine (vitamin B1), folic acid (vitamin B9) and cobalamin (vitamin B12). These are involved in several metabolic processes, where they act as coenzymes or cofactors.

Thiamin is essential for enzymes involved in energy production, being its cofactor thiamin pyrophosphate. B1 must be constantly supplied in the diet to avoid deficiency signs such as neurological disorders, haemorrhages and congested fins (NRC, 2011).

Folic acid intervenes in blood synthesis and carbon transfer making it essential in the synthesis of amino acids, purines and nucleotides (Halver, 2002; NRC, 2011). Macrocytic normochromic megaloblastic anaemia is, probably, the most characteristic symptom of vitamin B9 deficienc , however other deficiency signs include poor growth, anorexia and dark skin coloration (NRC, 2011).

Cobalamin is an essential vitamin for animals, however certain microorganisms present in the intestinal tract can synthesise trace amounts of this vitamin. It intervenes in hematopoietic processes and is a growth factor. (Halver, 2002; NRC, 2011). Deficiency of this vitamin can lead to abnormal development of erythrocytes, thus leading to megaloblastic anaemia, but also to reduced growth. Other signs are rare, possibly due to the synthesis which takes place by the intestinal microflora (NRC, 2011).

Despite their importance, requirements for these vitamins have not been stablished for gilthead sea bream, and in fact, information regarding their effects on commercial marine species is scarce. Thus, the aim of this study was to evaluate the effects of dietary vitamin B1, 9 and 12 supplementation in gilthead sea bream growth, productive parameters and health status when fed diets low in FM-FO.

Material and Methods

A practical plant-based diet (FM 10% and FO 6%) containing five increasing supplementation levels for vitamin B1, B9 and B12 was formulated (Table 1).

525 gilthead sea bream fingerlings, weighing 12.5 ± 0.27 g (mean ± S.D.) were randomly stocked into 15 tanks, in triplicate groups per diet, and manually fed until apparent satiation four times per day for 9 weeks. Water quality parameters, including temperature and oxygen, and feed intake was recorded daily. Fish performance, in terms of growth and productive parameters (including hepatosomatic HSI, and viscerosomatic VSI indexes) was monitored several times during the experiment. At the end of the trial all the fish were anesthetised, fish were individually weighed and measured, and samples were collected for proximate composition, vitamin content in different tissues, histology, molecular markers and haematology.

Results

Feed acceptance was good in all the treatments, and by the end of the experimental period fish almost tripled their weight. Sporadic mortalities were recorded, although they were limited to one individual in some tanks, thus not being significant. External signs of vitamin deficiency (including changes in behaviour, haemorrhages, loss of equilibrium, anorexia or dark skin coloration) were not observed in the fish.

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Table 1. Vitamin B supplementation levels of the experimental diets

	Diet number				
mg kg^{-1}	**1**	**2**	**3**	**4**	**5**
Vitamin B1	0.04	3.10	3.80	5.10	7.80
Vitamin B9	0.06	1.71	3.12	6.00	11.60
Vitamin B12	0.042	0.070	0.140	0.280	0.560

Table 2. Weight, HSI and VSI of gilthead sea bream and liver proximal composition (% dry weight) of gilthead sea bream liver fed increasing contents of vitamin B1, B9 and B12.

Diet	1	2	3	4	5
Weight (g)	42.3±6.2	42.4±5.7	41.1±5.5	42.4±6.0	41.7±5.5
HSI (%)	1.70±0.33	1.56±0.37	1.68±0.32	1.68±0.40	1.43±0.23
VSI (%)	11.0±1.7	9.9±1.3	10.0±1.2	10.0±1.1	10.4±1.6
Lipids (%)	45.0±3.6	44.6±2.9	41.9±2.9	43.5±3.5	48.6±0.6
Protein (%)	32.4±0.4	32.0±2.3	34.0±3.6	32.5±1.5	33.2±0.6

At the end of the experiment there was no significant effect of vitamin supplementation on weight, HSI or VSI or on liver proximal composition (Table 2).

Discussion and conclusions

These preliminary results show that vitamin B1, B9 and B12 supplementation on gilthead sea bream fingerlings is not required to maintain growth during a 9 week period, if fed a practical diet based on plant ingredients. In fact, B vitamin requirements are usually based on growth, survival, absence of deficiency signs, tissue accumulation and metabolic biomarkers (Hansen et al., 2015). According to the first three criteria, it may seem as though sea bream fingerlings do not require additional vitamin B supplementation when fed practical plan-based diets for 9 weeks, nevertheless, vitamin B deficiencies can be more subtle and produce an effect at another analytical level. Further analyses are being conducted to understand these effects.This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727610 PerformFISH project. This output reflects the views only of the authors, and the European Union cannot be held responsible for any use which may be made of the information contained therein.

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SIMPLE ANALYSIS OF THE CARRYING CAPACITY OF AQUACULTURE IN A SEMI-CLOSED SEA AREA

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Introduction

The environmental impacts of aquaculture wastes should be predicted for sustainable aquaculture when the site and production density are determined. Sustainable Aquaculture Production Assurance Act was established in 1999 to preserve the environment of the aquaculture area. However, the problems of algal blooming and hypoxic waters occur frequently especially in semi-closed bays, resulting in decreasing the production of cultured fish (Zhang and Kitazawa, 2016). The proper density of aquaculture has not been estimated because the reason for water pollution is complex. The pollution is caused by the wastes of cultured fish as well as by the external loading of nutrients. The tool for estimating the carrying capacity of the cultured area is required for sustainable aquaculture. The estimating tool must combine preliminary monitoring and numerical simulation. In the present study, however, a simple index is presented by dimension analysis to discuss the carrying capacity of the aquaculture site.

Materials and methods

An index considers the balance between the rates of waste excretion and water exchange. The local pollution of the seabed and hypoxic water tend to appear in case of shallow waters because the wastes fall directly to the sea bottom. Current velocity is one of the key parameters in a view of horizontal diffusion of wastes. The index can be proposed

$$I = \frac{\rho \cdot d \cdot S \cdot u}{E \cdot d_s} \quad (1)$$

where r (=1,025kg m^{-3}) is the density of seawater, d (m) the mean depth of the aquaculture site, S (m^2) the area of the aquaculture site, u (m s^{-1}) the mean current velocity, E (kg s^{-1}) the rate of waste excretion, and d_s (m) the vertical position at which the waste is produced. The mean depth of the aquaculture site, the area of the aquaculture site, the rate of waste excretion, and the vertical position of waste production are the parameters determined by the aquaculture planning. The mean current velocity is obtained from more than 2-week measurement. Otherwise, the current velocity can be estimated approximately by the equation of small amplitude wave theory for shallow waters.

$$u = \frac{gak}{\sigma}\cos(kx - \sigma t) \quad (2)$$

where g (=9.8m s^{-2}) is the acceleration due to gravity, a (m) the amplitude of tidal waves, k (m^{-1}) the wave number, s (s^{-1}) the angular frequency of tidal waves, x (m) the coordinate in the direction of wave propagation, and t (s) the time. The mean current velocity can be calculated by the integration of the equation (2).

Three aquaculture sites are selected for estimating the index. The information on the aquaculture site, approximate current velocity, and the estimated waste excretion rate are summarized for each site as represented in Table 1.

Results and Discussion

The numerical values of the indices are 78.9, 24.2, and 6.0 x 10^7 for Site A, B, and C, respectively. The indices versus waste excretion rate per square meters, i.e. the density of aquaculture, current velocity, and water depth are represented in Fig. 1. The d and d_s are set as 15m and 5m, respectively, in the left graph, and u and d_s are set as 0.1m s^{-1} and 5m, respectively, in the right graph. Naturally, the index increases with decreasing waste excretion rate, increasing current velocity and

(Continued on next page)

Table 1 Information on aquaculture at three sites.

Parameter	Site A	Site B	Site C
Production	4,500t year^{-1}	2,500t year^{-1}	1,000t year^{-1}
S	1.3 x 10^{6}m^{2}	2.5 x 10^{5}m^{2}	1.3 x 10^{5}m^{2}
u	0.2m s^{-1}	0.1m s^{-1}	0.1m s^{-1}
ρ	1,025kg m^{-3}	1,025kg m^{-3}	1,025kg m^{-3}
E	35,500t year^{-1}	10,000t year^{-1}	14,000t year^{-1}
d	50	15	30
d_s	15	5	15

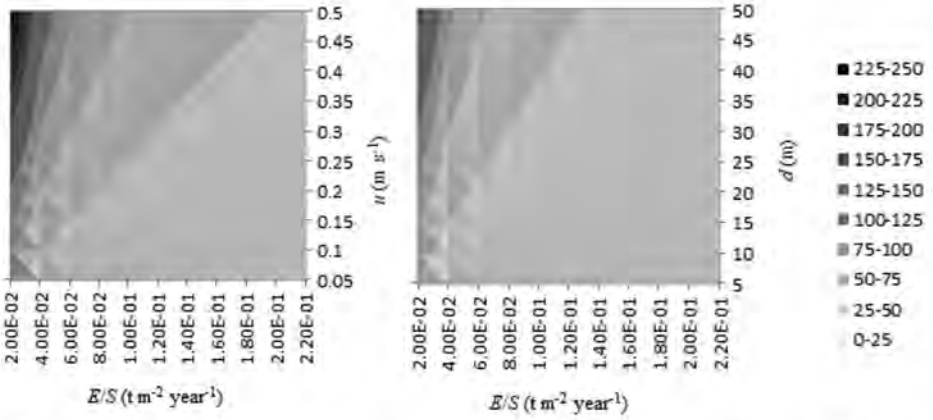

Fig.1 The index versus waste excretion rate per square meters and current velocity (left) and the index versus waste excretion rate per square meters and water depth (right).

water depth, so the aquaculture becomes sustainable if the value of the index is higher. The combination of the different parameters can represent the same value of the index. However, the threshold of the index for sustainable aquaculture cannot be still determined. Before determining the threshold, the accuracy of the index should be improved. For example, the definition of the area of the aquaculture site should be reviewed, whereas the licensed area is given in the present study. The relative importance of the parameters, i.e. the density of production, approximate current velocity, and water depth, should be considered, so each parameter will be weighed by factors. Also, the loadings of nutrients from neighboring aquaculture sites and land are not taken into account. Initial water quality before the installation of the aquaculture site may provide the information on the existing loadings of nutrients in the target area. The observations and the numerical simulation using a hydrodynamic and ecosystem coupled model can increase the accuracy of the index. Finally, the index should be applied to the aquaculture sites as much as possible in order to compare the index and the current environmental situation with each other.

Acknowledgement

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EVALUATION OF BIOACCUMULATION FACTOR (BAF) OF SOME METALLIC ELEMENTS IN ARAS RIVER FISH - EAST AZERBAIJAN PROVINCE

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The Aras River is one of the Border Rivers of Iran which flows 470 km from the border of Azerbaijan, Iran and Armenia to the Caspian Sea. Aras River water is used for domestic, agricultural, industrial and mining purposes. The entry of some environmental pollutants including metallic elements due to the activity of some mines at the margin of the river has contaminated the Aras River and because heavy metals are one of the most important environmental pollutants that cause harmful effects such as growth retardation, behavioral changes, genetic variations and fish mortality. In the present study, the amount of some heavy metals in the Aras River fish was investigated. The survey was conducted from 2015 to 2016 in four seasons at four stations in the Aras River from Jolfa County up to 25 km after the Nurduz border. Fish were captured using the electric fish shocker and cast net. The table below shows the changes in the average amount of bioaccumulation factor of copper, molybdenum, arsenic and mercury in the Aras River fish, and the total length and weight of fish. The bioaccumulation factor of molybdenum had the highest amount as compared to others, whereas the bioaccumulation factor of arsenic had the lowest amount as compared to others.

Among fish species, *Alburnoides bipunctatus* with the lowest total length and weight had the highest amount of bioaccumulation factor of molybdenum as compared to others, whereas *Barbus lacerta* had the highest amount of bioaccumulation factor of copper as compared to others.

FEEDING REQUIREMENTS OF THE TRITONS *Charonia seguenzae* (ARADAS & BENOIT, 1870)

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Introduction

Aquaculture is commonly considered an alternative for sustaining populations of endangered and intensively exploited aquatic species. In the case of te marine gastropod *Charonia seguenzae* (Aradas & Benoit, 1870), the population declined due to over-fishing for shell trade (Katsanevakis et al., 2008)but still exploited by Man, the present study attempts to gather and review the available scientific information on molluscs of minor commercial importance in order to assist in the adequate management and protection of their populations. Forty one species (18 gastropods, 13 bivalves, and 10 cephalopods and is protected in the Mediterranean Sea (Bern convention, Barcelona convention). Re-establishment of *C. seguenzae* populations in the depleted habitats requires knowledge of its biology and successful maintenance and breeding in captivity.

The diet of *Charonia seguenzae* is highly versatile with a wide range of alternative feeds (Doxa et al., 2012) supplementing natural preys. The knowledge of the feeding requirements of an organism is a prerequisite for the development of diet protocols for successful adaptation and husbandry in captivity with respect to welfare. Information about the optimal diet of an organism can derive from the study of its feeding preferences.

Materials and Methods

Fifteen wild individuals (641.9 ± 83.1g weight, 22.4 ± 1.4cm shell length) were submitted to three trials. For each trial one animal was placed at the center of a cylindrical tank (500L). Tritons were offered four different food types (sea cucumber, *Holothuria polii;* fish, *Boops boops*; squid, *Loligo opalescens;* shrimp, *Penaeus kerathurus*), placed at the circumference of the tank and allowed to consume *ad libidum* the preferred feed/s for 23h. The chemical composition of each food type was analyzed with the methods specified by the A.O.A.C, 1984 A.O.A.C., Official Methods of Analysis. In: S. Williams, Editor, Association of Official Analytical Chemists, Washington (1984), p. 1018.A.O.A.C. (1984). The energy contained in the food was calculated according to Garling and Wilson (1976). Preferred feed/s and food consumption (g) per type was measured and the content of consumed food in organics and energy was calculated.

Results

Sea cucumber was the most consumed feed reaching 80% of the trials (Fig. 1A), followed by fish (73.33%), squid (46.66%) and shrimp (44.44%). Tritons chose to consume more than one feed during 84.44% of the trials, consuming two types at 37.78%, three types at 33.33% and all of the 4 available options at 13.33% of the trials. Sea cucumber was also the feed that was consumed in higher quantity reaching 35.94±6.7g per trial (Fig. 1B) followed by squid (9.68±3.52g), fish (8.71±2.57g) and shrimp (0.96±.17g) with observed differences being statistically significant (Kruskal-Wallis, $p<0.001$). The consumed food content in terms of organics (Fig.1C) and energy content (Fig.1D) was higher when consuming more than one types, however the observed differences were not statistically significant.

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Discussion

Tritons showed a clear preference for sea cucumbers. It was the feed that was consumed during most of trials, in higher quantities. This is probably associated with the natural diet of the species that consists at echinoderms (Russo et al., 1990). Despite the high preference for sea cucumbers, tritons chose to consume more than one food types, indicating that sea cucumbers do not entirely cover their feeding requirements. Furthermore, the organics and the energy they acquired when consuming more than one species were higher. Summing up, an appropriate feeding protocol should integrate a variety of food sources including fish and squid to supplement sea cucumbe .

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EFFECTS OF HEAT KILLED *Lactobacillus plantarum* L-137 (HK L-137) SUPPLEMENTAL DIETS ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF BIGHEAD CATFISH (*Clarias macrocephalus*)

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Introduction

The bighead catfish, *C. macrocephalus* is one of the important fresh water culture species in the Mekong Delta of Vietnam because of its high market value and demand due to delicious meat and attractive yellow saffron meat color. For enhancing quality of the juvenile bighead catfish, this study was conducted to determine effects of HK L-137 on growth performance and immune response of the juvenile bighead catfish

Materials and methods

The feeding experiment was included four treatments with iso-nitrogenous (45 % crude protein) and iso-lipid (8 % lipid). The diets were formulated to contain different HK L-137 (It was made by House Wellness Foods Corporation in Itami, Japan) levels of 0 (as control diet), 10, 20, and 50 mg kg^{-1} diet. The feeding experiment was conducted in the 200-L composite tank (filled with 150 L of fresh water) in the recirculating aquaculture system. The juvenile bighead catfish (9.69±0.24 g/ind) were randomly stocked in previously prepared twelve tanks at a stocking density of 150 fish per tank with triplicates per dietary treatment, and were fed the respective diets for 60 days. The bacteria challenge with *Areomonas hydrophila* was conducted continuously at the end of the feeding experiment.

Results

At the end of the feeding experiment, the results showed that the fish was fed the diet at 10, 20, and 50 mg/kg HK L-137 significantly grew faster than control treatment. Similarly, significantly improved protein efficiency ratio, feed conversion ratio, survival rates, and serum lysozyme activity than those in without HK L-137 treatment. Total leukocytes count were significantly improved in fish fed 20 and 50 mg/kg HK L-137 compared to this in without HK L-137 treatment. Apparent digestibility coefficients (ADC) of crude protein and lipid were not significantly different among all treatments; however, ADC of dry matter and ash were higher significant in diet at 10 and 20 mg/kg HK L-137 compared to this in without HK L-137 treatment.

A bacterial challenge test with *A. hydrophila* revealed that the fish was fed the diet at 10, 20, and 50 mg/kg HK L-137 (~50, 100, 250 ppm of LP20) significantly had the lower cumulative mortality than those in control treatment

Discussion and conclusion

From previous studies, it has been suggested that enhanced growth performance of fish fed dietary HK L-137 might be attributed to elevate health status, digestibility, stimulation of gastric development and/or enzymatic secretion (Tovar-Ramirez et al., 2002; Nguyen et al., 2019). In additional, Dawooda (2015) suggested that HK L-137 helped farmed tilapia utilize the test diets efficientl , resulting in improved FER, which would be one reason for the faster growth of fish fed HK L-137. The improved feed utilization may increase the availability of protein and energy from food to be used for fish growth (Zaineldin et al., 2018). This study demonstrated that HK L-137 enhanced non-specific immune defense system of bighead catfish, providing them with higher resistance to with *A. hydrophila,* and better growth performance.

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Table 1. Growth parameters, survival rate and nutrient utilization in bighead catfish fed experimental diets for 60 days. W_i : initial mean weight, W_f : final mean weight, Wg: mean weight gain, SGR: specific growth rate, FI: feed intake, PER: protein efficiency ratio, FCR: feed conversion ratio, SR: survival rates, Y: yield

Parameters	HK-L137 (mg/kg)			
	0	10	20	50
W_i (g)	9.69±0.17^a	9.69±0.24^a	9.69±0.13^a	9.69±0.14^a
W_f (g)	72.9±2.62^b	79.5±2.05^a	76.5±2.01^a	77.5±1.81^a
Wg (g)	63.2±2.76^b	69.8±1.91^a	66.8±2.06^a	67.9±1.73^a
SGR (% day^{-1})	3.36±0.09^b	3.50±0.04^a	3.44±0.05^{ab}	3.46±0.03^a
FI (% day^{-1})	5.86±0.38^a	5.62±0.49^{ab}	5.21±0.11^{bc}	5.42±0.31^{ab}
PER	1.69±0.05^b	1.92±0.06^a	1.95±0.07^a	1.97±0.10^a
FCR	1.33±0.12^a	1.16±0.04^b	1.09±0.08^b	1.13±0.06^b
SR (%)	87.5±1.84^b	92.3±1.76^a	91.7±1.92^a	92.5±2.27^a
Y (kg per m^3)	63.8±1.20^c	73.4±3.16^a	70.1±1.16^b	71.7±1.72^{ab}

Values are means of three replicate group's ± SD. Within a row, value with the same letters are not significantly different (P>0.05).

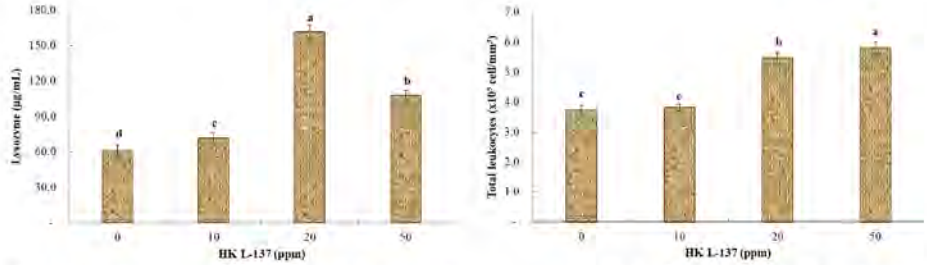

Fig.1. Serum lysozyme activity and total leukocytes count of bighead catfish after 60 days culture with dietary supplementation in different concentration of HK L-137.

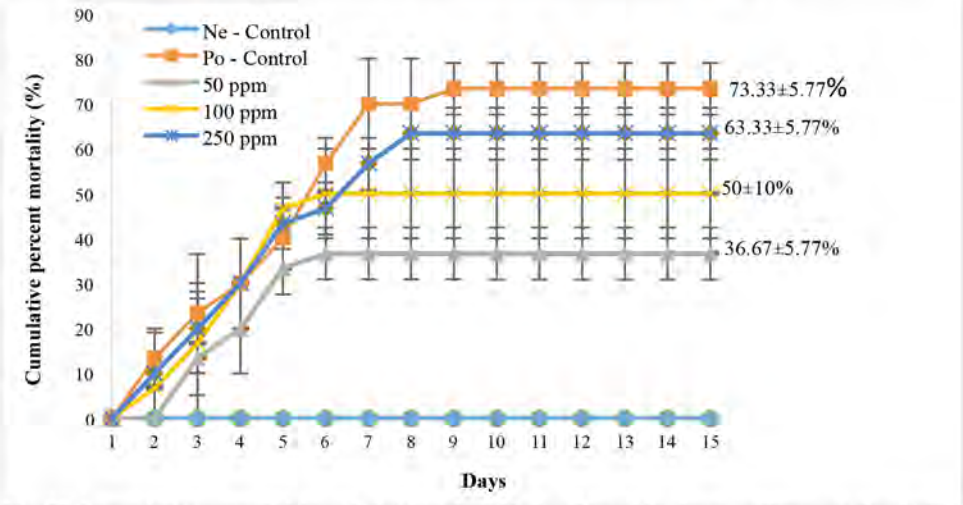

Fig.2. Cumulative mortality of bighead catfish after 60 days culture with dietary supplementation in different concentration of HK L-137 to challenge with *A. hydrophila*.

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DEVELOPING A CLINICAL DATABASE, MOBILE APPLICATION (APP), AND LEARNING GAMES FOR FISH DISEASE DIAGNOSTICS

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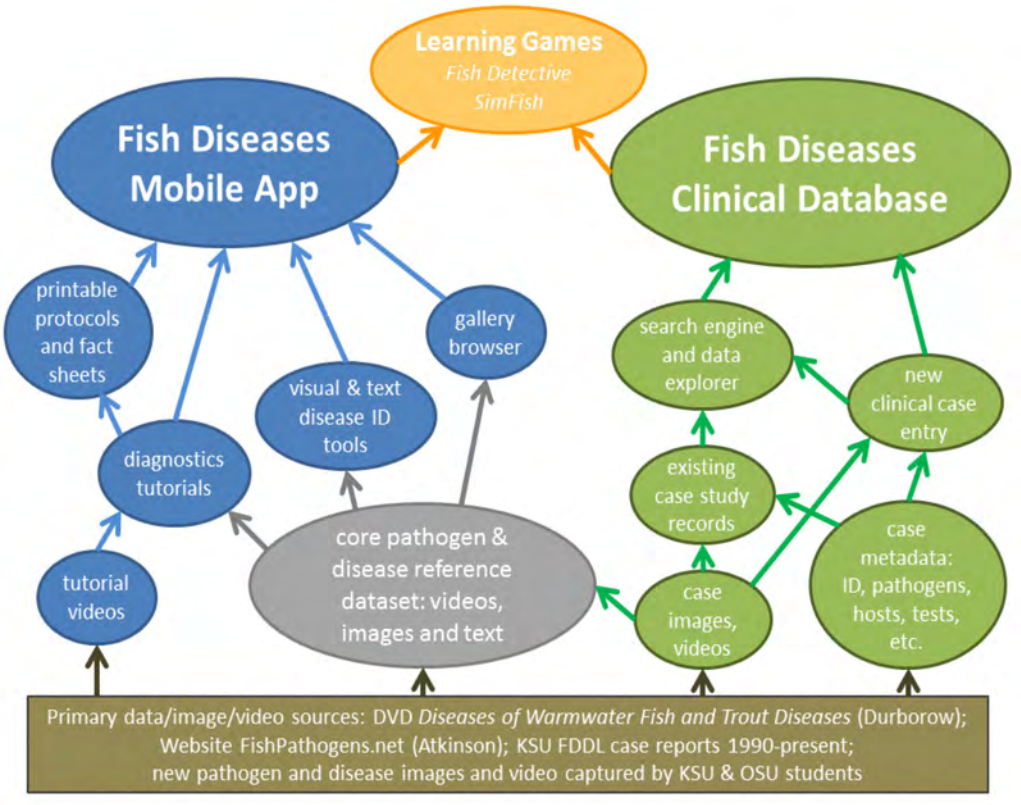
Kentucky State University and Oregon State University are developing a mobile application (App) with associated clinical Database and learning games to facilitate identification and treatment of fish disease cases encountered in the field. The clinical Database will enable fish disease diagnostic laboratories to organize disease records to allow for searches and epidemiology work; standardization of the Database among laboratories will facilitate capabilities for regionally coordinated epidemiological work. The App and learning games can be used as teaching tools to aid in fish disease education. The Database will complement the App by using learning machine technology (an artificial intelligence agent) to inform the App on patterns of disease occurrence and treatment efficac . This will aid App users to identify problems they find in the field and decide on treatment recommendations that have the highest likelihood of succeeding. The following flow diagram illustrates the objectives of this US Department of Agriculture funded Extension and teaching project.



DESIGN AND DEVELOPMENT OF RECIRCULATING LIFE-SUPPORT SYSTEM AS AN AMENDED LIVE FISH ROAD TRANSPORTATION FACILITY

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Introduction

Live fresh fish is often the most preferred and highly priced form of fish trading and has the largest amount of share for direct human consumption, 45% in 2016, followed by frozen (31%), prepared and preserved (12%) and cured (dried, salted, in brine, fermented smoked) (12%) (FAO, 2018). The main attraction in selling fish live and fresh is the increased return obtained because of their greater market value. Technological developments, improved logistics and increased demand improved the commercialization of live fish. Nevertheless, marketing and transportation of live fish is a challenging task. It involves complicated interaction between fish carrying activities, the environment and human factors as well as they are often subjected to stringent health regulations, quality standards and animal welfare requirements.

Orthodox systems for transporting live fish range from simple artisanal systems of plastic bags with an atmosphere supersaturated with oxygen, to specially designed or modified tanks and containers. All these conventional practices have technical issues and pragmatic research is lacking which identify the need of improvement for the levels of acceptability in all aspects of fish welfare (Ashley, 2007). In many developed countries, live fish transportation has been evolving from traditional methods to more advanced and sophisticated life support systems that regulate temperature, filter and recycle water, and add oxygen (King, 2009).

Design overview of the life support system

Designed life-support systems are typically composed of special purpose built insulated tank fitted with provisions for filtration of tank water to control the critical water quality parameters like temperature, dissolved oxygen (DO) levels, pH, carbon dioxide (CO_2) and ammonia (NH_3) concentrations. Recirculation of water is planned to be achieved by means of a hydraulic ram pump which uses kinetic energy of falling water. The potential energy of tank height holding water will govern the ram pump to recirculate the water through the filtrati n unit. Two water wheel is adequately designed in the path of flowing water in the system to add oxygen in the water. This system is continuous self-functioning and does not require any external energy to filter and recirculate the water during transportation

Conclusion

On-trip functioning life-support device can be a very effective and economic option to provide a continuous quality maintenance system to withstand all the suboptimal condition and shock loading during transportation. This amended transportation facility will also explore the distribution possibilities of fresh live fish to a distant market

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NOVEL INDICATOR TRAITS FOR FEED EFFICIENCY IN ATLANTIC SALMON (*Salmo salar)*

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Introduction

A measure of the efficiency of converting feed into growth is the feed conversion ratio (FCR), which is the amount of feed consumed per unit of growth. Improved feed efficienc , by improved growth or other means, will reduce production costs and reduce the environmental footprint per unit of product (Besson et al., 2016). Direct genetic selection for improved feed efficiency assumes that both individual growth and individual feed intake can be routinely recorded for a large number of individuals. However, since salmonids are kept in large units and fed communally by dispersing feed into the water, recording of individual feed intake is difficult at current. Individual feed efficiency in fish is, therefore, improved through indirect selection for a larger growth rate (Thodesen et al., 2001). Although growth and feed efficiency are correlated traits, the growth rate does not explain all variation in feed efficienc . Hence, it is time to address new indicator traits potentially more related to feed efficiency than growth in order to improve selective breeding for feed efficienc . The aim of the study was to identify novel indicator traits for the individual metabolic efficiency using stable isotopes to define the phenotypes.

Materials and methods

A 12-day experiment was conducted with 2281 Atlantic salmon parr, from 23 full-sib families that were allocated to 46 family tanks and fed an experimental diet enriched with the stable isotopes ^{15}N and ^{13}C. The fish were pit-tagged with a 2x12mm unique glass tag (RFID Solutions, Hafrsfjord, Norway), and a fin-clip was collected for genotyping. All fish were genotyped using AquaGen's custom Axiom®SNP genotyping array from Thermo Fisher Scientific (San Diego, CA, USA), which contains 56,177 single-nucleotide polymorphisms (SNPs). Muscle tissue samples were analyzed for the stable isotopes ^{15}N (AMN_i) and ^{13}C (AMC_i), and these were used to obtain individual indicator phenotypes for the metabolic efficiency (IFCR); and , defined as follows (takin ^{15}N as an example):

$$IFCR\_AMN_i = \frac{FW_i * APE_{Ni}}{FW_i - IW_i},$$

where FW_i and IW_i are final and initial weights of individual i, and is the excess atom percentage ^{15}N in muscle for individual i adjusted for the initial atom percentage (IA%) in the muscle tissue. The IFCR is a ratio of the "metabolic costs" (synthesis allocated to growth and replacement of nutrients) to total body growth, within the same time period. In addition, feed conversion ratio was recorded on a tank level (). Bivariate genetic analyses were conducted between and tank averages of $\overline{IFCR\_AMN}$ and $\overline{IFCR\_AMC}$.

Results and discussion

The metabolic efficiency is a major determinant in the conversion of feed into growth. Consequently, minimizing the energetic cost of maintenance is a strategic goal to obtain fish with enhanced growth and improved feed efficienc . Using feed enriched with certain isotopes (i.e., with altered ratios of ^{14}N/^{15}N and/or ^{13}C/^{12}C) and monitoring the subsequent rate of change in isotope profile in various tissues, the relative contribution of the nutrients to protein growth can be assessed (Le Vay & Gamboa-Delgado, 2011). After a dietary switch, the IFCR variables for nitrogen and carbon in the muscle are expected to be proportional to the mass of newly deposited nutrients in muscle and, as such, relate directly to the efficiency complex. Because the ratio is expected to be proportional to the amount of newly deposited body nutrients per g increase in body weight, fish that exchange a larger fraction of the body mass per unit of growth will be less feed-efficient. Efficient fish should be characterized by a low ratio between total synthesis (to replace degraded nutrients and synthesis of new tissue) and growth, i.e., as much as possible of the synthesis should be allocated to growth and as little as possible to replace

(Continued on next page)

degraded nutrients. The estimated genetic correlation between and was very high, to the extent that the estimate was fixed at the border of the parameter space (~ 1.0) for both nitrogen and carbon metabolism in muscle. The corresponding phenotypic (tank-level) correlations were 0.72 and 0.58, respectively. These results indicate that the mass of new nutrients in the muscle is closely genetically associated with FCR at the tank level. Since IFCR can be measured in individual fish, it is a promising indicator trait for individual feed efficienc . However, the heritability estimates of IFCR for both carbon and nitrogen were low (0.06 to 0.09). The indicator ratio traits, IFCR, are intuitively appealing and can easily be interpreted biologically. The IFCR variable allows for direct measurement of carbon and nitrogen fluxes by using stable-isotope profiling to trace the contribution and allocation of nutrients from feed to growth in animal tissue (Barreto-Curiel et al., 2018), and it is expected to have a universal relationship with FCR and could be of use independent of life-stage and species.

For complete results see Dvergedal et al. (2019).

Conclusion

Our findings show that the use of indicator ratio traits to assess individual feed efficiency in Atlantic salmon have great prospects in selection programs. Given that large quantities of feeds with contrasting isotope profiles of carbon and/or nitrogen can be produced cost-effectively, the use of stable isotopes to monitor nitrogen and carbon metabolism in various tissues have potential for large-scale recording of individual feed efficiency traits, without the requirement of recording individual feed intake.

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IFISHIENCI: INTELLIGENT FISH FEEDING THROUGH INTEGRATION OF ENABLING TECHNOLOGIES AND CIRCULAR PRINCIPLE

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Introduction

Today the majority of aquaculture production is done in land-based flow-through or pond systems, marine cages or recirculating aquaculture systems (RAS), with a range of production costs and environmental impacts. The increasing demand for fish has resulted in more costly, intensive aquaculture solutions with increasing sizes in rearing environments on land (RAS), coastal (semi-closed containment systems) and off-shore systems. This leads to new challenges to contain stress and maintain fish health and welfare in these production systems. Reducing stress will reduce its deleterious effects on fish behaviour, development and growth, feeding yield, reproduction, and immune function, improving overall health and welfare (Ebbesson and Braithwaite 2012). Some of the key preventative measures to maintain healthy populations is 1) to monitor the environmental and biological dynamics and limits in the systems through real-time monitoring, 2) the use of quality feeds with proper nutritional value, including functional additives to boost the immune system, reduce stress and aggression, and welfare, if needed (Sutili et al 2017).

Summary

The EU-funded iFishIENCi innovation action is bringing together 16 partners in a trans-disciplinary effort towards making genuine improvements to fish farming worldwide. Fish aquaculture is essential for providing healthy food to a growing world population, but its success depends upon our ability to find more sustainable farming practices. This means more effective ways of monitoring fish-health and welfare, as well as more efficient ways of feeding fish that reduce pressure upon the present sources of fish-feed ingredients, such as agricultural crops and wild-caught fish for fishmeal and oil. The ambition of iFishIENCi is developing and demonstrating disruptive IoT/AI based innovations, considering the feeding value chain as a whole, and addressing four commercially-important species (salmon, rainbow trout, sea bass and African catfish), with consumers demand as focus.

iFishIENCi aims to deliver breakthrough innovations supporting sustainable aquaculture, based on enabling technologies and circular principles, thereby providing the European aquaculture industry with the competitive advantage and growth stimulation needed to be a mover in revolutionizing global efficiency in fish production and meet society's needs for food from the ocean. This ambitious task will be achieved by providing to the market a flexible iFishIENCi Biology Online Steering System (iBOSS) that significantly improves production control and management for all fish aquaculture systems. iBOSS will maximise feed utilisation through smart feeding, provide continuous monitoring of fish behaviour, health and welfare and reduce response times to aberrations. iFishIENCi will target circular principles and zero waste by qualifying new and sustainable organic value chains for feeds, and valorisation of by-products. iFishIENCi´s innovations will provide important new assets to the consortiums SMEs, fish-farmers, feed producers and technology providers in the aquaculture sector, leading to market growth and job creation. Assets will be maximized through comprehensive sustainability assessments and engagements with the sector, regulators and consumers.

The overall strategy of iFishIENCi is to set a world standard on digital aquaculture: (1) by setting "the fish" at the heart of the decision-making in fish production, through joining forces between experts in fish and fish-farming and experts in digitization, IoT and AI; (2) by selecting the most promising emerging technologies on smart monitoring and control system, and pushing them forward through targeted development and integration; (3) by demonstrating the value of new and sustainable feed sources, contributing to the consolidation of a circular and blue bioeconomy; and (4) by proposing optimal value-chain for valorisation of waste from fish farming. To reach its ambition of improved competitiveness of the European aquaculture and greener society, iFishIENCi is built around five innovation pillars leading to four main products (Figure 1).

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STUDY OF THE EVOLUTION OF MICROORGANISMS COMMUNITIES IN AN AQUAPONIC SYSTEM OVER THE COURSE OF A FULL LETTUCE GROWTH CYCLE

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Introduction

Aquaponics is a combination of recirculating aquaculture and hydroponics techniques that enables the recycling of the nutrient-enriched aquaculture effluents whilst at the same time avoiding the use of mineral fertilisers for the growth of plants. Aquaponics is also a system based on a fragile equilibrium between 3 groups of living organisms i.e. fish, plants and microorganisms. Except for the nitrification process, little is currently known on the roles and properties of these microorganisms and even less when it comes to the bacteria and fungi which may be involved in plant beneficial interactions. The nitrification process is crucial in aquaculture and has therefore been studied in depth. The principal bacteria involved in the transformation of ammonia to nitrite are *Nitrosococcus*, *Nitrosospira* and *Nitrosomonas* while the bacteria responsible for the conversion of nitrite to nitrate are *Nitrobacter*, *Nitrospira* (Rurangwa and Verdegem, 2015), *Nitrococcus* and *Nitrospina* (Itoi et al., 2007). The aim of this work is thus to thoroughly study the microorganisms communities harboured by the closed-loop aquaponic system of Gembloux Agro-Bio Tech throughout a full lettuce cycle, in real conditions. To this end, high throughput sequencing is used to sequence microorganisms' DNA and bioinformatics tools are then used to assign taxonomic identification. Gaining a better understanding of the role of microorganisms in aquaponics could help foster the development of more viable and productive systems.

Material and methods

A first experiment was conducted between the 5th and 19th of May 2017 in the PAFF Box aquaponic system (Delaide et al., 2017) in Gembloux Agro-Bio Tech, which was at the moment hosting a variety of plants in the greenhouse compartment and Nile tilapias (*Oreochromis niloticus*) in the tank compartment. One sump sample and one biofilter sample per week were collected in the system. Bacterial DNA was then extracted from the samples and the 16S rRNA gene was sent for Illumina sequencing. The aim of this first trial was to gain a first insight of the evolution of the bacterial communities in our aquaponic system. To obtain a more in depth view, a second experiment was launched in 2019. 90 lettuces (*Lactuca sativa* var. Lucrecia) were sown on the 27.02.19 in rockwool plugs and were then transferred in the floating raft compartment of the PAFF Box (Delaide et al., 2017) aquaponic system on the 11.03.19. The system was at the moment hosting 34 Nile Tilapias (*Oreochromis niloticus*). Microbiota samples were collected twice per week during the first three weeks and then once per week during the last three weeks. Samples were harvested from the sump, the biofilte , the roots' rhizoplane and the roots' endosphere. DNA was then extracted with the FAST DNA Spin Kit (Mp, Biomedicals) and is currently being sequenced on an Illumina MiSeq machine. Data will be analyzed with the QIIME 1.9.1 program (Caporaso et al., 2010).

Results and discussion

The results of the first experiment showed that there seemed to be different communities harboured in the sump and in the biofilter with the biofilter community being significantly more diverse. No significant difference was observed in any community over the course of the three weeks.

Results from the second experiment are now awaited to answer our new hypotheses:

- Can we observe changes in the composition of the microbial communities when the aquaponic system is relaunched after the winter break?
- Do the bacterial AND fungal communities evolve over the course of a complete lettuce cycle of 6 weeks?
- What is the composition and the evolution of the microbial community present on the lettuces' roots?

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USE OF COPPER ALLOY MESH IN MEDITERRANEAN MARINE AQUACULTURE

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Introduction

Biofouling is a major problem in marine aquaculture with significant production impacts (Braithwaite & McEvoy 2005; Fitridge et al. 2012; Floerl et al. 2016). Nets made from nylon are susceptible to biofouling and require frequent maintenance with high costs. The use of copper alloy nets in aquaculture brings a promising solution to this problem (Chambers et al. 2012). In the present study, that was conducted for the first time in Greece, we demonstrate the comparative results between fish s microbial flora, length, weight and mortality being cultured in conventional net cage and those being raised in brass UR30® net cage. The wire UR30® is environmentally friendly, antifouling, has anti-microbiological properties, durability, is corrosion resistant and 100% recyclable.

Materials and methods

As part of the research process, two fish cages were used in Kefalonia Fisheries where the species *Sparus aurata* (Linnaeus, 1758) (sea bream) was cultured. The cage A-249 was made of copper UR30® mesh whereas the cage B-228 was constructed of nylon mesh. Video recordings of fish and algae growth were conducted with a camera GHOST-S, HD DRIFT at various stages in both cages. Seven samplings of 10 random fish from each cage took place between December 2014 and November 2015. The somatometric characteristics of fish (weight, length) were measured. For the microbiological research of fish, samples of the oral cavities, the gills and blood were examined. The evaluation of the microbial flora of fish tissue samples took place at the Biopathology Laboratory of the Medical School of the National and Kapodistrian University of Athens by using 5 different culture media (Blood Agar, Mac Konkey Agar, Chapman Agar, Sabouraud Agar, and SS Agar). The identification of the strains was made by the automated system VITEK2 of Biomérieux Company.

Results

No biofouling was recorded in the copper net cage A-249 in contrast to nylon cage B-228 during the study period (Photo 1). The fish in the copper alloy mesh cage demonstrated increased mobility. The weight and length measurements of the tested fish are shown on Figure 1. The fish from the copper alloy cage were of greater length and weight compared to those raised in the nylon cage.

The microbiological examination showed that the microbiological flora (*Aeromonas* spp, *Vibrio* spp, *Pantoea* spp., *Brevudimonas* spp., *Sphingomonas* spp., etc.) of fish from both cages (A-249 and B-228) ranged in normal levels (Figure 2). However it was clear that the samples from the gills and the mouth of fish coming from the copper cage showed lower microbe values compared to those of the nylon cage. No microbial growth was recorded in the blood of fish from either cages.

Discussion and conclusion

The increased mobility of the fish in the copper alloy mesh cage is an indicator of good health in fish, which was also confirmed by the reduced mortality in copper alloy net cage, in relation to that of the nylon cage. The greater length and weight of the fish from the copper alloy cage can potentially reduce the harvest time, leading to multiple economic benefits for aquaculture. The absence of biofouling from the copper cage can increase the oxygen levels in the cage and reduce the microbial load of the fish. Thus, the fish health is promoted and the operational costs associated with net cleaning are decreasing. Therefore the use of copper alloy nets can contribute to a sustainable aquaculture. Further long-term studies will reveal the impacts of copper netting in aquaculture.

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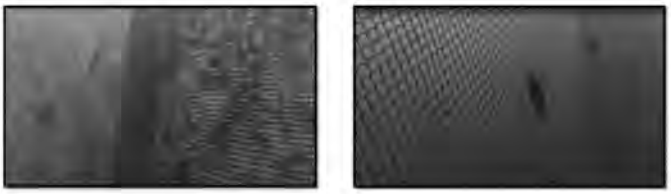

Photo 1. The obvious results of biofouling in nylon cage (on the left) in comparison to the copper cage (on the right).

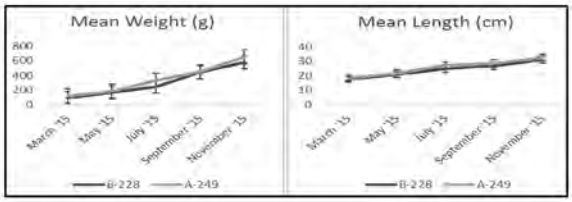

Fig. 1. The somatometric characteristics of the fish that were cultured in the nylon cage (B-228) and in the copper cage (A-249).

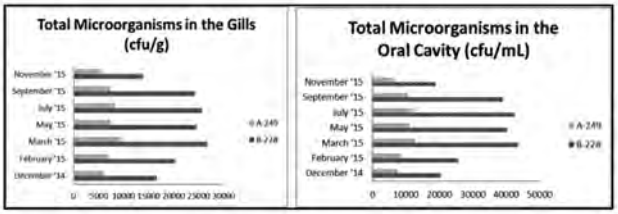

Fig. 2. The microbiological flora of fish from nylon cage (B-228) and from copper cage (A-249).

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ATLANTIC SALMON, SALMO SALAR, PARR GROW SUCCESSFULLY ON AN 80% PLANT PROTEIN DIET WHEN SUPPLEMENTED WITH FISH HYDROLYSATE

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Fishmeal, once a major component in aquafeeds, especially for carnivorous species, is now becoming an unsustainable ingredient. Efforts to replace fishmeal with alternative nutrient sources, such as plant proteins, are on-going but research has found that fish growth and health are often negatively affected. Fish protein hydrolysates (FPH) are supplemental ingredients that have been shown to have some positive effects on the health and growth in aquaculture species previously. This study investigated the effects of feeding an 80% plant protein diet, with and without FPH supplementation, on the growth and gut health of Atlantic salmon parr. A feeding trial was conducted with four different diets over a 12 week period. At the end of the trial it was found that fish on the plant protein diet grew significantly less than fish on the other diets. However, supplementing this diet with a partly hydrolysed fish protein allowed fish to grow as well as fish fed a traditional 35% fishmeal control diet. Blood amino acid results suggest that the fish hydrolysate supplements provided more bioavailable amino acids that were easily absorbed. In particular, fis on the PHP diet had significantly higher levels of blood essential amino acids. Furthermore, dietary protein source was found to have a significant effect on fish gut microbiota in terms of α– and β-diversity. This study has shown that Atlantic salmon parr can grow successfully on a very high (> 80%) plant protein diet when it is supplemented with a small quantity of high quality FPH.

EFFECT OF MAGNETIZED WATER ON THE GROWTH PERFORMANCE AND OXIDATIVE STATUS OF NILE TILAPIA (*Orechromis niloticus*)

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Introduction

Improving the efficiency of aquaculture could be achieved not only through the proper nutritional program but also through improving the ecological system, one method for improving the aquaculture system is Magnetism. Magnetic water is produced when water is exposed to magnetic field, which could affect fish growth performance parameters Cho and Lee (2005).

Therefore, we hypothesis that use of magnetized water could improve the growth performance of Nile tilapia.

Materials and methods

A total of 180 Nile Tilapia (*Oreochromis niloticus*) fingerlings weighing (11.7± 8.25) g were randomly allotted into two groups each has 6 replicates (15 fingerlings/ replicate). Fingerlings were reared in glass aquaria, the first group was supplied with clean de-chlorinated water (**CON**). The fish in the second group were reared in magnetized water (**MAG**). Magnetizing the water was achieved by water magnetizer that fixed at the main water source, the aquaria in the two groups were supplied with air pumping and filtration system applied. Fish in the two groups were fed the same commercial pelleted isocaloric, isonitrogenous basal diets that were prepared to cover the nutrient requirements of Nile Tilapia fingerlings according to NRC feeding standards (2011). The experiment lasted for 3 months after 2 weeks which served as acclimatization period during which the suitable feeding regime were suggested to be (4% of fish biomass). Body weight was recorded biweekly and at the end of experiment blood samples were collected for serum separation. Serum samples were used to analyze the MDA (malondialdehyde), glutathione, and glutathione peroxidase calorimetrically, data were statistically analyzed using Minitap software (REF)

Results and Discussion

Fish reared in magnetized water show enhancement in growth performance parameters. Body weight gain was significantly increased ($P < 0.05$), where it was 26.63 (±0.42) g in MAG and 23.76 (±0.42) g in CON group, our findings are similar to REF. The oxidative stress biomarkers were affected by magnetizing the water. The oxidative stress biomarkers are presented in Table I. MDA was significantly decreased, while, Glutathione and Glutathione reductase were significantly increased in MAG group compared to CON group.

Conclusion

It could be concluded that the use of magnetized water under intensive fish farming condition was advantageous as it has the ability to improve fish growth performance and minimize the oxidative stress

These findings are in line with REF

Item	CON	MAG	P
Malondialdehyde, nmol,ml	0.29±	0.13±	
Glutathione, ng/dl	0.12±	0.26±	
Glutathione peroxidase, ng/dl	0.11±	0.31±	

THE SEARCH FOR THE MATURATIONS INDUCING STEROID (MIS) IN EURASIAN PERCH *Perca fluviatilis*

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Introduction

In European aquaculture, Eurasian perch, Perca fluviatilis L., is perceived as one of the most highly valuable freshwater fish species and a strong candidate for the development of freshwater aquaculture. In the pursuit of improving the quality of reproduction in this domesticated species, investigating the hormones mediating the follicle oocyte maturation (FOM) is therefore indispensable. Up to date, 17α,20β-dihydroxy-4-pregnen-3-one (DHP) was postulated to be a possible MIS in Eurasian perch according to previous studies investigating the seasonal variations of this steroid in the blood. Using in vitro follicle incubations, this hypothesis was suggested to be true in a very close species to Eurasian perch, namely the Yellow perch. In other perciforms, THP or 20β-S (17,20β,21-trihydroxy-4-pregnen-3-one), was also recorded to be the possible MIS. However, some corticosteroids have shown a peak in their concentrations which synchronised with the peri-ovulatory period in some percids. This latter finding could signify the existence of a crucial role for these corticosteroids in the final stages of percid oogenesis.

Material and Methods and Results

Therefore, to further validate the existence in Eurasian perch of a maturation inducing activity behind the mentioned steroids, we tested in vitro a group of eight steroids namely, estradiol-17β (E2), testosterone, and the four main corticosteroids: cortisol, 11-deoxycortisol, corticosterone, and 11-deoxycorticosterone (DOC), in addition to the previously mentioned progestagens DHP and THP. The experiment was divided into two parts; in the first one, we tested all the mentioned steroids except for THP at three different doses on oocytes at the start of the FOM from sexually mature female perch. The cells were in-vitro incubated during 52 hours in Cortland media with and without the incorporation of human chorionic gonadotrophin (hCG) as an additional attempt to investigate the importance of hCG incorporation in such in vitro biological assays. This part of the experiment revealed that DHP was the only hormone showing to be a prominent MIS (with and without hCG) with highest proportion of GVBD and ovulation at the end of the hormonal exposures. On the other hand, hCG incorporation with the hormones showed to be of no significant importance. DHP was thereafter tested in the second part of this experiment in parallel with THP. Both progestagens were tested at five different doses on the oocytes of six other sexually mature female perch at the initial stage of FOM.

Conclusion

The results suggest that both DHP and THP trigger progress in FOM even at low doses in vitro and that progestagens are more credible candidates than the corticosteroids to be considered as the MIS in the Eurasian perch. Hence, the results of this experiment strongly show that both THP and DHP inductions should be taken into account when investigating the hormonal mediation of FOM and ovulation in Eurasian perch.

SITING GUIDELINES, MONITORING STANDARDS, AND ENVIRONMENTAL MODELS FOR COASTAL FINFISH AQUACULTURE

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The growth of strong coastal aquaculture industry in Morocco is expected in the near future given the national strategy, available suitable marine space, investor interests, and proximity to high demand seafood markets. Governments have made decisive efforts to increase their management capacities by implementing science-based strategies to monitor and control aquaculture's effects on the environment.

An aquaculture alliance was created between the National Oceanic and Atmospheric Administration (NOAA), Morocco's Department of Marine Fisheries (DPM), and National Aquaculture Development Agency (ANDA) with support from the U.S. Department of State to develop comprehensive guidelines for sustainable marine aquaculture.

Three written guidelines were developed, focused on siting, modeling, and environmental monitoring of marine finfis aquaculture along the Mediterranean and Atlantic coasts of Morocco, applying the most current management frameworks and tools available.

This scientific guidance is intended to assist the government agencies, coastal managers, industry, and scientists to make appropriate management decisions for the current industry as well as future development. Accomplishing the goals set forth in this guidance will encourage investment in the Moroccan green economy, promising international competitiveness and completion of the national strategy and challenging the aquaculture industry toward sustainability.

HIGH RESOLUTION COPERNICUS-BASED INFORMATION SERVICES AT SEA FOR AQUACULTURE**

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The increasing availability of data and technological advancements, such as the combination of Earth Observation and numerical model outputs, have been contributing to today's capability of better understanding the coastal environment and providing improved forecasts of future conditions.

Improved management of marine resources, with the assistance of forecast tools, is likely to enhance industry resilience and adaptive capacity under climate change. Among others, fishery and aquaculture activities are strongly influenced by environmental conditions and therefore can benefit from short-term forecasts of water quality. Short-term environmental fluctuations, including sea water temperature, chlorophyll concentration and turbidity, wind velocity and persistence, and current velocity, combined with long-term climate-related trends, can indeed impact growth rates of cultured animals and wild stock habitat distributions.

To this end, Earth Observation and models have proved to be complementary and effective to develop forecast tools. However, the accuracy and spatial resolution of the data play a crucial role in representing physical, chemical and biological processes. The EU funded HiSea project aims at tackling these issues by delivering accurate and reliable information, readily available, easily understandable and with a high resolution to fit seamlessly users' operation, planning and management requirements. The HiSea services will integrate high resolution Copernicus Services Products, such as Copernicus Marine Environment Monitoring Service (CMEMS) products, local monitoring data and advanced numerical modelling. The services offered as the end product will be based on the harmonization of different types of data and the added value is in their fusion and merging including estimates of the uncertainties and including data provided by the users/citiziens.

This allows improving operation, planning and management of different marine activities in aquaculture sectors. Such information services include among others early warning services, real-time crisis management, key performance indicators, information for planning operations, and a knowledge data base.

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QUANTIFYING METHIONINE REQUIREMENT OF AFRICAN CATFISH (*Clarias gariepinus*) USING A PLANT-BASED DIET

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Introduction

The increasingly use of plant ingredients to replace high cost fishmeal has motivated fish nutritionist to investigate the amino acid (AA) profile and their individual requirements in commonly cultured species (Rotili et al., 2018). Problems associated with the use of plant ingredients include the presence of anti-nutritional factors (NRC, 1993) and AA deficiencies (Cai & Burtle, 1996). Methionine is the first limiting AA in most leguminous plants (Ovie & Eze, 2010). It is a sulphur-containing essential amino acid that is required for the synthesis of cysteine in animal and humans (Niu et al., 2013). Methionine is an initiation codon required during protein synthesis for protein formation (Rotili et al., 2018). Inclusion levels of methionine below the requirements may cause growth reduction and decrease feed efficiency in fish (Harding et al., 1977). Therefore, adequate supply of methionine is essential in fish diets. Methionine requirements have been determined for several fish species, however, no reliable information is currently available for African catfish (*Clarias gariepinus*). Few studies (Fagbenro, Balogun, & Fasakin, 1999) conducted on methionine requirement of African catfish used purified ingredients as intact protein. Therefore, the goal of this study was to determine the methionine requirements of African catfish. It was hypothesized that AA utilization and protein retention can be improved by supplementing diets with appropriate levels of crystalline AA. This experiment was aimed at establishing the minimum level at which the supply of methionine results in maximum growth.

Materials & methods

A 7-week experiment was conducted to estimate the dietary methionine requirement of juvenile African catfish (initial weight 78 g), reared in a recirculation aquaculture system. A low-methionine (plant-based) diet was formulated using soy protein concentrate and faba beans as intact protein. Based on the methionine requirements for other species, which was obtained from literature (NRC, 2011), 7 diets were formulated, which all had the same basal composition but supplemented with different amount of crystalline DL-methionine: 0, 0.12, 0.24, 0.36, 0.48, 0.60, and 0.84%. At the start of the experiment, fish were weighed for initial body weight and randomly distributed among 21 aquaria (40 fish aquarium^{-1}). Experimental tanks were randomly assigned to each of seven diets with three replicates per diet. Fish were fed restrictively twice a day at 90% satiation. Apparent digestibility coefficient (ADC) of nutrients were measured by using settling tanks connected to the outlet of the aquaria in which African catfish are housed. The body composition of the fish were analysed both at the start and end of the experiment to determine the amino acid deposition, energy and nitrogen balance. At the end of the experiment, fish were weighed to determine the final body weight. Broken-line regression analysis (Robbins et al., 1979) was used to determine the quantitative methionine requirement by estimating the break-point for retained nitrogen and growth.

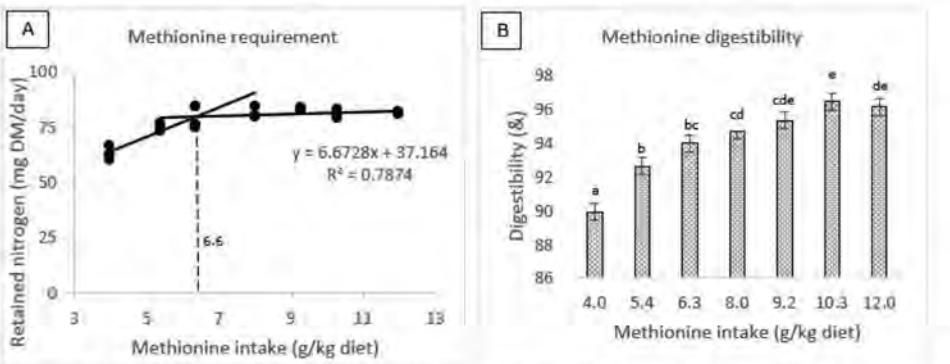

Figure 1: (A) Relationship between retained nitrogen and methionine intake and (b) apparent digestibility coefficient of methionine in African catfish fed diets containing different levels of crystalline methionine. Treatments sharing a common letter are not significantly different ($p<0.05$).

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Results

There were no significant difference in the ADC of protein among dietary treatments. Whereas, methionine digestibility increased with increase inclusion levels. Dietary methionine supplementation had a significant effect on branchial and urinary losses, retained nitrogen and protein efficiency (p-value<0.001). Increasing crystalline methionine significantly increased nitrogen retention up to 0.24% inclusion level, after which it remained before it declined at 0.84% methionine level. The mean weight gain values showed a slight increase in weight of fish as dietary methionine supplied (12-36g/kg protein) increases, which eventually levels up at the inflection point (requirement) then declined at higher inclusion level. The breakpoint of the growth response curve occurred at 6.55 g methionine /kg diet. The reported methionine requirement value in this study can be used to formulate least cost- diets using plants ingredients for production of African catfish

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NOVEL USE OF QUALITATIVE BEHAVIOUR ASSESSMENT TO MONITOR WELFARE IN FARMED ATLANTIC SALMON (*Salmon salar)*

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Introduction

Fish welfare is an important issue within aquaculture, not only for the health and well-being of stock but also for the sustainability and profitability of farms. To monitor the effect of husbandry procedures or the outcome of research designed to improve welfare, methods to assess fish welfare are required. These methods should be reliable, rapid, economical, easy to apply and preferably not require extensive training or expensive equipment. Experienced fish farmers can look at their stock and just know whether there are welfare issues evident within that tank or pond. They do this intuitively by observing the way fish interact with each other, how they move, how they feed, how they use the environment. It is not necessarily *what* the animal is doing but *how* it is doing it, which is often referred to as 'body language' or more scientifically as 'behavioural expression' (Wemelsfelder et al. 2012). Body language can therefore be used to infer an animal's physical or physiological state but potentially also its psychological (emotional or affective) state. In doing so this can give us insight into both negative and positive indicators of the welfare status of the animal.Qualitative Behavioural Assessment (QBA) is a scientific method for assessing the subjective experience of animals through the expressive qualities of behaviour. QBA is used extensively in the social sciences and is increasingly being applied in animal welfare science, where it bridges the gap between subjective judgements and scientific measurement approaches (Wemelsfelder, 2007). QBA uses a selective list of terms to describe qualitative behavioural expressions (e.g. tense, relaxed, calm, agitated). Observers use this list while viewing live animals or retrospectively using videos and assign a score based on how intensively they felt a particular expressive quality was evident. The scores are then used for statistical analysis. QBA has been validated as a welfare assessment tool for terrestrial animals and has been incorporated into EU Animal Welfare Assessment schemes for pigs, poultry and cattle. However, QBA has not yet been applied to fish, therefore this study seeks to evaluate whether QBA has potential for use as a welfare monitoring tool in fish

Material and Methods

Video footage, 17mins in duration, was recorded in 20 tanks at a commercial Atlantic salmon (*Salmo salar*) fish farm located in North-West Scotland. Ten tanks had environmental enrichment in the form of plastic strips to represent artificial kelp, while the remaining tanks had the standard production environment.

Fixed List Term generation
From the video footage, twelve short videos (45secs to 1min) were generated that showed a range of overt and contrasting behaviour such as darting, drifting, interacting with conspecifics or artificial kelp. The aim was to generate video clips that represented a number of expressive states, ranging from high to low valence and arousal. Experienced fish farmers viewed the clips and generated a number of terms to describe the behaviour. During a group discussion the preferred terms, considered most relevant in describing fish body language, were agreed and behaviours not evident in the videos added. The literature on fish emotion was also scrutinised to determine if any significant expressive terms were overlooked. This resulted in a list of twenty qualitative descriptors arranged in a fixed list to be used in subsequent QB sessions.

Qualitative Behaviour Assessment Sessions
A group of observers viewed 25 video clips, 1min in duration, created from the original footage. The video clips displayed contrasting expressive qualities and although the enrichment was visible in some clips it was not expressly selected for in this session. The observers were provided with a scoring sheet for each video clip. Each scoring sheet had the fixed list of twenty descriptor terms and each term had a 125mm horizontal line to be used as a visual analogue scale (VAS). For each video clip observers were instructed to make a single vertical line along the VAS, corresponding with how intensively they felt a particular expressive quality was evident in that clip and that all elements in the fixed list should be scored. Scoring was measured by the length of the vertical mark along the horizontal line. For intra-observer reliability a second session was held with the same observers and same video clips (in a different order) ten days later.

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A separate QBA session was held with different observers to determine whether there was a detectable difference in behaviours between tanks with and without enrichment. Twenty video clips (45 secs duration) were created from the original footage. Clips were selected for enrichment type although observers were unaware of the different enrichment. Expressive qualities were not selected for in this session. The same fixed list of descriptors was used and instructions for scoring was the same as previous session.

Quantitative Analysis
An ethogram was developed to quantitatively analyse the video clips used in the QBA sessions. Behaviours were either recorded as frequencies or as a duration.

Results

Principal component analysis, of the first QBA session identified four main principal components (PC), explaining 79% of the variation in the data. Many of the terms load strongly on the first PC, accounting for 56% of the total variation and range from anxious/tense to calm/relaxed. Only PC1 had a strong to moderate inter- and intra-observer reliability based on Kendall's coefficient of concordance (W= 0.68, chi-square= 335.31, p = <0.001) and partial correlation (r = 0.65, p = <0.001), respectively. Quantitative validation of QBA identifie strong correlations (r >= 0.68) between duration of calm movement and QBA scores within the first three Principal Components. PC1 (tense/calm) scoring was highly correlated with duration of calm (r = -0.68, p = <0.001) and of chaotic movement (r=0.62, p=<0.001).
Data analysis from the enrichment QBA session is still ongoing but will be available at conference

Discussion

Results here show that even observers with limited experience of fish show good agreement when scoring fish using QBA and are able to consistently observe differences between tense and calm fish. In addition, there was very good correlation between quantitative measures of behaviour and QBA which provides further validation of this methodology. More work is required to validate this method however initial results are encouraging.

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EFFICIENCY OF WITHIN-GROUP MASS SELECTION ON THRESHOLD TRAIT AND SUCCESSIVE MASS OR INDEX SELECTION ON CONTINUOUS TRAIT

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Introduction

Within-group mass selection consists in combining the efficiency of independent mass selection within groups and the simplicity of inbreeding management by rotational crossing of those groups. Previous simulations have shown that a first within-group mass selection on a threshold trait, such as disease resistance, is efficient to create genetic progress and that rotational crossing between groups could limit efficiently inbreeding (Enez & Haffray, 2018). The aims of this present *in silico* study were to simulate a successive 1st within-group mass selection on a threshold traits and a 2nd mass or BLUP (or index selection) on a continuous trait to evaluate the expected gains on the two traits depending on trait heritabilities, their genetic correlations and different selection pressures. The opportunity to get access to the pedigree by DNA parentage assignment was also investigated to optimize breeding program.

Materials and methods

Stochastic computations were used to simulate a breeding program for 10 generations. Factorial mating were generated with 160 parents distributed in 4 groups. Simulated progenies were reared in mixed family groups. True breeding value (TBV) was defined as the mean of additive value of sire and dam, modulo meiosis uncertainty. Individual phenotypic value for threshold (T-trait) and quantitative (Q-trait) traits was considered as the sum of genetic effect (weighted by heritability h^2), micro-environment effect (weighted by 0.1 for T-trait only), and residual effect. Each effect was sampled in Gaussian distribution. Threshold phenotypic value was determined by comparing underlying phenotypic value with a sensitive threshold dependent on eliminated phenotype prevalence. Individuals were available for selection only if their phenotypic value was larger than the threshold. Then selected individuals could be randomly sampled without Q-trait consideration, or selected by mass or BLUP selection on Q-trait with or without inbreeding management based on parentage assignment. Threshold on the T-trait was fixed at 50% in the reference situation and cumulated selection pressure on the two traits at 4% from 4000 individuals. HAN-rotational system was applied to cross groups and to produce the next generation. The effect of some heritabilities (0.1; .25; 0.50), genetic correlations r_g (-0.5; 0; 0.5) and selection pressure (0.50 and 0.25 on T-trait, and 0.32 and 0.10 on Q-trait) on inbreeding, phenotypic and genetic gains were estimated.

Results

Selection on T-trait only had no significant impact on TBV of Q-trait (Fig. 1). Adding mass selection on Q-trait, after mass selection on T-trait when genetic r_g in null, had no impact on genetic gain for T-trait.

Index selection with 32% selection pressure from estimated breeding values (EBV) on Q-trait didn't impact gain on T-trait but increased gain on Q-trait in the same order than mass selection with higher inbreeding. When Q-trait h^2 was limited (0.10), expected genetic gain on Q-trait was lower (1-2% vs 19%) than when h^2 was higher (0.5). Inbreeding also increased faster.

Introduction of inbreeding constrains limited up to 50% genetic gain on Q-trait compared to mass or index selection, whereas genetic gain on T-trait was quite similar. Inbreeding management seemed to be particularly useful when Q-trait heritability was more limited.

When index selection was considered with inbreeding management and the initial selection pressure on T-trait was the highest (0.25), genetic progress on T-trait was 50% higher than when initial selection pressure on T-trait was more limited (0.50), without effect on genetic gain on Q-Trait. (Fig. 2). Increase of selection pressure on Q-trait moderately impacted T-trait, but genetic gain on Q-trait rose by 50%. When r_g was closed to 0, then combined selection on T and Q-trait allowed similar genetic gain by trait than if each trait would have been selected independently.

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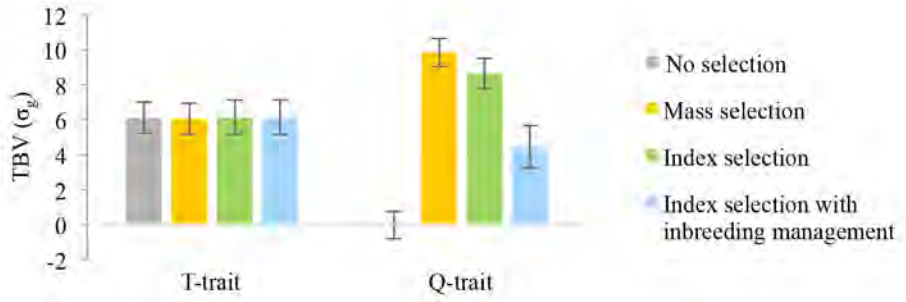

Fig. 1: TBV (in σ_g) of simulated individuals after 5 generations according to method of selection of Q-Trait. Heritability of T-trait and Q-trait is fixed at 0.25 and genetic correlation at 0.

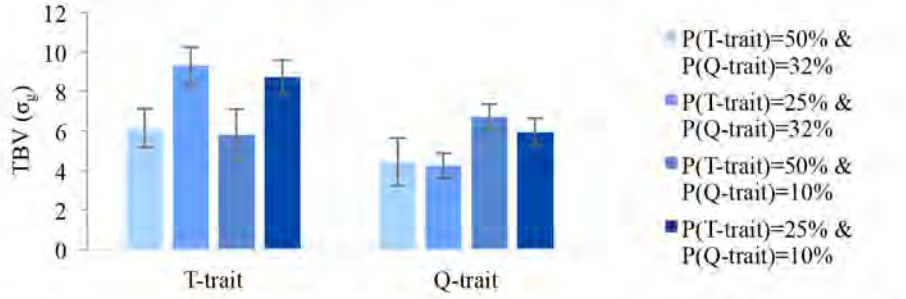

Fig. 2: TBV (in σ_g) of simulated individuals after 5 generations with index selection with inbreeding management according to selection pressure on T-trait and Q-trait. Heritability of T-trait and Q-trait is fixed at 0.25 and genetic correlation at 0.

Discussion

This work demonstrated that within-group multi-traits selection is an efficient strategy to improve simultaneously a threshold trait, such as survival, and then a second quantitative trait with low-cost inbreeding management when genetic correlation is limited. Results not reported in the abstract also shown possible simultaneous genetic gains when genetic correlation is -0.5, but more limited as also reported by Sonesson et al. (2005) with only one group.

The use of DNA parentage assignment in within-group multi-traits selection program may improve inbreeding management while guaranteeing the genetic variability of the selected individuals. The effectiveness of such selection program depends directly on the heritability of the traits, their genetic correlation, and selection pressure and/or number of assigned individuals used to estimate EBV.

Given its simplicity of implementation and effectiveness, both in terms of genetic improvement and inbreeding management, successive within-group multi-trait selection could be easily applied in breeding programs in aquaculture species.

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IMPACTS OF A DIETARY SUPPLEMENTATION OF RAINBOW TROUT (*Oncorhynchus mykiss*) BROODSTOCK WITH A LOW DOSE OF DRY GRAPE EXTRACT ON THE METABOLOMICS PROFILE OF THEIR EGGS AND THE GROWTH PERFORMANCES OF THEIR OFFSPRING

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Introduction

High quality gametes are those that have a high ability to fertilize or be fertilized and result in high survival rate during incubation as well as after hatching until the first feeding (Bobe and Labbé 2010; Valdebenito, C Gallegos, and Roldán 2013). Broodstock nutrition, genetics, and environmental conditions such as stress, temperature, handling and photoperiod are among the main parameters susceptible to modulate gamete quality (Bobe and Labbé 2010; Migaud *et al.* 2013; Valdebenito, C Gallegos, and Roldán 2013). Since nutrient content of the eggs is key to the good development of larvae, and directly linked to the broodstock diet, several authors have stressed the importance of this factor to ensure high quality offspring (Fernández-Palacios *et al.* 2011; Izquierdo, Fernández-Palacios, and Tacon 2001). To our knowledge, very little work has been published on the effect of antioxidants in rainbow trout broodstock nutrition. Indeed, whilst it is known that vitamin C and E are essential to obtain good quality gametes (Sandnes *et al.* 1984; Izquierdo, Fernández-Palacios, and Tacon 2001; Fernández-Palacios *et al.* 2011; Valdebenito, C Gallegos, and Roldán 2013; Bilguven 2014), study on other compounds, known for being involved in the antioxidant mechanisms are scarce.

The aim of this study was to evaluate the effect of a supplementation of rainbow trout (*O. mykiss*) broodstock with a small dose of dry grape extract on their reproductive performances, the impact on the eggs metabolome and on the offspring's growth performances.

Materials and Methods

Fish were divided in two groups of 80 females and 50 males each. The supplemented group's diet (NG, males and females) contained 80ppm of a commercial grape extract (Nor-Grape®), while the control group had none (CTL, males and females). Fish were fed the experimental diets for 5 months prior to spawning. Ova were collected and either analyzed for their metabolomic profile, or fertilized with the semen from males from the same group and incubated until hatch. Egg weight, embryonic survival rate and live fry after resorption were recorded. Fry from both groups was then raised for 8 weeks with the same diet.

Ova for metabolomic analysis were freeze-dried, then submitted to pressurized liquid extraction using sequential polar (dichloromethane) and polar (methanol) extractions. Liquid extract were then dried under nitrogen, before being re-solubilized in their respective solvent for LC-MS analysis. Mass spectra were then compared using MZMine2 software.

Results

Results did not show statistically significant differences between groups in egg weight (50.1mg vs.51.4mg. for CTL and NG respectively, ns.), embryonic survival (81.8% vs. 84.4% for CTL and NG respectively, ns.) or live fry at resorption (79.5% vs. 81.7% for CTL and NG respectively, ns.). However, fry from the NG parents had significantly higher growth at 6 weeks (1.78g vs. 1.80g, for the CTL and NG fry respectively, $p<0,01$, t-test) and at 8 weeks (3.25g vs. 3.42g for the CTL and NG fry respectively, $p<0,01$, t-test). Moreover, a general shift in the metabolomic profile of eggs was observed in the NG group, in both polar and non-polar fractions of the ova.

Discussion and conclusions

Whilst other research have shown a beneficial effect of supplementing broodstock with dietary antioxidants on the offspring's antioxidant status (Wischhusen *et al.* 2019), they did not study its impact on growth performances. Our findings show a benefic al effect of the supplementation with a standardized dry grape extract on the next generation's growth performances. They are thus in accordance with other research showing the beneficial effect of a supplementation with dietary antioxidants, thus demonstrating the interest of natural antioxidants such as grape polyphenols.

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The study of the impact of such a supplementation on the metabolomic profile of ova has, to our knowledge, not been studied before. Recent research has shown that a supplementation with certain dietary polyphenols could impact the metabolism of fish, more particularly the lipid metabolism (Torno *et al.* 2017; Welker *et al.* 2017). Whilst these studies showed modulation only at high doses of polyphenols, the present work evidenced a positive impact on the modulation of the egg metabolome at a low level of supplementation (80ppm) of a standardized dry grape extract.

Thus, the addition of the standardized dry grape extract in the diet of rainbow trout broodstock had a beneficial impact on their offspring performances, potentially due to a favorable change in the metabolomic profile of their eggs. More research is required to better understand the mode of action behind this phenomenon.

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IMPROVING GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES ADAPTABILITY TO ADVERSE CONDITIONS *VIA* NUTRITION

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Introduction

The Aquaculture industry is a key animal production sector to provide food and nutrition for direct human consumption, and by 2030 with the increasing population more 40 million metric tons of aquatic products will be necessary to maintain the actual seafood consumption *per capita*. Hence, it is important to study strategies to increase the production in aquaculture in a sustainable way. In winter, when water temperature drops below 13°C, gilthead seabream reduces activity, feed intake, metabolism, and growth. Therefore, it is essential to determine how nutrition may help the fish to cope with adverse conditions in order to establish predictability and ensure optimal feed utilization during the whole production cycle. The objective of the present study was to mitigate the impact of adverse water temperature during gilthead seabream juveniles' production through diet formulation.

Materials and methods

Gilthead seabream juveniles with an average body weight of 155g were distributed by nine 500L-tanks, at an initial density of 8.6 kg/m^3, in a flow-through system. Fish were fed once a day, *ad libitum*, with one of the three experimental diets: Control (43%Protein:17%Lipids); Low (41%P:17%L); Low$^+$ (41%P:17%L). A mixture of feed additives was included in the Low$^+$ diet. Each diet was assigned to triplicate tanks. The impact of experimental dietary formulations on several juvenile key performance indicators (growth, FCR, K, proximal composition, nutrient retention, diet digestibility, HSI and VSI) were determined at the end of the experimental period (84 days).

Results and Discussion

In order to ensure a sustainable growth, the Aquaculture industry needs to improve aquafeeds sustainability through a reduction in dietary fishmeal inclusion and/or an increase in feed efficiency by adding additives to the fish diets. At the end of the experimental period key performance indicators, like growth and FCR were similar between the fish fed Control and the Low$^+$ diet. In addition, energy digestibility was higher for fish fed Low$^+$ diet when compared to the other diets. This absence of significant differences in growth performance indicators implies a significant reduction of protein and fishmeal in juveniles' seabream diets. The results indicate that is possible to mitigate adverse conditions through a nutritional approach.

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ASSESSING OPTIMAL DIET FORMULATION TO HIGH QUALITY CLOWN ANEMONEFISH (*Amphiprion ocellaris*) JUVENILES

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Introduction

The ornamental trade has been growing in the last decade. Traditionally, fish are collected from the wild, causing the destruction of reef habitats and wild populations. Consequently, ornamental aquaculture is the viable strategy to a healthy and sustainable ecosystem. One of the most popular ornamental fish is the clown anemonefish (*Amphiprion ocellaris*). This species presents a bright orange body colour with three white bars, where the middle one has forward projecting bulge and bars with a very narrow to almost non-visible black margins. Fish value is mainly based on two quality parameters, colour and fish size. However, one of the main problems in reared fish is the loss of colour due to carotenoid deficiency in the diets. Therefore, nutrition appears as a valuable tool to improve the quality of the reared ornamental fish. One strategy could be the dietary inclusion of marine biomasses (microalgae, macroalgae, and zooplankton) and alternative new ingredients to overcome this situation. The main objective of the present study was to assess how innovative diet formulations could improve performance of *Amphiprion ocellaris* juveniles, as well as to standardize a feeding protocol that promotes fish quality, based on growth and colour.

Materials and methods

A stock of 180 juveniles with a mean initial body weight of 0.36g were divided in 9 rectangular tanks (10.5l) in a closed recirculation system (temperature: 26 ±2˚C; salinity: 30-31g.L^{-1}; dissolved oxygen: 97± 4%). Each diet was assigned to triplicate tanks. Fish were fed by hand to apparent satiety, several times a day with one of the three experimental diets. Diet A was used as control and was formulated with high levels of traditional marine protein sources (fishmeal, krill meal, squid meal, marine hydrolysates) and was supplemented with synthetic astaxanthin; diets B and C comprised a marked increase of various emergent raw materials such as natural Artemia biomass, microalgae (Nannochloropsis sp., Tetraselmis

Table 1- *Amphiprion ocellaris* juvenile's performance indicators and colour at 90 days after hatching (DAH).

	Diet A	Diet B	Diet C
Final Body Weight (g)	1.1 ± 0.32	1.0 ± 0.23	0.92 ± 0.24
Weight gain (%IBW/day)	150.57 ± 7.65^{a}	124.59 ± 18.25^{ab}	109.67 ± 6.24^{b}
Specific Growth rate (%/day)	1.77 ± 0.05	1.69 ± 0.11	1.61 ± 0.09
Feed Conversion Rate	1.99 ± 0.03^{c}	2.33 ± 0.09^{b}	2.49 ± 0.04^{a}
Voluntary Feed Intake (%ABW/day)	3.11 ± 0.10^{b}	3.44 ± 0.06^{a}	3.68 ± 0.14^{a}
Standard Length (cm)	3.65 ± 0.39	3.61 ± 0.29	3.51 ± 0.29
Condition index (K)	2.08 ± 0.68	2.10 ± 0.23	2.12 ± 0.31
Hepatosomatic Index	2.20 ± 0.75	1.93 ± 0.48	1.94 ± 0.55
Viscerosomatic index	7.04 ± 2.29	7.03 ± 1.9	7.20 ± 1.13
Colour (CIELab colour space)			
L*	20.07 ± 1.58^{b}	21.12 ± 1.94^{a}	20.49 ± 1.09^{ab}
a*	21.33 ± 1.76^{c}	25.83 ± 1.92^{a}	23.71 ± 2.22^{b}
b*	23.54 ± 1.63^{b}	25.99 ± 2.01^{a}	25.01 ± 1.78^{a}

Values are means ± standard deviation. Different superscript letters indicate statistical differences between replicates (Tukey's test, P<0.05).

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sp., Spirulina, Chlorella sp.) and insect meal, at the expenses of traditional marine protein sources and without the addition of synthetic astaxanthin. All diets were isonitrogenous and isoenergetic. The impact of experimental dietary formulations on several juvenile key performance indicators (growth, FCR, K, proximal composition, digestive capacity, colour) were determined at the end of the experimental period (90 days). Skin colour was measured through photo analysis and the L*, a* and b* coordinates from CIELab system were recorded. To estimate perceptible colour differences (ΔE*) among dietary treatments the CIE76 formula was applied. Hence, dorsal area of each fish was evaluated and average values of colour coordinates were used for ΔE* calculation (Diet A vs Diet B; Diet A vs Diet C and Diet B vs Diet C). Distance between colours were considered as being indicative of either an "irrelevant perceptual difference" (ΔE* < 1), a "slightly perceptual difference" (1< ΔE* < 2.3) or a "clean perceptual difference" (ΔE* > 2.3).

Results and Discussion

Regarding fish performance indicators, all the fish presented similar body weight (FBW), daily growth index (DGI), final length (ST), condition index (K), specific growth rate (SGR), viscerosomatic index (VSI) and hepatosomatic index (HSI) at the end of the experiment independently of the experimental diet. Weight gain was similar between Diets A and B. Experimental diets had impact on Feed conversion rate (FCR), with diet A having the lower FCR ($p<0.05$). Voluntary feed intake (VFI), was significantly higher in fish fed with diets B and C when compared to die A ($p<0.05$).

Fish skin colour was positively influenced by the inclusion of novel and emergent ingredients (Diets B and C) ($p<0.05$). The skin colour in the dorsal area showed that fish fed lower level of novel and emergent ingredients (Diet B) had higher redness (a*), followed by a higher incorporation in Diet C. The lower redness (a*) was observed in the fish fed traditional marine protein sources and supplemented with synthetic astaxanthin (Diet A). There was a clear perceptible colour difference between Diet A and Diet B (ΔE* Diet A vs Diet B = 5.23), while between Diet A and C, and Diet B and C (ΔE*=2.82 and ΔE* = 2.42, respectively) values were similar.

During this study it was observed that the innovative diets formulations positively improved the colour of *Amphiprion ocellaris* juveniles. Novel and emergent ingredients, such as microalgae, Artemia biomass and insect meal, supported an overall growth performance similar to that of a commercial ornamental feed with high levels of traditional marine derived proteins. In addition, natural carotenoids from microalgae and Artemia successfully pigmented clownfish attaining a higher redness (a*) pigmentation efficacy than synthetic astaxanthin

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SOURCES AND SINKS OF BACTERIA IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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Introduction

Recirculating aquaculture systems (RAS) provide numerous niches for suspended and attached bacteria, collectively addressed as the microbiome of a RAS. Bacteria perform important functions in water purification of a RAS but they also compete with fish for oxygen and some may even challenge the immune system of the animals. Biofilters for nitrification and denitrificati n are indispensable for sustainable RAS technology (Martins et al., 2010). The bacteria in these filters convert nitrogenous fish excretions into gas, which can leave the system, and in bacterial biomass. However, the terminal denitrification process is complicated and not easy to control (Stavrakidis et al. 2019). Bacteria are a significant part of the solid waste produced in RAS. While fish, feces, not-consumed feed and biofilters are sources of bacteria, water exchange and physical filters, which reduce or remove solid waste, are sinks of bacteria. Modern RAS, which abstain from periodic water exchange but rather replenish losses by evaporation and flushing out of solid waste (Orellana et al., 2014), operate with very high hydraulic retention times of the process water. Hence, also bacteria may stay in a RAS for very long time. Little is known about the (taxonomic) composition and temporal changes and development of the microbiome of RAS (Llewllyn et al., 2014, Rud et al., 2017). In this study we analyzed the microbiome and studied effects of sources and sinks on the microbiome in a RAS, stocked with *Sparus aurata*, in the first commercial, marine fish farm operated without connection to the sea.

Materials and methods

The marine fish farm in Völklingen, Germany, operates four marine RAS (4 x 2500m³). Water quality (pH, temperature, salinity, nitrate-N, total ammonia-N,nitrite-N, phosphate) is monitored three times per week in the fish production tank using standard methods. For microbiome analyses, process water was sampled at nine positions in the RAS. Sampling positions started with water from the fish production basin entering the water treatment unit, comprised seven positions inside the water treatment and a sample of process water leaving the treatment towards the production basin. Samples were frozen at -20° and transferred to -80°C. A commercial service provider (SeqIT GmbH, Kaiserslautern, Germany) analyzed the microbiome using Illumina sequencing of the V3-V4 region of the microbial 16S rRNA gene. Sequences were aligned with sequences in the SILVA database (https://www.arb-silva.de) using the Burrows-Wheeler Aligner (Li, 2009). To facilitate recognitionof pattern, we combined operational taxonomic units (OTUs) of the same phylum. Sources and sinks of bacteria were inferred from changes of fractions of phyla rather than from bacterial counts.

Results and conclusions

During the first year of operation, the RAS was successively stocked with 4 cohorts of seabream (*Sparus aurata*), each cohort adding about 100 000 fingerlings to the RAS. Fish harvest started 12 months after first stocking

The microbiome was analyzed 16 times during a period of 2 years, starting 1.4 years after first stocking. At 12 of 16 sampling dates, the relative contribution of γ-proteobacteria was smaller in samples taken at the exit of the water treatment (clean water) than in water leaving the production basin (soiled water). This indicated that the production basin, likely the fishes, were sources of γ-proteobacteria.

Samples of particles removed from RAS by first drum filtration, were greatly enriched in flavobacteria and α-proteobacteria compared to process water passing the filte . Only on 3 of 16 dates, the fraction of γ-proteobacteria removed from RAS was higher than in the passing water. After 3.4 years of continuous RAS operation, planctomycetes, clostridia and fusobacteria, which showed low abundance in the second year of operation had increased their shares on expense of flavobacteria, α-and γ-proteobacteria.

ϒ-proteobacteria were more efficiently removed by the flotati n apparatus (foam fractionation) than by drum filtration. This is a significant observation because this taxon not only increased in the fish basin but also comprises several important fish pathogens. The result shows that the two filter systems complemented each othe .

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Eye-catching was the contribution of a number of uncultivated/unknown OTUs assigned to ε-proteobacteria. At 11 of 16 sampling dates they dominated the microbiome of water entering the fish tank. Although their relative abundance decreased in the fish tank, dominance was restored during passage of the water treatment by preferential removal of other phyla. We were unable to identify a source of these bacteria, but high abundances of members of this phylum were reported in cod larvae (Vestrum et al., 2018). From our observation, we can infer that these bacteria proliferate slower than other proteobacteria in the fish tank. These bacteria have hydrophilic surface structures that prevent enrichment in the liquid-vapor interface of the flotation apparatus and the formation of larger aggregates that could be removed by the drum filte . Apparently, they were not harmful to fish

- It is obvious that a better sustainability of RAS requires a prolonged hydraulic retention of process water.
- A longer retention time requires a denitrification biofilter in RAS that could be an additional source of bacteria if the effluent is not treated by flotatio
- This study proofed that drum filtration and ozone enhanced flotation are complementary filter process
- Nevertheless, an inefficient removal of solitary bacteria may necessitate additional sinks to comply with animal welfare.

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VITAMIN AND MINERAL COMPOSITIONS OF THE ROTIFER *Brachionus plicatilis, müller* 1786 CULTURED WITH DIFFERENT FEEDS

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Introduction

The rotifer Brachionus plicatilis is the first live prey feed to marine fish larvae (Hamre et al., 2013). It is commonly fed commercial diets or freshly cultured microalgae species (Ferreira et al., 2008), which may differ in their biochemical composition and physical properties (Hamre, 2016). Despite their importance for larval performance, there is scarce information on micronutrients contents in live feeds from commercial hatcheries (Hamre et al. 2013; Izquierdo et al., 2017). The objective of the present study was to determine the effects of two different forms of *Nannochloropsis oculata* and common commercial rotifer diets on the vitamin and mineral compositions of rotifer.

Materials and methods

Rotifers (Brachionus plicatilis, L-type, lorica size 250 μm) were cultured in 1000 L circular tanks with baker's yeast (Saccharomyces cerevisiae; Pakmaya, Turkey) at Akvatek Aquaculture Company, İzmir, Turkey. The rotifer stocking density was 600 rot/mL in all experimental tanks. Rotifers were fed one of five different types of food: Algome® (dried Schizochytrium sp.), ProteinPlus® (PP), Inactive Beaker's Yeast® (INBY), spray-dried Nannochloropsis oculata (SDN) and freshly cultured N. oculata (FN). The experimental diets were given 4 times a day during 16 days of semi-continuous culture.

Results and Discussion

At the end of the experiment, eight essential vitamins (Vit A, Vit E, Vit C, Vit B1, Vit B6, Vit B2, Niacine and Folic acid), nine minerals (Ca, Mg, P, I, Co, Se, Mn, Cu and Zn) and three antioxidant (Lutein, Likopen and β-carotene) compositions of rotifers were determined. Ca, Zn and I levels were found higher in INBY fed rotifers (P<0.05). P and Mn levels were significantly higher in SDN fed rotifer groups (P<0.05). Se and Cu levels of rotifers were increased by utilization of fresh *N.oculata* biomass (P<0.05). Co and Mg levels were found higher in rotifer fed Algome diet (P<0.05). In terms of vitamin composition of rotifer, PP supported Niacine, B2 and folic acid levels (P<0.05). B6 level of rotifer was increased by utilization of fresh *N.oculata* (P<0.05). Vitamin E and A were found higher in rotifer fed SDN diet (P<0.05). Vitamin B1 level was found higher in INBY group. Vitamin C was not detected among groups. Lutein and β-carotene were found higher in rotifer fed SDN diet (P<0.05) (Table 1). Overall, the results indicated that vitamin and mineral compositions of rotifers could largely vary depending on the diet selection.

Rotifer nutritional quality and production success are main concerns for commercial hatcheries. The present study showed that vitamin compositions of rotifers differ innrelation to the diets used. The results could suggest a benefit from the combination of SDN and PP diets according to rotifers vitamins and carotenoids contents. However, according to our results, rotifers should be enriched vitamin C and vitamin A after rotifer cultivation. Regarding mineral compositions results are more similar among rotifers fed the different diets, excluding Mn.

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CtrlAQUA SFI – CONTRIBUTION TO FUTURE AQUACULTUURE

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Content

Norwegian government aim for a 5-fold increase in Atlantic salmon aquaculture production within 2050. At the same time aquaculture industry has to be sustainable and has a responsibility towards many of the UN Sustainable Development Goals. The goals regarding growth have to be achieved at the same time as salmon industry is still facing challenges with e.g. sea lice infestation and escapes. These challenges have resulted in zero growth in salmon farming in Norway the last years. A sustainable growth therefore enforces alternative and innovative production methods, with collaborative forces.

Already, there are several alternative production methods, besides the traditional open cages, and some of these methods originate in the belief that in order to avoid sea lice and escapes, you should keep the fish longer on land. Salmon start their life on land, either in flow through systems, or in systems using recirculation technology (RAS). After smoltification, there are several alternative ways of post-smolt and grow out production. After Norwegian regulations from 2016 allowed land-based production all the way to slaughter, this is a growing production method in Norway, and world-wide. Additionally, some farmers move their post-smolt to semi-closed containments (Fig 1), before transferring them to open cages in the last production phase. In addition, several different prototypes for on-growing production off-shore are developed.

CtrlAQUA is an SFI (centre for research driven innovation) that has been given eight years to contribute to the development of closed (RAS) and semi-closed containment (S-CCS) aquaculture. CtrlAQUA shall develop technological and biological innovations to make closed containments a reliable and economic sustainable technology, and contribute with knowledge transfer and innovations within the research fields Technology and Environment, Production and Welfare, and Preventive Fish Health. In focus is the post-smolt, since this is a sensitive life stage for the salmon in which their health and welfare are highly dependent on optimal environment and treatment. The centre goals are achieved with joint forces between seven R&D institutions, and thirteen industry partners representing fish farmers, suppliers and biotechnology companies, in addition to an impressive amount of master - and doctoral students.

Among research questions that CtrlAQUA is working with are to facilitate innovation of CCS technology, hydrodynamics, water treatment processes, and sensors, to achieve a high level of production control. Further, we are working to identify physiological and environmental requirements for post-smolts in CCS, optimize environmental parameters, innovate new welfare and robustness indicators and maximize output from each CCS platform without compromising welfare and robustness. Finally, we are working towards innovations to prevent, detect and control disease in closed production systems, strengthen the fish s robustness and disease resistance with focus on barrier functions and cardiovascular capacity, strengthen pathogen control and management of disease outbreaks in CCS, and developing new or improved vaccines and protocols for pathogens that represent a particular threat in CCS.

During the presentation, highlights of research results will be shown.

Acknowledgement

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Fig 1. Sketch describing the principle of semi-closed containment with water inlet deeper than the lice belt (purple) (Illustration: Oddvar Dahl

FutureEUAqua – FUTURE GROWTH IN SUSTAINABLE, RESILIENT AND CLIMATE FRIENDLY ORGANIC AND CONVENTIONAL EUROPEAN AQUACULTURE

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Content

European aquaculture production has reached 1.25 million tonnes of seafood annually, with a value of over 4 billion euro. Of this amount, 4% is certified as organic, amounting in 2015 to a total of approximately 50,000 tonnes (EUMOFA, 2017). In 2015, EU consumers spent 54 billion euro for buying fisheries and aquaculture products, reaching the highest amount ever recorded (EUMOFA, 2017). Nevertheless, Europe is still heavily dependent on external markets to cover this demand. The increased demand for aquaculture products has to be covered at the same time as food production need to be more sustainable, climate friendly and supporting the UN Sustainable goals.

The newly started EU project FutureEUAqua aims to effectively promote sustainable growth in aquaculture that is resilient to climate changes, and environmental friendly organic and conventional aquaculture of major fish species in Europe. It is a well-documented assumption that aquaculture that will meet future challenges with respect to the growing consumer demand for high quality, nutritious and responsibly produced food. FutureEUAqua will promote innovations in the whole value chain, including genetic selection, ingredients and feeds, non-invasive monitoring technologies, innovative fish products and packaging methods, optimal production systems such as IMTA and RAS, taking into account socioeconomic considerations by the participation of a wide spectrum of stakeholders, training and dissemination activities.

To achieve these ambiguous goals, 32 partners from R&D, industry and associations, originating from nine countries will collaborate in research, training, dissemination and contact with stakeholders through e.g. stakeholder events.

FutureEUAqua will contribute with innovations that will arrive Technology readiness level (TRL) ranging from five to nine. Innovations will result from all research topics, including sustainable genotypes, feeds and farming management solutions; smart tools to monitor the farming environment that guarantee aquatic animal health and welfare, tailor-made aquaculture fresh/processed foods and packaging, IT tools and information packages to improve consumer's awareness about aquaculture products and related markets.

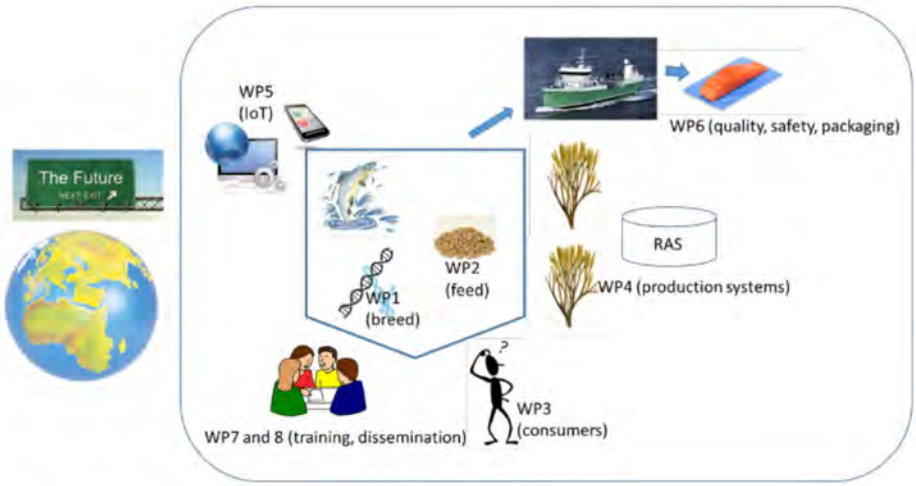

Figure 1. Structure of FutureEUAqua

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The results and innovations will have impacts by improving resilience and sustainability of aquaculture farming systems and practices. The results will have impact on a diversity of end users representing the whole value chain from breeding companies to processing plants and intelligent packaging, including e.g. digital farming solutions for improved animal health and welfare, retailers and customer care providers. We intend to gather stakeholders to contribute to the professional skills and competences of those working and being trained to work within the blue economy and support the implementation of the EU Common Fisheries Policy (CFP) and contribute to policymaking in research, innovation and technology.

The outcomes and recommendations will be presented at the two planned stakeholder events, and discussed at the dedicated conference at the end of the project's timeline. These meetings will address all stakeholders and interest groups affected by the scope of FutureEUAqua. A final roadmap for the exploitation of results, including efforts for technology transfer will be presented at the final conference.

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DOSE-EFFECT RESPONSE OF A BLEND OF ORGANIC ACID AND NATURE-IDENTICAL COMPOUNDS ON GILTHEAD SEABREAM (*Sparus Aurata* L.)

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Introduction
Organic acids (OA) and botanicals are widely used in terrestrial animals as zootechnical feed additives and they gained an increasing interest as alternative to reduce antibiotic usage. Usually they have been used as feed additives with the aim of improving performance, intestinal health, immune status and as antimicrobials. To the best of our knowledge, the available studies in aquaculture are very limited and little is known about their impact on the fish immune system. The aim of this study was to determine the effect of AviPlus®Aqua (Vetagro SpA), a blend of OA (sorbic and citric acid) and nature-identical compounds (NIC, thymol and vanillin) on gilthead seabream (*Sparus aurata*) growth performance and immune status. Gilthead seabream was selected as a fish model due to their interest in marine aquaculture

Materials and Methods
One hundred and sixty gilthead seabream specimens of an average weight of 50g were obtained from a local farm (Spain), distributed randomly into eight aquaria (20 fish per aquarium) and acclimatized for 15d. Fish in each aquarium were fed one of the following experimental diets (control, CTR, or AviPlus®Aqua 250ppm, 500ppm and 1 000ppm). Fish were fed twice daily *ad libitum* during 4 weeks and after 2 and 4 weeks of the feeding trial, 5 fish of each aquarium were sampled to determine growth performance and immune status. Two humoral immune parameters (alternative complement activity and IgM values) and two cellular immune parameters (phagocytosis and respiratory burst activities) were determined for each sampled fish. Data were analyzed with one-way ANOVA.

Results
Growth performance. Fish fed diet supplemented with AviPlus®Aqua 500ppm for 4 weeks showed higher body weight ($P<0.05$). Regarding the specific growth rate (SGR) and weight gain (WG) of the fish, no significant differences were detected among the different groups after 2 weeks of the experimental trial whereas after 4 weeks, fish fed AviPlus®Aqua 500 and 1 000ppm had higher SGR ($P<0.05$).

Immune status. The complement activity found in sera from fish fed the different diets for 2 weeks was always lower than the values recorded for fish fed the same experimental diets for 4 weeks. Regarding IgM, fish fed AviPlus®Aqua 250, 500 and 1 000ppm for 2 weeks showed higher values compared to CTR, although the observed differences were only statistically significant with AviPlus®Aqua inclusion at 1 000ppm ($P<0.05$).

Regarding phagocytosis of head-kidney (HK) leucocytes, fish fed diet with AviPlus®Aqua 500ppm showed higher significant phagocytic capacity after 2 weeks ($P<0.05$), but not after 4 weeks. No significant differences were observed about phagocytic ability. Finally, respiratory burst activity of HK leucocytes of fish fed AviPlus®Aqua 500ppm and 1 000ppm was significantly increased after 2 weeks, but not after 4 weeks $P<0.05$).

Conclusions
In conclusion, the present data showed that AviPlus®Aqua was able to improve growth performance in seabreams starting from 500ppm of inclusion, after 30d of feeding. Moreover, the same inclusions showed an increase in immune parameters, such as respiratory burst and serum IgM levels, after 15d. These data highlight a potential immuno-boosting effect at d15, that seems to prime the immune response of the fish. This primary boosting seems to have a positive effect on the general health status of seabreams, that leads to improved performance after 30d of feeding. Further immune-parameters needs to be studied to better understand the mechanism of action.

Acknowledgments
This research was funded by Vetagro S.p.A. and POR-FESR 2014-2020 Emilia Romagna grant.

INTITIAL UPTAKE CAPACITY OF AMONIUM IN *Saccharina latissima* IN AN IMTA CONTEXT

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Introduction

Norway is the world's leading producer of Atlantic salmon (*Salmo salar*) (FAO 2018). Further strategic development and research is needed to realize the Norwegian government's long-term aim to increase fourfold the current production volume by 2050 (Olafsen et al., 2012, Parliament message nr. 16 (2014-2015) p.15). Through the growing concern about the environmental impact of increasing waste load from salmon production a stronger focus on environmental sustainability of the production, including improved resource and energy utilization is required.

Approximately 39% of the total nitrogen content from feed given to salmon, is released as dissolved inorganic nitrogen (DIN), and Ammonium (NH_4^{+}) (Wang et al 2013). The sugar kelp *S. latissima* has been presumed to have a DIN removal rate of 5-40% from salmon farm emission (Broch and Slagstad, 2012 Forbord et al., 2012; Sanderson et al., 2012; Sjøtun 1993; Subandar et al., 1993). The major objective of this study was to investigate uptake of Ammonium and Nitrate in *S. Latissima* from an IMTA perspective and if the pulses of waste released from fish farms are accessible for S. Latissima in during growth phase.

Material and Methods

Nitrate depleted and saturated *S. latissima* (~10cm) were exposed to a gradient of Ammonium (0.25-16 µmol), for 5 hours. By following the depletion of substrate concentration at 8 different point of time, uptake rates (µmol L^{-1} gDW^{-1} h^{-1}) were determined.

Results

Interestingly, S. latissima was observed to rapidly adapt to different concentrations of Ammonium (NH_4^{+}), with a swift increase in uptake rates. Maximal uptake rates were observed within 50 minutes as shown in figure 1. Furthermore, uptake of NH_4^{+} increased linearly with augmenting concentration, regardless of nutritional history. The nutritional history of *S. latissima* appeared to affect the uptake, as nitrogen depleted specimens had a significant faster uptake of NH_4^{+} than nitrogen saturated specimens as shown in figure 2

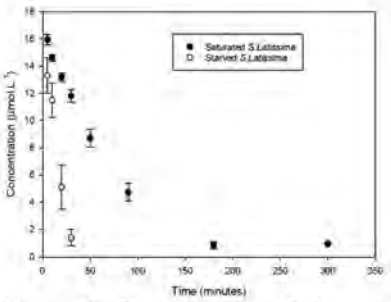

Figure 1: Time course of decrease in ammonium concentration (initially16µmol L^{-1}) in culture medium with juvenile plants of *Saccharina Latissima* for nitrate saturated and depleted plants (n=5±SD)

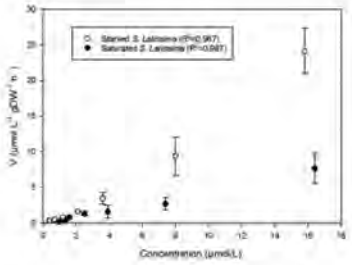

Figure 2: Ammonium uptake rates for *Saccharina Latissima* from 5 to 50 minutes related to available concentration for both nitrate saturated and depleted plants (n=5±SE, linear regression with R^2=0,987 for depleted and saturated).

(Continued on next page)

D**iscussion**

With an increase in initial substrate concentration, the uptake rates were also increasing. Furthermore, the uptake rates were consistently higher for the nitrate depleted *S. Latissima* than in the experiment with the saturated sugar kelp. The rates for nitrate depleted *S. Latissima* were also increasing to a higher degree with an increase in initial concentration. This shows that the depleted *S. latissima* had a higher capacity to take up NH_4^+ than saturated *S. latissima*. The difference increased with increased available concentration. This confirms that the nutritional history influences the NH_4^+ uptake for *S. latissima*, as is generally believed to be the case for seaweed (Hurd et al., 2014). The results from this experiment indicate that juvenile *S. Latissima* have the ability to utilize pulses of Ammonium released from the fish farms for growth, independent of nutritional state.

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EFFECT OF *Plukenetia voluvilis* AS ALTERNATIVE TO FISHMEAL ON GROWTH AND DIGESTIVE ENZYMES AND BODY COMPOSITION IN WHITE LEG SHRIMP (*Litopenaeus vanameii*)

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Introduction

Shrimps need high percentage of protein in diet ranged to 25% to 33% (%, dry matter) of which minimum 12% is fishmeal (Suarez et al., 2009). Fishmeal is an essential ingredient in fish feed, although it is not a sustainable source so that, it is foreseen a decrease of fishmeal inclusion in shrimp feed and an increase of alternative protein used, in fact, aquaculture depends less and less on fishmeal. The FIFO for crustacean 0.91 in 2000 and 0.46 in 2015 (IFFO 2018). *Plunkenetia volubilis* belongs to Euphorbiaceous family native to the Peruvian jungles. The flour and oil from the seeds contain approximately, 48% oil and 27% proteins which are rich in cysteine, tyrosine, threonine and tryptophan. The fatty acids of sacha are mostly unsaturated, about 85% polyunsaturation, comprised of approximately 34% linoleic acid (18:2*n*6) and 51% linolenic acid (18:3*n*3). Given the above considerations, the objective of this work was to evaluate the effect of different dietary levels of *P. volubilis* as an alternative protein source on growth performance, body composition and digestive enzyme activities of *L. vanamei* juveniles.

Material and methods

A feeding trial was carried out at a shrimp farm located at Santa Rosa provincia de El Oro – Ecuador (79° 57' 21'' 3° 23' 45'') in cages of $1m^3$ arranged in 1 hectare ponds. Fifteen *L. vanameii* juveniles with an average initial weight of 3.41 ± 0.31 g were randomly distributed and stocked into 8 cubical cages of $1m^3$ capacity at a density of 15 individuals per cage. Shrimps were fed during 10 weeks three times daily (08:00, 12:00, 16:00) at 15 % of their body weight with four iso-nutritional (35% and 9% DM, respectively) experimental diets designed with increasing levels of *P. voluvilis* biomass: 0% (D-0), 15% (D-15), 25% (D-25) and 50% (D-50) substitution. Every week, the total weight of shrimp in each tank was recorded to adjust the daily amount of feed. At the end of the feeding trail, 8 morphometrics traits and daily gain, Specific Growth Rate (SGR) and Feed Convertion Ratio (FCR) were determined. Crude protein and total lipid contents of abdomen samples were determined by standard methods. Finally, for enzymatic activity analysis, hepatopancreas from 10 shrimps per treatment were pooled to obtain enzymatic extracts in order to determine trypsin (Erlanger et al., 1961) chymotrypsin (Del Mar *et al*., 1979) and total alkaline protease (Alarcón *et al*., 1998) activities.

Table 1. Growth performance, morphometric traits and nutrient utilization of juvenile *L. vanamei* at the end of the feeding trial.

	D-0%	D-15%	D-25%	D-50%
Total weight	14.34 ± 2,36	14.48 ± 1,64	16.01 ± 1,33	16.75 ± 2,70
Cephalothorax weight	3.99 ± $0,59^{ab}$	3.65 ± 0.36^{a}	4.48 ± $0,41^{b}$	4.40 ± $0,82^{b}$
Abdomen weight	8.74 ± $1,17^{a}$	8.86 ± $1,04^{ab}$	9.75 ± $0,99^{ab}$	10.32 ± $1,71^{b}$
Abdomen weight + exoskeleton	10.20 ± $1,77^{a}$	10.24 ± $0,95^{a}$	11.34 ± $1,07^{ab}$	12.21 ± $1,98^{b}$
Hepatopancreas weight	0.42 ± $0,13^{a}$	0.45 ± $0,11^{a}$	0.60 ± $0,09^{b}$	0.48 ± $0,15^{ab}$
Total lenght	12.76 ± 1,40	13.10 ± 0,39	13.30 ± 0,59	13.65 ± 0,53
Cephalothorax lenght	5.65 ± 0,30	5.65 ± 0,30	5.49 ± 0,31	5.30 ± 0,42
Abdomen lenght	8.96 ± 0,61	8.96 ± 0,61	8.95 ± 0,28	9.13 ± 0,52
SGR (%)	2.07 ± $0,03^{b}$	2.16 ± $0,06^{c}$	1.97 ± $0,04^{a}$	2.06 ± $0,04^{b}$
DAILY GAIN (g/dia)	0.17 ± 0,01	0.13 ± 0,06	0.16 ± 0,01	0.15 ± 0,01
FCR	0.94 ± 0,08	0.95 ± 0,03	0.96 ± 0,08	1.04 ± 0,04

Values are expressed as mean ± SD (n = 10). Values in the same row with different lowercase letters indicate significant differences ($p< 0.05$).

Table 2. Digestive enzyme activities (U mg soluble $protein^{-1}$) measured in hepatopancreas extracts of juvenile *L. vanamei* at the end of the feeding trial.

	Trypsin (U/mg prot)	Quimotrypsin (U/mg prot)	Basic protease (U/mg prot)
Control	0,09 ± 0,02	0,195 ± $0,025^{b}$	295,2 ± $65,5^{b}$
15% replac.	0,07 ± 0,02	0,167 ± $0,010^{ab}$	203,7 ± $22,9^{ab}$
25% replac.	0,07 ± 0,005	0,130 ± $0,004^{a}$	156,1 ± $4,03^{a}$
50% replac.	0,07 ± 0,005	0,139 ± $0,031^{ab}$	139,0 ± $16,1^{a}$

Values are expressed as mean ± SD (n = 10). Values in the same row with different lowercase letters indicate significant differences ($p< 0.05$).

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Results and discussion

The results obtained indicate that the inclusion of *P. volubilis* up to 39% in practical diets did not cause noticeable negative effects on growth performance of *L. vanamei* juveniles. Total weight and abdomen weight in shrimps fed with 25 and 50% sacha inchi were elevated, although significant differences from those fed the D-0% diet were only observed in abdomen weight (**Table 1**). This increase in the abdomen weight have a positive effect on shrimp quality. None of the treatments produced any clear adverse effects on tail composition (data not shown). Regarding digestive enzyme activities, fish fed *P. volubilis* supplemented diets showed lower chymotrypsin and alkaline protease activities compared to shrimp fed D-0% diet, although significant differences were only observed in D-25% and D-50% groups. Trypsin activity remained unaffected by *P. volubilis* inclusion. This fact could be related with the presence of diverse antinutritional factors including trypsin inhibitor in Sacha inchi seeds and in the cake (46.19 UTI/mg urinary trypsin inhibitor) (Lázaro Aguilar, 2015).

Conclusion

The results of this experiment indicates that *Plukenetia voluvilis* is very interest as alternative source to fishmeal in shrimp feeding that could be studied more deeply.

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CHANGES IN THE AMINO ACIDS CATABOLISM BY INSECT MEAL INCLUSION ON DIETS OF *Oncorhynchus mykiss, Sparus aurata* AND *Tinca tinca*

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Introduction

In the last years, the interest for the insect as protein source has been increased. The insects are being studied as food as humans and as protein source for animals. In this sense, its use as alternative protein source to fishmeal is one of the main applications of insects.

The inclusion of *Tenebrio molitor* and *Hermetia illucens* meal has been studied in several fish species mostly focused in growth and nutritive parameters, digestive efficiency and effect on the quality of the product, but the effect on amino acid metabolism has not been studied. This experiment study the changes in the intermediary metabolism of amino acids of three commercial fish species Trout (*Oncorhynchus mykiss*), Gilthead (*Sparus aurata*) and Tench (*Tinca tinca*).

Material and methods

Five diets isonegetic, isolipidic and isoproteic; one control and four experiments were designed for this experiment: H30 (30% of fish meal was replaced by *Hermetia* meal), H15 (15% of fish meal was replaced by *Hermetia* meal), T 30 (30% of fish meal was replaced by *Tenebrio* meal) and T15 (15% of fish meal was replaced by *Tenebrio* meal).

Table 1. Km (mM) of enzymes for Gilthead seabream. Trout and Tench.

Km		C	H15%	H30%	T15%	T30%
GDH	Gilthead	0.44 ± 0.05	0.47 ± 0.03	0.41 ± 0.06	0.56 ± 0.06	0.61 ± 0.05
	Trout	0.32 ± 0.06a	0.55 ± 0.06b	0.40 ± 0.02ab	0.48 ± 0.06ab	0.47 ± 0.04ab
	Tench	0.46 ± 0.05	0.37 ± 0.03	0.30 ± 0.03	0.42 ± 0.06	0.43 ± 0.08
ALT	Gilthead	2.42 ± 0.29	1.58 ± 0.36	1.94 ± 0.17	2.25 ± 0.09	2.01 ± 0.17
	Trout	0.89 ± 0.17	0.86 ± 0.22	1.33 ± 0.15	1.41 ± 0.23	0.64 ± 0.11
	Tench	4.05 ± 0.27	3.72 ± 0.19	3.82 ± 0.41	3.48 ± 0.20	4.03 ± 0.37
AST	Gilthead	1.06 ± 0.11ab	1.29 ± 0.06abc	0.84 ± 0.05a	1.65 ± 0.30bc	1.93 ± 0.11c
	Trout	1.49 ± 0.32	2.53 ± 0.33	2.28 ± 0.32	1.51 ± 0.24	1.70 ± 0.19
	Tench	1.19 ± 0.15	1.26 ± 0.05	1.13 ± 0.05	1.06 ± 0.03	1.14 ± 0.10

Values are expressed as mean ± SD (n = 10). Values in the same row with different lowercase letters indicate significant differences (p< 0.05).

Table 2. Vmax (U/mg prot) of enzymes for Gilthead seabream. Trout and Tench.

Vmax		C	H15%	H30%	T15%	T30%
GDH	Gilthead	552.04 ± 54.92a	448.48 ± 48.10ab	254.11 ± 15.70cd	388.32 ± 23.34bc	230.46 ± 23.14d
	Trout	331.84 ± 22.92a	1425.36 ± 88.58c	979.97 ± 85.36b	1049.93 ± 76.99b	1673.24 ± 78.98c
	Tench	471.03 ± 37.63a	506.24 ± 29.56a	885.05 ± 33.92b	676.34 ± 88.20ab	628.19 ± 43.44a
ALT	Gilthead	172.83 ± 23.12a	317.74 ± 37.12b	271.56 ± 16.90ab	373.68 ± 35.80b	300.32 ± 14.29b
	Trout	94.81 ± 12.17a	202.21 ± 22.59c	120.67 ± 13.69ab	142.02 ± 11.01abc	155.58 ± 9.18bc
	Tench	1066.00 ± 82.95ab	868.42 ± 45.34a	1366.51 ± 118.54b	898.21 ± 55.70a	835.05 ± 90.36a
AST	Gilthead	1037.05 ± 93.26	953.89 ± 111.92	758.98 ± 103.55	912.51 ± 91.39	752.87 ± 49.92
	Trout	237.95 ± 22.54a	551.31 ± 50.79b	610.26 ± 40.91b	613.64 ± 32.59b	613.42 ± 12.34b
	Tench	1297.73 ± 179.10ab	1287.25 ± 147.35ab	1585.28 ± 101.51b	960.91 ± 153.35ab	820.84 ± 136.50a

Values are expressed as mean ± SD (n = 10). Values in the same row with different lowercase letters indicate significant differences (p< 0.05).

Table 3. Catalytic efficiency of enzymes in Gilthead. Trout and Tench

EC		C	H15%	H30%	T15%	T30%
GDH	Gilthead	1393.02 ± 88.05a	1002.10 ± 163.68ab	718.08 ± 79.58bc	637.02 ± 56.53bc	405.55 ± 51.46c
	Trout	1140.98 ± 208.51a	2467.96 ± 200.11bc	2266.93 ± 211.19b	2269.37 ± 268.72b	3220.71 ± 95.18c
	Tench	1194.22 ± 229.03a	1373.20 ± 144.28a	3248 ± 170.85b	1508.81 ± 116.13a	1895.39 ± 364.62a
ALT	Gilthead	64.70 ± 3.09a	216.76 ± 25.45b	142.60 ± 7.75c	191.23 ± 11.45c	149.13 ± 9.82c
	Trout	133.16 ± 19.37ab	234.90 ± 35.23b	84.20 ± 5.20a	97.83 ± 18.40a	233.78 ± 34.95b
	Tench	265.04 ± 25.21ab	236.01 ± 17.96a	411.42 ± 57.69b	270.26 ± 32.97ab	215.93 ± 15.68a
AST	Gilthead	987.09 ± 215.41a	733.62 ± 69.36ab	940.88 ± 111.53a	779.89 ± 151.33ab	390.89 ± 18.99b
	Trout	173.79 ± 26.68a	220.84 ± 21.66ab	255.09 ± 24.44abc	399.60 ± 64.47c	376.54 ± 32.16bc
	Tench	824.82 ± 123.32ab	1190.38 ± 89.57a	1358.49 ± 96.98a	908.65 ± 154.20ab	629.29 ± 152.94b

Values are expressed as mean ± SD (n = 10). Values in the same row with different lowercase letters indicate significant differences (p< 0.05).

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The fish were feed with experimental diets until triplicate the weight. After experimental period, the fish were sacrificed and the liver were quickly removed and frozen in N_2 liquid and kept at -80ºC until enzymes determination. The method used for the determination of enzyme activity is based on the spectrophotometric measurement of the decrease in optical density caused by the oxidation of nicotinamide adenine dinucleotide (NADH). All enzymes were measured using methods described by Bisswanger (2011). Alanine aminotrasferase (EC 2.6.1.2, ALT) was measured at different concentrations of L-alanine (0.01, 0.1, 1, 10, 25 and 50 mM) as substrate. Aspartate aminotransferase (EC 2.6.1.1, AST) was measured at different concentrations of L-aspartate (0.01, 0.1, 1, 10, 25 and 50 mM) as substrate. Glutamate dehydrogenase (EC: 1.4.1.3, GDH) was measured at different concentrations of α-ketoglutarate (0.01, 0.05, 0.1, 0.5, 1 and 10 mM) as substrate.

Results

In the table 1, 2 and 3 show the data obtained for kinetic parameters (Km, Vmax and EC) obtained in the three fish species studied. The data show that the enzyme activity change depending on fish and insect species

Discussion:

Rainbow trout and seabream are considered as carnivore species, with a crude protein requirement of 43% (FAO 2019a) and 45-50% (FAO 2019b) for juveniles respectively. The tench feeding habit are not completely known, although it is considered omnivore (Benzer et al. 2009, 2010). The data obtained for kinetic parameters are in agreement with the feeding habit of these species, tench displays a high amino acids degradation probably due to an excess of dietary amino acids. Regarding insect inclusion, in tench any diets seem to have a great effect except for H-30. However, in trout the insect inclusion increased Vmax in the three enzymes assayed, which could be consequence of amino acids utilization for gluconeogenesis o energy purposes. In Gilthead seabream GPT and GOT show different mechanisms of adaptation to the insect inclusion, while GOT modified the Km, GPT modified Vmax. The changes of GDH it is considered a good indicator of amino acids utilization (Sánchez-Muros et al., 1998) because all the glutamate produced in the transamination is deaminated by GDH, with the formation of NH_4^+. In this sense, the catalytic efficiency of GDH, the inclusion of insect don´t affect except for H-30 while in seabream decreased and in trout increased.

In conclusion, the results show that the inclusion of insect lead changes in amino acids catabolism through the modification of kinetic parameters. In general term, inclusion of insect increased the efficiency of oxidative deamination in trout and decrease it in seabream, without changes in tench, except for H-30 diet.

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EFFECTS OF SQUID (*Loligo* sp.) INK EXTRACT TO WHITE SHRIMP (*Litopeneus vannamei*) *CypA* GENE POLYMORPHISM IN WHITE FECES SYNDROME (WFS) CASE

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Introduction

White Feces Syndrome (WFS) is a disease problem in shrimp farming along with increasing production through intensification. Early indications appear in feed trays and water surface, where abundant floating white fecal strings were observed. In severely affected shrimp, hepatopancreas and gut become white and pale yellow in color (Mastan, 2015). WFS was caused by interaction between gregarine (protozoa) and *Vibrio* sp. (Limsuwan, 2010).

WFS caused decreasing of immunity respond which related to immune gene *CypA* (Muhammad *et al*., 2017). One ingredient of Squid (*Loligo* sp.) ink extract is oleic acids which has an antibacterial function (Fadjar *et al*., 2016).

The objective of this research was to know the effect of squid ink extract to *CypA* gene polymorphism in white shrimp (*L. vannamei*) under WFS condition.

Material and Methods

Squid ink was taken from squid (*Loligo* sp.). Maseration was carried out with 96% methanol solvent with a ratio of 1: 3 and then stored for 7 days. Evaporation was carried out using 200 ml of maceration results with a rotary evaporator. The shrimp in WSF condition and pond water was taken from the shrimp pond with an average weight of 2.18 grams and fed with shrimp feed mix with : 6 ppm (treatment B), 8 ppm (treatment C), and 10 ppm (treatment D) squid ink extract , while WFS shrimp as positive control (treatment E) and healthy shrimp as negative control (treatment A). Shrimp was reared for 2 weeks in laboratory. After 2 weeks, shrimp samples were taken from every treatment aquarium and counted for survival rate.. Shrimp meat were taken as much as 0.05 grams and chopped until smooth then added with pH 7 TE-buffer for quantitative testing with NanoDrop Spectrophotometry (Fatchiyah *et al*., 2011). DNA amplification was done using thermal cycler and PCR mix with F primer (5'-CTGTAAAGTTTCAGAACATTCCCCC-3') and R primer (5'-GGACACCTATCTTGTTTCACCACCT-3'). Electrophoresis was done using gel agarose electrophoresis methods. Result of DNA extraction was sent for DNA sequencing and the result was confirmed using BLAST program. Survival rate was analyzed using ANOVA.

Result

Nucleotide composition was dominated by T (36.3%), A (24.8 %), C (21.9%), and G (17.0 %). WFS shrimp (E) have very different nucleotide bases compared to *CypA* of healthy shrimp (A) and WFS shrimp which had treated with squid ink extract (treatment B, ,C, D) but there were still mutation at base pair number 30 and 33.

125 number of sites was investigated resulting 15 polymorphic sites number and 17 mutation sites. Percentage of polymorphism can be seen as treatment B= 8 %, C= 9.6 %, D= 9.6% and WFS shrimp (E) = 10.4 %.
Maximum Likelihood Estimation resulted treatment B (8.5 %), C and D (10.3 %), and control positive/WFS shrimp (E) (11.3 %).

No significantly different between healthy shrimp (A) and treatment shrimp (B,C, and D), but highly significantly different with WFS shrimp (E).

Discussion and conclusion

Domination of nucleotide T and A make the gene mutate due to weak bonds. CypA gene in vannamei shrimp is categorized as rich in A-T which has a weak bond because it has 2 hydrogen bonds compared to C-G (Lee and Luo, 1977).

Polymorphism occurs because the percentage of polymorphism is more than 1% as mentioned by Lusiastuti *et al*. (2015) that polymorphisms (changes in one changing nucleotide in the genome) can occur at any genetic location and can be found at least in two different sequences. At least 1% cut off is classified as polymorphism and if the frequency is smaller than 1%, the allele is considered to have a mutation.

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The smallest polymorphism is obtained in treatment B (6 ppm squid ink extract) which means that only a few genetic changes in the *CypA* gene.

Based on survival rate analysis, using squid ink extract can increase SR of white shrimp and can be related to *CypA* gene in immune system.

The conclusion of this study was polymorphism was happen in WFS on white shrimp and the use of 6 ppm squid ink extract is the best because it causes the least change in the *CypA* gene.

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GENETIC AND GENOMIC PARAMETERS FOR VNN RESISTANCE, BODY WEIGHT AND CORTISOL CONCENTRATION IN EUROPEAN SEA BASS (*Dicentrarchus labrax* L.)

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Introduction

Viral nervous necrosis (VNN) is a serious pathology and one of the major threats for European sea bass (*Dicentrarchus labrax* L.) hatcheries and farms, having severe impacts, both in terms of animal and economical losses (Munday et al., 2002). Recently, interest has been directed towards selective breeding for improved genetic resistance, supported by the estimated magnitude of additive genetic variation (Doan et al., 2017; Palaiokostas et al., 2018). The aim of this study was to estimate the heritability for mortality after a VNN challenge test in a sea bass experimental population derived from a commercial stock, as well as for cortisol concentration after stress exposure and body weight at a fixed age. Genetic and phenotypic correlations among traits were also estimated, with the aim of identifying possible indicator traits to be used in indirect selection approaches to improve VNN resistance.

Materials and methods

A sea bass experimental population (N = 650) derived from a commercial breeding stock (Valle Cà Zuliani Società Agricola s.r.l.) and reared in external facilities (open sea-cages, Valle Cà Zuliani client) was subjected to a confinement stress firs and to a VNN challenge test later. All the fish were individually weighed (body weight, BW, at 548 days post-hatching) and tagged. The mortality (MORT) was recorded daily throughout the 29 days of the challenge test. Post-stress cortisol concentration (HC as ng ml-1, later normalized through the square root transformation of the original data, SRHC) were measured through radioimmunoassay technique from a blood sample collected from each fish before infection. A genome-wide SNP dataset for sea bass was generated through a high-throughput sequencing approach (2b-RAD), obtaining 16 075 SNPs. All the experimental fish and their parents were genotyped; the parentage and a high-likelihood pedigree were reconstructed for each tested individual. We estimated the genetic parameters of the traits using Bayesian procedures: univariate (to estimate heritability) sire-dam model for MORT (binary trait) or animal models for BW and SRHC; bivariate (to assess the genetic and phenotypic correlations between traits) sire-dam models when MORT was one of the trait, animal models for the other traits. To estimate the genomic heritability, five different regression models were implemented: Bayes A, Bayes B, Bayes C, Bayesian LASSO and Bayesian Ridge Regression.

Results

The survival rate at the end of the VNN challenge test was 52.2%. Genetic heritability and correlations are presented in Table I and II. Genomic heritability estimates for each of the five Bayesian models are presented in Table III.

Discussion and conclusion

Our results evidenced the presence of a genetic basis for the traits under investigation. The heritability of MORT estimated through a univariate model was low, but when it was estimated through bivariate models, the estimates were higher and comparable to estimates on larger datasets (Doan et al., 2017; Palaiokostas et al. 2018). Non-trivial genetic correlations among the traits (except for MORT and SRHC) suggest the possibility to develop indirect selective strategies to enhance VNN disease resistance. The genomic heritabilities were in general lower than the genetic estimates obtained using sire-dam or animal models (except for SRHC), probably due to the "missing heritability" phenomenon and the inability of the SNP effects to pick up all the variation due to additive genetic effects (De Los Campos et al., 2015). Despite the high number of iterations (2 000 000), the Bayesian LASSO was the only model that failed to reach convergence, and such estimates were not consistent with those obtained with the other genomic models. In conclusion, our results suggest the feasibility of selective breeding approaches to improve resistance to VNN, resistance to stress and body weight in European sea bass, also directing the interest to genomic prediction tools in the view of developing strategies to implement such selection.

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Table I Genetic heritability (h^2), probability of h^2 being greater than 0.1 and the highest posterior density intervals (HPD 95%) of h^2 for the investigated traits

Trait	h^2	$P(h^2 > 0.1)$	HPD 95%
MORT	0.136	0.697	0.016, 0.307
BW	0.571	1.000	0.331, 0.838
SRHC	0.189	0.933	0.065, 0.342

Table II Genetic heritability (h^2), correlations (genetic, r_a, and phenotypic, r_p) and probability (P) of r_a being positive (in case of a positive estimate) or negative (in case of a negative estimate) from bivariate analyses

Trait 1	Trait 2	h^2 trait 1	h^2 trait 2	r_p	r_a	P
MORT	BW	0.228	0.451	-0.084	-0.388	0.884
MORT	SRHC	0.223	0.237	0.020	-0.078	0.578
SRHC	BW	0.193	0.592	-0.023	0.115	0.652

Table III Heritability estimated with genomic models and probability of the estimates being greater than 0.1

Trait	Bayes A	Bayes B	Bayes C	Bayesian LASSO	Bayesian Ridge Regression	$P(h^2 > 0.1)$
MORT	0.08	0.08	0.09	0.02	0.10	0.34
BW	0.44	0.43	0.44	0.54	0.44	1.00
SRHC	0.19	0.20	0.19	0.20	0.19	0.98

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SPATIAL FRAMEWORK FOR SITE SELECTION AND REGULATION OF FRESHWATER CAGE AQUACULTURE IN EUROPE

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Introduction

The use of cages for fish production in freshwater lake systems has declined in recent decades as production has moved to land-based systems in many locations. In the past, lake systems in northern European countries were important for intensive aquaculture as cages were used for production of Atlantic salmon (*Salmo salar*) smolts, rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta)*. There are a number of reasons for this migration including the desire to have more control over production, technological advances, environmental concerns, and regulation and policy. For salmon aquaculture in particular, Scotland is now the only country where there are high numbers of smolts produced in freshwater cages. However, there are many freshwater lake systems throughout Europe and, if planned and managed appropriately, cages in freshwater systems can be an efficient and cost-effective production method. Site selection is of vital importance, as this will underpin the success and sustainability of a farm. Geographic Information Systems (GIS) can be used to identify the most suitable locations based on selected criteria. However, to date, the use of GIS to investigate spatial aspects of freshwater cage culture has been limited, particularly in comparison to the marine sector. There is a need for a spatial framework that can be used to identify suitable locations for culture, supporting sustainable growth of aquaculture in Europe in line with Blue Growth strategies.

Methods

A spatial framework was developed to assess the physical suitability and carrying capacity of freshwater lake systems and tested on a freshwater lake (loch system) in the north of Scotland. Environmental and socio-economic data layers were developed from fieldwork, earth observation data and secondary datasets. The modelling approach uses a number of GIS-based sub-models which are then combined to produce the final result.

Results and discussion

The model identified a number of sites that could potentially be suitable for aquaculture. Physical parameters were a key constraint, but socio-economic factors also affected overall suitability. The results also highlight the importance of the wider catchment and land-based activities, which must also be considered when selecting a site and managing farm operations as these influence carrying capacity. The framework can be applied to freshwater lake systems throughout Europe to assess suitability for cage production.

Acknowledgements

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SURVEY OF WATER QUALITY AROUND RAINBOW TROUT (*Oncorhynchus mykiss*) CAGES IN THE SOUTHERN CASPIAN SEA

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Introduction

Water status is determined using the water quality index (WQI), which is presented to simplify the reporting and interpretation of water resources information (Rubio-Arias *et al*., 2012). Water quality index is one of the important tools for summarizing information on water parameters as well as transferring water quality information to authorities (Sanchez, 2007). This index is used to understand water quality issues by aggregating complex data and creating a single body to describe water quality (Bhargava, 1983).

Materials and methods

This study was carried out in two rainbow trout aquaculture farms in 5 floating cages in two regions of Mazandaran province (klarabad and Abbas Abad) on the southern coast of the Caspian Sea in the January to August 2015. The Iranian surface water quality index (IRWQIsc) for was determined around the floating cages using water parameters including turbidity, temperature, pH, electrical conductivity, dissolved oxygen, nitrate, ammonium and phosphate during *Oncorhynchus mykiss* aquaculture and the factors above plus biochemical oxygen demand of water, three months after aquaculture (APHA, 2005). The water quality index around the cage was determined by separating the different sampling periods in two modes: 1. Close the cage (cage shadow and distances of 50 and 100m); and 2.Distance of 1000m away from the cage (Karakassis *et al*., 2000). In order to calculate the water quality index of Iran, I-value was determined using standard tables for each parameter and then the IRWQI value was determined by the following formula.

$$IRWQI_{sc} = [\prod_{i=1}^{n} I_i^{w_i}]^{\frac{1}{\gamma}}$$

$$\gamma = \sum_{i=1}^{n} W_i$$

Wi: Weight of parameter i, Ii: Quality index value for parameter i of rating curve
n: number of water quality parameters

Results

The results showed that there was no significant difference between the two fish breeding centers and the distances to and from the cage ($P < 0.05$). However, the calculated values of water quality index in the study period in August (outside the fish farming period) were higher and significantly different than other periods ($P < 0.05$), but the water quality status of the sampling area during the period. According to the Iranian Surface Water Quality Index (IRWQIsc), it was in moderate condition.

Discussion and conclusion

The results seem to be influenc d by the natural conditions of the region. These results indicate that the quality and status of water around fish farms in cage culture are more dependent on the natural environment. Because, far from the cage and outside the fish farming period, the impacts of rainbow trout cage culture on water quality are not observed

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Table1. Iranian Surface Water Quality Index (IRWQIsc) and Water Quality Status (WQS) around rainbow trout breeding cages in two regions of Clarabad and Abbas Abad in the southern Caspian Sea (2015)

Marine Farm (fish cage culture)	Months of sampling	IRWQIsc Close to cage (up to 100m)	IRWQIsc Away from the cage (1000m)	WQS
Kelar Abad	January	46.39b	46.39b	moderate
	March	46.93b	46.93b	moderate
	May	46.96b	46.80b	moderate
	August	51.03a	50.82a	moderate
Abas Abad	January	46.69b	46.27b	moderate
	March	46.75b	45.91b	moderate
	May	46.71b	46.08b	moderate
	August	50.32a	49.51a	moderate

* The Latin letters represent a significant difference in each column between the 5% sampling periods under the F test.

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THE IMPACT OF A PISCINE ORTHOREOVIRUS (PRV) INFECTION ON THE RESPIRATORY PERFORMANCE AND CAPACITY OF ATLANTIC SALMON AND SOCKEYE SALMON IN BRITISH COLUMBIA, CANADA

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Concern exists over the high prevalence of piscine orthoreovirus (PRV) in farmed Atlantic salmon (*Salmo salar*), which is now approaching 100% in British Columbia, Canada because PRV is known to be associated with heart and skeletal muscle inflam ation (HSMI). Even though the HSMI-associated mortality of farmed Atlantic salmon in British Columbia is reported to be negligible, the risk of the virus spreading to and harming wild salmon is considered to be very real given the suggestions that adult sockeye salmon (*Oncorhynchus nerka*) infected with PRV are failing to successfully swim up the Fraser River to reach their natal spawning areas. Given that PRV replicates in red blood cells of salmonids and can be associated with cardiac inflam ation, we reasoned that the respiratory capacity of an infected salmon should be impaired if they had been deliberately infected with a strain of PRV from British Columbia and viremia had become established. Therefore, we monitored the respiratory capabilities in sockeye salmon smolts for 9 weeks after they were injected with purified virus and in Atlantic salmon smolts for 21 weeks after they were injected with PRV-infected red blood cells. Comparisons were made with time-matched measurements on control fish that had received sham saline or non-infected red blood cell injections. Well-established performance indices (maximum, routine and standard oxygen uptake; recovery from exhaustion; performance in a hypoxic exposure) were measured over 4-days during a whole-animal respirometry trial, along with *in vitro* measurements of red blood cell oxygen affinity and hemoglobin concentration. None of the Atlantic salmon injected with a strain of PRV from British Columbia died during the respirometry experiment despite being chased to exhaustion and were made severely hypoxic, even though had developed severe viremia, a transient cellular antiviral response and minor heart pathology. Furthermore, when compared with sham-injected controls, none of the respiratory indices that were measured, including red blood cell oxygen affinity and hemoglobin concentration, changed appreciably or consistently at weeks 3, 9, 18 and 21 post-infection. Similar results were found for sockeye salmon smolts that were tested at weeks 3 and 9 post-infection. In contrast, an IHNV injection, which caused 34% mortality, initially suppressed energetic demanding cell proliferation and protein synthesis pathways while the IHNV load was being cleared in the surviving smolts (up to day 28). Also, the IHNV load in the survivors was associated with reduced standard (19%; p=0.015) and routine (19%; p=0.019) oxygen uptake, as well as a reduced ability to tolerate hypoxic water (15%; p=0.0007). Thus, while standard respirometry practices detected sub-lethal consequences of an IHNV infection, the viremia produced by a PRV infection did not impair respiratory functions of sockeye and Atlantic salmon smolts.

THE OPTIMAL DIETARY γ-Aminobutyric ACID (GABA) LEVEL IN JUVENILE OLIVE FLOUNDER, *Paralichthys olivaceus*

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Due to the potential benefits of γ-Aminobutyric acid (GABA) in marine finfis culture, an 8-week feeding trial was conducted to determine the optimal dietary level of GABA and its effects in Juvenile olive flounde , (*Paralichthys olivaceus*). Eight groups of fish were randomly distributed into 24 rectangular 40 L tanks. Triplicates of 30 fish averaging 4.90 ± 0.1 g (mean ± SD) were fed one of the eight experimental diets; a basal diet without GABA as a negative control (CON), a positive control composed of CON + 4g/kg oxytetracycline (CON_{OTC}), and six other diets prepared by adding 50,100, 150, 200, 250, and 600 mg/kg GABA. Parameters including growth performance, non-specific immune response, disease resistance, enzyme activity, histology, and genetic expression will be measured after the trial is concluded. Results from this experiment will be reported in conference.

EFFECT OF LIGHT POLLUTION ON DIURNAL RHYTHM OF MELATONIN IN *Catla Catla* AND *Labeo Rohita*; COMPARATIVE STUDY IN FARM AND INDOOR FACILITY ILLUMINATION CONDITIONS

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Introduction

Use of artificial light at night (ALAN) for outdoor illumination has been exceeded in metropolitan, semi urban areas and animal farms including aquaculture research facilities. worldwide. ALAN has caused disruption in natural light and dark periods. These modified day lengths have profound effects on internal body clocks regulated by melatonin. ALAN at indoor research facilities might interfere with the results of scientific trials which have not be fully realized yet.

Materials and Methods

Present study investigated the effects of ALAN on diurnal profile of melatonin at indoor facility/laboratory (artificial light group: AL) compared with that at outdoor farm (natural light group: NL) in *Catla Catla* and *Labeo rohita*. Light intensity was measured to be 5 and 156 lux at farm and indoor facility, respectively. Blood samples were collected over the period of 24 hours.

Results

Levels of melatonin in NL group of both species started increasing at sunset (107.44 ± 0.10 pg/ml in labeo; 103.05 ± 1.23 pg/ml in catla). Highest levels were observed at midnight (180.90 ± 0.21 pg/ml in labeo; 175.06 ± 1.30 pg/ml in catla). Hormone profile started decreasing after sunrise and the minimum values were observed at mid-day. Diurnal profile of melatonin did not show any significant variability ($P > 0.5$) in AL group in both species and remained low over the period of 24 hours. This study concluded that ALAN in laboratories and indoor aquaculture facilities disrupts the hypothalamus-pituitary-pineal (HPP axis) and might affect the physiological activities regulated by melatonin in fish.

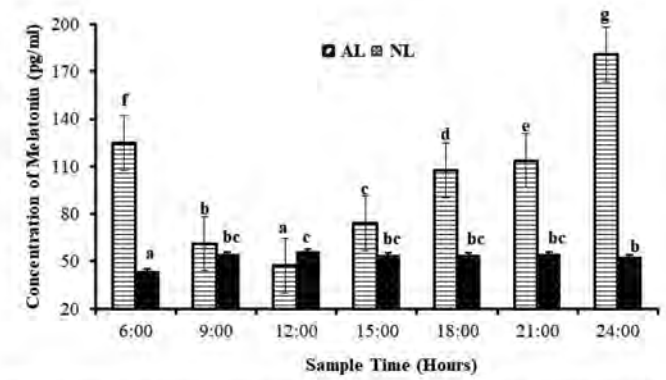

Fig. 1 Profile of melatonin (mean ± SE) in *Labeo rohita* exposed to natural (NL) and artificial photoperiod (AL) under farm and indoor facility conditions. Study was conducted on 27.03.2018 and 30.03.2018, respectively. Timings of sunrise and sunset were 6:00 and 18:00, respectively. Data Labels represents the homogenous subsets by Tukey's post hoc test.

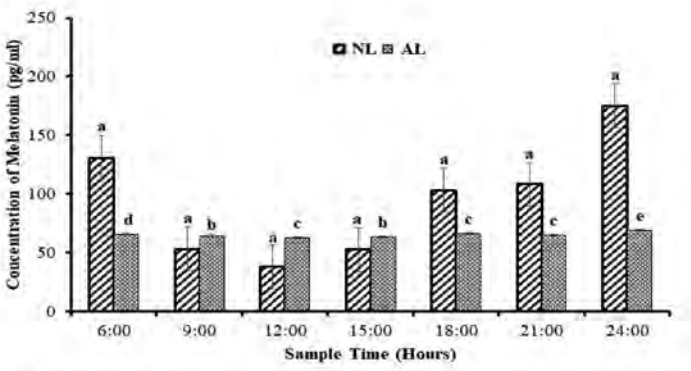

Fig. 2 Profile of melatonin (mean ± SE) in *Catla catla* exposed to natural (NL) and artificial light (AL) under farm and indoor facility conditions. Study was conducted on 27.03.2018 and 30.03.2018, respectively. Timings of sunrise and sunset were 6:00 and 18:00, respectively. Data Labels represents the homogenous subsets by Tukey's post hoc test.

EFFECT OF BIOLOGICAL AND PHYSICAL PRE-TREATMENTS OF *Ulva rigida* IN THE QUALITY OF ON-GROWING EUROPEAN SEABASS *Dicentrarchus labrax*

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Content
A growth trial with European seabass was performed to assess the effect of the dietary inclusion of *Ulva rigida* as is or with technological pre-treatment with ultrasounds (US) or solid-state fermentation (SSF) with *Aspergillus ibericus*. Promising results of the pre-treatment of *U. rigida* prior to dietary inclusion on growth performance and feed utilization efficiency of seabass were obtained, however, the effect of these treatments on fillet nutritional and sensory quality was not yet evaluated.

Introduction
Macroalgae show high productivity and do not compete for arable land to be cultivated as terrestrial alternatives (Bikker *et al*., 2016)a biorefinery approach aimed at cascading valorisation of both protein and non-protein seaweed constituents is required to realise an economically feasible value chain. In this study, such a biorefinery approach is presented for the green seaweed Ulva lactuca containing 225 g protein (N\\thinspace{\\texttimes}\\thinspace4.6. *U. rigida* is a green macroalgae with promising applications in food and feed industries (Biancarosa *et* al., 2018). However, its high fiber content may impair its inclusion in aquafeeds as fish lack the digestive enzymes able to hydrolyze it. Therefore, pre-treatment can be

Table I: Plasma metabolites (mg dl^{-1}) levels and muscle fatty acid profile (% total identified fatty acids) of European seabass at the end of the experiment.

	FM-based diet	Untreated *U. rigida*	Ultra-sound *U. rigida*	Solid state fermented *U. rigida*
Glucose	117.2 ± 39.3	126.7 ± 14.5	111.2 ± 31.7	118.3 ± 14.4
Cholesterol	118.1 ± 32.5^{ab}	159.3 ± 34.3^{c}	151.6 ± 50.4^{bc}	108.6 ± 40.1^{a}
Triacylglycerides	185.9 ± 73.4	179.7 ± 76.0	144.1 ± 73.2	157.2 ± 43.8
Total proteins (g dl^{-1})	4.27 ± 0.41^{a}	4.77 ± 0.50^{b}	4.40 ± 0.13^{ab}	4.73 ± 0.58^{ab}
Total SFA	25.9 ±0.43	25.3 ±0.93	24.6 ±0.99	26.2 ±0.27
Total MUFA	38.2 ±1.01	37.8 ±0.57	38.9 ±0.47	37.5 ±1.60
Total ω3	20.3 ±1.23	20.7 ±0.84	19.8 ±0.99	21.1 ±1.37
Total ω6	13.4 ±0.49	13.9 ±0.73	14.4 ±0.64	13.2 ±0.83
ω3/ω6 ratio	1.52 ±0.10	1.50 ±0.13	1.38 ±0.08	1.60 ±0.08

Means (± standard deviation) in the same row with different superscript letters are significantly different (Duncan test; $p<0.05$).

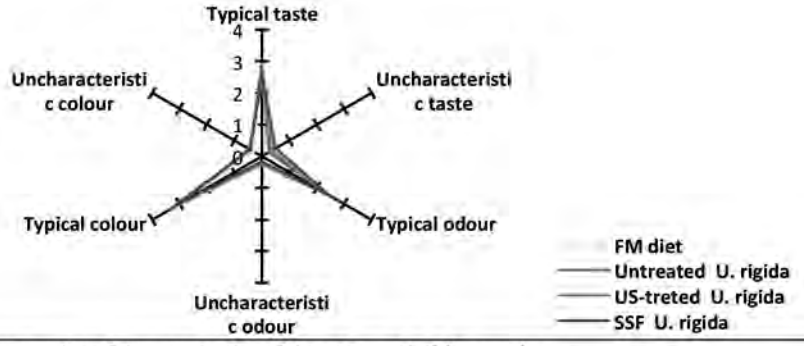

Fig. I: Sensory quality of European seabass fillets at the end of the experiment.

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a helpful strategy to disrupt the complex macroalgae cell-wall and promote the release of fermentable sugars and other valuable compounds and facilitating the access of digestive enzymes to the cell content (Fernandes *et al*., 2019a)and new applications for macroalgae should be considered. In this work, sequential biological treatments as solid-state fermentation (SSF. In a previous growth trial, it was observed that dietary replacement of fish meal by 5% untreated *U. rigida* reduced growth performance and feed efficiency of on-growing European seabass, while the dietary inclusion of *U. rigida* pre-treated with ultrasounds or solid-state fermentation with *Aspergillus ibericus* did not affect growth performance nor feed efficiency (Fernandes *et al*., 2019b). However, the influenc of these biological and physical pre-treatments of *U. rigida* on the nutritional and sensory quality of commercial-size European seabass was not yet evaluated.

Materials & Methods

For the biological pre-treatment, dry micronized *U. rigida* was fermented with *A. ibericus* (MUM 03.49) for 7 days at 25°C, in tray-type bioreactors; for the physical pre-treatment, *U. rigida* was submitted to ultra-sounds (US) during 1 hour in protective containers, using a high-intensity ultrasonic processor at 50-60 Hz. A control diet containing 25% fishmeal (FM) (D1); untreated and pre-treated *U. rigida* were incorporated in test diets replacing 5% (w/w) FM, as follows: D2: untreated *U. rigida*; D3: US-treated *U. rigida*; and D4: SSF-treated *U. rigida*. All diets were isoproteic (45%) and isolipidic (18%). Triplicate groups of European seabass with 108 g IBW were fed to apparent visual satiation for 64 days. At the end of the experimental period (FBW averaging 240 g), plasma metabolites and fillet fatty acids profile and sensory properties were assessed.

Results & Discussion

Plasma glucose levels were lower in fish fed ultra-sound treated *U. rigida* diet relatively to fish fed the untreated *U. rigida* diet. Dietary inclusion of *U. rigida* increased plasma cholesterol levels, which were restored to the control levels in fish fed the SSF *U. rigida* diet (Table I).

Even though fillet polyunsaturated fatty acids (PUFA) content was similar among groups, fish fed *U. rigida* diets presented 8 to 13% more α-linolenic than fish fed the FM-based diet. The ω3/ω6 ratio was 8% lower in fish fed ultra-sound *U. rigida* diet but 7% higher in fish fed solid state fermented *U. rigida* diet than with the untreated *U. rigida* diet.

Concerning the organoleptic properties of European seabass, no significant differences in typical odor, color, and taste between groups were found (Fig.1).

Conclusions

U. rigida pre-treatment by solid-state fermentation or ultra-sounds did not compromise fillet FA profile and the sensory quality of European seabass. Such results support the potential for inclusion of pre-treated *U. rigida* in sustainable diets for European sea bass.

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VITAMIN K IN FISH NUTRITION: AN INTEGRATIVE UPDATE OF FUNCTIONS AND REQUIREMENTS

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Introduction

Vitamin K (VK) is a fat-soluble molecule known to be essential for blood coagulation and bone metabolism by two different pathways: (i) the gamma-carboxylation of different VK dependent proteins (VKDPs) and (ii) the transcriptional control of several genes through the pregnane X receptor (PXR) signaling. Recently, new biological functions of VK have been suggested in mammals. In this regard, dietary VK levels regulates testosterone synthesis in testis, promoting male fertility (Karsenty and Ferron 2012). Furthermore, nutritional status of VK has been also associated with neural development and cognitive capacities (Ferland 2012).

In fish species, a relative limited impact of VK on fish physiology, with low nutritional requirements, were reported for blood coagulation and bone development/homeostasis (reviewed in Krossoy et al., 2011). This could be related with an extensive use of menadione (VK3; either as menadione sodium bisulphite or menadione nicotinamide bisulphite) as the nutritional source of VK in commercial diets. Interestingly, several reports evidenced that fish growth and development might depend on the source of VK-used.

Here, we will review the molecular basis of VK metabolism and the recent literature on this issue, showing how VK has a higher impact on fish hemostasis and skeletal system than previously considered. Furthermore, new reports suggest a broader role in another biological functions such as brain and visual organs development, as well as reproductive status.

Results and discussion

A specific nutritional requirement of VK during fish larval development for a better skeletal development, reducing the incidences of skeletal deformities when supplementing live prey enriching emulsions with phylloquinone (VK1), was first shown in 2014 by Richard and co-workers. In the same study, the initial evidence of VK having new roles was provided through a comparative proteomic approach, where proteins involved in muscular contraction, energy metabolism and/or osmorregulation were found differentially expressed in fish fed with VK1 supplemented diets. Afterwards, we characterized the effects of VK deficiency and expanded the biological roles where it might be involved through the localization and quantification of the genes involved in the VK recycling and the PXR signaling. Chemically induced VK deficiency ¡during fish larval development caused hemorrhages in brain, skeletal deformities and triggered ectopic calcifications (Fernández et al., 2014). The genes encoding the enzymes responsible for VK recycling during VKDPs carboxylation, the VK epoxide reductases (VKORS: VKORC1 and VKORC1L1), were found differentially expressed along larval development and in adult tissues through qPCR, suggesting a high requirement of VK during fish early development (embryonic and endotrophic larval period), but also at visual organs, brain and ovary in adult fish (Fernández et al., 2015). Through *in situ* hybridization procedures, PXR was found to be specificaly expressed in cells from the granular layer, which has been related with cognitive capacities; and at inner nuclear and nerve fiber layers, regions related with night blindness and other retinal disorders (Marques et al., 2017).

More recently, and following previous results, we explored how a dietary VK supplementation affected the reproductive status and the early development in fish species through high throughput transcriptomic analysis (RNASeq). VK1 supplemented diets increased testosterone levels and sperm quality (reducing sperm DNA fragmentation) in fish (Fernández et al., 2019). In addition, using an innovative approach, we were able to identify some small non-coding RNAs (sncRNAs) within blood plasma directly associated with nutritional and reproductive status, highlighting the potential use of these sncRNAs as an integrative and less invasive biomarkers in aquaculture (Fernández et al., 2019). In addition, through a chemically induced VK deficien y we have revealed its high impact on early fish development (embryonic and endotrophic period), inducing mortality, hemorrhages and retinal atrophy, and reducing skeletal development (Granadeiro et al., 2019). To gain insights into the underlying mechanisms a RNASeq analysis identified 766 differentially expressed (564 up- and

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202 down-regulated) genes related with particular cellular components (cytoplasm, extracellular matrix, lysosome and vacuole), biological processes (mainly amino acid and lipid metabolism and response to stimulus) and pathways (oxidative stress response and apoptosis signaling pathways). Last, but not the least, dietary VK level might have an additional effect on the intestinal microbiome (Fernández et al., unpublished).

Altogether, these research studies highlighted the need for a reformulation of the nutritional requirements of VK in commercial diets used in farmed fish species along the whole production cycle (from gametes to broodstock)

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SKELETOGENESIS OF TENCH (*Tinca tinca*) REARED IN EXTENSIVE AQUACULTURE: TOWARDS A HIGH QUALITY PRODUCTION STANDARDS

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Introduction

Tench (*Tinca tinca*) is a freshwater fish that belongs to the Cyprinidae family inhabiting stagnant waters with abundant vegetation of central and southern Europe. Although it has been cultured for centuries, there is a limited production in quantity (around 1400 t) and value (4.5 M USD) per year, mainly located at France, Check Republic, Germany and Poland (http://www.fao.org/fishery/e). In Southern countries, Spain (Extremadura and Castilla y León) and Italy (Piedmont), it has been traditionally reared with a preferred commercial size of 80-120 g with a high value (16-18 € kg^{-1}; Parisi et al., 2014).

Tench is considered a promising new species for aquaculture diversification since it can tolerate low levels of oxygen, a wide range of temperatures (10-34 ºC), vegetable sources on their diets and the regular manipulation during rearing procedures. The main bottlenecks are: (i) the use of Artemia nauplii during the early development in indoor systems; (ii) the climatic variability on extensive rearing systems with the slow growth and feeding stop under 10 ºC, requiring 2-4 years to reach the commercial size (120-500 g, respectively); (iii) the lack of specifically formulated commercial feeds; and (iv) the high rate of skeletal deformities (Parisi et al., 2014).

It is long known that skeletal deformities hinders production efficienc , decrease animal welfare and product value if deformed specimens reach the market (Boglione et al., 2013).

Although some research effort has been placed to determine the optimal nutritional composition of diets for tench (one of the main factors determining skeletal development in fish species), there is a knowledge gap on the sequential development of its skeleton and the required nutrients for its successful development. In this sense, describing the ontogeny of skeletal development (when and how skeletal structures are formed) will pave the future studies to optimize rearing conditions during early development in order to mitigate and/or avoid the appearance of bone deformities.

The aim of the present study was to implement an acid-free double staining protocol, providing a detailed description of the skeletogenic process during tench larval development as well as assessing the skeletal quality (types and incidence of skeletal deformities) of juveniles reared in traditional extensive systems.

Materials and methods

At a local producer facilities (Tencas Mateo), fish gametes were obtained by manual abdominal striping of tench females and males and an *in vitro* fertilization was performed. Embryos were maintained at 22 ºC for two days, and newly hatched larvae were released in a small natural pond. Sampling of tench larvae/juveniles was performed at 2, 5, 7, 30, 42, 65 and 85 dpf using a 60, 100, 200 or 1000 µm sieve according with fish size (10-15 fish per sampling point). Fish were euthanized with a lethal dose of tricaine methanesulfonate (MS-222, Sigma-Aldrich), its standard length (SL) measured, rinsed in distilled water, fixe in 4% buffered (pH 7.4) formaldehyde and stored at 4 ºC until the skeletal analysis. To evaluate the degree of mineralization of the skeleton, and to identify and quantify the incidence of skeletal deformities, animals were stained for bone and cartilage in whole mount preparations using an optimized acid-free staining protocol (Fernández et al., 2018). Skeletal structures were identified and named according to Cubagge and Mabee, 1996; Bird and Mabee, 2003). The development of skeletal structures was categorized as cartilaginous, on its onset of ossification and with and advanced ossification in order to mathematically represent the ossification process along larval developmen

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Results

Tench larvae in extensive systems shows an exponential growth in standard length although showing a large variability. The skeletal system started to be represented by a low developed cartilage structures at the fish skull, only being identified structures for breathing and preys capture at 4-9 mm SL. First structures to be ossified at 9-16 mm SL were cleithrum, maxilla, dentary and operculum. Posteriorly, while cranial structures underwent extensive ossification, caudal fin complex start to be formed through chondral ossification (from 14 mm SL onwards). Finally, axial skeleton was progressively formed through intramembranous ossification, being fully mineralized at 25 mm SL. Early juveniles (20-48 mm SL; 85 dpf) have a considerable incidence of skeletal deformities, mainly located at the end of the vertebral column (around 50%), although the first type of deformity clearly identified along the development was the absence of pectoral fins (at 14 mm SL).

Discussion and conclusions

The implementation of an acid-free staining protocol allowed us to perform an accurate description at which size each skeletal structure appeared and ossified since such procedure avoid the loss of calcium in incipient ossified structures, typical of acid cartilage staining procedures. Furthermore, the present detailed description of how the skeleton is formed will represent a basic knowledge to apply in regular monitoring procedures to compare how nutritional regimes, rearing conditions and systems might improve skeletal quality of produced tench.

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NARBONNE VETCH (*Vicia narbonensis*) AS AN ALTERNATIVE RAW MATERIAL TO SUBSTITUTE FISH MEAL IN RAINBOW TROUT (*Oncorhynchus mykis*) DIETS

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Introduction

The sustainable use of natural resources is a key issue to ensure food safety, nutrition and the sustainable development of aquaculture (FAO, 2018). Among other factors, fish nutrition is one of the most critical in fish farming, representing 40–70% of the production costs and determining survival, growth potential and quality of farmed fish. Nowadays, the future of aquaculture relies on the identification of suitable and alternative raw materials to substitute fishmeal (FM). Different sources and percentage of inclusion have been evaluated in order to develop Fish-Free Feeds (F3) and/or alternative diets (with important but not total FO and FM substitution). Although meal from insects and other invertebrates, algae, microorganisms, feather and/or byproducts from other agrifood industries are also currently considered, FM replacement is currently done with protein vegetable sources, considered as the best candidates. Soybean meal (mainly produced in USA, Argentina and Brazil) is still the most used alternative raw material to substitute FM, although it hampers fish physiology by unknown. In order to reduce the import of soybean meal, European aquaculture needs to explore, identify and promote local vegetable proteins sources in order to reduce its dependence of third countries.

Narbonne vetch *(Vicia narbonensis)* is a legume crop widely available and abundant in several regions of Southern European countries (France, Turkey and/or Spain), with seeds containing 20-30% protein and a partially balanced amino acid profile. Although a low inclusion level has been already implemented in diets for pork and broilers, little is known about their effects on aquatic animals. Thus, the present study aimed at evaluating the effects on growth, digestive system and nutritional value of increasing levels of inclusion of Narbonne vetch in diets for rainbow trout (*Oncorhynchus mykis*).

Materials and methods

After acclimatation, 25 fish (26.8 ± 0.7 g and 13.4 ± 0.1 cm) were randomly allocated into 9 cylindrical fibe glass tanks (500 L) connected to a recirculating aquaculture system. Water conditions were 15 ± 1 °C, > 7 ± 1 mg/L of dissolved oxygen, and 12:12 h light:dark cycle photoperiod.

Fish were hand-fed to apparent satiation once a day (until a maximum of 3% daily feed intake) for 63 days with experimental diets containing graded levels of Narbonne vetch (*Vicia narbonnensis*). The presence of anti-nutritional factors (ANFs) in Narbonne vetch was previously determined *in vitro* through the assessment of rainbow trout alkaline proteases inhibition (Alarcón et al., 1999). An analysis of essential amino acids was also performed to identify potential deficiencies was also done. Finally, three isoproteic (44%) and isolipidic (18%) diets with increasing levels of inclusion of Narbonne vetch (*Vicia narbonnensis*; 0 (Control), 10 (A10) and 30% (A30)) were formulated.

Growth performance (weight and length) was evaluated at 21, 42 and 63 days, being fish previously fasted during 24 h. Fish were anesthetized with MS-222 (180 mg/mL), SL was assessed using a graduated ictiometer (± 0.1 mm) and wet weight with a GRAM S3R-6KD balance (± 0.1 g). Apparent digestibility analysis was carried out during the last two weeks of the growth trial. At the end of the experiment 3 fish from each tank were randomly sampled and sacrificed with an overdose of MS222 in order to: i) biochemically assess the blood plasma composition (glucose, cholesterol and triglycerides) as well as the aspartate aminotransferase and alanine aminotransferase activities (Bio-Science Medical S.L.) using a microplate reader (ELx800TM; BioTek Instruments); ii) histological characterization of key tissues (liver, proximal and distal intestine) by HE and alcian blue staining as described by Bancroft and Stevens (1990); and iii) determine the fatty acid composition of fillet samples through HPLC analysis

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Results

Narbonne vetch has a low content of methionine, isoleucine, leucine, phenylalanine, histidine and lysine as alternative raw material to substitute FM. Furthermore, the presence of ANFs has been evidenced by a progressive inhibition of alkaline proteases with the increase of inclusion levels (with a 28% and 46% inhibition in A10 and A30 diets, respectively). In agreement with these results, a 30% inclusion of Narbonne vetch in rainbow trout diets reduced the final fish growth, SGR and FCR; while a 10% inclusion only reduced the FCR. Fish from A30 group had a higher hepatosomatic index, reflecting a histological disorder on their hepatocytes. Among the parameters evaluated regarding the histological organization of the intestine (villi folds height, density of goblet cells, height and nucleus position of enterocytes, etc.), some slight effects were also observed when increasing the levels of Narbonne vetch in fish diets. Fatty acids composition of the muscle was also affected, particularly the content on Docosahexaenoic acid (DHA), but only in fish fed A30 diet.

Conclusions

Present results showed as 30% Narbonne vetch inclusion in fish diets affected rainbow trout growth and physiology. Similar results were previously reported in another freshwater fish species, the tilapia (*Oreochromis niloticus*), were inclusion of > 20% provided lower growth (Buyukcapar et al., 2010). The present poor growth in fish fed high inclusions of Narbonne vetch seemed to be due to the presence of ANFs that had an impact on the digestive system. Future studies will explore the inclusion of another locally produced legumes and/or different varieties of Narbonne vetch as potential alternatives to soybean meal in fish feeds

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GENE EXPRESSION ANALYSIS AS AN INDICATOR OF EGG QUALITY IN ATLANTIC COD *Gadus morhua*

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Introduction

Atlantic cod (*Gadus morhua*) is a key fish in the marine trophism of the Northern Hemisphere and an emerging species in aquaculture. Cod is characterized for being iteroparous with synchronous oocyte development and spawning up to 19 batches over several weeks (February-May) (Kleppe et al., 2014). Viability of eggs and embryos is unpredictable and mortality as well as malformations in early-life stages are high. Currently, egg quality and survival is determined by fertilization rates and cleavage patterns. Although those measures provide reasonable indication of egg quality, knowledge of the molecular basis of embryonic development would improve current practices of egg quality determination. Gametic transcripts are essential during early developmental stages (Lawrence et al., 2006) since zygotic gene transcription is not activated until several cell divisions had been completed (Skjærven et al. 2011). Even though the Atlantic cod genome is now sequenced and more transcripts are available (Dale et al., 2019) a limited number of studies have analysed its early transcriptome. This study opens up the possibility of finding links between six stage specific genes and egg quali .

Materials and methods

In order to develop molecular criteria to assess egg quality in Atlantic cod we stripped gametes from 12 parental pairs and obtained 4-7 egg batches from them during the 2018 spawning season. Eggs and sperm were stripped every 48-72h, fertilized and incubated until full batch hatching. Samples were taken at six embryonic developmental stages during the incubation period (unfertilized egg, zygote, blastula, gastrula, early somatogenesis and late somatogenesis) as well as newly hatched larvae for further molecular analyses. Conventional egg quality determinants such as fertilization rate, normal cleavage pattern and egg mortality were also recorded.

Seven out of thirty-three genes were selected upon their effici ncy values for gene expression analysis to delve into the temporal transcriptome activity during cod embryogenesis and twelve housekeeping genes were included to normalise mRNA levels between samples.

Results and discussion

Standard egg quality indicators have revealed significant egg quality variation throughout the spawning season within female, being significantly higher in the middle of the period. Besides, egg quality seems to differ among females, what is an additional source of variation to be explained in this study using gene expression analyses. According to the efficiency values of the tested genes they seem to be adequate to explore early gene expression in cod.

Gene expression comparison among females and among spawns could allow the development of egg quality-associated markers for cod aquaculture improvement.

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THE EFFECT OF PRICE AND PRODUCTION CHANGES ON FIRMS' PROFITABILITY AND RISK: A SIMULATION WITH EUROPEAN PRODUCERS OF CULTURED SEA BASS AND SEA BREAM

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Introduction

European sea bass (*dicentrarchus labrax*) and gilthead sea bream (*sparus aurata*) are both an economically important cultured fish species along the Mediterranean coast. Both species represent respectively 10.9% and 13.5% of the total value of the European aquaculture sector. The EU is one of the largest producers of sea bass and sea bream in the world, being Greece the largest producer within the EU followed by Spain. However, the Turkish sea bass and sea bream industry has been steadily increasing production volumes for the last decade to the point where Turkey is now the world's major producer of seabass, competing with European producers with lower prices (Globefish, 2015). As consequence, during this period of time, European firms have been struggling to maintain profitability of their farm

The main aim of this work, which is part of the MedAID project funded by the European Commission (H2020, GA727315), is to carry out a sensitivity analysis to assess the impact of changes in output quantities and prices on firms profitability and operating risk of European producers of cultured sea bass and sea bream in the Mediterranean Sea using the classical cost-volume-profit (CVP) model. This model is an important and well-known managerial tool that attempts to specify a firm s cost and revenue functions, allowing to business owners and managers to perform break-even analyses as well as to predict profit behavior in different conditions in order to evaluate business viability (Ingram, Albright, & Hill, 2003; Weygandt, Kieso, & Kimmel, 1999).

Methodology

To perform the sensitivity analysis, the CVP model employed in our study is as follows:

$$TR = pq$$

$$TC = c_0 + c_1 q$$

where TR = Total operating revenues; TC = Total operating costs; p = Sales price per unit; q = Number (quantity) of units to be manufactured and sold during the period; c_0 = Constant parameter of the total cost function (total fixed expenses for the period), being $c_0 >= 0$; and c_1 = Slope parameter of the total cost function (unit variable cost), i.e. variable expenses to manufacture and sell a single unit of product, being $c_1 >= 0$.

The *margin of safety* (MOS)**,** which is the difference between the actual sales and the break-even sales of a firm, is an important figure for any business because it tells management how much reduction in revenue will result in break-even serving as a measure of operating risk (Ingram, Albright, & Hill, 2003; Weygandt, Kieso, & Kimmel, 1999). The margin of safety can be also expressed in the form of ratio, which is calculated by using the following formula:

$$\text{MOS ratio} = 100 \times (\textit{Actual sales} - \textit{Break-even sales}) / \textit{Actual sales}$$

Thus, the larger is the ratio the lesser is the risk in reaching the breakeven point and the risk of business to have losses.

Table I. Representativeness of the sample used in this research

Country	Number of firms			Number of employees		
	Population	*Sample*	*%*	*Population*	*Sample*	*%*
Croatia	5	4	80.0	374	327	87.4
Cyprus	4	2	50.0	171	149	87.1
France	7	2	28.6	191	50	26.2
Greece	36	7	19.4	3,971	3,025	76.2
Italy	18	10	55.6	339	232	68.4
Slovenia	2	1	50.0	11	8	72.7
Spain	28	4	14.3	951	212	66.6
All countries	**100**	**30**	**30.0**	**6,008**	**4,003**	**66.6**

Source. Authors' elaboration using AMADEUS database with data of the year 2014.

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Economic variable	Variation (%)	Proportion of firms with losses (%)	Average operating profit (th. €)	Average ROA (%)	Average variation in MOS ratio (% units)
Price (p)	***+10***	10.0	2,737.6	9.79	17.23
	+5	26.7	1,644.2	5.75	10.58
	+1	30.0	769.5	2.53	2.63
	0	40.0	550.8	1.72	0
	–1	43.3	332.1	0.91	–3.03
	–5	70.0	–542.6	–2.31	–16.70
	–10	86.7	–1,636.0	–6.34	–613.93
Production (q)	***+10***	30.0	832.2	2.73	4.88
	+5	30.0	691.5	2.23	2.56
	+1	36.7	579.0	1.82	0.53
	0	40.0	550.8	1.72	0
	–1	40.0	522.7	1.62	–0.54
	–5	43.3	410.2	1.22	–2.82
	–10	50.0	269.5	0.71	–5.96

A representative sample of the 30 main firms producing cultured sea bass and sea bream in the Mediterranean Sea from 7 European countries was employed in our analysis (see Table I). The period of time analyzed ranges from 2005 to 2014 (10 years). The *TR* curve was estimated with a linear function by ordinary least squares (OLS). The *TC* curve was estimated through nonlinear regression with parameter constraints using linear, quadratic, and cubic functions although, finall , we have employed in the results section a simple linear function because the fit obtained with it was the best. The variables used to estimate the two curves for each firm were *TR* = yearly total operating revenues of the firm (in thousands of euros) obtained from the AMADEUS database, *TC* = yearly total operating costs of the firm (in thousands of euros) obtained from the former database, and *q* = yearly sales of the firm (in tons), which was estimated using the yearly total operating revenues of every firm divided by the yearly average of sea bass and sea bream ex-farm prices in the firm s country. In this case we hypothesized that the firm is producing and selling in the same proportion both species since we cannot observe the firms actual level of production and sales. The yearly sea bass and sea bream prices in each country were obtained from the EUMOFA database.

Results

In Table II we present the results of the sensitive analysis where we have simulated how different changes in economic conditions (variation of market prices) or decisions (variations in production levels) have affected on the profitability (operating profit) and risk of the sampled firms. We have increased and decreased those factors in different proportions (1%, 5%, and 10%) to estimate the effects. The largest positive and negative effect on firms operating profits has been obtained with the price variable (*p*) although this variable is not over firms control (competitive market). On the other hand, changes in the level of production (*q*) had the lowest effect on firms profits. Likewise, similar results have been obtained with the firms operating risk (average variation in the MOS ratio) although the negative effect of a reduction in price is stronger than the effect obtained with the opposite variation.

Conclusion

Regarding the sensitive analysis that we have performed to analyse the effect of price and production changes on firms profitability and operating risk with European producers of cultured sea bass and sea bream, we can conclude that the changes in market prices have had the largest effect (positive or negative) on firms operating profits and risk, whereas changes in production levels have had the lowest effect on those variables.

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ASSESSMENT OF FEED DOSES TO BE ADMINISTERED TO *Garra rufa* (HECKEL, 1843) UNDER AN ICHTHYOTHERAPY REGIMEN

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Introduction

Garra rufa (Heckel, 1843) is the only recommended fish species to be used in ichthyotherapy, an activity in which the fish are used to clean the skin, wounds or treat other skin conditions. Notwithstanding, this activity raises several questions regarding fish welfare. One of the main constraints regards the fact that some fish keepers are accused of starving the animals, as to motivate them to interact with humans and feed on their dead skin cells. In order for the fish to be interested in removing cells from the corneal layer of the epidermis, it will be necessary to provide them with good quality feed, in adequate quantities, as to avoid a decline in the animals' health and physical condition.

An experimental trial was conducted to determine the quantity of food that could be adequate to establish a commitment between fostering the interest of *G. rufa* in interacting with humans and avoid compromising the fish welfare. Accordingly, fish submitted to a daily chthyotherapy session were given four different food doses. At the end of 3 weeks, *G. rufa* were evaluated in what regards: survival; growth in terms of total length and individual weight, specific growth rate (SGR), absolute growth rate (AGR), feed conversion rate (FCR) and body condition index (K), plus the fish nibbling interest throughout the experimental trial.

Materials and Methods

During 3 weeks, *G. rufa* were subjected to a single ichthyotherapy session of 30min.day^{-1} with a different human subject. The fish were maintained in 2 recirculating aquatic systems (RAS), from which were used 15 aquaria of 22L, with 9 individuals each. Five sets of 3 aquaria, randomly distributed in the RAS, were assigned with a different quantity of a commercial granulated feed (energy content of 4230Kcal.Kg^{-1}), given at 05:00PM of each day. The food supplied to each treatment corresponded to 25% (dose 1), 50% (dose 2), 75% (dose3) and 100% (dose 4) of the daily dose, corresponding to an *ad libitum* circumstance (≈0.065g.fis $^{-1}$). The fifth set of aquaria portrayed a reference situation (RS), in which *G. rufa* received 100% of the daily dose and were not subjected to ichthyotherapy procedures. On weekends, all the fish were given 100% of the daily dose of food and were left quiet in the RAS to simulate the conditions of a commercial establishment practicing ichthyotherapy.

The fish were transferred to 12L aquaria 30min before the ichthyotherapy sessions. The aquaria contained only tap water, left to warm by means of water heaters with the thermostat set for 28ºC and strong aeration to eliminate chlorine disinfectant residues, since the previous day. People's hands were inspected for the absence of active lesions, carefully washed, disinfected with alcohol and passed thoroughly through clean water, for the health safety of both humans and fish. During these sessions, the number of times that the fish came in contact with humans, nibbling the hands of each volunteer.

At the beginning and at the end of the experimental trial, all *G. rufa* were measured for total length with an ichthyometer and weighed on an analytical balance.

Results

By the end of the trial, only 2 fish died because they tried to escape the RAS and not in consequence of the ichthyotherapy procedures. All *G. rufa* grew in terms of total length, without significant differences between treatments (Kruskal-Wallis test H_4=6.18, p>0.05), but those who were given dose 1 gain more weight than those from dose 4 (One-way ANOVA $F_{4,126}$=3.35, p<0.05, Bonferroni test p<0.05). *G. rufa*'s behaviour evolved with time. The number of times that fish invested in exploring hands increased from 8.15±6.66 to 21.85±11.15incursions.fis $^{-1}$ from the 1st to the 3rd week of ichthyotherapy. Although, the most active fish were always those who were given less food. Accordingly, fish fed with dose 3 invested less time in nibbling hands (12.01±2.31incursions.fis $^{-1}$) and those from dose 1 were the most interactive (32.60±2.40incursions. fis $^{-1}$) in the last week of the trial.

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The performance parameters SGR and AGR showed that *G. rufa* fed with dose 1 showed values almost as good as to those from the reference situation (RS), while those from dose 4 showed the worst results. Regarding DGI, the values obtained did not differ between the fish treatments (H_4=8.85, p>0.05), while *G. rufa* from dose 4 presented a significantly lower K than those from RS ($F_{4,126}$ =2.87, p<0.05, Bonferroni test p<0.05).

Discussion

The present work was able to perform daily sessions of ichthyotherapy without registering mortality of *G. rufa* fish. For reasons of hygiene, fish were transferred daily between their RAS and the treatment aquaria, disinfected and with new water. Although this procedure induced stress for the animals, *G. rufa* shown to be a resilient fish. Their behaviour evolved during the experimental trial, evidencing a learning capacity related to daily routine procedures. This was manifested by the increased frequency of contact between fish and humans over the course of three weeks, regardless of the amount of feed given to them. However, the most interactive fish were always those to whom less food was given. All fish grew under the experimental conditions, in terms of total length, but not in terms of individual weight. Fish submitted to daily ichthyotherapy sessions and fed with the whole daily dose showed more reluctance to nibble human skin and decreased in body weight. This result reflected as a decrease in the body condition index, probably as a consequence of the daily stress to which they were subjected. Although the skin of vertebrates in aquatic habitats (including humans and other fish) reveals itself as an alternative food source, easier to obtain (Sayili et al., 2007), epidermal cells appear not to be part of the typical diet of *G. rufa* (Yalçin-Özdilek and Ekmekçi, 2006). Therefore, the welfare of the fish is put into question when adopting this type of procedure. Without an adequate diet, fish growth can be delayed and may even cause the animals' death (Wildgoose, 2012). For this reason, it is recommended that a good quality commercial feed be supplied to satisfy the fish nutritional requirements. The amount of feed recommended as a daily dose will be between the 50% and 75% of the dose *ad libitum*, which corresponded to an energy content among 0.14 and 0.21Kcal.fis $^{-1}$. In this way, a compromise can be found between the interest of *G. rufa* to interact with humans and ensure the fish welfare

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ASEXUAL REPRODUCTION OF SEA ANEMONES FOR ORNAMENTAL PURPOSES

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Introduction

Sea anemones *Actinia equina* (Linnaeus, 1758) and *Actinia fragacea* Tugwell, 1856 have relevant characteristics to be exploited as ornamental species or as potential organisms for the extraction of bioactive compounds. There are currently some successful records of asexual propagation of anemones in captivity. Generally, this propagation is done by longitudinal cutting healthy specimens in several portions (Olivotto et al., 2011; Scott et al., 2014). However, there is a limited number of studies with sufficient details on the optimization of the process and it has been reported only for a few species

The main objective of this work was to evaluate the viability of propagating sea anemones *A. equina* and *A. fragacea*, by longitudinal fission of the animals in two halves. It was evaluated the survival rate of these anemones, the time it took them to regenerate and evaluate the growth of the new sectioned anemones.

Materials and Methods

The anemones were collected manually at Praia do Portinho da Areia Norte (in the middle Atlantic coast of Portugal), transported to laboratory and acclimated in a recirculation aquatic system (RAS) for 1.5 months. Sixty anemones of each species were selected, based on their similar size. The animals were left to fix on a small identified pebble, with a known weight, and kept at 10ind.aquarium^{-1}. The *A. equina* anemones were distributed by 6 random 18L aquaria in the RAS, while *A. fragacea* anemones were inserted in other 6 aquaria of the same RAS. For the trial, all anemones were forced to contract at their maximum capacity, in order to minimize variations of water content within the gastrovascular cavity and body shape. Then, each individual was gently dried with absorbent paper, to remove excessive water, weighed on an analytical balance and measured for height and diameter. In 3 aquaria of each species, the animals were kept intact (control), while in the other 3 aquaria, the individuals were cut longitudinally in half (treatment). Afterwards, all the anemones were monitored for 20 weeks, regarding their survival, size (height of the body column and diameter of the pedal disc), biomass and regeneration of the sectioned individuals (how long it took them to join the ends of the cut, protecting the gastrovascular cavity; the number of individuals who did fully regenerated; and the time it took them to achieve it, with the disappearance of the scar).

Results

None of the anemones died during the acclimation or the experimental periods. Although anemones *A. fragacea* were initially larger than *A. equina* (in terms of biomass, diameter and height), both species showed similar behaviour throughout the experimental trial. At the end of 20 weeks, all the anemones lost biomass and became smaller in height and diameter, independently of the species and of being cut in half or not.

In what concerns the severed anemones, *A. equina* were faster to curl and seal the cut zone than the anemones *A. fragacea* (41.75±2.45 versus 54.5±2.23days; Mann-Whitney test U_{118}=4385.00, $p<0.001$), re-establishing their shape and the gastrovascular cavity. Almost all *A. equina* were able to fully regenerate (90%), whereas only 71% of *A. fragacea* were able to eliminate their scar during the experimental period (Pearson's chi-square test χ^2_1=5.79, $p<0.05$). Moreover, *A. equina* also took lesser time to achieve this accomplishment than *A. fragacea* (Mann-Whitney test U_{95}=2580.00, $p<0.001$; 98.89±1.01 days *versus* 105.00±0.00 days). Indeed, the first *A. equina* to fully regenerate was observed at day 75, whereas the first individual *A. fragacea* without a scar was detected at day 90.

Discussion and conclusion

The methodology of dividing anemones longitudinally in half has proven to be effective as an asexual propagation method, both for *A. equina* and *A. fragaceae*. It employs procedures of low technological exigency and, consequently, implies low economic costs. The anemones *A. equina* and *A. fragacea* were able to regenerate without any mortality. Despite the damage inflicte on the anemones, they must not have suffered pain due to the lack of a developed nervous system. They

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have only clusters of sensory neurons, as essential structures of a diffuse network, without a central information processing organ (Hickman et al., 2014; Castro & Huber, 2016). However, the fact that all specimens decreased in size, during the experimental procedure, evidenced the need to investigate in the future: 1) the best environmental conditions to be supplied to these organisms in captivity; and 2) what is the best diet, which will allow them to regenerate and grow as fast as possible.

As the sea anemones *A. equina* and *A. fragacea* gather potential as ornamental species and scientific research subjects, the asexual propagation process will help to reduce the impact on their native populations and ecosystems. The process of obtaining two organisms from a single one is feasible. Thus, the need to capture natural specimens of anemones, for ornamental, scientific research or biotechnological application purposes, would be reduced. This process can be carried out in captivity, at a desirable frequency, to obtain an intended number of individuals with similar genotypes, all year long, without the need of collecting wild specimens.

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THE ABC MODEL—A PARADIGM SHIFT IN INTEGRATED CARRYING CAPACITY MODELLING

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Introduction and Modelling Approach

Carrying capacity for aquaculture has been defined in different ways, and can be considered to be the lowest common denominator of physical space, production capacity, ecological sustainability, and social aspects (McKindsey et al, 2006; Ferreira et al., 2013). The latter includes factors such as social license and governance, which are not amenable to mathematical modelling.

The ABC (Aquaculture, Biosecurity, and Carrying Capacity) model focuses on four key pillars of sustainability: physics, husbandry, environment, and pathogens.

Previous work on ABC has described innovations with respect to combining production and pathogen modelling, through a combination of Individual-Based Models (IBM) that simulate physiological processes in a quasi-deterministic manner, and a state-of-the-art stochastic approach to host-pathogen interactions. One of the key features of this model is the ability to simulate disease events triggered by accumulation of pathogen in the host through uptake from the environment (i.e. from the surrounding water), rather than through contact between animals.

The disease component can only be used stochastically to simulate scenarios and does not provide predictive modelling on the timing of disease outbreaks. Nevertheless, risk assessment, which is key for licensing, insurance, and investment decisions can benefit significantly from the application of such combined models. The model has been used to examine the effects of climate change on the onset of disease, production, and mortality, but also to look at the effect of stocking density on water quality, by means of indicators such as oxygen depletion and build-up of ammonia in farm areas.

Results and Discussion

Fig. 2 shows the results of an ABC model run for gilthead bream (*Sparus aurata*), considering two different mixing conditions for a farm with five culture areas 100 m apart. The upper pane shows results for dissolved oxygen and ammonia with high lateral dispersion, whereas the lower pane considers a situation where the effect of turbulence is minimal.

The troughs (oxygen) and peaks (ammonia) are shown in Fig. 2, and illustrate how site selection can significantly affect water quality. At present, the onset of disease events in ABC is determined only by water temperature, and not by e.g. low oxygen, but as better data become available on the triggers for pathogen outbreaks, ABC can be used to analyse interactions between environmental degradation and disease.

The effect of pathogens on aquaculture production is shown in Fig. 3, using as a host-pathogen pair Atlantic salmon (*Salmo salar*) and Infectious Hematopoietic Necrosis virus (IHNv). Under the appropriate temperature conditions, and provided the virus concentration in the water is above a certain threshold, fish become infected and can subsequently either recover or die. The biological FCR (Fig. 3) is acceptable for an early stage pathogen outbreak (top panel), but untenable for a late-stage outbreak (bottom panel) because of the amount of money already spent by the farmer on feed.

The IBM component of the ABC model means that every individual animal (finfis or shellfish) in a simulated population can be traced. This is used in ABC to simulate traceability of a fish crop, using the principles of blockchain analysis (and other DLTs) to illustrate how the trajectory of an individual animal can be described in terms of growth rate, welfare with respect to environmental conditions, and exposure to pathogens during its culture cycle.

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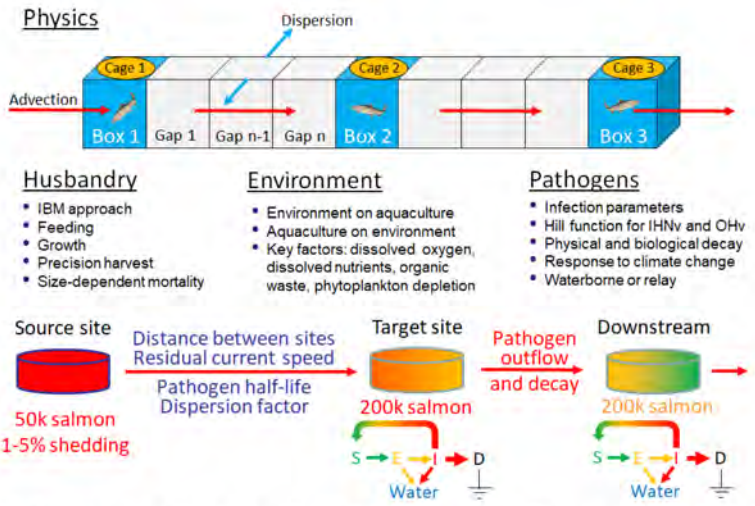

Fig. 1 – Diagram of the four pillars of the Aquaculture, Biosecurity, and Carrying Capacity (ABC) model, with details of the individual components of each.

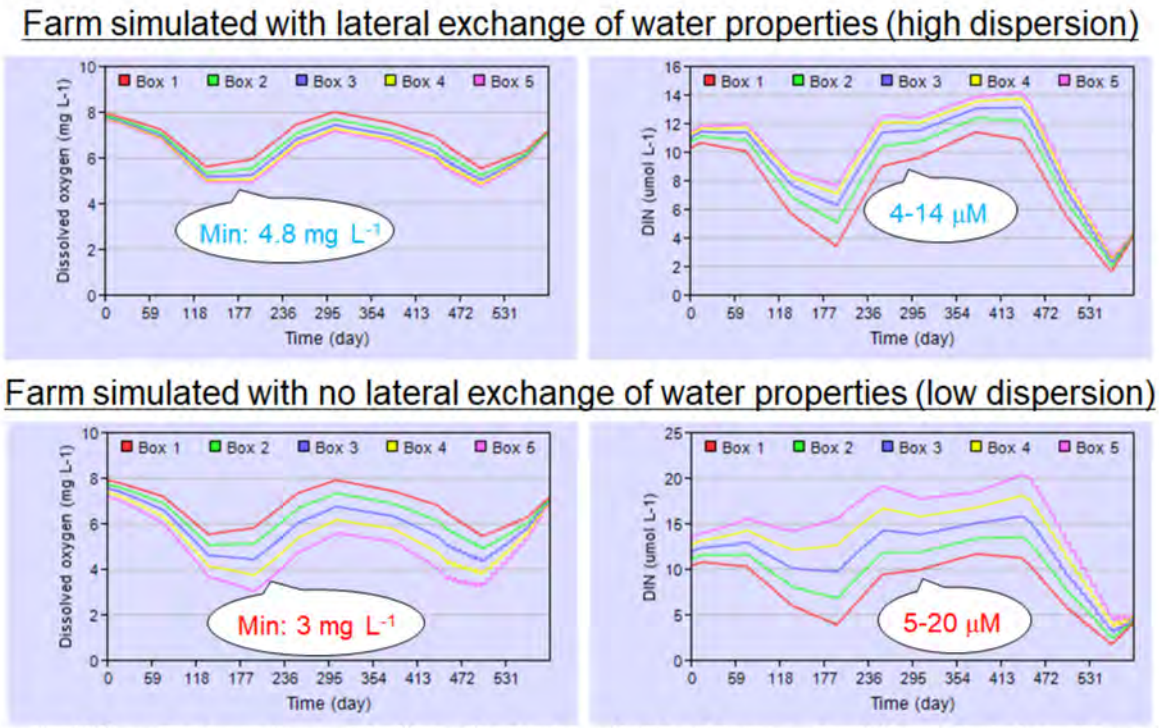

Fig. 2 –ABC results for environmental effects of cultivation of gilthead bream under different physical conditions. The graphs show cages (boxes) from upstream (Box 1) to downstream (Box 5) for a net residual current flowing from Box 1 to Box 5.

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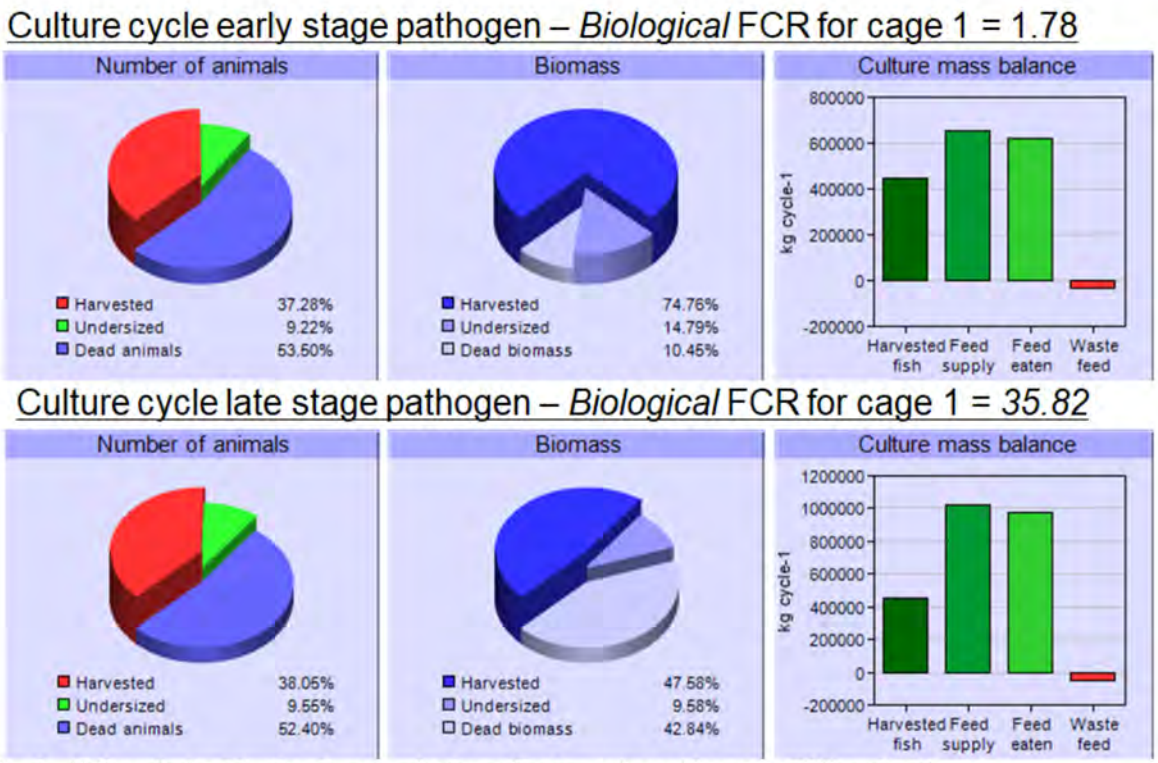

Fig. 3 –ABC results for Atlantic salmon production subject to early- and late-stage IHNv outbreaks.

EFFECTS OF VEGETABLE DIETS ON THE GROWTH, GAMETOGENIC AND OFFSPRING DEVELOPMENT OF THE SEA URCHIN *Paracentrotus lividus* (LAMARCK, 1816)

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Introduction

The increasing demand for sea urchin roe and consequent rising prices have resulted in a worldwide escalation of sea urchin fisheries, which are causing a depletion of the wild stocks. Therefore, a change is needed to preserve this resource, which may be achieved through the development of sustainable echinoculture. Moreover, this activity can also contribute to improve the roe nutritional content and quality, supplying this product all year long. For these reasons, the European attention has focused on the edible sea urchin *Paracentrotus lividus* (Lamarck, 1816).

The present study intended to evaluate the growth, gametogenic and offspring development of *P. lividus* fed with three artificial diets, formulated with vegetables. The determination of sea urchins' daily feed intake was made for each diet and their performance was estimated through the individuals' somatic growth (daily growth rate, weight gain and linear growth rate), gonadosomatic index (GI) and gametogenic development stage (GDS) of their gonads. Moreover, the effect of broodstock feeding was evaluated in their offspring (concretely, in what concerned the time of occurrence and duration of the embryonic and larval development stages).

Materials and Methods

Paracentrotus lividus (N = 234) were harvested in the intertidal zone of Praia de São Marcos (Portugal; 39º19'08.9"N, 009º21'18.9"W), with a test diameter of 3.72±0.03cm and a total weight of 22.23±0.31g.

The sea urchins were randomly distributed by three recirculating aquaculture systems (RAS) and fasted for 44 days at 16°C, with a photoperiod of 12h, being measured and weighed afterwards. Then, 18 individuals were sacrificed, had their gonads weighed for determining the GI (Carboni et al., 2015) and further analysed histologically for their GDS, according to Byrne (1990).

Afterwards, the remaining sea urchins were individually hand fed *ad libitum* for 120 days, at 20°C, with a known amount of the formulated diets, using agar as a biding agent: diet A with maize (*Zea mays*) and spinach *(Spinacia oleracea)*; diet B with maize, spinach and acorn (*Quercus ilex*) and diet C with maize, spinach and pumpkin (*Cucurbita maxima*). At the beginning of each day, the feed remaining on the tanks was weighted to measure ingestion rates. At each 30 days, 9 random individuals of each diet were measured, weighted and then dissected for assessing the GI and the GDS. At the end of the trial, all the remaining specimens underwent the same procedures. Furthermore, 12 random individuals per diet were induce to release gametes at the end of the 3rd and 4th months of the trial, by injecting 40µL.g^{-1} of potassium chloride 0.5M through the peristomial membrane. Each batch of resulting embryos were distributed by 3 cylindroconical tanks of 50L, with an initial density of 2larvae.mL^{-1} and fed using a mix of frozen microalgae *Tetraselmis suecica* and *Phaeodactylum tricornutum*. Samples of 15mL were collected in triplicate, in order to assess the embryos density and development, at 12, 24, 36, 60 and 84 hours after fertilisation.

Results

Diet B was the least consumed diet (0.75±0.70g.ind^{-1}.day^{-1}), followed by diet A (2.44±1.21g.ind^{-1}.day^{-1}) and then diet C (3.40±1.28g.ind^{-1}.day^{-1}).

In what concerns test diameter, sea urchins grew on average 0.88mm.month^{-1} with diet A and 0.52mm.month^{-1} with diet C, while for diet B the individuals decreased 0.28mm.month^{-1}. The same pattern was observed for individual wet weight, in which sea urchins gained 41.60mg.ind^{-1}.day^{-1} with diet A, 11.90mg.ind^{-1}.day^{-1} with diet C and those from diet B lost 45.56 mg.ind^{-1}.day^{-1}.

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At the end of the starvation period, the GI was 2.48±1.12%, plus 72% of *P. lividus* were at the spent stage and 28% were in the recovery stage. In the end of the trial, the highest GI was attained by individuals fed with diet A (8.71±2.18%), followed by diet C (4.44±2.20%), whereas those fed with diet B showed a GI below the value obtained from the starvation period. Diet A led to a relative frequency of 14% of sea urchins in the mature stage, 36% in premature stage, 11% in growing stage, 25% in recovery stage and 14% in spent stage. Meanwhile, diet C resulted in a relative frequency of 28% of the specimens in mature stage, 17% in premature stage, 14% in growing stage, 25% in recovery stage and 17% in spent stage.

Individuals fed with diet B did not made any progress in the gametogenic cycle, with 100% individuals in the spent stage reason for which it was not possible to induced them to reproduce and obtain gametes. In general, larvae from broodstock fed with diet A reached advanced stages faster than those from broodstock fed with diet C and showed a better survival response, as well.

Discussion and conclusion

Maize, spinach and agar (diet A) was the most successful feed, leading to higher values of test diameter, individual wet weight and GI, followed by diet C (maize, spinach, pumpkin and agar). Both diets A and C promoted more advanced gametogenic stages of the gonads. Diet A also allowed obtaining better survival and faster embryonic/larval development of the sea urchins offspring than diet C.

Although, *Q. ilex* acorn seemed a promising ingredient, due to its nutritional composition (namely, its fatty acid profile), the presence of tannic acid eliminated the possibility of success of diet B. In the future, this substance should be eliminated, before considering using acorn as an ingredient in formulated feeds for aquatic organisms.

The results obtained in this study showed that the use of land vegetables and their by-products can improve gonadal growth of *P. lividus*, proving to be efficient food resources to obtain sea urchin roe. Accordingly, jellified diets may improve adult sea urchins rearing, as well as aiding in the development of sea urchin larvae, due to the improvement of necessary energy reserves present in the oocytes.

Acknowledgements

This project has the financial support of Operational Programme MAR2020, through the project 16-02-01-FMP-0004: Ouriceira Aqua: Aquaculture and Enhancement of Gonad Production in the Sea Urchin (*Paracentrotus lividus*). Also, this study had the support of Fundação para a Ciência e Tecnologia (FCT), through the strategic project UID/MAR/04292/2019 granted to MARE - Marine and Environmental Sciences Centre.

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BIO-FORTIFICATION OF GILTHEAD SEABREAM (*Sparus aurata*) WITH DIETS CONTAINING ALGAE AND SELENISED YEAST

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Introduction

The seafood industry seeks healthy and sustainable solutions to meet consumer trends that are increasingly moving towards healthy and natural eating habits. The replacement of the traditional fish meal and fish oil in aquafeeds with environmentally sustainable ingredients like algae has instigated interest due to their high nutritional value and richness in bioactive compounds. In this context, natural feed ingredients (macroalgae, microalgae and yeast biomass) were exploited as alternative to fish meal (FM) and fish oil (FO) sources, as well as vehicles for bio-fortification of fish in iodine, selenium and n-3 PUFAs.

Material and methods

Gilthead seabream (370g) were fed for 10 weeks at EPPO-IPMA facility (Olhão, Portugal) with four diets in triplicate tanks: a commercial-based diet (FMFO) and three experimental diets with a 33% replacement of fish meal by a microalgae biomass (*Chlorella, Tetraselmis* and *Schizochytrium*) (AB diets). A further replacement of 20% FO was tested in ABVO diet. All AB rich diets were supplemented with both *Laminaria* and Se-yeast: diets $ABVO_{\mathbf{I8+Se1}}$ and $ABFO_{\mathbf{I8+Se1}}$ provided equivalent levels of iodine (8mg.kg^{-1}) and selenium (1mg.kg^{-1}), whereas an extreme diet $ABFO_{\mathbf{I13+Se}1.3}$ was further enriched with I (13mg.kg^{-1}) and Se (1.3mg.kg^{-1}). All diets had similar EPA+DHA contents.

At the end of the trial, growth performance, whole body and muscle composition, nutrient retention and gain were evaluated. The expression of genes related to fatty acids, iodine and selenium metabolism were also evaluated in liver and muscle.

Results

Final body weight of fish fed the bio-fortified diets was similar to those fed the FMFO (622-625g) diet, with the exception of the $ABFO_{\mathbf{I13+Se}1.3}$ that resulted in a significantly lower weight (589.46g). This diet also showed a significantly lower protein efficien y ratio (PER) than all other diets. The specific growth rate (SGR) and feed conversion ratio (FCR) remained similar among dietary treatments. Lipid intake was significantly affected by the dietary treatments: $ABVO_{I8+Se1}$ = FMFO < $ABFO_{I8+Se1}$ < $ABFO_{I13+Se1.3}$ but did not affect whole-body lipid (including EPA+DHA), protein or I content. Whole-body content and gain of Se increased significantly with the inclusion of Se-yeast, reaching the highest values in fish fed $ABFO_{I13+Se1.3}$ diet. The DHA content in the fillet increased significantly in fish fed diets $ABFO_{I8+Se1}$ and $ABFO_{I13+Se1.3}$ compared to the FMFO diet, whilst the I content was highest in the $ABFO_{I13+Se1.3}$ diet. The Se fillet content increased with the inclusion of selenised yeast: $ABFO_{I13+Se1.3}$ > $ABFO_{I8+Se1}$ > $ABVO_{I8+Se1}$ > FMFO (figure 1)

Discussion and conclusion

The present results indicate that the concomitant replacement of 33% FM and 20% of FO by a blend of microalgae biomass (*Chlorella, Tetraselmis* and *Schizochytrium)* can be achieved without any significant impact on growth performance or whole-body composition in large-sized *S. aurata*. The Se content increased concomitantly with increasing dietary inclusion of Se-yeast. Fish were able to selectively retain DHA and I in muscle resulting in its fillet fortification in DHA, I and Se. However, the highest dietary levels of both Se and I resulted in growth impairment.

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In conclusion, the bio-fortificati n of gilthead seabream fillets in Se, I and DHA through dietary supplementation with Se-yeast and algae is possible but requires a cautious selection of ingredients at adequate level to avoid growth impairment.

Acknowledgements

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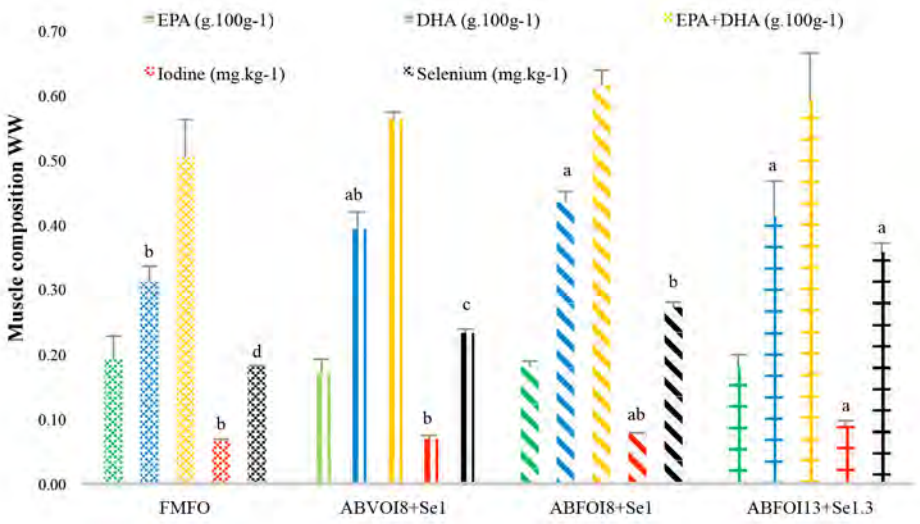

Fig. 1. Muscle EPA, DHA, iodine and selenium content of *S. aurata* fed the experimental diets for 10 weeks.

APPLICATION OF ENVIRONMENTAL SUSTAINABILITY INDICATORS AND INDICES IN TWO NILE TILAPIA (*Oreochromis niloticus*) NET-CAGE CULTURES

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Introduction

The theme about environmental sustainability is increasingly gaining the attention of the population, becoming a global concern. Environmental sustainability indicators can be used in the management of environmental externalities. Indicators provide values that simplify more complex phenomenon and summarize characteristics of a system in a simplified way. These indicators are combined into an index that can be used in the comparison of different systems or the same system over time (Valenti, 2008; Valenti et al., 2018). In this context, the present study aimed to provide information on the environmental sustainability of two tilapia net-cage production system in the state of São Paulo, Brazil, by using a set of environmental indicators.

Materials and methods

Samples of water, sediment, fish, feed provided and greenhouse gases were necessary to measure the environmental sustainability from each farm. These samples were collected three times throughout the production cycle of each property (189 days on farm A; 263 days on farm B), representing the beginning, middle, and end of cultivation. Environmental sustainability was measured using the indicators defined by Valenti et al. (2018). The environmental indicators were divided into five criteria: the use of natural resources; efficienc in the use of resources; release of pollutants and unused by-products to the environment; pollutants accumulated on the bottom of the water body; and conservation of genetic diversity and biodiversity.

Each indicator was converted into a performance scale, with scores ranging from 0 to 100. The treatment with the best indicator value (more sustainable when compared to the others) was arbitrary scored as 100, and the others were determined by proportion.

Results

Table I Environmental sustainability indicators

Indicator	Farm A	Farm B
1. Use of Space (ha.t-1)	0,0017	0,0014
2. Dependence on Water (m^3.t-1)	199	248
3. Use of Energy (MJ.t-1)	30 378	30 376
4.Proportion of Renewable Energy (MJ.t-1)	0	0
5. Use of Nitrogen (kg.t-1)	90	87
6. Use of Phosphorus (kg.t-1)	20	20
7. Efficiency in the Use of Energy (%)	22,8	22,0
8. Efficiency in the Use of Nitrogen (%)	20,7	21,4
9. Efficiency in the Use of Phosphorus (%)	16,5	16,4
10. Production Actually Used (%)	100	100
11. Potential of Eutrophication (kg P.t-1)	11	6
12. Potential of Eutrophication (kg N.t-1)	71	70
13. Potential of Organic Pollution (kg.t-1)	983	371
14. Potential of Global Warming (kg.t-1)	-334.50	97.81
15. Pollution by Hormones (kg.t-1)	0	0
16. Pollution by Heavy Metals (kg.t-1)	0	0
17. Accumulation of Phosphorus (kg.t-1)	5	11
18. Accumulation of Organic Matter (kg.t-1)	157	759
19. Risk of Farmed Species	4.0	4.0

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Discussion and conclusion

The environmental sustainability index for fish farms A and B were 93 and 81, respectively and influenced by the higher accumulation of pollutants in fish farm B. This farm had almost 5 times more accumulation of organic matter and 2 times more accumulation of phosphorus. When comparing the values of this indicators with the one published by Moura et al. (2016), farm B showed 11 times more amount of organic matter accumulated per ton than the farm evaluated by these authors (759 kg.t-1 and 67.20 kg.t-1, respectively). In this scenario, using the environmental sustainability index was possible to identify which criteria is the main concern on the farm. The use of these index and indicators allows to, in an easier way, plan and apply changes to the management, minimizing more effectively the impacts of aquaculture on the aquatic ecosystem, becoming a more sustainable production.

(FAPESP, CNPq, Capes)

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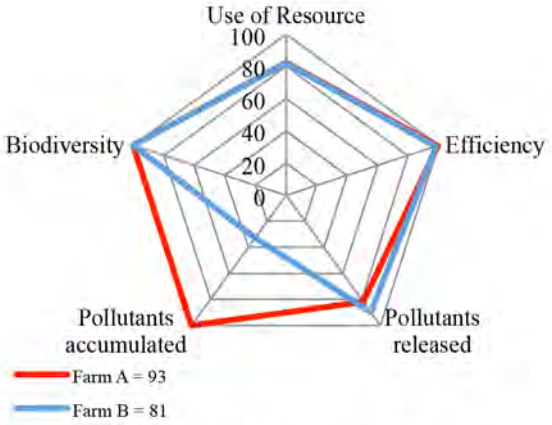

Fig. 1. Environmental sustainability index.

EFFECTS OF COMPLEXED TRACE MINERALS AT DIFFERENT INCLUSION RATES IN COMMERCIAL SEA BASS (*Dicentrarchus labrax*) DIETS

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Introduction

Trace minerals are key elements for activation and function of hormones and hundreds of enzymes. They are essential for proper development and function of bone, nervous and reproductive systems, being critical to epithelial tissue production and maintenance and thus affect health through enhanced skin, gill, fin, scale and gastrointestinal integrity. By playing essential roles in activation and modulation of several processes involved in fish immune response, optimal trace mineral nutrition is very important in helping fight stress and disease. Among these, zinc is known to exert beneficial effects beyond growth, namely through modulating immune response and resistance to disease development of muscle and bone, reduction of cataract incidence and oxidative stress (Gammoh and Rink, 2017). In addition, zinc plays an essential role in wound healing, and speeds re-epithelialization processes in both humans (Lin et al., 2018) and fish (Ogino and Yang 1979; Hughes 1985; Jensen et al., 2015; Gerd et al., 2018). Interestingly, metal-amino acid complexes have proven to be more efficient than inorganic minerals in reducing skin lesions of Atlantic salmon after infestation with *Caligus* (Figueiredo-Silva et al., 2019), indicating enhanced barrier defense mechanisms against pathogens. Major objectives of this study were to evaluate effects of metal-amino acid complexes (Availa®Zn, Availa®Fe, Availa®Mn, Availa®Cu, Availa®Se), supplemented at half the level of inorganic or in combination with inorganic minerals (sulfates of Zn, Fe, Mn and Cu, and Se in the form of selenite), on growth performance, gut and skin morphology, hepatic enzyme activity and zinc content in skin of European sea bass.

Material and methods

Quadruplicate groups of European sea bass, with an initial body weight of 15 g, were daily fed one of 3 diets, formulated to vary in trace mineral source and/or level to apparent satiety, for 4 months. A Control diet was formulated to include an inorganic trace mineral premix of 100 ppm Zn (ZnSO4), 80 ppm Fe (FeSO4), 24 ppm (MnSO4), 6 ppm Cu (CuSO4) and 0.24 ppm Se from (Na2SeO3). A second and third diet were formulated to include metal-amino acid complexes as a 50:50 combination with inorganic minerals or at one-half the dose of inorganic minerals in the Control diet, respectively. In order to magnify response to trace mineral source and level, fish were submitted to a temperature challenge in the second- half of the feeding period (last 2 months), with feed restricted by 50%, from the pre-stress period intake, in the last month of the feeding period.

Results and discussion

Metal-amino acid complexes supplemented at one-half the level of inorganic sources maintained growth performance of European sea bass (Fig.1). Performance results indicate metal-amino acid complexes are a more effective or bioavailable source of trace minerals than inorganic sources in European sea bass, as demonstrated previously in Atlantic salmon (Figueiredo-Silva et al., 2019) and catfish (Paripatananont and Lovell, 1995a,b). In work by Paripatananont and Lovell (1995a), zinc methionine complex (Zn-Met) was shown to be 3 to 5 times more bioavailable than inorganic Zn ($ZnSO_4$), in meeting growth requirements in purified and practical diets containing phytic acid, respectively. In addition, benefits of supplementing channel catfish diets with metal amino acid complexes vs. inorganic minerals were observed to go beyond growth performance, with Zn-Met being 3 to 6 times more effective than $ZnSO_4$ in protecting channel catfish against *Edwardsiella ictalurid* (Paripatananont and Lovell 1995b).

Increased hepatic activity of glutathione peroxidase (GPx) found in European sea bass supplemented with metal-amino acid complexes at one-half the level of inorganic trace minerals (Fig.1), indicate metal-amino acid complexes are more effective in promoting the antioxidant capacity of fish. Partial or complete replacement of inorganic trace minerals with metal-amino acid complexes had a clear impact on the number of goblet cells, in both intestine and skin of European seabass. As part of the mucosal immune system, goblet cells play an important role in protecting fish against pathogens, especially in aquatic animals that are in close contact with their environment. Enhanced antioxidant capacity (i.e. GPx) and barrier defense lines (i.e. goblet cells) are expected to translate to better response of fish to disease, and thus result in healthier fish, especially when grown under commercial farmed conditions. Outcomes of this project are expected to contribute to the development of more efficient diets for European seabass, through the supply of trace minerals that are highly available and efficiently meet seabass performance targets, contributing toward their welfare status.

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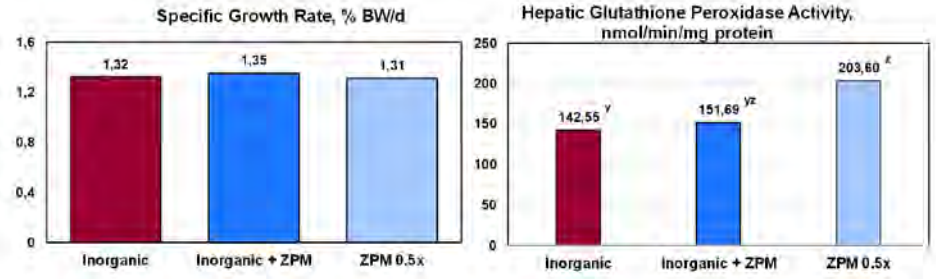

Fig. 1. Specific growth rate (left) and hepatic glutathione peroxidase activity (right) in European seabass, at the end of the feeding trial.

COHABITATION CHALLENGE REVEALS LOW EFFICACY OF A COMMERCIAL VACCINE AGAINST *Piscirickettsia salmonis* IN ATLANTIC SALMON

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Introduction

Fish vaccination is considered an optimal strategy for the prevention of infectious diseases in the global farmed salmon industry. Failure in the protection of vaccines against the intracellular bacteria *Piscirickettsia salmonis*has been proposed as the cause of the increased use of antibiotics in the Chilean farmed salmon industry.The salmon farming industry in Chile during the year 2017 produced 791103 tonnes of fish, which generated exportation of $ 4800 million USD, corresponding to 6% of the country's total exports. *P. salmonis* is the main bacterial pathogen that infects to salmon during its culture in the sea; it affects more frequently the Atlantic salmon being the cause of the mortality of 69.4% of fish grown in seawater (Salmon Farming Health Report, 2017). *P. salmonis* generates large economic losses in the salmon industry, estimating annual losses of $ 700 million USD Maisey et al. (2016).There are several vaccines used in Chile for prevention of *P. salmonis* in atlantic salmon, however does not exist studies for analyse of their efficac .The main of this work is to evaluate the efficacy of an injectable pentavalent commercial vaccine against *P. salmonis* in fish exposed to cohabitation challenge and to evaluate the efficacy of pentavalent plus live attenuated injectables vaccines against *P. salmonis* in fis intraperitoneally inoculated.

Materials and Methods

5,586 Atlantic salmon fish were separated between three main groups called Controls (Mean weight = was 331.7g): group without infection, Cohabitants (Mean weight = 331.4g): to evaluate *P. salmonis* dissemination by water and Trojans (Mean weight = 152.4g): to evaluate the intraperitoneal inoculation of *P. salmonis*. Controls and cohabitants were distributed between unvaccinated and vaccinated with a pentavalent inactivated commercial vaccine. Whereas Trojans were distributed in four treatments: Unvaccinated, Pentavalent vaccinated, Live attenuated commercial vaccine, Pentavalent plus Live attenuated vaccine. We evaluated the survival probability by Kaplan-Meier analysis and Relative percentage survival (RPS). Further, Total IgM and Specific IgM against *P. salmonis* was evaluated by ELISA immunoassay.

Results

The survival probability of vaccinated and unvaccinated Controls were 100%, Unvaccinated Cohabitant was 60.3% and Vaccinated Cohabitant was 56.7%.Whereas survival probability of Trojans were Unvaccinated 0%, Penta-Vaccinated 1.3%, Live-Vaccinated 0.1%, Penta-Live-Vaccinated 0.5% (Figure 1).Thus, in Vaccinated fish exposed to the cohabitation

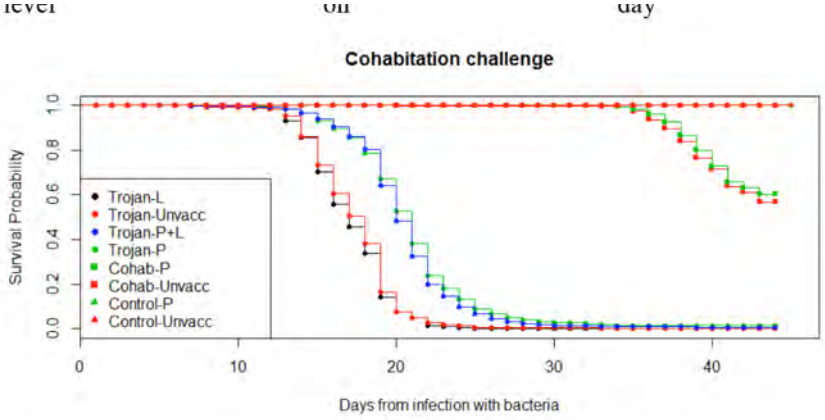

Figure 1. Cohabitation challenge. Survival analysis by Kaplan Meier.

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challenge 45 days after trojans inoculation, the relative percent survival (RPS) was -13.9%. In trojans fish exposed to intraperitoneal inoculation of *P. salmonis*, the RPS_{60} was -0.91% in the group that received Live attenuated vaccine, 75.5% in the group that received Pentavalent vaccine and 71.8% in the group that used both Pentavalent plus Live attenuated vaccines (P+L).The RPS decreased in all groups of Trojans after 45 days post inoculation, the final RPS was 0.10% in the group treated with Live attenuated vaccine, 1.3% in the group treated with Pentavalent vaccine and 0.5% in the grouptreated with both Pentavalent plus Live attenuated vaccines (P+L). Levels of Total IgM in vaccinated cohabitants and vaccinated controls were lower than unvaccinated controls on day 21. At day 41 at the end of challenge, vaccinated cohabitants and vaccinated controls showed higher levels of Total IgM than unvaccinated controls. Levels of specific IgM were similar for all groups of cohabitants and controls on day 21, whereas vaccinated cohabitant fish decreased the specific IgM level on day 41.

Discussion and conclusion

Our results conclude that the use of pentavalent vaccine in Atlantic salmon does not prevent the mortality of fish on a cohabitation challenge. Respect to the cohabitation challenge, we conclude that the injection of Pentavalent vaccine against *P. salmonis* reduces the IgM antibody level at 21 days of challenge, but increases this on day 41. Also we observed that fish exposed to cohabitation infection increases the level of total IgM antibody in comparison with fish without infection. Our results indicate that in the case of the natural infection of PS, fish does not need vaccination against PS, because we observed the same mortality between both groups vaccinated and unvaccinated, despite we found increased level of IgM antibody at the end of challenge. These results suggest the implementation of vaccination trials evaluating the efficacy of vaccines, with the purpose of to prevent high mortality and to reduce the use of antibiotics in salmon industry.

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MUSSELS OR TUNICATES: THAT IS THE QUESTION. EVALUATING EFFICIENT AND SUSTAINABLE RESOURCE USE BY LOW-TROPHIC SPECIES IN AQUACULTURE SETTINGS

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Introduction

The growing demand for aquaculture products can only be maintained by increasing the production of lower trophic species such as bivalves and tunicates. Low trophic species avoid the energy losses during trophic transfers to build animal protein, making them ideal candidates to exploit available resources in coastal waters. In the particular case of fjords, forced upwelling of deep nutrient-rich waters can promote phytoplankton growth, or in other words, the growth of bivalve and tunicate food. However, the density at which bivalves and tunicates are cultured can compromise phytoplankton populations and consequently, marine food chains. A highly configurable environmental model was constructed to study the ecosystem effects and potential biomass production of hypothetical bivalve and tunicate aquaculture scenarios in a Norwegian fjord under forced upwelling conditions.

Materials and methods

A multi-box spatial model which included utrients, phytoplankton and zooplankton, with the addition of a suspension-feeder and detritus sub-models was created for Lysfjord following Filgueira et al. (2010). A new Dynamic Energy Budget model was parameterized for the tunicate *Ciona intestinalis*. All farming scenarios were initialized based on the size and length of natural populations of *Mytilus edulis* and *C. intestinalis* and densities were adjusted according to current and expected aquaculture practices for mussels and tunicates, respectively. A series of 7 farming scenarios were defined for each species (Table I), which resulted from the combination of a different degree of aquaculture development, characterized in terms of area occupied with farms (2.5, 10, 20, 30 and 50%), and the possibility of activating/deactivating the forced upwelling (only in 2.5 and 30% scenarios).The effects of farming scenarios on phytoplankton populations has been evaluated in terms of chlorophyll depletion (*Chl depletion*) using the following calculation:

$$Chl\ depletion\ (\%) = \frac{Chl_{background} - Chl_{farming}}{Chl_{background}} \times 100$$

where $Chl_{background}$ and $Chl_{farming}$ are the chlorophyll concentrations in background and farming scenarios, respectively. This approach assumes that chlorophyll concentration is a good proxy for phytoplankton abundance. Positive values of this index suggest a reduction or depletion of phytoplankton in the farming scenario compared to the background. Similarly, negative values indicate an enrichment in phytoplankton.

Result and discussion

The simulations objectively determined the level of aquaculture development that maximizes the sustainable utilization of resources towards bivalves and tunicates biomass production (Table I). The model also highlighted the positive effect of the forced upwelling on both cultured production and phytoplankton abundance under aquaculture scenarios. Finally, the model predicted that tunicates would be more efficient than mussels at extracting resources (Table I) due to their lower metabolic cost and higher filtration capacity. Although a full economic analysis would be required to decide on the preferred species to be cultured, these results encourage current pilot studies in which tunicates are explored as a sustainable way to efficiently exploit marine resources for aquafeed production

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Table I. Total production (t) and chlorophyll depletion (%) in different aquaculture scenarios in terms of species, percentage of area in the fjord allocated for aquaculture, and the presence of forced upwelling in the fjord.

Species	Area (%)	Upwelling	Production dry weight(t)	Chlorophyll depletion (%)
Mussels	2.5	Yes	0.66	-47.7±22.2
Mussels	10	Yes	2.53	-32.7±19.3
Mussels	20	Yes	4.72	-15.1±17.0
Mussels	30	Yes	6.56	0.9±14.9
Mussels	50	Yes	9.19	25.9±12.5
Mussels	2.5	No	0.54	3.1±0.6
Mussels	30	No	5.25	31.9±5.2
Tunicates	2.5	Yes	2.69	-36.1±22.6
Tunicates	10	Yes	9.39	7.9±31.0
Tunicates	20	Yes	14.27	43.5±32.6
Tunicates	30	Yes	16.21	61.9±27.2
Tunicates	50	Yes	17.00	78.7±17.9
Tunicates	2.5	No	2.41	11.2±4.7
Tunicates	30	No	13.82	73.1±15.7

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HYDROPONIC CULTURE IN NFT USING EFFLUENTS FROM BIOFLOC TECHNOLOGY IN THREE DIFFERENT TROPHIC CONDITIONS

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Introduction

Biofloc technology (BFT) has shown to be a beneficial technique implemented in the production activity in many countries, this production system is increased in the last years, given that it represents a low cost in equipment and a versatile way to transform aquaculture waste into valuable products that can be used in horticulture (Gallardo-Collí et al., 2019). The benefits of BFT were noted by Pinho et al. (2017) who used this water for lettuce in aquaponics units. Despite the favourable attributes of BFT, its use has not been assessed at different trophic modes and its application in hydroponics has not been evaluated yet. Diversifying the production system is clearly a necessity in developing countries, which is why this investigation was focuses on the characterisation of macro- and micronutrient residuals from *O. niloticus* BFT in three different trophic modes and their relationship with the NFT hydroponic horticulture with five green leaf plant species: lettuce (*Lactuca sativa*), pak-choi (*Brassica rapa* subsp. *chinensis*), rocket (*Eruca sativa*), spinach (*Spinacia oleracea*), and basil (*Ocium basilicum*) during the complete nursery and grow-out tilapia culture.

Materials and methods

This study was conducted in Centro de Investigaciones Biológicas del Noroeste (CIBNOR) located in La Paz, Baja California Sur, México. A greenhouse (35.3 m x 13 m) was designed. It included two areas as follows: (a) Aquaculture: 15 culture tanks of 1 m^3 (1.4 m x 0.7 m); (b) Hydroponics NFT: 12 hydroponic beds (204 holes per bed). The experiment was divided into two phases (nursery and grow-out) used Nile tilapia (*Oreochromis niloticus*) (40 weeks). The cultivation method was BFT and the effluents were used in Hydroponic experiment with six treatments with two replicates: Control HO= Hoagland, H = Heterotrophic, Q = Chemoautotrophic, CS = *C. sorokiniana* 2805, CV = *C. sorokiniana* 2714 and M = *C.* spp., with the following plant species: lettuce (*Lactuca sativa*), pak-choi (*Brassica rapa* subps. *chinensis*), rocket (*Eruca sativa*), spinach (*Spinacia oleracea*), and basil (*Ocium basilicum*). The effluent from every fish tank (200 L) was transferred to an anaerobic tank (1 m^3) (only one per treatment) without light or aeration. The content of each tank was mixed and left to stand for settlement process for 24 h. After this period, the residual water for every treatment was transferred to each hydroponic 200-L tank, each one corresponding to one hydroponic bed. This experiment was performed for five weeks; plants were harvested after this period. Every day the parameters were analysed also a set of 50-ml samples (liquid fraction) were obtained from each tank once a week for evaluated the nitrogen residuals and every 10 weeks to evaluate 16 elements (B, Ca, Co, Fe, K, Mg, Mn, Mo, N, Na, Ni, P, S, Se, Si, and Zn) by optic spectrophotometry (ICP–AES VARIAN® model Liberty II; Mulgrave, AUST). At the end of the experiment the growth parameters were evaluated in the plants. One-way ANOVA was performed followed by Tukey's tests to detect significant differences between treatments (significance level of 0.05) (Minitab 17 Statistical Software, 2010).

Results

Conductivity in Q and H showed the lowest level during the first weeks, and after week 11 they increased to the highest level (> 6 dS m^{-1} maximum level) until week 21. In the liquid fraction, the dominant macro elements were Na and Ca at all treatments. Potassium, N, P and Si accumulated during all the experiment in all treatments ($p < 0.05$). Regarding micronutrients, Fe and Mn were detected after week 20 ($p < 0.05$). During the experimental period, the photoautotrophic treatments showed the highest levels of micro and macronutrients among all treatments. Rocket (*E. sativa*), and spinach (*S. oleracea*) showed the highest wet growth with treatment Q ($p < 0.05$), and lettuce (*L. sativa*) reached the highest growth with Hoagland solution. According to growth percentage, lettuce (*L. sativa*) and pak-choi (*B. rapa*) did not show significant differences ($p > 0.05$) while for basil (*O. basilicum*) and spinach (*S. oleracea*), the best results were obtained with treatment CS ($p < 0.05$), and rocket (*E. sativa*) with treatment Q ($p < 0.05$).

Discussion and conclusion

The physicochemical parameters obtained in *O. niloticus* rearing were ideal for hydroponics cultivation. The nitrogen level that could be retained in the biofloc system was optimal for plant cultivation when compared with the hydroponics. The ideal conductivity for hydroponics was 1.5 to 2.5 dS m^{-1} (Trejo-Téllez and Gómez-Merino, 2012). In this case Q and H had the best conductivy profile for sensitive plants, so photoautotrophic treatments were ideal for moderate tolerance. The

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effluents from aquaculture are rich in N and P (Lazzari and Baldisserotto, 2008)the amount of residue deposited into the rearing tanks has increased significantl . Nitrogen (N. Rakocy et al. (1997) reported that aquaculture effluents supplied at least 10 nutrients required by plants. In this research, only five (P, Mg, Mo, B and Mn) essential macro and micronutrients were detected in optimal quantities. Pinho et al. (2017) implemented heterotrophic biofloc and obtained favourable results for butter lettuce (17.7 SGR); in this study, the SGR value obtained for lettuce (*L. sativa*) was 8.9-17.4 SGR ($p > 0.05$). In addition, Castillo-Castellanos et al. (2016) detected a wet weight of 18.8 g plant^{-1} for lettuce (*L. sativa*) with a yield of 47.9 g m^2, using effluents from aquaponics with *O. niloticus*; in this study, lettuce (*L. sativa*) wet weigh was 320 g plant^{-1} with Hoagland, and values from 35.1-112.9 g plant^{-1} were obtained with the rest of the treatments ($p > 0.05$); thus, lettuce (*L. sativa*) growth was irregular and highly variable. The best combination was with spinach (*S. oleracea*) and/or pak-choi (*B. rapa*) and/or lettuce (*L. sativa*). Therefore, *O. niloticus* BFT rearing in the photoautotrophic mode and its integration with NFT hydroponics horticulture in a non-recirculating system is beneficial in coastal arid zones where water is scarce and there is a need to improve aquaculture performance, reuse water, BFT nutrients and increase profit.

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AROTEC-G® A DIETARY AND SUSTAINABLE STRATEGY FOR THE CONTROL OF THE ECTOPARASITE *Sparicotyle chrysophrii* IN GILTHEAD SEABREAM *Sparus aurata*

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For many years, fish farmers have struggled in the battle against ectoparasitic infestations, which are generally due to the intensification of the production process. The traditional use of chemotherapeutic agents to prevent and control fish diseases in aquaculture, such as antibiotics and disinfectants, have been heavily criticized due to the emerging resistant pathogenic strains, the environmental impact of its application and the accumulation of residues in fish, leading to an increased interest towards the developments of alternative sustainable treatments. In the last years, nutritional strategies focused on the use of feed additives as modulators of the immunological and physiological responses, as well as anti-stress and antioxidant therapies, have aroused great interest in the aquaculture industry. Thus, the use of functional feeds in aquaculture is a reality due to their numerous productive, welfare and environmental advantages.

In gilthead seabream farming the use of chemical solutions on-site sea cage treatments to fight against *Sparicotyle chrysophrii* (a monogenean gill parasite specific for the gilthead seabream (*Sparus aurata*)) is common. These treatments include formalin baths and other chemicals used in routine disinfections, in addition to net removal and cleaning (Sitjà-Bobadilla et al. 2006). However, the environmental impact of chemical treatments and the apprehension about human health risks have to be taken into consideration. This raised the importance of developing alternative treatments which are necessary (Wooster et al., 2005).

Essential oils as feed additives

Essential oils (EOs) have been evaluated in the past two decades in several farmed and domestic animal species due to their antimicrobial, antiparasitic, anti-inflammatory and antioxidant properties. Regarding aquatic species, abundant evidences of their benefici l properties and efficacy on fish performance and health have been demonstrated (Encarnação, 2016). The inclusion of EOs in aquafeeds, reported positive results as dietary additives, such as appetite stimulants, feed utilization and growth, boost of the innate immune system with enhancement of the resistance against bacterial and parasitic diseases (Sutili et al. 2017). Besides, the microencapsulation of EOs in fish diets is a suggested strategy to ensure their stability, to prevent ingredients interactions with the host and with the environment. So, it allows a sustained release throughout all the gastrointestinal tract where their effects can be predicted more effectively. The use of garlic thymol and/or carvacrol essential oils in functional feeds for aquaculture have been tested and demonstrated for its effectiveness in the fight against bacterial and parasitic challenges, both in in vitro and in vivo trials.

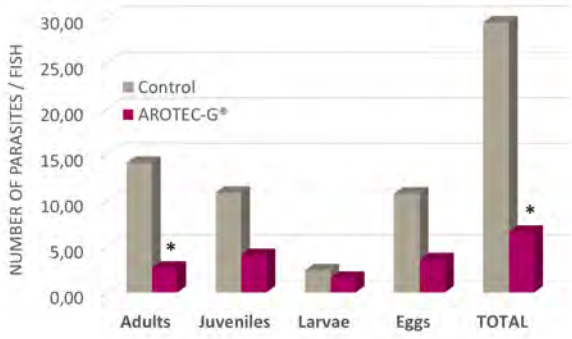

Figure 1: Number of S. chrysophrii parasites per fish fed with the AROTEC-G® and control experimental diets (mean ± standard deviation). Three parasite categories according to morphological characteristics (adults, juveniles and larvae) are represented, as well as the total prevalence of the ectoparasite. Different letters indicate significant differences between dietary groups (P < 0.05).

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Evaluation of AROTEC-G® inclusion in feed

Under this context, recently a study was conducted to evaluate the response of juvenile gilthead seabream (40.26±0.14 g) to the dietary administration of AROTEC-G from TECNOVIT-FARMFAES (a microencapsulated combination of garlic, thymol and carvacrol (EOs). The aim of this trial targeted immune response and effect over ectoparasites. Tissues such as gills and skin were analyzed so asses the effect that *Sparicotyle chrysophrii* had in the immune system, target for most ectoparasites. For this purpose, a 65-day nutritional assay was performed to evaluate the biological processes activated in the skin and gills of fish fed with additive

Skin mucosal response to AROTEC-G®

After the nutritional assay, the effect of the dietary additive in skin was analyzed. Results obtained from epidermal mucus as health status indicator revealed a significative decrease in the classical stress biomarkers, such as cortisol, lactate and glucose, when animals were fed with AROTEC-G®, suggesting an improvement in the overall fish health condition and welfare. Moreover, the mucus from fish fed with AROTEC-G® also significantly inhibited the growth of other fish pathogenic bacteria. Those results were supported by the transcriptomic analysis of the skin-associated lymphoid tissue (SALT).

AROTEC-G® antiparasitc efficiency

Further effect of AROTEC-G® in the control of the parasitization by *S. chrysophrii* was also assessed through an in vivo co-habitation trial for 39 days. The outcomes of in vivo parasitization support the results obtained from the nutritional trial, where a significant decrease in the *S. chrysophrii* total number per fish fed AROTEC-G® (*Figure 1*).

Conclusions

Throughout this study, the microencapsulated additive AROTEC-G® demonstrated to be an effective natural compound with immunostimulant properties designed to be included in functional fish diets to protect gilthead seabream against infestations by ectoparasites such as *S. chrysophrii*. In seabream, the effect of the additive is still being evaluated in other mucosal tissues, such as the intestine, in order to access its global immune effect and consider further applicability. The positive results obtained showed a promising potential for several marine cultured species in the treatment against other ectoparasites as well.

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QUANTIFICATION OF SULFOLIPIDS FROM DIFFERENT MARINE ORGANISMS

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Introduction

For many years, algae and cyanobacteria play an important part in the human nutrition in many cultures. *Spirulina* sp., for example, serves as aliment in Mexico and African countries. In Asian countries, filamentous cyanobacteria (e.g. *Nostoc* sp.) are wildly used as food. Besides the use of cyanobacteria, there are numerous records of historical usage of algae (Gantar and Svincev, 2007). *Pyropia* sp. and *Undaria* sp., for example, are commonly used in Asian cuisine (Wells et al., 2017).

Nowadays the use of macro- and microalgae as well as cyanobacteria becomes increasingly important for human nutrition even in Western diets. Mostly they are being consumed for their functional benefits beyond the traditional considerations of nutrition and health (Wells et al., 2017). In Europe, many algal and cyanobacterial species are legally on the market as food or ingredient. Furthermore, even some products have been already legalized according to Regulation (EU) 2015/2283 as novel foods such as algal oil from the microalgae *Ulkenia* sp. or *Odontella aurita*. The positive effects on human health, hypothesized to result from certain macro and especially minor nutrients are widely discussed in the literature. On the other hand, there is little known about the negative aspects or about minor components with bipolar properties. Sulfolipids (SQDGs) represent one of these minor components.

SQDGs consist of sulfoquinovose and a diacylglycerol, whereas sulfoquinovose is a sulfonated hexose analogous to D-glucose, but features a stable carbon-sulfur bond. The meaning of sulfolipids within the human diet is controversially discussed. On the one hand, they are attributed to some health promoting effects and are known to have antiviral properties. On the other hand, sulfur compounds can have a negative impact, because some microorganisms of the lower gastrointestinal tract are able to produce H_2S as a (toxic) metabolite during the biodegradation in the gut (Burrichter et al., 2018). Therefore, knowledge about the impact of SQDG on human health is a crucial factor. To investigate such effects and due to the fact that SQDGs are not commercially available, it is essential to obtain these standards in very high and pure amounts. Therefore, within this study it was aimed at the extraction and isolation of the sulfolipid fraction and the characterization as well as quantification of the chemical structures in di ferent marine organisms.

Materials & methods

Fresh plant material was dried in liquid nitrogen prior to lyophilization, followed by homogenization using mortar and pestle. The extraction of the crude lipid extract was achieved with four steps. First, the powder was grinded in a ball mill, then extracted on a vortex and ultra-sonication. Then the extract was centrifuged and the supernatant was removed and collected. The extraction steps were repeated three times. The supernatants were combined and evaporated to dryness under nitrogen stream.

The residue was re-dissolved in chloroform/methanol (3/2, v/v). The crude lipid extract was cleaned using a solid phase extraction.

SQDGs were analyzed on an Agilent 1260 Infinity Quarternary LC System (Agilent Technologies Germany GmbH, Waldbronn, Germany) coupled to a triple quadrupole API 4000 QTrap mass spectrometer (SCIEX, Darmstadt, Germany) equipped with a turbo spray source, operating in negative ion mode. MRM was performed by using mass transition between specific parent ions into corresponding fragment ions for each analyte.

Results

The sulfolipid extraction, clean up, and quantification, using a targeted-HPLC-ESI-QqQ-MS/MS multimethod, were developed and validated based on a cyanobacteria *Spirulina* sp powder model matrix. This method allowed the simultaneous determination and quantification of 30 SQDGs. The analysis of SQDGs was validated in terms of linearity, precision, lower limit of quantification. The results indicate that the method is accurate and precise for the quantification of SQDGs in dried plant material. Using this method, different marine organisms were screened on their SQDG content. The marine organisms varies in their SQDG content and in the fatty acid distribution.

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Discussion & conclusion

As a result, up to 17 different sulfolipids were detected and quantified in the different species. The results showed similarities in the sulfolipid composition of the different species. The SGDG with the molar mass of 816 g/mol was identified as the predominant sulfolipid. In addition, the comparison between the samples showed that the lower plants had a higher sulfolipid diversity than tested cormophytes.

With this new developed method, SQDGs can be characterized and quantified. Subsequently the effect of cultivation parameter such as composition of the culture medium, light or temperature on the content of SQDGs shall be investigated. Furthermore, it should be enquired if negative metabolites in human digestion occur there.

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ACCUMULATION OF BIOACTIVE COMPOUNDS IN RAINBOW TROUT AND PIKE-PERCH FILLETS AFTER FEEDING WITH A MICROORGANISM ENRICHED DIET

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Introduction

Foods based on aquaculture production are still on the rise in human diets and nutrition. In order to minimize the need for fishmeal and oil in fish feed in the sense of sustainable aquaculture and to counteract the disturbed ecological balance, the joint project "FENA – fishmeal and oil substitute for sustainable aquaculture" was created and supported by the BMEL/BLE. On of the aims of that project was to develop a high-quality fish food based on yeast, cyanobacteria, and microalgae to reduce the need for fishmeal and oil, which are currently the main ingredients of many fish feed

This study aimed at characterizing the changes in antioxidant activity from the fish feed production to the accumulation of (potential health-beneficial) antioxidants in the fish fillets of *rainbow trout (Oncorhynchus mykiss)* and pike-perch *(Sander lucioperca*), when being fed with an innovative microorganism-based fish feed. On the one hand, this study looks at the change in the antioxidant profile from the raw materials via the extrusion process to the final product the fish feed. On the other hand, the accumulation in the fish fillet over the timeframe of 16 weeks was considere

Material & methods

In this study, 70 fishes per species were studied over a 16 week feeding period. Besides the new sustainable fish feed, a commercially available control diet with fish meal and oil were tested. A further common industrial diet was purchased from Aller Aqua GmbH (Golßen, Germany) as gold standard for the health parameters. The antioxidant activity was analyzed at hand of the total phenolic content, the well-known TEAC assay, and electron paramagnetic resonance spectroscopy.

Results

Schafberg et al (2018) already showed that this kind of feed formulations, consisting of a mix of the cyanobacterium *Arthrospira* sp., the microalgae *Crypthecodinium cohnii*, and the yeast *Rhodotorula glutinis*, led to good feed performances, but the present study showed a more efficient and balanced growing behavior of the fish. Antioxidant activity in the fish filets was improved

Discussion & conclusion

At first, the present study showed that the enrichment with bioactive compounds, stabilizing primarily the feed, was successful. Furthermore, there was a significantly enhanced bioactivity in the rainbow trout filets at the end of this study, while the pike perch filets did not show any significant enrichment

This recipe can serve as a basis for a sustainable fish feed that can also compete with conventional fish feeds for rainbow trout mast. The enhancement of antioxidant activity of the fish filets might consequently also improve product's shelf-life.

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OPTIMIZING WALLEYE *Sander vitreus* AND HYBRID WALLEYE *S. vitreus* X *S. canadense* TANK STOCKING DENSITY AND PERFORMANCE IN A TRADITIONAL RECIRCULATING AQUACULTURE SYSTEM (RAS) FOR COMMERCIAL FOOD FISH PRODUCTION

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Introduction and Discussion

Walleye is a prized sport fish and food fish and is often on the menu of white tablecloth restaurants throughout the Great Lakes region of the U.S. Walleye has been recognized as a species with substantial aquaculture potential because of its high market value and limited supply from traditional commercial sources (National Aquaculture Development Plan; Summerfelt (1996). Researchers at the University of Wisconsin -Stevens Point Northern Aquaculture Demonstration Facility (UWSP NADF) has experienced substantial success rearing walleye and hybrids to a food market size in a recirculation aquaculture system over the past 10 years and have built upon a wealth of existing knowledge with this species, Summerfelt and Johnson (2014); Bristow et al. (1996); Barrows et al. (1993); Bulkowski et al. (1983); Johnson and Summerfelt (2014). Despite the existence of a strong market demand and the existence of aquaculture methods for raising walleye, commercial production is still constrained by several factors. Making production methods commercially efficient which include understanding tank stocking density, swimming speed, flow rates and fish performance are critical to move walleye forward in the aquaculture food fish movement. Researchers at the UWSP NADF have recently made strides in understanding these critical components to rear walleye and hybrids successfully in RAS and will provide an overview.

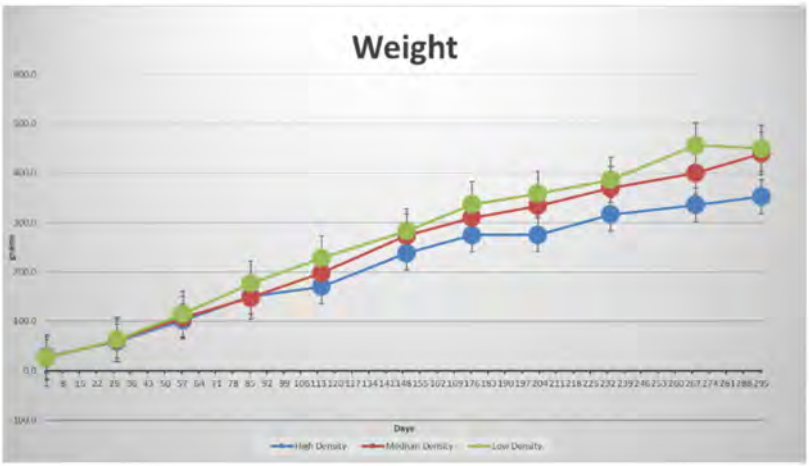

Figure 1. Weight gain of walleye reared at different densities in RAS system.

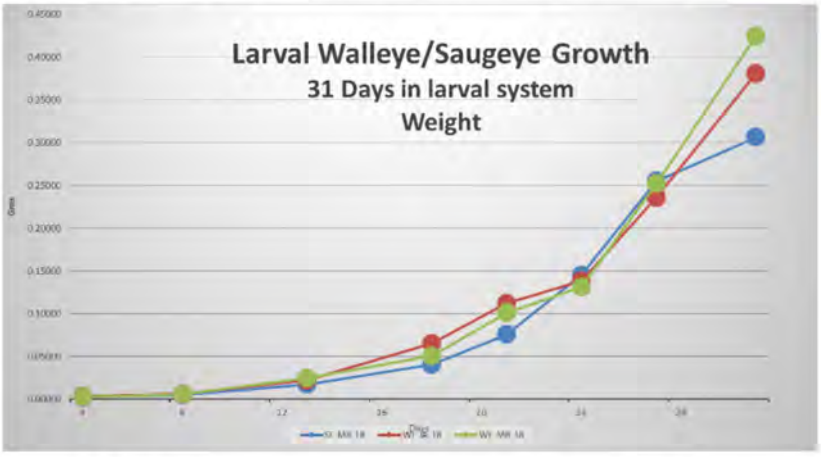

Figure 2. Larval walleye and saugeye growth rates for 31 days reared in UWSP NADF larval system.

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ORGANIC AND CONVENTIONAL MARINE SHRIMP FARMS IN BRAZIL: MACROBENTHOS OCCURRENCE INSIDE AND IN THE OUTLETS OF THE PONDS

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The Guaraíra Lagoon, Rio Grande do Norte State, Brazil, is surrounded by a large number of marine shrimp farms under the semi-intensive and intensive management. Some of these farms started developing as marine fish farms after in 1924 an artificial connection between the former freshwater lake and the nearby sea had been made while others were established only recently. Despite of the concerns in the public about the ecological sustainability of the shrimp industry in Brazil and decreasing prices on the international market the marine shrimp industry around Guaraíra Lagoon is growing steadily.

In 2001, one conventional shrimp farm located on Guaraíra Lagoon started to develop an organic management, using the natural pond biota as the main food source in contrast to the conventional system where the feed is the main food source. In 2004, this farm was certified as the first ganic shrimp farm in Brazil. Due to the lower stocking density and the dispense with feeding, organic farming is claimed to be more environmentally friendly, the organic shrimp farming has significantly lower variable cost, can produce larger shrimp and catches higher prices on national and international markets.

In order to compare the pond ecology and environmental impact of different shrimp farming systems, the organic farm, and shrimp farms using semi-intensive and intensive management around Guaraíra Lagoon where investigated during a rearing cycle at the first semester of 2005. Macro-benthos organisms, aquatic animals living on the bottom of the body of water, are considered as good indicators of environmental changes inside and outside the marine aquaculture ponds and were therefore chosen as one parameter to be analysed.

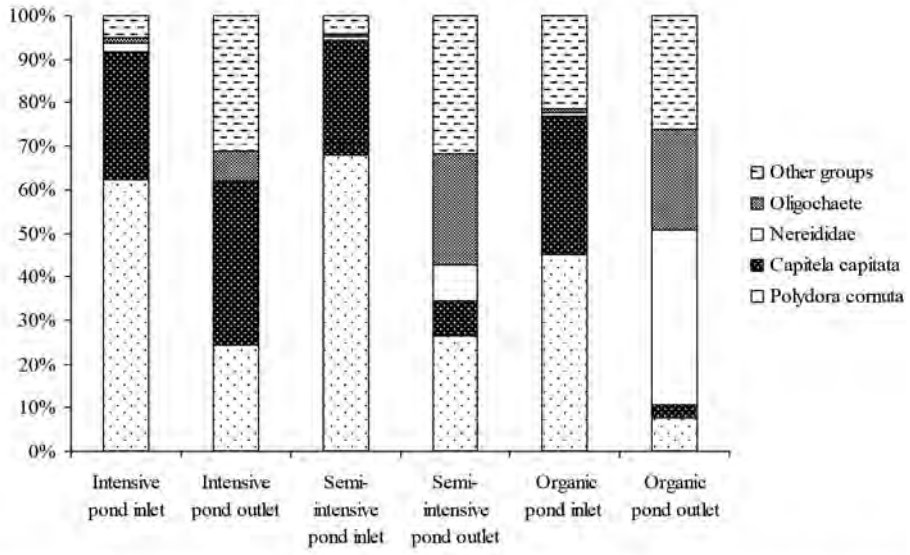

Figure 1. Occurrence (%) of dominant macrobenthos groups inside and at the outlet of the intensive, semi-intensive and organic marine shrimp ponds.

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Benthos samples were collected with a corer of 100 mm diameter, sieved with 1.0 mm mesh size and the organisms fixed in 5% buffered formalin for later analysis. Samples were analysed for the occurrence of different taxa and their abundance.

The results showed an increase of the macro-benthos density during the rearing cycle mainly in the intensive and semi-intensive system where artificial feed was provided. The initial abundance of the macro-benthos inside the ponds was higher at the organic system. The polychaetes dominated in all the systems as the main macro-benthos group but crustaceans, oligochaetes and nemertines were also found. In the semi-intensive and intensive system, the polychaetes of the family Spionidae were predominant (Fig. 1). Only in the organic system the biodiversity was higher inside the ponds than outside. Results of macro-benthos analyses will be related to the organic matter in the soil, water nutrients, redox potential and pH in the soil.

HEMATOLOGICAL AND BIOCHEMICAL BLOOD PROFILE OF ADULT GYNOGENETIC SIBERIAN STURGEON *Acipenser baerii* BRANDT

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Introduction

The aim of the present study was to investigate the hematological indices, morphological characteristics of peripheral blood cells and biochemical indices of mature gynogenetic diploid Siberian sturgeon *Acipenser baerii*.

Although some data about the blood cells or hematological analyses in gynogenetic offspring and juvenile forms of Siberian sturgeon was published, there is no information regarding mature fish. Moreover, there is no information about biochemical indices of blood of gynogenetic of Siberian sturgeon.

Materials and methods

The gynogenetic diploids of Siberian sturgeon (average body weight - 5,78 kg) and control diploids (average body weight - 7,76 kg) were used in the present study. The meiotic gynogenesis was conducted based on methods described by Fopp-Bayat (2007).

Hematological indices were determined according to Svobodova et al. (1991) and covered haematocrit, red blood cell count, white blood cell count, hemoglobin concentration, mean cell volume, mean cell hemoglobin concentration, mean cell hemoglobin and differential white blood cell count.

Biochemical indices were determined with Catalyst Dx chemistry analyzer (Idexx Lab., USA) and covered blood concentration of albumin, globulin, total protein, ammonia, glucose, triacylglycerols, calcium, phosphates and activity of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and creatinine kinase.

Results and discussion

Gynogenetic fish were significantly ($p<0.05$) shorter and lighter when compared to control diploids. Mean erythrocyte volume in this group was slightly bigger. No other differences (based on studied indices) between studied groups of fish were recorded.

In our study, differences between gynogenetic and control diploids were limited to significantly weaker growth of gynogenetic fish only. Although differences in erythrocyte indices were noticed in gynogenetic fish of several species, the results of our study suggest that morphological erythrocyte changes in gynogenetic Siberian sturgeon are noticeable only in larval / juvenile fish

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EXPLORING NEW TECHNOLOGIES FOR DETECING STRESS IN FARMED SALMON: INITIAL TRIALS IN TANK BASED FACILITIES

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Introduction

Monitoring the fish during production is an essential element in realising Precision Fish Farming (PFF) methods in commercial aquaculture, as the ability to apply control engineering principles to a process always depends on the ability to observe the dynamics of that process.

There exist several different methods for collecting data on fish kept in production facilities, ranging from those targeting the fish at group/population level (e.g. sonars, echo sounders) to purely individual-based techniques such as biotelemetry. Although group/population level data may be useful to assess large scale dynamics in the system, the ability to harvest data on the individual level may be crucial in determining the status of the fish during production. This is particularly important during critical operations such as crowding, as the fish are subjected to external stressors that may affect different individuals in several ways. To assess various aspects of the fish responses during such operations, it is therefore beneficial to look at individual based data rather than focusing on the population as a whole.

Biotelemetry has previously been used to monitor both behavioural (activity) and physiological (heart rate) aspects in full-scale crowding operations on farmed salmonids. Although these studies demonstrated that there were relationships between heart rate and activity, and the crowding pressure, it is still unknown how this relates to the actual stress levels of the fish. We therefore conducted a controlled small-scale experiment to investigate the links between activity and heart rate, and the stress levels/physiological states of the fish

Materials and methods

The experiment was conducted in six outdoor tanks of ca. 4 m^3. Twelve fish carrying data storage tags measuring heart rate (Star Oddi Inc.), four of which also carried transmitter tags measuring activity (Thelma Biotel AS), were distributed equally between four of the tanks, meaning that each of these contained three tagged and four untagged fish. The two remaining tanks contained only untagged reference fish. After tag deployment, the fish were allowed to recover for two weeks before they were subjected to an intensive stress regime. Although it is not possible to recreate the stressors experienced during industrial crowding procedures, the regime was designed to approximate such processes as much as possible in both duration and method by sequentially reducing the water level in the tanks four times over four hours. After the stress test, the fish were allowed to recover for 1.5 weeks before the experiment was finally terminated. Data was collected throughout the experimental period, while blood samples were collected to assess the physiological status and stress of the fish before tagging, before and after the stress trials, and after the final recovery period.

Results and discussion

Data processing is still ongoing, but some initial results from the study are ready. In the first period after tagging, the fish carrying heart sensors reported a gradual decrease in heart rate until it eventually stabilised into a circadian rhythm (higher heart rate during daytime) after a few days. This may indicate that the tagging procedure induced a stress response in the fish, but that this response declined as the fish were allowed to recover. The heart rates of the fish changed during the stress tests, with the circadian rhythm breaking down and generally higher heart rates than in the preceding period. After the stress tests, the heart rate subsided again and stabilised at a circadian rhythm after a few days. This suggests that induced stress affected the heart rate of the fish, which in turn may indicate that heart rate can be a useful stress indicator for farmed fish.

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There was also a response in the activity data, but it was less clear than that seen in heart rate. As for heart rate, there was a circadian rhythm in swimming activity in the period preceding the stress testing (higher activity during daytime). This was expected as salmon are known to be more active during daytime than at night, which also probably explains why the heart rate had a similar diurnal variation. During stressing, measured activity levels dropped to lower values than in the preceding period. This was probably because the primary stressor in the experiments (lowering the water level in the tanks) also led to the fish having less volume available for movement. Less movement in turn leads to lower accelerations which again leads to the tags computing lower activity scores. After the stress period had concluded, activity levels increased to higher values than before the stress testing, implying a similar response as that seen in commercial crowding. This may suggest that increased swimming activity also may be a potential stress indicator for salmon in sea-cages, although measured activity was lower during stressing due to the reduced volume for movement.

Although both these sensor principles are possible to apply at a full-scale farm level, further analyses correlating the sensor values with physiological values from the blood samples will be carried out in the SalmonInsight project to establish the validity of data provided by the use of such sensors as operational welfare indicators.

HYDRODYNAMIC LOADS ON EXPOSED FISH FARMS

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Introduction

Fish farming at exposed locations requires robust and reliable structures that facilitate sustainable, safe and efficient production. Performance under increased exposure must be evaluated for structural components and the complete fish farm. During the previous years, there has been a focus on increasing the quality of input for structural analysis, including design values for environmental loads and dimensions of netting (solidity). More precise design input values will also require more precise load models: Input parameters and analysis methods are closely linked and will combine yield loads on and response of the structures. It is thus insufficient to change the input parameters without changing the analysis methods accordingly.

The net is the main barrier preventing escape of fish, and the design and dimensioning of nets is therefore of crucial importance in the development of safe and robust fish farms. Analysis of fish farms shows that for a traditional fish farm, drag loads on nets are the major contribution to hydrodynamic loads on exposed fish farms. For new types of fish farms, established and validated load models exist for rigid parts of the structure, while loads on the nets will be a major source of uncertainty.

Methods and results

Methods for establishment of design values for water currents with 10- and 50-year return period were developed based on measurements of current velocity at 22 different locations over periods between 7.5 months and 2.5 years.

It was found that estimating design values for current velocities based on physical measurements for one month, which is the current standard, is not sufficient. It is recommended that design values are found using extreme value analysis based on at least one year of measurement data. If this is not possible in practice, the highest value found during at least three months of measurements can be used with a given multiplication factor.

A new method for measuring solidity of netting was developed. This includes a rig to apply a constant pretension in the netting, backlight illumination, a machine vision camera and custom photogrammetry software.

Moreover, a method for model testing of netting materials was developed. This involved towing of net panels. Loads and relative velocity between fluid and the net panels were measured. The results will be applied to establish drag coefficients and drag loads on traditional nets. Initial tests on traditional netting materials were performed.

THE EFFECT OF DENSITY ON GROWTH IN RED CUSK EEL (*Genypterus chilensis*) – A NEW SPECIES FOR CHILEAN LAND-BASED AQUACULTURE

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Introduction

The red cusk eel (also known as Congrio colorado) is a promising new species for the Chilean aquaculture industry. The fish is in high demand in the Chilean market, but landings have declined rapidly due to overfishing. During the last ten years, a pilot-scale commercial company (Colorado Chile SA) has closed the lifecycle for culture of this species and is now producing offspring and juveniles from cultured broodstock fish. With a stable and predictable production of juveniles, the next step is to optimize juvenile on-growing, and to select a rearing system that fulfils the requirements of the species. As the fish are bottom-dwelling and negatively phototactic, a low-light shallow-raceway system was tested, and the present study investigated growth in such a system at three different densities of fish

Material and methods

Six raceways (2.5 x 0.6 m) were used in the experiment. In each raceway, a restricted area was assigned for the fish, and juvenile red cusk eels (100-110 g) were placed in these chambers at three different densities (replicate treatments); Low (~30 kg/m^3), Medium (~45 kg/m^3) and High (~60 kg/m^3). In each raceway, 20 fish were individually tagged using Trovan® Passive Transponder tags. At the start of the trial (24 January 2018), each fish (tagged and untagged) was weighed (g) and measured (cm). The study lasted for 266 days, and fish were measured on approximately monthly intervals throughout the experiment. Water (RAS) was supplied at a constant temperature of 17 °C and oxygen levels were kept above 80% in the water outlet. The fish were kept in the dark apart from during the period of daily feeding and cleaning routines

Results

At the end of the experiment, mean weight was not statistically different between fish from the three treatment groups. However, due to an initial (non-significant) lower mean weight in the high density group, overall SGR in the high density group was significantly higher (13% and 10%, respectively) than the low and medium group. (SGR high density=0.47, medium density=0.43 and low density=0.42). Mean density in each group increased from the initial densities of 30, 45 and 60 kg/m^3 to 77, 132 and 193 kg/m^3, respectively.

Discussion and conclusion

The experiments demonstrated that red cusk eel can be reared at very high densities without compromising growth. In fact, high densities seem to be an important prerequisite for the species to thrive. These fish are negatively phototactic and hides in burrows and between rocks in nature. In culture, a high density may function as a potential shelter, where fish are hiding underneath each other, only leaving their hiding place to feed. Shallow raceways were evaluated as promising rearing units for juvenile on-growing of this new aquaculture species.

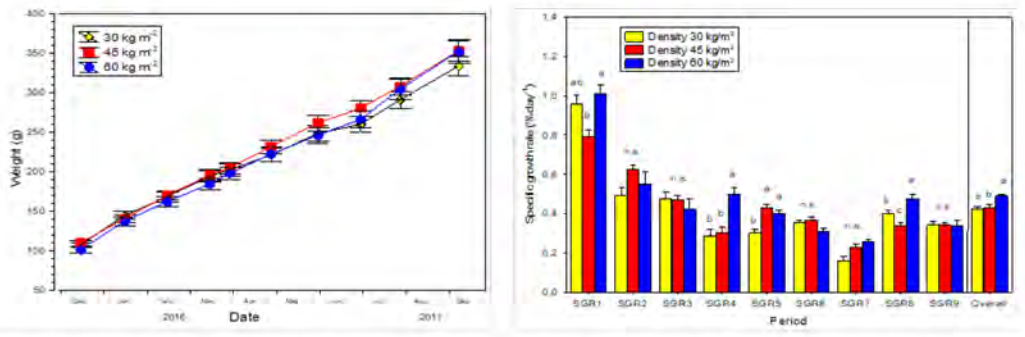

Fig. 1. Mean weight and periodic and overall SGR of juvenile red cusk eel reared at three densities for 266 days. Different letters denote significant differences (two-way nested ANOVA, $P < 0.05$) between treatments in each growth period.

MICROBIAL COMMUNITY DYNAMICS IN RECIRCULATING AQUACULTURE SYSTEMS REARING ATLANTIC SALMON PARR *(Salmo salar)* WITH REDUCED ORGANIC LOADING THROUGH MEMBRANE FILTRATION

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Introduction

A key challenge in recirculating aquaculture systems (RAS) is the accumulation of particulate organic matter. This can stimulate growth of bacteria, influence nitrification kinetics, general water quality, and fish health (Chen et al., 1993). Integration of a membrane for ultrafiltration in the water treatment loop has been proposed to reduce the amount of organic matter build-up, thereby lowering bacterial substrate and other negative effects of turbid water (Wold et al., 2014). An environment with rapid escalation of bacterial numbers, and frequent fluctuations in the microbial community dynamics could reflect an environment rich in opportunistic unfavorable bacteria (Skjermo et al., 1997). The supply of organic matter is typically the limiting resource determining the carrying capacity of heterotrophic bacteria in the system. Thus, a low and stable carrying capacity is suggested to be a strategy for securing an optimal microbial environment with less bacterial blooms and stable community dynamics (Attramadal et al., 2014). This experiment was conducted in order to study the effects of membrane filtration and varying levels of organic matter on the microbial community dynamics in RAS water and biofilm.

Materials & methods

The work was funded by the ERA-NET COFASP (Europe 2020 strategy) and the Norwegian Research Council project "Water treatment technology for recirculating aquaculture systems to increase efficiency by reducing the negative effects of organic matter (RAS-ORGMAT)" (260872/E40). The experiment was set up at NTNU/Sintef Sealab with two replicate small-scaled (4.2m^3) RAS rearing Atlantic salmon parr (*Salmo salar)*. One control system (cRAS) and one system with membrane filtration (mRAS) were run for 72 days. The experiment was separated into three different periods (P) where the water exchange rate was manipulated (40 – 90% recirculation d^{-1}) in order to ensure variations in organic load. P1 had increasing organic load↑, P2 decreasing organic load↑ and P3 increasing organic load↑. The ultrafiltration membrane (Compact 4.0G X-flow/Pentair) had an average pore size of 30nm and filtrated 10% of the total water flow (120 l min^{-1}) prior to the biofilte . Water samples and moving bed biofilm reactor (MBBR) carriers were collected for microbial analysis at two samplings each period. The V4 region of the bacterial 16S rRNA gene was targeted for taxonomic classification through Illumina sequencing. Bacterial concentrations and bacterial growth rates in the water were measured with flow cytometry and thymidine incorporation, respectively.

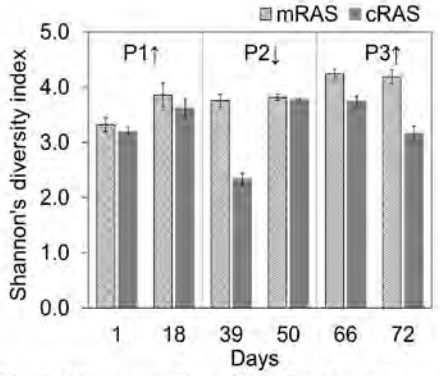

Fig. 1, Shannon's diversity index for water samples

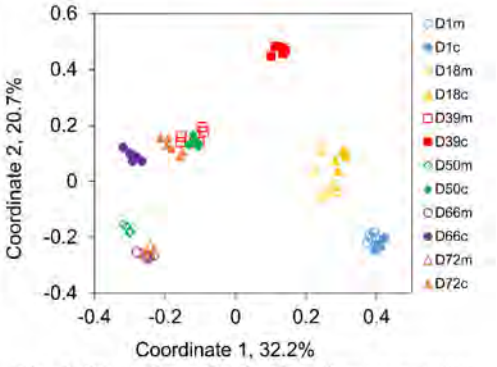

Fig. 2, PCoA Bray-Curtis plot of water samples D=day of sampling, m=mRAS, c=cRAS

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Results & discussion

Flow cytometry and thymidine incorporation revealed that both systems had higher bacterial densities and growth rates in the water after the periods of increasing organic load (P1 & P3). However, cRAS had throughout the study significantly higher numbers for both variables except after P2. Thus, the membrane filtration had a clear effect on densities and growth rate of the bacteria with increasing organic load, whereas the effect was not that evident with decreasing organic load. Illumina sequencing showed different microbial community compositions in the two systems. The Shannon's diversity index (Fig. 1) shows more diverse and even communities in mRAS in P2 and P3. The difference is especially evident at Day 39, which was about two weeks after the systems had gone from high to lower organic loading. Principal Coordinates Analysis (PCoA) based on Bray-Curtis similarities (Fig. 2) shows development of the microbial communities over time through the periods. The plot indicates that after the systems experienced the first change in the environment (by Day 39), the communities evolved in separate directions. At the end of the study, the microbial communities of mRAS overlapped more than cRAS. A change in the environment can be a window for bacterial blooms (De Schryver and Vadstein, 2014), which could be seen in the flow cytometry data for cRAS in the beginning of P2 and a genus of *Gemmobacter* dominating the microbial communities at Day 39. The results could indicate that the membrane filtration stabilized the environment by keeping the organic load more constant. At times with increased organic pressure on the system, mRAS was less affected than cRAS. In the MBBR biofilm, the diversity was similar between the systems, and little variation in microbial community dynamics was observed. The growth and mortality rates of the fish were not significantly different between the two systems.

Conclusion

Different organic loading on the systems affected the microbial community dynamics of the RAS water. The applied membrane filtration kept the organic load more constant in the periods of increasing organic loading, and this resulted in a higher diversity and more stable microbial communities with fewer bacterial blooms. Variations in organic loading did not affect the microbiota of the biofilm in the MBBR

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ORGANIC VS INORGANIC MINERALS PREMIX INCLUSION IN PLANT MEAL-BASED DIETS FOR JUVENILES SEA BASS, *Dicentrarchus labrax*; EFFECTS ON GROWTH PERFORMANCE INDICATORS AND IMMUNOLOGICAL RESPONSE

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Introduction

Micronutrient requirements such as Fe, Cu, Mn, Zn, and Se are essential to fish (Kaushik S. 2002). A lower or higher than optimum supply of dietary minerals can affect the biochemical and physiological responses in fish. Fish meal (FM) is a rich source of micro minerals and has been the major protein source in aqua feeds for fish over the years. However due to the limited supply, its use in fish feeds has been significantly reduced and replaced to a large extent by plant ingredients (Tacon et al., 2008). The later has a direct effect on the availability of minerals in fish. The knowledge of the requirements for these micronutrients in European sea bass, *Dicentrarchus labrax.* were restricted to limited data from the NRC, 2011 while promising data have been arisen from the European research project "ARRAINA" concerning the inclusion of inorganic minerals in low FM based diets. The availability of the minerals depends on various factors among which the chemical form of the minerals. The aim of this study was to examine the effect of different forms of minerals (inorganic vs organic) on the growth, immune system and antioxidant capacity of European sea bass, *Dicentrarchus labrax*, when fed on a low FM diet. Lower levels of mineral premix than optimum were also investigated in order to assess if lower levels would be sufficient to cover the needs of this fish species. This was accomplished through a feeding trial at the end of which both growth, humoral and mucosal immunological parameters, antioxidant capacity and tissue mineral concentration were assessed. In the present abstract preliminary results will be presented, concerning growth, protein efficiency and some immunological parameters.

Materials and methods

European sea bass juveniles (~20g) were fed the 6 experimental diets. The selection of mineral level addition was based on the optimal levels of selected minerals reported by the ARRAINA project. Feeds were formulated as described on the table below. Low fish meal diets contained as main ingredients, wheat and corn gluten, soya protein concentrate, fish oil and rape seed oil. All diets were isonitrogenous and isoenergetic.

Results

After 94 days feeding, juveniles sea bass showed significantly improved growth performance (SGR) and protein efficiency (PER) in fish fed the ORG diet (Fig 1). This difference was significant when compared to diet INORG. Immunological parameters were assessed in blood, serum and in skin and gut mucus. Hemoglobin was improved in ORG diet fed fish. Ceruloplasmin, an indicator of inflammation, was elevated in the blood of fish fed the deficient (CTRL-) and INORG plant-based diets. Blood respiratory burst activity was significantly reduced in the blood of fish fed the INORG diet compared to all the other tested diets. In contrast with the growth parameters, serum and gut lysozyme activities were significantly reduced in ORG-fed fish

Discussion & Conclusions

The growth parameter and protein efficiency results showed a significant improvement of the fish performance when fed the organic minerals enriched plant based diets even when compared to the FM based diet (CTRL+). This improvement was not apparent with levels below optimum or with excess of organic minerals, the latter suggesting a potential toxicity of the high concentrations of dietary minerals. The use of organic mineral premix was also more potent than the inorganic premix at the same dietary dose. The same improvement of some hematological/immunological parameters in fish fed with the optimum organic mineral premix was evident concerning the hemoglobin level and respiratory burst activity and reduction of the inflammatory response but the effect was not so clear concerning many other immunological parameters assessed in the present study. The results on the antioxidant capacity of the fish may give more information about the beneficial e fects of the organic mineral addition to sea bass diets based on plant protein sources.

On the whole, the use of organic mineral premix at the optimal level seem to improve fish performance and some immunological parameters when these fish were fed plant meal based diets up to or above levels obtained in FM fed fis

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Raw materials	CTRL+	CTRL-	ORG	INORG	ORGlow	ORGhigh
FM	30	10	10	10	10	10
KRILL		2.5	2.5	2.5	2.5	2.5
MINERALS premix	Inorganic minerals*	No mineral	Organic minerals*	Inorganic minerals*	65% of ORG diet minerals	150% of ORG diet minerals
*concentration in ppm: Se 0.67, Fe 248, Cu 17.3, Mn 59.7, Zn 169 considered as optimum in previous project (ARRAINA)						

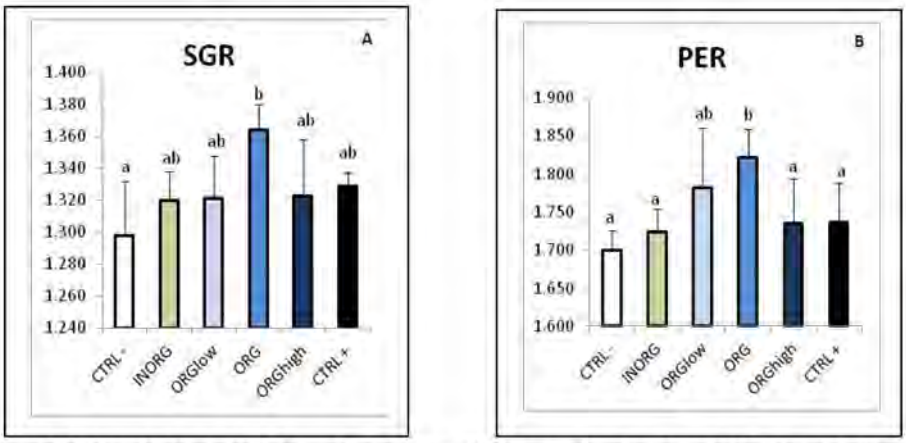

Fig 1 A) Specific Growth Rate and **B)** Protein efficiency ratio of sea bass fed the tested diets . Different letters show significant differences between dietary treatments (ANOVA P<0.05, Duncan t-test). (n=4).

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APPLICATION OF RESPONSE SURFACE METHODOLOGY TO OPTIMIZE THE GROWTH AND FEED EFFICIENCY RESPONSE IN EUROPEAN SEABASS FED GRADED LEVEL OF FISH MEAL AND SHRIMP HYDROLYSATE

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Introduction

Marine protein hydrolysates are promising ingredients that can bring critical functionalities to fish feeds, formulated to meet fish nutrient requirements. Palatability and digestibility are known to be driven by the peptide profile of the dietary hydrolysate. However, interactions with fish meal are expected to be dose and quality dependant making any model, or prediction, about final dietary palatability and digestibility a bit uncertain. This is where statistical tools, such as full factorial design, may help us quantifying ingredient individual effects, along with their interactions, at graded inclusion levels, with optimization of zootechnical performance as the final ta get.

Materials and methods

Two full factorial experimental trials were designed in juvenile European seabass (5-10g initial body weight) reared in 100l tank and fed 24 different diets with graded levels of fish meal (0, 5, 10 and 20%) and shrimp hydrolysate (0, 2.5, 5, 7.5 and 10%), including 4 repetitions. Fish (40 per tank) were fed either *ad libitum* or at 80% satiety for 7 weeks using an automatic feeding system. At the end of the trial, specific growth rate (SGR) and feed conversion ratio (FCR) were calculated and estimated response surfaces were then calculated for both parameters using Statgraphic 2.0 software.

Results and discussion

Results showed that at low level of inclusion, shrimp hydrolysate performed better than fish meal for growth and for FCR. From 8% dietary inclusion, a quadratic effect was observed mostly due to the too high level of peptides supplied by hydrolysate. The statistical model gave us a optimal combination of 20% fish meal and 6.6% shrimp hydrolysate then 20% fish meal and 3.9%% shrimp hydrolysate for maximizing growth rate and feed utilization respectively. Higher dietary level of fish meal would have to be studied to check the benefit of dietary hydrolysate at higher level of fish meal. Other variables such as the quality of fish meal as well as the origin and the peptide profile of hydrolysate would deserve to be evaluated too.

ESTABLISHING METHODS FOR DETERMINATION OF FISH QUALITY IN DOMESTIC FISH SPECIES IN THE AQUACULTURE OF MECKLENBURG-WESTERN POMERANIA

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Introduction

Aquaculture is the fastest growing area of food production with a steady expansion of more than 8% per year since the 70s (Burbridge et al. 2001; Knaus 2012). Besides this, the fish flesh quality has gained importance among the consumers because it is directly related to human health and nutrition. But so far, fish flesh quality of only few species (e.g. *Oncorhynchus ssp., Oncorhynchus mykiss, Salmon salar)* has been examined and this mostly in regard of the chemical composition. These results exhibited that lipid and protein composition in fish muscle vary greatly between species and are depending on age, sex, environment and season (Johnston 2001). Presently, there are no established methods and regulation to measure the physical properties of fish flesh. Therefore, the aim of this study was to transfer the protocols used for beef, pork and chicken to fish for analyzing the physical parameters of fish flesh. For a first description and to ensure improvement of fish flesh quality in future due to adaptation of fish cultivation in aquaculture, two economically important fish species, the European perch (*Perca fluviatilis*) and marena whitefish *Coregonus* maraena), were analyzed.

Methods

Fishes were cultured in a recirculation system in the Institute for Fisheries in Born, Germany. Adults of both sexes from *Perca fluviatilis* (PFL, n = 15) and *Coregonus maraena* (CMA, n = 15) were slaughtered by standard procedure in accordance to the German Animal Welfare Act (§ 4(3) TierSchG). Morphometric measurements of animals and filets as well as the physical measurements were taken immediately after death. Quality parameters of muscle like pH, isoelectrical conductivity and impulse-impedance were measured at 5min and 1h post-mortem using pH-Star (Matthäus), LF-Star (Matthäus), and Meat Check 150 (Matthäus), respectively. The filet firmness was measured 0h post-mortem by Texture Analyser TA.XTplus (Winopal) with Warner Bratzler blade in caudal central to the lateral line of the filet, in triplicate. Color of muscle divided by dorsal and ventral filet was measured three times with CR-300 Chroma Meter (Minolta). The water holding capacity (WHC) was determined by filte -press-method via Hypress (Grau and Hamm, 1953), in triplicate. Morphometric and quality parameters were statistical analyzed using SAS (v.9.2).

Table I: Physical parameters of fish quality in European perch (*Perca fluviatilis*, PFL) and marena whitefish (*Coregonus maraena*, CMA) in aquaculture of Mecklenburg-Western Pomerania

	PFL (n = 15)		CMA (n = 15)	
Physical parameter	mean	SEM	mean	SEM
lightness	42.70	0.70	43.64	0.91
pH (5min)	6.68	0.04	6.87	0.04
isolectrical conductivity (5min)	4.79	0.26	4.05	0.17
impulse-impedance (5min)	70.93	1.54	60.33	1.30
water holding capacity [%]	16.45	0.60	20.90	1.26
shear force max. [N]	53.89	1.76	24.74	0.65

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Results

The morphometric data of PFL, member of Percidae and CMA as member of Salmonidae differs significantly ($p \leq 0.001$) in species-specific manner. At slaughter PFL has a total length of 37.58 ± 0.37 cm and a weight of 777.07 ± 0.30g. CMA exhibited a total length of 32.58 ± 0.27cm and a total weight of 327.07 ± 7.59g. Carcass weight (exclusion of head and internal organs) of PFL and CMA has been 469.07 ± 14.41g and 237.27 ± 11.21g. For the consumers, especially the amount of free water in the fish filet, defined as WHC and texture, represented by the maximal shear force (SF), is of interest. Under the present fish cultivation condition, the results show a firmer texture (53.89 ± 7.76N) with lower WHC (16.45 ± 7.76%) in the PFL filets. Compared to this, the CMA filets exhibit a softer texture with 24.74 ± 0.65N and a higher WHC (20.90 ± 1.29%). Furthermore, also the impulse impedance showed higher values in PFL filets (able I).

Discussion

In the meat production, firm regulations for pork, beef, and poultry meat quality have been existing. Due to the high diversity of fish species, regulations for fish muscle quality has not been defined thus far. With the present study, we could show that an adaptation of the used physical methods can be transferred to fishes as well. Therefore, the obtained results are a first step in order to develop consistent regulation and statements regarding the physical values of fish filet to define the quality of fish fles

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GWAS REVEALS SEVERAL GENOMIC REGIONS GOVERNING SPONTANEOUS XX-MALENESS IN RAINBOW TROUT

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Introduction

Rainbow trout (*Oncorhynchus mykiss*) is a male heterogametic (XY) sex-determined species and the master sex-determining gene, sdY, has been identified (Yano et al., 2012). Surprisingly, a small proportion of phenotypically male individuals is regularly observed in all-female trout livestock (produced by crossing sex-reversed XX-males with standard XX-females). Maleness has also been described in gynogenetic families (expected to be all-XX females) (Quillet et al., 2002). In further studies we showed that the maleness ratio could depend on both genetic background and rearing temperature (Valdivia et al, 2014) and found several QTL associated with spontaneous masculinization (Guyomard et al., 2014). Those results indicate the existence of a complex genetic and environmental control of gonad differentiation underlying the major sdY determinism. In this study, we aimed at further describing the genetic architecture of spontaneous maleness in a French all-female trout population using a GWAS approach based on a medium-throughput SNP chip.

Materiel and methods

Fish were produced at the trout farm "Les Fils de Charles Murgat" in June 2017 from 5 factorial mating designs (50 dams and 50 sires in total). Eggs were separated into two batches and were kept at 12°C until the end of yolk resorption. At that time, temperature was increased to 18°C for one of the batches for a period of 1100 degree-days, covering the expected window of gonad differentiation. The two groups were then reared at the same temperature (between 12 °C and 14.5°C) until they were sexed. Sex was determined by visual observation of both gonads for 10 000 fish per temperature. In each group, about 6 500 fish were examined at 10 months post-fertilization and the 3 500 remaining 5 months later. Fish were distributed in 3 sex classes: female (2 ovaries with no visible sign of testis area), male (two testis, with no visible female area/ovocyte) and intersex (presence of both male and female areas in at least one gonad) and encoded as 1, 2 and 3, respectively. A fin clip was sampled from all males (n=163), intersex (n=132) and from 858 females randomly chosen for genotyping using the 57K SNP Axiom™ Trout Genotyping Array. Sex was analyzed as a linear trait modelled by GBLUP accounting for the fixed effect of the rearing temperature and the random effects of all retained SNPs. Using BLUPF90 software, genomic variance, phenotypic variance and heritability were estimated using AIREMLF90 program and GWAS were based on the POSTGSF90 program (Aguilar et al., 2014). Different options of POSTGSF90 were used to derive QTL significan e, QTL effect and proportion of genomic variance explained by QTL, considering 1Mb-sliding windows of adjacent SNP and weighted GBLUP (wGBLUP).

Results and discussion

The overall maleness rate (males + intersex) was 1.99% at 12°C and 0.87% at 18°C. After quality controls, 30 811 SNP and 1 139 fish were retained.

Genomic heritability was estimated at 0.51 (± 0.05) with genomic and phenotypic variances estimated at 0.22 (± 0.03) and 0.43 (± 0.02), respectively. With the GBLUP model, we detected 5 sex- QTL on 4 chromosomes (see Figure 1). One QTL on Omy1 was highly significant and explained 1.92% of genetic variance; the suggestive QTL on Omy7 explained 2.98% of genetic variance. The other QTL explained between 0.83 and 0.91% of genetic variance, whether they were significant at the chromosome level or not (Figure 1, B). Using the wGBLUP model with two iterations to better quantify the QTL effect, we confirmed the role of the two QTL on Omy1. Interestingly, none of the QTL was located on the male-specific Y-chromosome.

This study paves the way for the identification of the causal mutation(s) and the gene(s) responsible for this spontaneous maleness of XX female rainbow trout.

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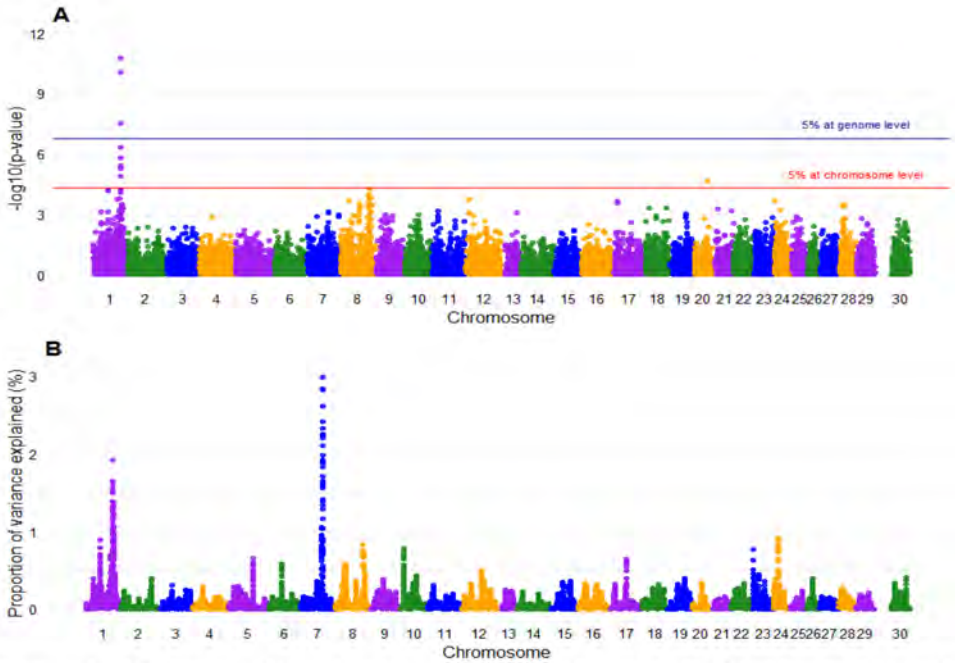

Figure 1. Manhattan plots showing associations between SNP and sex (A) and percentage of genetic variance of sex explained by 1Mb-sliding windows (B)

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FORECASTING THE IMPACT OF FUTURE CLIMATE SCENARIOS ON CULTURED MUSSELS GROWTH IN THE GALICIAN RÍAS (NW SPAIN)

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Introduction

The socio-economic role of mussel aquaculture in the Galician rías (NW Spain) and its dependence on environmental conditions have generated an increasing concern about the impact of future climate scenarios on this production system.

ClimeFish is an on-going H2020 project committed to the study of the impacts of climate change on freshwater and marine fisheries and aquaculture across Europe. Within this framework, the aim of this work is to forecast the effects of future climate scenarios on mussel growth in the Galician rías,

Methods

We have simulated mussel shell and soft tissue growth using the dynamic model proposed by Fuentes-Santos et al. (2019) under observed (2006-2015) and projected (2016-2055 at decadal intervals) climate conditions under the RCP4.5 and RCP8.5 emission scenarios.

Our growth model is driven by sea surface temperature, solar irradiance and food availability, which in turn is driven by the coastal wind regime and river discharges (Aguiar et al., 2015). We have used three climate models (POLCOMS-ESM, CSIRO-Mk3.6.0, and the NorESM1-ME) to account for model uncertainty in SST projections. RCP projections for coastal winds, river discharges and solar irradiance were downloaded from the EURO-CORDEX MPI-ESM-LR model. We have generated 1000 bootstrap realizations of 3-year cycles for each environmental driver for the observed conditions and future decadal projections to feed the growth model.

We have simulated the growth of mussel seed (initial shell length = 15mm) during 450 days considering different seeding periods: winter (February), spring (June), summer (September), autumn (November). We have simulated the impact of climate change on mussel growth in terms of the time needed to reach the optimal market size (target flesh weight = 8g)

Results and discussion

SST projections vary across climate models. SST barely increases according with POLCOMS-ESM, suffers approximately a 1ºC increase according with CSIRO, and increases gradually up to 1.5-2ºC in 2046-2055 under RCP8.5 according with NorESM. The MPI-ESM projections predict a decrease in solar irradiance during summer and a subtle increase in spring and autumn. Finally, we do not predict any climate related change in food availability during the next 3 decades.

Fig. 1 highlights the important role of the seeding time on mussel growth. Mussels seeded in February need less time to reach the target weight than those seeded in summer and autumn, while those seeded in June have the largest culture length. Fig. 2 shows that the culture length shall suffer a slight increase under POLCOMS conditions but shall decrease with CSIRO and NorESM projections. However, climate change has a minor impact on mussel growth in comparison with the seeding schedule.

Our results suggest that climate change shall barely affect mussel growth in the Galician rias over the next three decades. However, a proper analysis of the impact of climate change on mussel aquaculture requires addressing other factors, such as seed availability and harmful algal blooms, which determine the seeding and harvesting schedule. The settlement cycle is controlled by solar irradiation (Fuentes-Santos et al., 2016), which shall not suffer relevant changes in the next decades. Thus, we may not expect significant shifts on seed availability. Harmful algal blooms, which cause harvesting closures of culture areas, may have an important negative impact on production. Álvarez-Salgado et al. (2011) found that rainy springs produce long-lasting closures in summer and windy summers favour long-lasting closures in autumn. A preliminary analysis of future wind regimes and continental runoffs in the area suggest that harvesting closures shall decrease by 10% in summer and increase by 10% in autumn.

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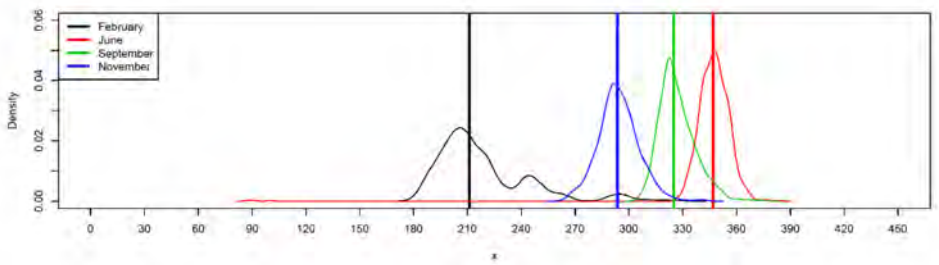

Fig. 1 Time (days) to reach the optimal flesh weight (8g) under observed environmental conditions (2006-2015) in the Ría de Ares-Betanzos. Vertical lines indicate the median.

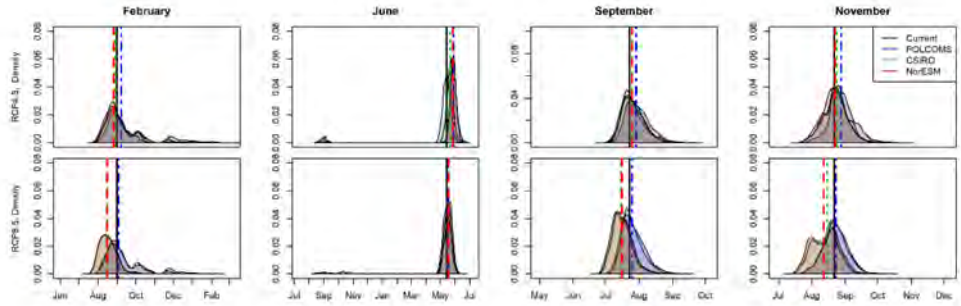

Fig. 2: Density distribution of the harvesting dates for the target size (8g flesh weight) under current (black) and RCP4.5 (top) and RCP8.5 (bottom) conditions for the three climate models in 2046-2055. Vertical lines identify the median of each distribution.

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THE CRITICAL SUCCESS FACTORS OF AN OPEN OCEAN FARMING OPERATION

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Innovasea Systems Inc. delivers a wide array of equipment and services to the aquaculture industry. Offerings range from land-based hatchery/nursery technology, wireless instrumentation collection, and analysis to open ocean farming platforms. Innovasea supports its clients from egg to harvest. With a staff of more than 200, some with 20+ years' experience, Innovasea has witnessed a range of successes through their clients and industry. As a result, the company has identified several critical success factors required for a productive open ocean farm as well as a phased approach business model.

Decisions such as site selection and choice of species in the nascent stages of a greenfield venture will significantly impact managing costs in a commodity price-driven industry. Prudent staffing decisions, adhering to best management practices, reliable sources of feed and finally access to quality fish health support are crucial to averting disasters which can escalate quickly in a hatchery or open ocean farm. Equipment suitable for the environments of the farm is vital to the longevity of the operation. Hatcheries must be designed with the final requirements in mind. Farming equipment must be able to withstand the rigors open ocean yet provide for reliable operations such as stocking, feeding, mortality retrieval, and harvesting.

Finally, the startup must be adequality capitalized to support the inevitable learning curve. Innovasea works with existing and potential clients on a three-stage growth model that enables a phased approach to build out a new farm. The incremental investment provides validation of systems, processes and the market at each phase. Phase one demonstrates site viability, costs, the effectiveness of the team, processes and market demand. Phase two proves the scalability of the farm and market. Phase three provides volume and profitability based on lessons learned in the two earlier phases

NUTRITION MEETS GENETICS: GROWTH COMPARISON OF EIGHT RAINBOW TROUT *(Oncorhynchus mykiss)* STRAINS DEPENDING ON PROTEIN SOURCE OF THREE DIFFERENT EXPERIMENTAL DIETS

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Introduction

In times of often depleted wild fish stocks and increasing consumer awareness, research is being carried out on protein sources that are suitable as an alternative to fish meal in animal feeds for carnivorous fish species (Naylor et al., 2009). However, high proportions of fish meal substitutes, such as plant and insect proteins, can have a negative impact on growth and fish health. Selective breeding of fish populations for adaptation to fish meal-reduced or fish meal-free diets could make a valuable contribution to the more sustainable production of high-quality edible fish. Le Boucher et al. (2012) were able to show that after only one generation of selection for adaptation to pure plant diets in rainbow trout, an improved survival rate and biomass was achieved. In the present study, a communal testing design was used to investigate the impact of different protein sources on growth in the offspring of several rainbow trout strains.

Materials and methods

The offspring (average weight 31.2g; age 262 days post fertilization) of 28 full-sib families from eight different rainbow trout strains were distributed equally among nine uniform tanks. Three experimental groups were fed in triplicates for 90 days with one of three experimental diets. All diets were administered at the same percentage of biomass. The isoenergetic and isonitrogenic experimental diets were based on either fish meal, microalgae meal or insect meal. All experimental fish were individually tagged using PIT-tags. Body weight and length were individually recorded at the beginning and the end of the experiment. Based on the individual weight gain, the performance of families was ranked within the different environments (diets) and examined for an existing genotype-environment-interaction. Breeding values for weight gain were estimated for each sire based on offspring performance. In addition, heritability was estimated.

Results

Preliminary results show high correlation coefficients between the rankings of genotypes (families) within the observed environments (Table I).

On average of all experimental diets, rainbow trout exhibited a weight gain of 84.3g ± 28.4g over the entire trial period. Breeding values indicated significant differences in performance between the families (figure 1), which seemed to occur independently of the experimental diet administered. Estimated breeding values for weight gain ranged from 14.42g to -23.23g. Heritability for weight gain was calculated as 0.38 (standard error = 0.06).

Discussion and conclusion

None of the observed rank correlation coefficients fall below the threshold of 0.8 for a possible genotype-environment interaction as defined by Robertson (1959). These observations suggest that the adaptability to the experimental diets used, with their different protein sources, is well developed and to a large extend regardless of genetics. Thus, breeding values might be considered consistent irrespective of the feed protein source used during performance testing, which would greatly facilitate breeding. The breeding values calculated under this assumption show a clear variability between the families. The investigated genotypes are subject to a pure conservation breeding without any selection - this likely contributed to the high heterogeneity in performance. Nevertheless, the proportion of weight gain attributable to genetics seems high with a heritability of 0.38 (SE 0.06). The type of protein source in the experimental diets used was insignificant for the observed performance levels of the different genotypes. Therefore, fish meal was well substitutable under experimental conditions. The exchange of further components of industrial aquafeeds such as fish oil should be included in the future in order to assess the possibilities of breeding rainbow trout for adaptation to aquafeed which is independent of marine capture fisheries input.

(Continued on next page)

Tab.I. Rank Correlation Coefficients between the rankings of 28 rainbow trout families depending on the protein source used in the diet administered (environments 1 - 3).

	Fish meal	Insect meal	Microalgae meal
Fish meal	1.000	0.895	0.916
Insect meal	0.895	1.000	0.907
Microalgae meal	0.916	0.907	1.000

(All correlation coefficients are significant at $p < .0001$)

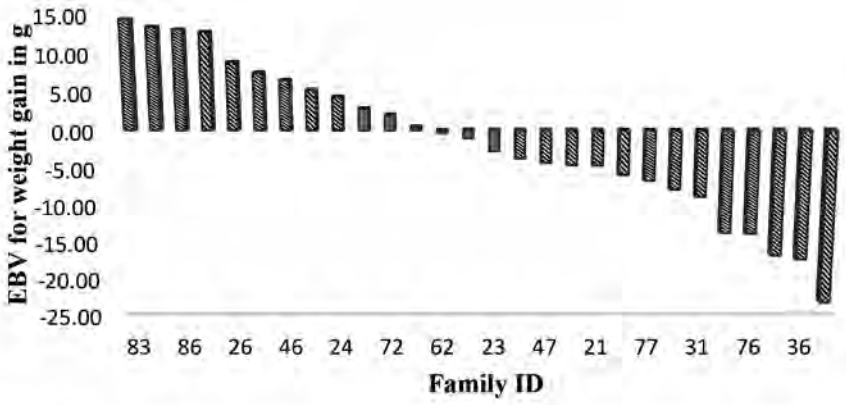

Fig. 1. Estimated Breeding Values for weight gain varying between 28 tested families.

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ENHANCEMENT OF NEUTRAL LIPID PRODUCTION IN MARINE MICROALGAE *Dunaliella tertiolecta* EXPOSED TO SODIUM SELENITE

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Introduction

Constant usage of fossil fuels resulted in considerable ecological and economic problems. Because of the fossil fuels non-renewability, rapid acceptance of renewable energy sources as their replacement is of the pivotal importance. Third generation biofuels, which are also represented by microalgae-derived biodiesel, are a partial solution to the mentioned problems. A direction towards the sustainable development of microalgae-derived biodiesel is development of feasible strategies responsible for microalgal lipid accumulation (Zhu et al., 2016). It has been shown that microalgae have the capability to alter their lipid biosynthetic pathway under unfavorable environmental conditions towards the neutral lipids accumulation (Sharma et al., 2013). The objective of this study was to evaluate the potential application of sodium selenite as neutral lipid inductor in marine microalgae *Dunaliella tertiolecta* which could favor the production of potential microalgae derived-biodiesel.

Materials and methods

Sodium selenite (Na_2Se0_3) stress experiment was performed according to the OECD 201 (2011) guidelines. Microalgae *D. tertiolecta* were grown under different Na_2SeO_3 concentrations (0.0, 1.8, 3.6, 7.2, 14.4, 28.9, 57.8, 115.6 and 231.3µM) 15 days of exposure. Microalgal growth was monitored by optical density measurements (680 nm). Lipid peroxidation was determined in the context of malondialdehyde (MDA) quantity (Pancha et al., 2015), while a Nile red method was used for quantitative measurement of neutral lipids in *D. tertiolecta* (Yilancioglu et al., 2014). Oil bodies (droplets) in algal cells were observed under a fluorescent microscope using blue light as the excitation wavelength

Results

At selenite concentrations up to 28.9µM algal growth rate were similar to the control group during 15 days of exposure. With selenite increase to 57.8µM at the 15th day of exposure algal cell number dropped by 35% compared to control indicating an inhibition in cell growth. The MDA levels were significantly higher in 115.6 and 231.3µM Na_2SeO_3 at the 15th day of exposure (0.39 and 1.39µM OD^{-1}) compared to control (0.22µM OD^{-1}). The highest lipid productivity (6.16 10^5 cell^{-1} day^{-1}) was obtained on 115.6µM Na_2SeO_3 at the 15th day of exposure (Fig 1; a). Neutral lipid content (yellow fluorescence) increased with the increase of Na_2Se0_3 concentration (Fig 1; b).

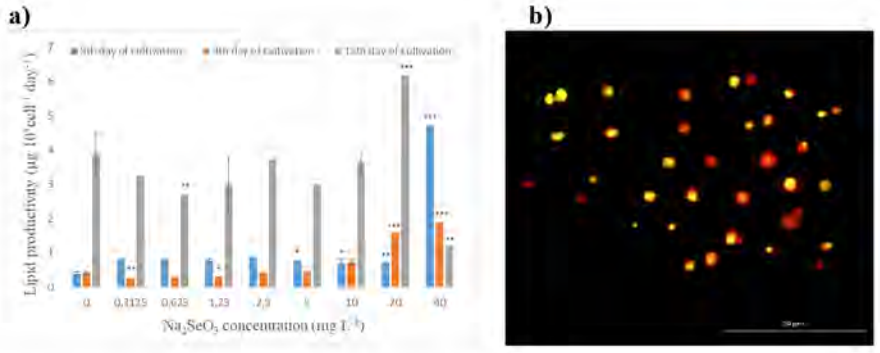

Fig 1. (a) Effects of different sodium selenite (Na_2SeO_3) concentration on neutral lipid productivity of *D. tertiolecta*. (b) Nile Red fluorescence of *D. tertiolecta* with 115.67µM of Na_2SeO_3 at 15th day of exposure. Values are expressed as means±SD. Asterisks indicate a significant difference (*p<0.05, ** p<0.01 and ***p ≤ 0.01).

(Continued on next page)

Discussion and conclusion

Selenium, as an important trace element, acts either as an essential micro-nutrient or as a toxicant which depends on the dose in biological systems (Zhong and Cheng, 2017). This study showed that *D. tertiolecta* in comparison to other tested microalgae (Sun et al., 2014) has different sensitivity to selenium, suggesting that selenium tolerance can vary significantly between different microalgal species (Sabatini et al., 2009). Increased MDA concentration is an indicator of membrane lipid peroxidation which implies an increase of reactive oxygen species concentration. Results obtained using Nile Red method pointed out that exposure of microalgae cells to different concentrations of Na_2SeO_3 had a remarkable effect on neutral lipids accumulation in *D. tertiolecta*. Since lipid accumulation is partly mediated by oxidative stress (Yilancioglu et al., 2014) it can be concluded that oxidative stress induced by Na_2SeO_3 is an effective factor for neutral lipid induction in *D. tertiolecta*, which can be utilized in biodiesel production.

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ANTIOXIDANT POTENTIAL OF NEW MICRO AND MACROALGAE PRODUCTS FOR LIVE PREY ENRICHMENT

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Introduction

Brachionus plicatilis and *Artemia* are commonly used in aquaculture as live food for fish larvae. However, these preys are deficient in long chain polyunsaturated fatty acids (LC-PUFA) and need to be enriched in order to improve their nutritional value. These fatty acids (FA), particularly docosahexaenoic acid (DHA), are essential nutrients for the normal development of several larval tissues, especially nervous system (brain and visual perception). However, they are readily oxidable, being susceptible to suffering lipid peroxidation due to the presence of free radicals causing oxidative stress. During both, early stages of larval development and live prey enrichment protocols, there is a pro-oxidant environment due to the high metabolic activity and the aeration systems, respectively, compromising the appropriate supply of LC-PUFA to fish larvae (Viciano et al., 2017). Algae are rich in omega-3 LC-PUFA and bioactive compounds with antioxidant potential, such as carotenoids. Thus, new potential enrichment products obtained from microalgae and macroalgae were tested in the presence or absence of a pro-oxidative LC-PUFA environment in order to evaluate their potential as enrichment and antioxidant compounds.

Material and methods

Three different enrichment experiments were carried out. In the first one (E1) rotifers and *Artemia* were enriched with a commercial lipid emulsion based on DHA (Incromega DHA500) without supplementation (C) or supplemented with an astaxanthin rich commercial product (NatuRose®; NR); a fucoxanthin extract from *Lobophora variegata* (>97% purity) (FU); or a spray-dried *Isochrysis galbana* (ISD). In the second experiment (E2), different emulsions based on Incromega DHA500 (*B. plicatilis*) or on a DHA-rich marine lecithin (LC-60) (*Artemia*), were assayed without supplementation (C) or supplemented with ISD or three different formats of *Navicula salinicola:* fresh (NFRE), frozen (NFRO), or spray-dried (NSD). Finally, in a third experiment (E3), baker yeast or PhytoBloom Prof were used as controls (C) for *B. plicatilis* and *Artemia*, respectively, adding as experimental enrichment products: *I. galbana* fresh (IFRE), frozen (IFRO) or ISD. After 6 (E1 and E2) or 24 (E3) hours of enrichment, survival rate, peroxides index (PI) (Shantha and Decker, 1994) and malondialdehyde (MDA) content (TBARS) (Ohkawa et al., 1979) were determined.

showed the lowest PI and TBARS (15.05±3.85; P=0.014 and 1.76±0.16; P=0.013, respectively) in E3.

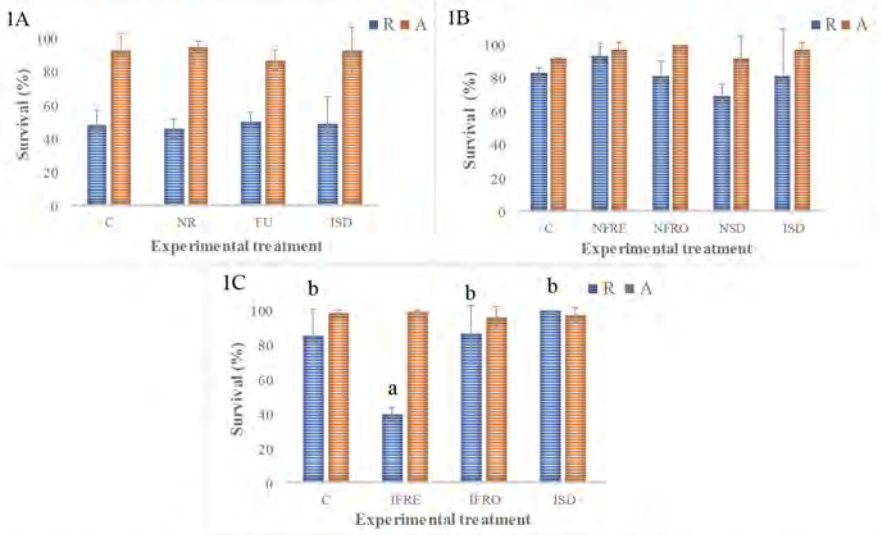

Fig. 1. Survival (%) of *Artemia* (A) and rotifer (R) in E1 (A1), E2 (A2) and E3 (A3). Different letters denote significant differences (P<0.05)

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Results

Regardless of the enrichment experiment, survival of live preys was not affected by the treatment, although it was higher in *Artemia* than in rotifers. Only IFRE treatment negatively affected *B. plicatilis* survival (P=0.001) (Figure 1). Overall, E1-PI ($mEqO_2$ kg^{-1}) was lower in the crustacean (17-41) and surprisingly high for ISD-rotifers (170.27±15.51), whereas E1-TBARS (nmol MDA mg $protein^{-1}$) remained stable between treatments in both species. E2-PI and E2-TBARS did not differ between treatments in *Artemia* (35-44 and 0.84-0.96, respectively) while rotifers given ISD showed the lowest MDA content (1.14±0.90; P=0.001). Interestingly, ISD-*Artemia* showed the lowest PI and TBARS (15.05±3.85; P=0.014 and 1.76±0.16; P=0.013, respectively) in E3.

Discussion and conclusion

According to the obtained survival rates, the new ingredients formats and concentrations assayed as single enrichment products or in combination with lipid emulsions, do not adversely affect the population status compared to the control commercial treatments. The generally lower survival rate of E1-rotifer could be related to the higher culture density used in this trial compared to that of E2 and E3 (470 *vs* 200 and 87 ind mL^{-1}, respectively). Both PI and TBARS were lower in *Artemia* than in rotifers, with the E3 ISD treatment showing even reduced amounts than the commercial product. This is probably due to the higher content of the carotenoid cantaxhanthin in the crustacean (D'Abramo et al., 1983), which may have been acting as an antioxidant in this specie. The main objective of live prey enrichment studies is the improvement of their nutritional value. In this sense, it is much easier to modify the FA profile of rotifers because of theirs passive filter feeder condition, whereas *Artemia* rapidly modifies the dietary FA profile (Viciano et al., 2017). However, the lower PI and TBARS in *Artemia* seems to indicate a better antioxidant defense and a higher potential for the effective use of the micro and macroalgae products of this species. Marine larval feeding experiments employing the best treatments of the current study will be developed in the near future.

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FAMILIAR VARIATION EXPLAINS REDUCED PROTECTION OF COMMERCIAL VACCINES AGAINST BACTERIAL PATHOGENS IN ATLANTIC SALMON

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Introduction

Vaccination is considered crucial in world aquaculture for being one of the most important methods of preventing and controlling illness. Every year, millions of farmed salmon are vaccinated to prevent different bacterial and viral diseases. However, the control of some infections still remains the main problem affecting the top salmon producing countries: Norway, Canada and Chile. Piscirickettsiosis has been infecting Chilean salmon for almost 40 years and is currently considered the main bacterial threat to this industry. *Piscirickettsia salmonis* is an intracellular bacterium that vaccines have failed to control in Chile. *P. salmonis* causes hematocrit decrease, pale liver, pale and enlarged kidneys and spleen, and necrosis of all lymphoid tissues, resulting in high mortality rates.

There are 33 vaccines commercially available against piscirickettsiosis including, inactivated bacterial vaccines, live attenuated vaccines and vaccines based on recombinant proteins. Protection failure of vaccines against this bacterium has been proposed as the cause of the increased use of antibiotics in Chile. The reason for the failure of these vaccines is currently unknown, but it has been hypothesized to be the result of the decline of circulating levels of antibodies over time and coinfection with sea lice *Caligus rogercresseyi* (Figueroa et al. 2017). In this study, we provide evidence that supports that the effectiveness of a commercial vaccine against *P. salmonis* in Atlantic salmon mainly depends on the host's genetic variation.

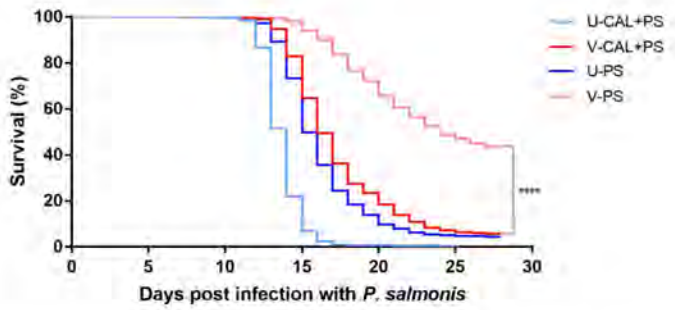

Fig. 1. Atlantic salmon survival curve after challenge with *P. salmonis*(PS) or *C. rogercresseyi*(CAL) plus *P. salmonis* on vaccinated (V) or unvaccinated (U) fish.

Table I. Heritability of resistency against *P. salmonis* and Coinfection *P. salmonis* and *C. rogercressegy* in two populations of Atlantic salmon. For abbreviations see fig. 1.

Population	U-PS	U-CAL+PS	V-PS	V-CAL+PS
1	0.48 ± 0.06	0.23 ±0.06	0.38 ± 0.07	0.65 ± 0.07
2	0.34 ± 0.06	0.26 ± 0.04	0.36 ± 0.07	0.50 ± 0.06

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Materials and Methods

This study was carried out in accordance to the guide for the care and use of experimental animals of the Canadian Council on Animal Care. Fish from two populations were challenged against *P. salmonis* and *C. rogercresseyi* as described by Figueroa et al. (2017). Of the total, 2.926 fish were vaccinated and 2.816 were not. The fish were intraperitoneally vaccinated using two commercial vaccines, one being Pentavalent, vaccine against IPNV (infectious pancreatic necrosis virus), ISAV (infectious salmon anaemia virus), *Aeromonassalmonicida*, *Vibrio ordalii*, and *P. salmonis*; and the other, Live attenuated vaccine against *P. salmonis*. Control fish were injected with PBS (phosphate-bu fered saline).

Results

Preliminary results showed that vaccinated fish challenged with *P. salmonis* (V-PS) had an optimal RPS_{60} of 84.8 % and a mortality of 9.8 % at day 16 post infestation. Conversely, vaccinated fish under a coinfection challenge (V-CAL+PS) had a RPS_{60} of 89.1 % and a mortality of 5.3 % at day 13 post infestation (Fig. 1). Nevertheless, at the end of challenge at day 27, the RPS and mortality rate dramatically decreased for V-PS (41.2 % and 56.3 %, respectively) and for VCAL+PS (5.8 % and 94.2 %, respectively, Fig. 1).

We state that there is high family variability in the response to vaccination in two domesticated populations of Atlantic salmon as illustrated in table 1. A positive genetic correlation but of medium magnitude was observed between natural resistance and resistance mediated by vaccines (range rg= 0.30-0.60), so that both processes are not totally synergistic as previously hypothesized. Low response to vaccination was associated with high mortality, lower growth, higher bacterial loads, and higher clinical signs, particularly when fish were co-infected with the sea lice *Caligus rogercresseyi*. Conversely, high response to vaccination was associated with low mortality, lower bacterial load, better growth performance and reduced clinical signs of the disease.

Discussion

The manufacture of vaccines for salmon should move towards a strategy of precision medicine, including the genetic variation of the host as a key element for the development of effective vaccines against *P. salmonis*.

Acknowledgements

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ROBUST SALMON SKIN – IS THERE A GENETIC COMPONENT?

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Introduction

Wound related problems account for large losses in Norwegian Atlantic salmon aquaculture and is commonly linked to fish management procedures or pathogens, including lice (Takle et al. 2015). The delicate outer mucosal barrier of fish is made of living cells and is easily damaged during mechanical handling, which may facilitate entry of pathogens or cause osmotic problems. Fish skin morphology and function is affected by more than 500 genes in zebrafish (*Danio rerio*) (Li et al. 2011), but little is known about this for Atlantic salmon. Minuscule damage to the outer epithelial layer of the skin has previously been linked to winter ulcers in salmon, caused by *Moritella viscosa*. In this study we have investigated the effect of genetics on wound healing and resistance to this bacterium, in order to improve skin robustness in Atlantic salmon.

Materials and methods

A total of 1800 Atlantic salmon (mean weight of 105g) from the AquaGen breeding nucleus were transported from Kyrksærterøra to VESO Vikan (Norway) in September 2018. Upon arrival, the fish were distributed evenly to two tanks with 34ppt sea water. After one week of acclimatization, all fish in tank 1 were anaesthetised and a 2.75 mm circular wound was made in their tail fin with an ear punch pincer (considered a gentle handling procedure). The fish were left to heal for one week before images of the wounds were captured with a stereo microscope. All fish in both tanks were subjected to a *Moritella viscosa* bath challenge test (Karlsen et al. 2017) 16 days post arrival. Dead and moribund fish were collected from the tanks daily, and the challenge test was terminated after 20 days.

Wound healing (tank 1) was measured as the difference in size between the original hole and the hole after 7 days (Fig. 1) and resistance to *Moritella viscosa* (both tanks) was measured as survival time (time until death) after the onset of the challenge test. In total 1645 fish were genotyped, of which 884 were from tank 1 and 761 from tank 2. The parents of these fish had previously been genotyped, enabling calculation of heritability for the di ferent traits.

Results

Wound healing and survival time in the challenge test varied significantly between families. Heritabilities were moderately good for wound healing (h^2=0.18±0.06) and for survival time (h^2=0.16±0.06). There were no significant genetic correlations between the two traits. Cumulative mortality during the challenge test was higher (95.5%) in tank 1 than in tank 2 (84.9%).

Discussion and conclusion

Wounds are caused by mechanical injuries and/or pathogens and create welfare issues for farmed fish and economic problems for fish farmers (Takle et al. 2015). The present study showed that even gentle handling increased the pathogenicity of *Moritella viscosa*, as seen by a higher cumulative mortality in tank 1 than in tank 2.

The present study also showed that healing of mechanical wounds and *Moritella viscosa* mortality can be improved by breeding (h^2 0.16-0.18). However, no correlation was found between wound healing and *Moritella* resistance. The results are very promising and will be supplemented with genomic selection, fine mapping and transcriptome studies, and studies of the combined effects of genetic selection, optimal diets and optimal vaccines on improved salmon skin robustness.

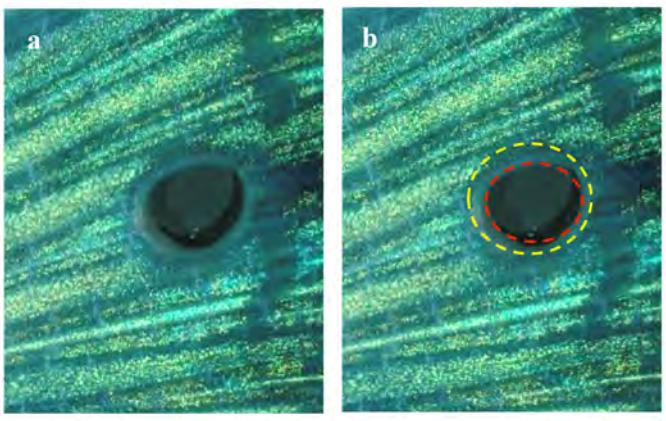

Fig. 1. Photographs showing healing of the wound 7 days after making a hole in the tail fin of an Atlantic salmon (a) and the difference in size between the original hole (yellow line) and healed hole after 7 days (red line).

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MOLECULAR APPROACHES FOR THE GENETIC MANAGEMENT OF OYSTERS ALONG THE SOUTH COAST OF BRAZIL

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Introduction

Oysters represent an important fishery and aquaculture resource worldwide and are an important source of income for the coastal communities. In Brazil, the main native oyster species economically important for fisheries and mariculture are *Crassostrea brasiliana* (= *C. gasar*) and *C. rhizophorae*. Currently, *C. brasiliana* is the one use in aquaculture operations due its higher size. However, the presence of exotic oysters in native oyster banks are jeopardized the native oyster genetic resources. This study aimed at identifying native and exotic species in the estuarine areas of Southeast coast of Brazil as well as to develop a panel of microsatellite loci of *C. brasiliana* by next generation sequencing for further studies on genetic diversity and population structure of this native oyster.

Material and Methods

The oysters were collected in mangrove roots and rocks in three estuaries of Southeast Coast of Brazil (Paraty, Bertioga and Cananeia). Due the high plasticity phenotypic in oyster, we used 16S and COI sequences and PCR-RFLP to identify unambiguously the different oyster species. The DNA of *C. brasiliana* was employed to develop microsatellite markers by next-generation sequencing (NGS). A genomic library was constructed following the manufacturer protocol of Illumina® Nextera DNA Library Preparation kit and the sequencing was conducted in a HiSeq2500 (Illumina, San Diego, USA). The reads were analyzed in *CLC*^{Bio} software with seven covers, generating contigs above 300bp. The reads were cleaned with *Seq Clean* and *Perl Script-Velvet* tool. Microsatellite loci were identified from dataset by *BatchPrimer3 V.10.* Primer sets were designed in the same software and selected using *OligoAnalyzer 3.1 IDT*. After selection, the PCR conditions were standardized for each pair of primer.

Results

Among 233 samples collected in the three estuaries, 55 were *C. brasiliana,* 54 *C. rhizophorae* and 124 were *Saccostrea* sp., an exotic species. *C. brasiliana* was found nor in Bertioga neither in Paraty. *Saccostrea* sp. comprised 92%, 65% and 15% of the sampled individuals collected in Paraty, Bertioga and Cananeia, respectively, while *C. rhizophorae* 8%, 35% and 41%. The PCR-RFLP using the *Alu*I enzyme was effective to discriminate *C. brasiliana* and *C. rhizophorae*, two cryptic species, while *Saccostrea* sp. was easily identified by its shell's morphological characteristics. The largest fragment generates by *Alu*I have 410bp for *C. rhizophorae* and 205bp for *C. brasiliana*

Microsatellite markers were isolated and selected from a genomic library obtained by NGS. After a first selection, we identified 359 loci, of which 157 are dinucleotides, 45 trinucleotides and 157 tetranucleotides. The dinucleotides motives more abundant were AG/TC with 72%, followed by AC/TG (16%) and AT/TA (12%). Among trinucleotides prevailed the AAT/TTA motif with 53%. The repeat number ranged from 6 to 34, 4 to 8 and 3 to 9 for di, tri and tetranucleotides, respectively.

A set of 30 primers were selected, 10 for each repeat type (di, tri and tetranucleotides) based on size of PCR product, primers size, melting temperature, content of CG and formation of secondary structures. Thirty primers were synthesized and the conditions for PCR were established for each pair of primer (annealing temperature 56 – 60ºC and $MgCl_2$ concentration 2,0 – 2,5 mM).

Discussion and conclusion

The first record of *Saccostrea* sp. date on June 2014, in Bertioga, SP (Galvão et al., 2018). Over the last past years, this species has expanded along the coast as shown herein. *C. brasiliana*, otherwise, has decreased in places where it used to be abundant as in Bertioga and Paraty locations. In Cananeia, a relevant area for native oyster's production, local fisher and oyster farmers reported the presence of *Saccostrea* sp. in the later 2017. These facts raise the concern about its invasive potential, becoming a threat to native populations.

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A rapid cutback of population size has serious consequences on population health and survival. This reduction may induce the loss of genetic diversity (Frankham et al., 2014).

The impact of the expansion of this exotic species in the long-term survival of the native oyster should be investigated. Against this background, this new novel of microsatellites (SSR) markers will be useful to assess genetic variability in remaining populations.
The PCR conditions were standardized for 30 loci SSR developed by NGS in the present work. After polymorphism analysis in a capillary sequencer 4300 *DNA Analyser Li-Cor* and validation of selected primers, they will be used to evaluate the genetic diversity and structure of wild populations of *C. brasiliana* oysters of the Southeast coast of Brazil.

This study will contribute for further studies on hatchery production associated with genetic improvement programs as well as in restoration programs (Hornik and Plough, 2019). As verified in this work, *C. brasiliana* has been reduced in number and almost disappeared in places where it was abundant in the past. Therefore, the reestablishment of the natural population with their genetic variability will be crucial for the continuing sustainable use of their marine genetic resources.

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BIOFILM AS TOOL TO EVALUATE ORGANIC AND CHEMICAL DISPERSION FROM MARINE FISH FARMS

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Introduction

Marine biofilms are organized communities of mixed micro-organisms, typically surrounded by a matrix of extrapolymeric substances (EPS) which facilitates the attachment of the community to any surface (Characklis and Marshall, 1990). Diatoms and bacteria constitute the major components of biofilms occurring in the marine environment. The moment a clean surface is submerged in the sea, biofilm-forming micro-organisms rapidly colonize and form highly complex, dynamic three-dimensional (3D) surface structures (Davey and O'Toole, 2000). Using these communities on artificial surfaces facilitates the direct comparison between sites without confounding environmental and physical variables (Webster and Negri, 2006). The analysis of biofilms in test substrates enables medium term (days) rather than momentary states of the studied ecosystem (Brummer et al., 2003). The aim of this work was to: (1) quantify fish farm particulate organic carbon, particulate organic nitrogen and total phosphorus, and (2) to correlate them with the structural, trophic and element accumulation changes in the biofilm community at an o fshore fish farming zone from Mediterranean

Materials and methods

The Western Mediterranean case study of the TAPAS Project Horizon 2020, offshore Fish Farming Zone of San Pedro del Pinatar (Murcia, Spain), is one of the most aquaculture intensive areas in Spain of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) production, with an average production of around 11.000 tonnes per year. The fish farming is exclusively carried out in open sea cages. The changes in the biofilm community due to organic matter enrichment and trace elements contamination derived from fish farming were studied. The biofilm biomass and chlorophyll *a* were quantified along an environmental gradient of fish farm wastes. Total organic carbon (TOC), total organic nitrogen (TON), total phosphorus (TP), trace elements and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were measured. The field assays were performed using glass slides as the artificial substrate for biofilm community development. Glass slides were supported by slide holders. The slide holders, in turn, were maintained 3 m below the water surface by an anchoring system and a buoy. Particulate sedimentation rates were measured by means of sedimentation traps composed of four attached cylinders (100 cm height and 12 cm diameter). Each cylinder had a funnel at the bottom, which guided the particulate matter into a 250 ml polyethylene tube. Biofilm slides and sediment traps were deployed from fish cages located at the edge of the fish farms facility along horizontal transects at 0, 25, 75, 175, and 650 m from the fish cages.

Results

Our results indicate that organic pollution influenced carbon and nutrient accumulation of biofilms as it has been previously reported. PCA analysis grouped stations following the environmental gradients, indicating that the accumulation pattern of trace elements in biofilm was consistent along the fish farm influence. Cu, Zn and Cd were the main metals released to the environment due to fish farm, which had similar accumulation dynamics as TOC, TON and TP along the environmental gradients. Concentration of these chemicals in biofilms showed higher values for samples close to the fish cages. The isotopic signatures clearly differentiated the biofilm communities along transects (Fig. 1). Diatom abundance, biomass,

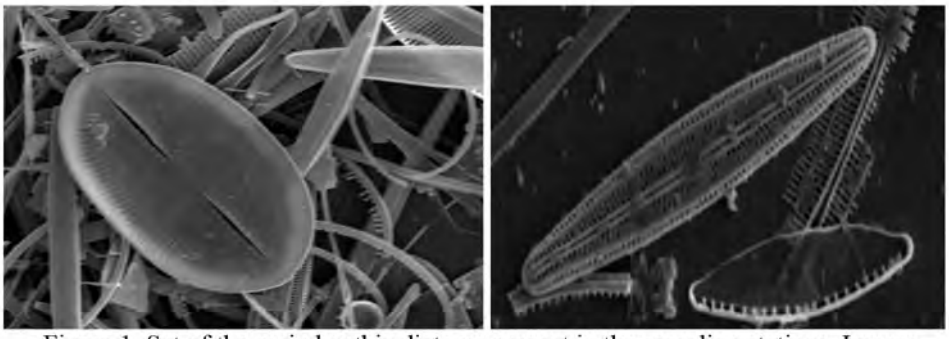

Figure 1. Set of the main benthic diatoms present in the sampling stations. Images captured by scanning electron microscope

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species richness and H´ showed a very similar trend with distance, greatly increasing close the fish farm and stabilizing further from the fish farm. The nMDS plot of diatom assemblages showed an environmental gradient between sampling stations, and that the within-station similarity among samples increased with distance from the fish farm. The regression model showed that the community structure descriptors reached their asymptotic point further from the fish farm than the POC, PON and TP sedimentation rates.

Discussion and conclusions

Benthic diatoms that grow on hard substrates represent the type of community and, therefore, are potential bioindicators (Desrosiers et al., 2013). The results of this study suggest that benthic diatoms are potential indicators to detect the emission of antibiotics in the marine environment. Therefore, it is suggested the incorporation of benthic diatoms incubated in hard substrates in coastal waters monitoring programs, as they are a simple and easy method to evaluate changes in water quality.

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STUDY OF SEDIMENT ALONG AN ENVIRONMENTAL GRADIENT TO EVALUATE THE IMPACT OF MARINE FISH FARMS

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Introduction

In recent decades, seafood production from marine aquaculture has undergone almost exponential growth worldwide in terms of cultured biomass and is expected to follow the same trend in the future (FAO, 2007). A combination of factors, including production levels, feed characteristics and feeding efficienc , influences the quantity and quality of the wastes released by fish farming (Islam, 2005). The main negative impact of finfis aquaculture is the resulting organic enrichment derived from these wastes, which mainly consist of fish faeces and uneaten food and which may spread from tens to hundreds metres from the fish farm (Brown et al., 1987, Hall et al., 1990, Iwama, 1991). Such wastes take two forms: particulate and dissolved. In the water column, the levels of dissolved wastes rapidly reach background levels, whereas particulate wastes tend to sink and accumulate on the seabed. This process may produce important changes in sediment geochemistry and in the benthic communities (Brown et al., 1987, Weston, 1990, Karakassis et al., 2000, Holmer et al., 2005).

Materials and methods

The Western Mediterranean case study of the TAPAS Project Horizon 2020, offshore Fish Farming Zone of San Pedro del Pinatar (Murcia, Spain), is one of the most aquaculture intensive areas in Spain of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) production, with an average production of around 11.000 tonnes per year. The fish farming is exclusively carried out in open sea cages. The changes in the biofilm community due to organic matter enrichment and trace elements contamination derived from fish farming were studied (Fig.1). Sampling stations were located close to the mooring system of the sediment traps (~2 m) used for measuring waste dispersion (0, 20, 120 and 600 m). All distances were sampled once during the period that the sediment traps were deployed, taking four replicates at each sampling station. The top 2 cm of the sediment were used for the physico-chemical analyses. Macrofaunal samples were taken using a hand grab (400 cm2) that penetrated to a depth of ~10 cm.

From the cores taken at each sampling station the following parameters were measured. Four cores were used to measure the redox potential with an Orion ORP 91-80 electrode that was previously calibrated using a redox buffer solution (220 mV at 25 °C). The same cores were used to measure the rest of the physico-chemical parameters. Sediment grain size was assessed by dry-sieving with a mechanical shaker through a series of sieves (2, 1, 0.5, 0.25 and 0.064 mm mesh), in accordance with the Wentworth scale (Wentworth, 1922). The fine fraction of the sediment (i.e. silt/clay) was taken as the sediment percentage that passed through the 0.064 mm mesh. The organic matter content was measured by weight difference after heating the dried sediment at 450 °C for 5 h. POC, PON and TP percentage of the sediment were determined following the same protocol as used for the samples from the sedimentation traps.

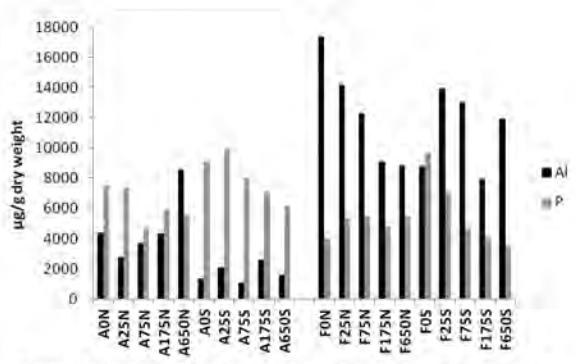

Figure 1. Al and P concentration in the sediment measured along an environmental gradient of two fish farms

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Macrofauna was used as a surrogate of the ecological benthic status. For the macrofaunal analysis, sediment samples were washed through a 1 mm sieve with sea water. The remaining sediment was fixed in a 4% formalin buffered solution, separated into major faunal groups and stored in a 70% alcohol solution for later identification. The determination of benthic groups was made to the lowest possible taxonomic level using a binocular dissecting lens. Macrofauna ash-free dry biomass was determined separately for each of the four sediment samples taken for the macrofaunal analysis at each station by weight difference after drying to constant weight at 60 °C and subsequently heating at 450 °C for 5 h.

Results

Trace elements on sediment below an open water fish farm were obtained. Fish farming modified the benthos not only in a physico-chemical and biological way, but also functionally, lowering the number of trophic groups and the evenness among them.

Discussion and conclusions

The present study demonstrates that fish farming not only influences physico-chemical and biological parameters but also alters the functioning of the ecosystem from a trophic point of view, affecting mainly the grazers and the evenness among the trophic groups.

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REGULATION OF DIGESTIVE AND ABSORPTIVE PROCESSES IN TWO MEDITERRANEAN FISH: GILTHEAD SEA BREAM AND SEA BASS

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Introduction

One of the greatest costs incurred during fish aquaculture is that of the feed, especially in carnivorous fish species. Replacing fish meal and oil by plant feed affects digestive and absorptive processes and has an impact on growth. Therefore, different ingredients of plant origin have been tested for decades. Better knowledge of how those processes are regulated would help in the design of high-quality diets leading to optimal growth and food efficiency; minimizing food loss and contributing to sustainable aquaculture. In this communication, we report different compensatory mechanisms in sea bream and sea bass that we believe could help maintain growth in response to changes in raw materials, time of food administration, dietary composition and rations.

Material and methods

Gilthead sea bream and sea bass at different stages of growth (from juveniles to adults) were fed diets in which fish meal or oil were substituted for plant-based ingredients in different percentages (from 38% to 100%) and under different culture conditions. Activity of digestive enzymes (pepsin, alkaline protease, amylase and lipase) was analysed, the trypsin/chymotrypsin activity ratio was characterized, activity of enzymes present in the intestinal membrane (aminopeptidase and alkaline phosphatase) was measured, and the capacity for absorption of essential and non-essential amino acids and D-Glc in different regions of the intestinal tract (pyloric caeca, proximal intestine and distal intestine) was determined under both post-prandial conditions and 24 h after ingestion. The SPSS Statistics 22.0 software was used to perform statistical analysis.

Results and discussion

Gilthead sea bream and seabass are capable of modulating their digestive and absorptive processes in such a way that their growth is maintained when they are fed diets in which protein of fish origin is replaced by either plant proteins or lipids and starch, as long as the diet meets their nutritional requirements and the animals can tolerate the antinutritional factors present (García-Meilán, 2015). The mechanisms reported for this modulation are dependent on the main composition of the diet, the ingredients used in its formulation and the quantity in which it is supplied, as well as the administration method (satiety, controlled or restrictive) (García-Meilán, 2015). Here, we report three levels of regulation of the digestive and absorptive processes: 1) fish modulate intestinal digestive activity and nutrient absorption capacity, 2) they regulate the increase of pancreatic enzyme synthesis and secretion and 3) as necessary, they increase voluntary feed intake.

Proteolytic activity is affected by an excess or deficit of protein in the diet, which causes an increase in anticipatory activity, and may alter the trypsin/chymotrypsin ratio during digestion (García-Meilán et al., 2013; 2016b). Modifications of this ratio affect the availability of certain amino acids, dipeptides and tripeptides, the capacity the fish have for the absorption of which is up-regulated in different intestinal regions; with up-regulation of distal absorption capacity being an indicator of a compromised situation for the fish (García-Meilán et al., 2016a, b). Proteolytic activity is also affected by increased lipid and starch content of the diet: lower protease activity while the trypsin/chymotrypsin ratio is unaffected (García-Meilán et al., 2014; 2016b).

α-Amylase activity is less sensitive to changes in diet composition than protease; although it also responds to them. Thus, a diet with a low starch content causes an increase in the synthesis of α-amylase and in its activity, possibly as a compensatory mechanism to balance the low availability of glucose. This mechanism is normally accompanied by an increase in the capacity to take up this monosaccharide (García-Meilán et al., 2013; 2014; 2016b).

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Modulation of lipase activity due to dietary composition is more controversial. Some experiments show an increase in this enzyme activity depending on lipid or fibre content and due to a high protein content (García-Meilán et al., 2013, García-Meilán, 2015); while other experiments produce no changes in lipase activity. Meanwhile, fish fed low-energy diets showed an increase in lipase synthesis and anticipatory activity, accompanied by up-regulation of capacity to absorb D-Glc and L-Ala. In diets with a very low lipid content (12%), even the α-amylase activity increased depending on fis development and energy requirements (García-Meilán et al., 2016b).

It has also been found that length, morphology and intestinal histology are strongly influenced by both diet and eating habits, affecting the activity and distribution throughout the intestine of nutrient absorption mechanisms. Thus, the presence of plant ingredients or the general composition of the diet tends to increase intestinal transit rate. In such a situation, sea bream digestion processes are improved by an increase in the anticipatory release of pancreatic enzymes. This compensation mechanism may be accompanied by a post-prandial increase in enzyme release and even an increase in the relative intestinal length. Despite the increase in intestinal transit, all this allows the absorption of nutrients necessary to maintain growth, while saving protein whenever possible (García-Meilán et al., 2013; 2014; 2016 a, b).

Moreover, in sea bream, dietary inclusion of highly digestible carbohydrates improves digestion and absorption processes when they are administered in the morning, leading to a protein-sparing effect and producing growth comparable to that of fish fed with a commercial diet (García-Meilán et al., 2014). In addition, it has been reported that in gilthead sea bream the pH of the intestinal content, digestive activities and nutrient absorption capacities all decrease as the animals grow (García-Meilán et al., 2016a).

Finally, the dietary fatty acid profile also modulates digestive and absorptive processes. Juveniles are not capable of adapting their digestive or absorptive activities to changes in this profile in the short term (4 weeks), while adult animals can adapt (García-Meilán et al., 2016a).

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BENEFITS FROM WITHIN-FAMILY GENOMIC EVALUATION IN AQUACULTURE SCHEMES WITH SMALL FAMILY SIZES

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Introduction

Many economically important traits in aquaculture selection programmes, such as disease resistance and slaughter traits, cannot be directly recorded on the selection candidates. For these traits, recording is typically performed on the sibs of the candidates (and so they are known as sib traits). Selection is based on sib performance and thus, the within-family variation is not taken into account when phenotypic or standard BLUP evaluation are performed. In contrast, with genomic evaluation, selection can take into account both between and within family variation and thus the accuracy of evaluation and the response to selection can be increased (Meuwissen et al., 2001; Nielsen et al., 2009). However, given the large number of selection candidates and tested individuals involved in aquaculture breeding programmes, standard population-wide genomic evaluation may be too expensive to be applied if the low economic value of selected fish is considered.

A particular cost-efficient genomic evaluation approach for aquaculture breeding programmes proposed by Lillehammer et al. (2013) is within-family genomic evaluation (WFGE). With this approach only the within-family genetic component of the breeding value is estimated using molecular information. Then the estimated within-family component is combined with an estimate of the family breeding value that can be obtained from traditional BLUP. With WFGE, the marker density required for obtaining higher accuracies than with BLUP is much lower than the density required for population-wide genomic evaluation, given the high levels of linkage disequilibrium that exist within families, reducing thus the genotyping costs.

Using computer simulations, Lillehammer et al. (2013) found up to 15% of benefits with WFGE (using 50 or 100 SNPs per Morgan) than with BLUP for salmon breeding programmes with large family size (200 fish per full-sib family). However, given that the genomic evaluation is performed only within families the accuracy of this approach would depend on the family size that for species other than salmon is usually much smaller.

The objective of this study was to predict, through stochastic simulations, the genetic gain for schemes with small family sizes when WFGE is applied. Selection was for two traits including a candidate and a sib trait that mimicked growth and disease resistance, respectively.

Material and methods

The base population was created in a two-step process. Firstly, a large population in mutation-drift equilibrium was generated by randomly selecting 500 males and 500 females across 4,000 discrete generations. The genome was composed of 20 chromosomes and the total genome size was 15 Morgans (approximates that of seabream, seabass and turbot). Each chromosome carried 10,000 evenly spaced biallelic loci that included 10, 50 or 100 SNPs per Morgan. Initial frequencies were 0.5 for all loci. Once the equilibrium was reached, 200 males and 100 females per replicate were sampled and mated (each female with 2 males) at random to create a base population with family structure.

Table I. Cumulative genetic gain for the index at generation five from BLUP and within-family genomic evaluation (WFGE) using different number of SNP markers per Morgan (n_{SNP}), for schemes with different numbers of tested fish for the sib trait (n_{ts}).

		WFGE		
n_{ts}	*BLUP*	$n_{SNP} = 10$	$n_{SNP} = 50$	$n_{SNP} = 100$
5	3.90	3.95	3.97	3.98
10	4.01	4.08	4.11	4.10
20	4.24	4.30	4.35	4.39
40	4.38	4.44	4.49	4.53

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Two uncorrelated traits were simulated and both were affected by 1,000 loci that were randomly sampled from all loci at t = 0. The initial phenotypic variance (V_p) was 1 for both traits and the heritabilities (h^2) were 0.4 and 0.2 for growth and disease resistance, respectively. Phenotypic values were obtained by adding a normally distributed individual environmental effect with mean zero and variance V_E to the genetic value. All selection candidates were recorded only for growth. The disease resistance trait was recorded on the sibs and the number of sibs tested varied across scenarios (5, 10, 20 or 40 per family). Thus, the total number of fish created per generation was 6,000 (the selection candidates) plus 1,000, 2,000, 4,000 and 8,000 fish recorded for disease resistance

Two different evaluation methods were considered: standard BLUP and WFGE. In WFGE schemes, EBVs are combinations of a conventional family estimated breeding value and a genomic within-family breeding value. In particular, the EBV for individual i of family l is $EBV_{il} = ½\ EBV_{si} + ½\ EBV_{di} + w_{il}$. The composite term ($½\ EBV_{si} + ½\ EBV_{di}$) was estimated as the average EBV for family l obtained from standard BLUP using the full data set. The within-family breeding value w_{il} was obtained from SNP-BLUP evaluation using only information from family l. Different scenarios varying in the density of markers (10, 50 and 100 SNPs per Morgan) used in WFGE were considered. The GS3 software (Legarra et al., 2011) was used for carrying out genomic evaluations.

The EBVs for growth and disease resistance were combined in a selection index that gave the same weight to both traits. Each generation the 200 males and the 100 females with the highest index value were selected. Each selected female was mated with 2 males producing 30 fish per mating (i.e., the schemes had 200 full-sib families). Selection was practised for 5 discrete generations. A total of 100 replicates were simulated.

Results and discussion

Table I shows the cumulative genetic gain for the index obtained from both evaluation methods. Increasing the number of markers used in WFGE had a very small effect in genetic gain. In agreement with the results of Lillehammer et al. (2013), 50 SNPs per Morgan seem to be enough to fully benefit from this genomic method. Doubling the marker density from 50 to 100 SNPs per Morgan increased genetic gain by less than 1% at t = 5. The cumulative genetic gain for the index increased when the number of tested sibs increased. The benefit of WFGE over BLUP was 3 - 4% at t = 5.

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EFFECT OF CITRONELLA ESSENTIAL OIL (*Cymbopogon nardus*) ON GROWTH AND METABOLISM OF GILTHEAD SEA BREAM (*Sparus aurata* L.)

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Introduction

Modern aquaculture tried maximizing productive yield of the installation, but usually induces chronic stress that affected growth, immunological status and reproduction of the cultivated specimens. In addition, the use of antibiotics in aquaculture has become abusive inducing a negative impact on the environment and generating antibiotic-resistant pathogens. For this reason, the inclusion of additives in cultivated fish food are been proposed to compensate stress states as well avoid antibiotic use. The essential oil extracted from *Cymbopogon nardus* has a high content of geraniol and citronellal. Citronellal is used as a basic material for the synthesis of important chemical compounds called ionones. This oil presents insect repellent activity, fungicidal and bactericidal action.

The objectives of this study are to determine the effects of inclusion of *C. nardus* essential oil in the gilthead sea bream (*Sparus aurata*) food, with the following specifics objectives: i) to assess its effect on growth, and ii) to establish its influence on metabolism. In addition, our study also evaluated the antimicrobial potential of this essential oil as a method of control of bacterial diseases in aquaculture.

Materials and methods

Immature juveniles of gilthead sea bream (*S. aurata*, N = 162, 10,2 ± 0,3 g body mass) were provided by *Servicios Centrales de Investigación en Cultivos Marinos* (SCI-CM, CASEM, University of Cádiz, Puerto Real, Cádiz, Spain; Operational Code REGA ES11028000312) and were randomly distributed into nine 80-L tanks (for each tank N = 18), with flow-through water system at 18-19 °C, under natural photoperiod (May-July 2017). Three feeds containing different doses of citronella oil were tested: i) control, ii) dose 1.0ml.kg^{-1} feed, iii) dose 2.0ml.kg^{-1} feed in triplicate during 60 days. All fish were fed a daily ration of 2.0 % body mass distributed in two meals. Biometric analyzes (length and weight measures) were performed every 15 days, and, at the end of the experiment, blood and liver samples were collected and the weight of the liver was taken. Hepatosomatic index was determined as: HSI = 100*(liver weight/body weight). Plasmatic and hepatic metabolites levels were performed. Differences were tested by one-way ANOVA ($p<0.05$). To determine the *in vitro* antimicrobial potential of citronella essential oil its capacity to inhibit three fish pathogens (*Vibrio harveyi*, *Photobacterium damselae subsp. piscicida* y *Aeromonas hydrophila*) was evaluated. The potential toxicity was calculated by the observation of the decrease of the bioluminescence of *Vibrio fischeri*.

Table I: Biometric, plasmatic and hepatic parameters assessed in different experimental groups. Different letters indicated significant differences (one-way ANOVA ($p<0.05$).

	Parameters	Control	Dose 1	Dose 2
Biometric	Initial length (cm)	10.4 ± 0.05	10.4 ± 0.05	10.3 ± 0.05
	Final length (cm)	13.3 ± 0.07	13.2 ± 0.09	13.1 ± 0.07
	Initial weight (g)	17.2 ± 0.3	18.5 ± 0.3	16.9 ± 0.3
	Final weight (g)	44.8 ± 0.9	44.5 ± 1.0	44.2 ± 0.8
Plasma	Glucose (mM)	3.1 ± 0.2	3.6 ± 0.2	3.3 ± 0.2
	Triglycerides (mM)	2.4 ± 0.1^{a}	2.1 ± 0.1^{b}	2.0 ± 0.2^{b}
	Lactate (mM)	2.2 ± 0.3	1.4 ± 0.1	1.1 ± 0.1
	Hematocrit (%)	32.2 ± 2.0	29.1 ± 1.3	28.3 ± 1.3
	Hemoglobin (mg.dl-1)	3.1 ± 0.3	3.1 ± 0.2	3.2 ± 0.1
Liver	Glycogen (µmol.gww-1)	285.4 ± 29.5	267.6 ± 20.5	248.9 ± 20.6
	Glucose (µmol.gww-1)	36.3 ± 3.8	37.9 ± 4.5	41.2 ± 1.9
	Triglyceride (µmol.gww-1)	$7.2^{a} \pm 0.5$	$3.9^{b} \pm 0.4$	$5.1^{b} \pm 0.6$
	HIS (%)	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1

(Continued on next page)

Results and discussion

The addition in the feed of citronella essential oil was tolerated by *S. aurata* specimens without affecting (positively or negatively) growth parameters. Also, the addition of this essential oil did not cause alterations or significant changes on metabolism of *S. aurata* specimens (Table I).

However, this oil appears to modulate lipid metabolism, reducing plasma and hepatics levels. Similar data have been found in *Oreochromis mossambicus* fed with ginger oil (Immanuel et al., 2009) and in *O. niloticus* fed with essential oil of *Citrus sinensis* (Metwally, 2009) and garlic oil (Acar et al., 2015). Our results suggest that the inclusion of citronella oil could be beneficial by modulating lipid metabolism

The citronella essential oil inhibited the bacterial growth of *V. harveyi*, *P. damselae subsp. piscicida* at a concentration of 31.25µl.ml.-1 and *A. hydrophila* at 62.5µl.ml.-1. The median effective concentration (EC50) to inhibit the bioluminiscence of *V. fischeri* was 0.766µl.ml.-1. The *in vitro* tests confirmed that the citronella essential oil possess antimicrobial activity against several fish bacterial pathogens (Singh et al., 2011), although it is necessary to carry out *in vivo* studies to verify if these potential remains without generate adverse effects on fish

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INHIBITION OF GONADAL DEVELOPMENT OF YELLOW PERCH, *Perca flavescens*, GROWN AT CONSTANT TEMPERATURE AND PROLONGED PHOTOPERIOD

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Introduction

Yellow perch, *Perca flavescens* (Michill, 1814), is one of the most abundant species of the *Percidae* family in the inland waters of the northern regions of the North American continent. It is a highly valued food fish in the United States with additional markets in Canada and Europe. Yellow perch commands a high market value for meat texture and taste, low percentage of fat and phospholipids, long shelf life, resistance to freezer damage and for its traditional popularity around the Great Lakes region. The market demand is for product of only a 150 gram harvest weight. The reduction in the wild catch due to overfishing and devastation of the natural habitat (Clapp and Dettmers, 2004) has resulted in an increased interest in research of the biology of this species, and the development of appropriate culture technologies. To date, significant impediments have limited the commercial production of yellow perch in the United States. The limited duration of seasonal optimal ambient conditions for pond and cage culture in the Great Lakes region was found to be unacceptable for commercial production. Grown at ambient temperatures, most of the females of this fish reaches sexual maturity at the end of the second year, when their medium weight is greater than 60g. Males can be sexually mature by the end of the first year, while weighing only about 15g (Malison, 1999). The development of the gonads has a negative effect on somatic growth, as also reported in the European perch (Rougeot, 2003). Controlled conditions within recirculating systems, without seasonal temperature fluctuations, enhance growth and inhibit sexual maturation (Melard et al., 1996). The prolongation of the daylight period also inhibits the development of the gonads, especially in males (Jourdan et al., 2000). The aim of this study is to identify and quantify the advantages of controlling photoperiod and temperature to suppress the gonadal development of yellow perch.

Materials and Methods

Yellow perch fingerlings were raised from 16.5 grams to market size in an indoor recirculating system. For the purpose of this study, a total of 6000 fish were divided into two separate tank units. Fish were raised at constant temperature of 24-25^{o}C. Dissolved oxygen level was maintained at saturation using oxygen injection. Calcium hardness was maintained between 80-150 mg/l using calcium chloride, and pH was maintained between 7.2 and 7.4 with the addition of sodium bicarbonate. During the entire experimental period perch were fed continuously with commercial slow sinking pelletized feed ("Ziegler Brothers", PA), containing 45% of crude protein and 12% fat, using automatic belt feeders. Fish were fed 5% of their body weight per day in the first month, 4% in the second, 3% in the third and fourth, 2.5% in the fifth and sixth month, and 1.5% in the last four months of the growing period (Jug-Dujakovic and Van Gorder, 2002). Both groups were kept at constant temperature of 25-26°C and a diurnal photoperiod of 16 hours at 300 lux. At the end of the experimental period, the total weight of the fish as well as that of the gonads and the gonadal somatic index was determined using a Mettler balance.

Results and Discussion

Yellow perch grew from 16.82 and 16.13 g respectively to 181.21 and 173.49 grams in eight months. At the end of the experiment most of the fish gonads were in the initial stage of development, with an average weight of 3.28 and 3.14 (Table 1). The gonads account for 1.81% of the body weight in both groups. In the natural environment, gonads can account for 20-30% of the total weight in females and 8-15% in males. (Melard et al., 1996), significantly affecting the growth and dress-out percentage.

Table 1. Growth of yellow perch during eight months of the experiment, and the weight of gonads at the end of experiment

Tank	Initial weight (g)	Final weight (g)	Gonads (g)
1	16.82	181.21	3.28
2	16.13	173.49	3.14

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Grown at ambient temperatures, most of the female American perch reach sexual maturity at the end of the second year, when their medium weight is greater than 60g. It has been shown that under natural conditions, females grow faster than males (Piferrer, 2001). In natural conditions of lower temperature in late autumn and early winter, more energy is used for the development of the gonads than for the somatic growth (Henderson et al., 2000). If the gonads are not fully developed, 150g Yellow Perch will yield 55-60g of edible flesh (Malison,1999)

Maintaining a one-year production cycle at constant temperature and prolonged photoperiod slows down the development of the gonads and increases the percentage of the edible portion of the fish (Malison, 1999; Jug-Dujaković and Van Gorder, 2002). The culture of yellow perch within indoor controlled-environment recirculating aquaculture systems with continuous prolonged photoperiod and constant temperature elevated to 24-25°C can result in improved growth rates and the subsequent grow-out of yellow perch to harvest size within an acceptable time frame, and an increased dress-out percentages.

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POPULATION-SPECIFIC IMMUNE RESPONSE OF *Perca fluviatilis* TOWARDS *Aeromonas hydrophila:* PILOT STUDY

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Introduction

European perch is being described as a candidate species for European aquaculture diversification due to high market demand especially in Alpine countries. In past decades, the species has been well adapted in recirculating aquaculture system (RAS) allowing year-round intensive production (Policar et al., 2015). However, bacterial diseases by *Aeromonas* sp. are frequently causing low growth and mass mortality in intensively cultured fish including European perch (Rupp et al., 2019). Based on geographic origin, it can be expected that individual populations have been exposed to different pathogenic pressure in their localities, which in turn has led to different sensitivity to the bacterial or viral diseases. To test this hypothesis, we tested the extent of the immune response in different populations of European perch populations following the stimulation with gram-negative *Aeromonas hydrophila* bacterium.

Materials and methods

In present experiment, we used juveniles (body weight 93.03±25.05 mg) from six European perch populations originating from: **FV**, Valkea-Kotinen Lake (61° 14′ 32.1″ N, 25° 03′ 47.1″ E Finland); **PS**, Stary Dwór Lake (53° 44′ 51.6″ N, 20° 27′ 11.8″ E Poland); **IS**, Sorgà Pond (45°11’02.5”N, 10°59’59.8”E Italy); **CN**, Nové Hrady Pond (48° 47′ 33.8″ N, 14° 48′ 32.3″ E Czech Republic); **SV**, River Váh (49° 07′ 29.7″ N, 18° 27′ 26.2″ E Slovakia); and **SL**, Liptovská Mara Reservoir (49° 5′ 20.1″ N, 19° 34′ 38.3″ E Slovakia). Fish were kept in 60-L light grey tanks (tank size of 22.5 × 30 × 89 cm) at 23 °C ± 0.5 set up in RAS. Oxygen saturation (> 90%) and pH level (7 ± 0.5) were monitored twice a day with a multimeter (Hach Lange HQ40d, Germany); photoperiod was constant at 12L:12D. During the experimental period (7 days) fish were not fed.

At the start of the experiment, we injected 15 individuals per population with PFA inactivated *A. hydrophila* saline solution. In addition, one group (15 individuals from SL, noted as **C**) was used as a negative control and injected with saline solution only. At each sampling day (1, 3, and 7), peritoneal leukocytes were isolated from five individuals per population and their total number and ratio of myeloid and lymphoid leukocytes were analyzed by flow cytometry and microscop .

Results

The increase in the number of peritoneal leukocytes indicates early onset of peritoneal inflammation accompanied by dramatic changes in the ratio of white blood cell lineages. In general, one day after injection of the inactivated *A. hydrophila*, 1-7 million leukocytes were isolated from the peritoneal cavity of European perch. The first day was also the only time point, where most of the cells were of myeloid character *i.e.* neutrophilic and monocytes (approximately 55-66%). Following days, the number of cells gradually decreased and 7 days after injection ranged from 1 to 4 million cells of which 80-90% were lymphocytes. In the C group, we recorded no increase in the number of cells.

With respect to different European perch populations, it can be noted that the *A. hydrophila* injection induced two types of reactions. While the fish originated from FV, SL and SV showed a dramatic influx of leukocytes during the first 24 hours (approximately 6-7 million cells; 4 million myeloid cells), the fish from CN, IS, PS reached only 1-2 million. However, in these fish populations most of these cells were neutrophil and monocyte lineages. Remarkably, while the fish from FV, SL, and SV group reduced myeloid cell amount to just 500,000 on the third day, the fish from PS, IS and CN were up to three times higher (1-1.5 million). In contrast to the apparent polarity in myeloid cell response, no significant differences between groups were observed in lymphocyte numbers.

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Discussion and conclusion

The ability to eliminate a pathogenic agent and prevent its spreading through the body is critical to the infection survival. Knowledge from other fish models (*i.g.* rainbow trout) suggests that pathogens are recognized by the resident macrophages, which recruit neutrophils and monocytes. These cells eliminate the threat through phagocytosis and subsequent respiratory burst. The acute phase of inflammation is followed by activation of acquired immunity, particularly a population of B cells. The rate of acute inflammation induction as well as the number of cells which need to be recruited into the infected tissue, provide insights into the body's ability to defend itself against infection. In our pilot experiment, we demonstrated that peritoneal activity undergoes remarkable changes. During the first 24 hours after injection, the highest influx of leukocytes was found in perch populations from FV, SL and SV, where neutrophils and monocytes were dominant. Our preliminary results represent the first comprehensive description of the inflammatory processes in the peritoneal cavity of European perch, and present important information for future selective-breeding programs.

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DESENVIRONMENTAL AND ECONOMIC ASSESSMENT OF A PILOT AQUAPONIC PRODUCTION: A LIFE CYCLE APPROACH

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Conventional agricultural practices are often pointed out for their heavy environmental burdens such as soil and water pollution, greenhouse gas emission and natural resource depletion. Reducing these impacts is one of the main issues to ensure sustainable food production in a near future. Thanks to the efficient use of water and the nutrient recycling and valorizing, aquaponics is usually presented and perceived as an environmentally sustainable method to produce fish and vegetables.

Indeed, the combination of these two food productions bring up noteworthy advantages and limits the effluent discharge in a local environment, however, it does not erase the drawbacks inherent to culture intensification associated to aquaculture production in RAS or hydroponic culture in closed environments. The main environmental impacts of aquaponics are linked to high energy use (electricity), fish feed addition and infrastructure needs (Boxman et al., 2017; Forchino et al., 2017; Jager et al., 2019). These categories could also be responsible for the main operational economic burdens.

According to the life cycle approach, the Life Cycle Assessment (LCA) and the Life Cycle Costing (LCC) represent flexible and performant tools to assess both the environmental and economic sustainability of a given process. These tools were previously performed on the set-up phase of an indoor aquaponic system, giving a picture of the economic and environmental burdens associated to the construction of a pilot production facility (Forchino et al., 2018).

This coupled aquaponic system (total volume: 19 m³), hosted in a 104 m² building, produces tilapia (with an expected yearly production of 1 t), lettuce, rocket salad, basil, coriander and parsley (expected vegetable yearly production: 2 t).

The aim of this study is to evaluate both the environmental and economic sustainability of fish and vegetable production in this aquaponic system through the combined application of LCA and LCC.

Giving a comprehensive vision of the environmental impacts of the aquaponic production, the results will highlight bottlenecks and efficient development levers to improve the system. Moreover, this study will be coupled with a mass balance analysis to assess the nutrient flows between the different compartments of the system and measure the efficiency of nutrient recycling. This functional analysis would help to put into perspective the environmental impacts of the production with the internal functioning of the system and effluent discha ge.

Finally, the economic approach will enable a scaling-up simulation to evaluate the opportunity to generate profits

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REAL-TIME BASED AUTOMATED BIOMONITORING FOR FOOD AND WATER QUALITY CONTROL

Gerhardt, A.

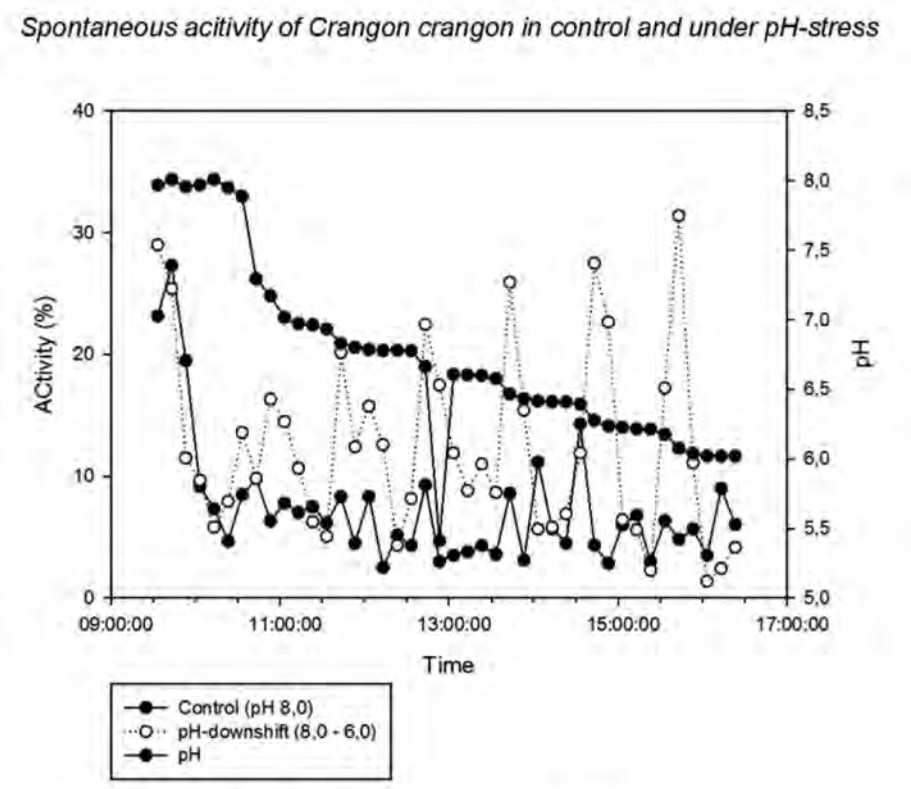
Aquaculture represents an increasing market worldwide including both freshwater and marine fish, cruastaceans, molluscs and other species. Next to sharp regulations on fish health food safety and quality as well as water quality monitoring are important issues in aquaculture farming.

The Multispecies Freshwater Biomonitor (MFB) is a so-called real-time based automated online biomonitor recording quantitatively the fitness of all types of aquatic animals in a continuous manner. Changes in fitness, e.g. due to changes in water quality can be detected immediately and countermeasures taken at the earliest to prevent economic failures in food production. The MFB has successfully been used to monitor rivers (e.g. Rhine), wastewater treatment plants and waterworks.

The application in aquaculture is new. Therefore, we tested different freshwater and marine fish (e.g. *Sparus aurata*) and crustaceans species regarding their signal quality, handling and performance in the MFB. Toxic pulses were simulated (e.g. acid stress, metal stress) and the responses of shrimps (Marine: *Crangon crangon*; freshwater: *Macrobrachium lanchesteri*) studied. Fig. 1. shows the immediate response of increased avoidance in *C. crangon* to stepwise pH-downshifts in seawater.

Moreover, the MFB was tested in situ, such as at the effluent f a wastewater treatment plant with *Poecilia reticulata* and in a shrimp farm with *Penaeus monodon* (both in India).

The first results demonstrate the great potential of future application of the MFB in the aquaculture sector



(Gerhardt, A. & S. Bamber (2013): JEP 4, 61-69)

HIGH RESOLUTION SATELLITE REMOTE SENSING OF RED TIDES IN SHELLFISH FARMING COASTAL WATERS

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Introduction

Harmful algal blooms (HABs) are worldwide deleterious ecological phenomena that can severely impact shellfish ecosystems, fisheries, and human health. Several species of the genus *Dinophysis* are known to produce lipophilic toxins, which can accumulate in suspension feeding bivalves and intoxicate consumers of contaminated shellfish. A striking feature of the *Dinophysis* toxic events documented along the French Atlantic coast in the last decades was the low concentration of *Dinophysis* in seawater samples (Delmas et al., 1992; Belin and Soudant, 2018), which prevented its observation from satellite remote sensing. Since *Dinophysis* is an obligate feeder of *Mesodinium rubrum*, the detection of *M. rubrum* could be used as an early warning system. *M. rubrum* is a globally-distributed mixotrophic marine ciliate known to form ephemeral and massive red tides in coastal areas, such as estuaries, fjords, and upwelling zones. Detection, sampling, and quantification of such red tides is notoriously challenging, however, due to the speed at which the ciliate can grow, swim, aggregate, disaggregate, and/or be consumed. Here, we present a novel detection and quantification method based on high spatial resolution satellite remote sensing.

Materials and methods

A culture of the cryptophyte *Teleaulax amphioxeia* was used to feed the mixotrophic ciliate *M. rubrum* (Fig. 1). The inherent optical properties of *M. rubrum* were measured from the culture, and a radiative transfer model was used to simulate its optical signature in terms of remote sensing reflectance (R_{rs}), which is the optical parameter available from satellite data. A detection and quantification algorithm was then developed based on the optical signature of *M. rubrum*, and applied to a three-year archive of high-resolution satellite data over a shellfish farming area located along the French Atlantic coast.

Results and discussion

The particulate absorption and backscattering coefficients of *M. rubrum* were characterized for the first time using laboratory cultures, and were consistent with *in situ* observations (Guzmán et al., 2016). Radiative transfer simulations allowed us to characterize the optical signature of *M. rubrum*s over a wide range of chlorophyll-*a* concentrations. The simulated R_{rs} spectra exhibited unique spectral features, including a near-infrared (NIR) peak associated with the presence of chlorophyll-*a* and a green trough associated with phycoerythrin absorption. An algorithm based on the amplitude of these spectral green and NIR anomalies was then proposed and applied to the archive of Sentinel-2 data from 2017 to 2019 over a shellfish farming area located in southern Brittany (France). Several red tides were successfully detected, allowing us to study the spatio-temporal dynamics of *M. rubrum* blooms at high spatial resolution (20 m) over a wide coastal area (Fig. 2). Massive blooms of other species (e.g., *Lepidodinium chlorophorum*) were also observed in shellfish farming sites, and successfully discriminated. Our results demonstrate the advantage of using Earth Observation, not only to monitor HABs in aquaculture sites, but also to better understand their spatiotemporal dynamics, and link their formation and duration to environmental forcing mechanisms.

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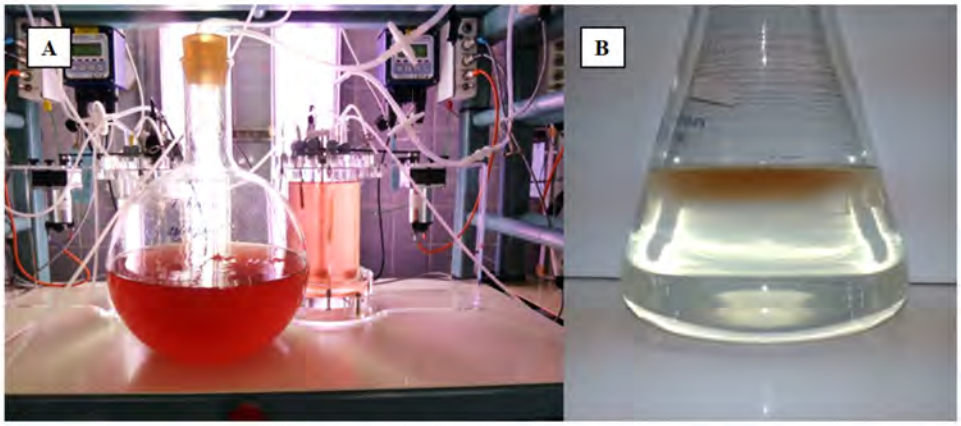

Figure 1. Laboratory cultures of *Teleaulax amphioxeia* (A) and *Mesodinium rubrum* (B). The cell number was approximately 10^7 and 10^5 cell ml^{-1} respectively. Temperature was 18°C, PAR was 150 µmol Photon m^{-2} s^{-1}, and nutrient concentration was not limiting.

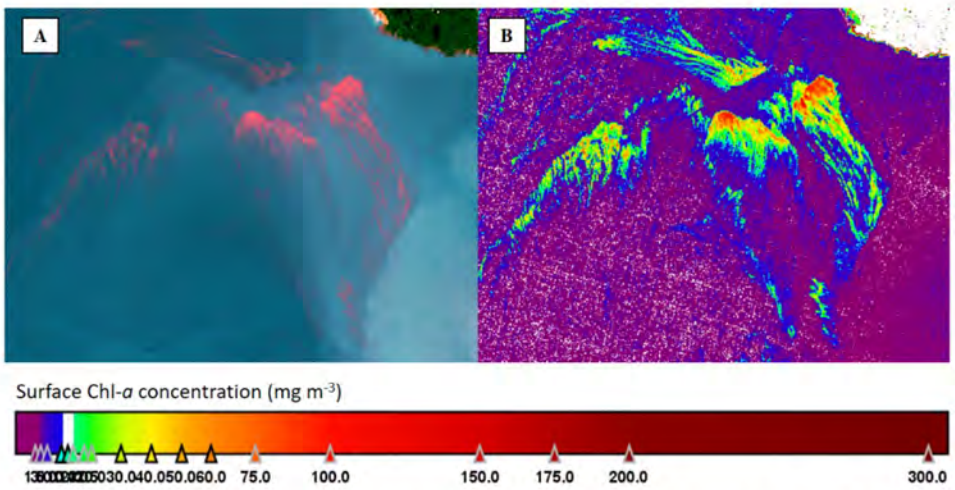

Figure 2. A) Sentinel-2 true colour image of a *M. rubrum* bloom detected off the Loire River estuary on 12 April 2017. B) From the same image, a computed map of chlorophyll-*a* concentration.

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BALLAN WRASSE (*Labrus bergylta*) LARVAL REARING, A COMPARATIVE STUDY OF DIFFERENT FEEDING REGIMES AND MICRO DIETS BASED ON THE COPEPOD *A. tonsa* AS START FEED

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Ballan wrasse as a cleaner fish for sea lice is becoming more and more popular in the Norwegian salmon industry. Never the less, the industrial cultivation of this species still has challenges and the availability of high quality juveniles remains a major bottleneck during the production cycle.

Two key challenges are faced during juvenile production. One being the first feeding of the larvae and the second being the weaning on dry pellets.

To tackle these challenges, a multifactorial experiment with three different feeding regimes and four different commercial micro diets was conducted. All feeding regimes were based on Copepods (*Acartia tonsa*, C-Feed AS) as start-feed. Approximately 200 000 Ballan wrasse larvae were equally distributed among 24 60-liter tanks and reared for 55 days. Eight treatments (three replicate tanks per treatment) were included and split in four early weaning treatments and four late weaning treatments.

In the early weaning treatments Copepods were fed to the larvae from 4-24 days post hatching (dph). A co-feeding period of 4 days followed, in which four different commercial micro diets (one for each treatment: Otohime, Reed Mariculture; AgloNorse Extra, Trofi; Molofeed; Gemma Micro, Skretting) were introduced to the experimental tanks.

In the late weaning treatments live feed was fed from 4-37 dph. Two of the four treatments were fed with Copepods while the other two treatments were fed with enriched (Multigain, BioMar) Artemia. A co-feeding period of 7 days followed until 44 dph when two commercial micro diets (Otohime, Reed Mariculture and AgloNorse Extra, Trofi) were introduced.

Larval length and weight were assessed for 15 larvae per Tank every fifth day. Furthermore, daily mortalities were counted individually per tank. At the end of the experiment, all remaining larvae were counted. Additionally, 25 individuals per Tank were sampled for malformation analyses.

All early weaning treatments had reduced survival rates, compared to the late weaning treatments. This was likely due to a mortality peak two days after the last Copepod feeding at ca.30 dph. Never the less groups fed with Trofico and Skretting showed the best growth performance in the early weaning approach.

In the late weaning treatments, the overall survival rate was higher, compared to the early weaning treatments. The long fed Copepod groups had an improved survival rate, resulting in twice as many surviving larvae at the end of the experiment compared to the late weaning group fed with Artemia. This result could be linked to a mortality peak in the Artemia fed groups shortly after they were weaned on the micro diets. Pure Trofi feeding reduced the mortality slightly but was still increased in comparison to almost no mortality in the weaned Copepod group.

However, the growth of the larvae exclusively fed with Copepods was reduced between 35-45 dph due to reduced Copepods prey size. This deficit was superseded by an increase in growth of the larvae in both Copepod treatments, as soon they were fed with micro diets.

REGULATION OF THE RHYTHMICITY OF PROTEOLYTIC DIGESTIVE FUNCTION IN GILTHEAD SEABREAM UNDER DIFFERENT DAILY FEEDING PROTOCOLS

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Introduction

Digestion consists of a group of complex processes, involving physical, chemical, and biochemical transformations of the ingested food. All of them are interdependent and finely tuned by a number of molecular, hormonal, and nervous signals, many of which are driven by the circadian system. This system is composed of several interconnected oscillators, for which light-dark and feeding-fasting cycles are the most powerful synchronizers. An increased knowledge of Food Anticipatory Activity (FAA), an out of Food-entrainable oscillators (FEO), may help to optimize the utilization of nutrients by a given species adapting feeding protocols to these internal rhythms. The present work aimed to evaluate the rhythmicity of some of the gastrointestinal tract (GIT) factors that are related to digestive efficiency in gilthead seabream (*Sparus aurata*) juveniles as well as to determine how these factors are affected by different feeding protocols.

Materials and methods

Juveniles of *Sparus aurata* were randomly distributed into four groups with three replicates (for each group N = 124, body mass 17.91 ± 0.2 g), in 250-L tanks, with flow-through water system at 19.5 ± 1.0 °C, and a photoperiod of 11L/13D. All groups were fed a daily ration of 2.5 % body mass, with the same experimental diet but different daily feeding protocols (P) during the daylight: P1) one meal at 08:30 h (local time); P2) three meals at 08:30, 13:30, and 18:30 h; P3) five meals at 08:30, 10:00, 13:30, 16:00, and 18:30 h; and P4) continuous feeding from 8:30 to 18:30. After two-weeks, fish were sampled (six individuals, two per tank) every 4 hours during a 24 h cycle. Relative expression of genes related to appetite regulation (ghrelin, *ghrl*), nutrient transport (Peptide transporter 1, *slc15a1*), and circadian rhythms (period circadian

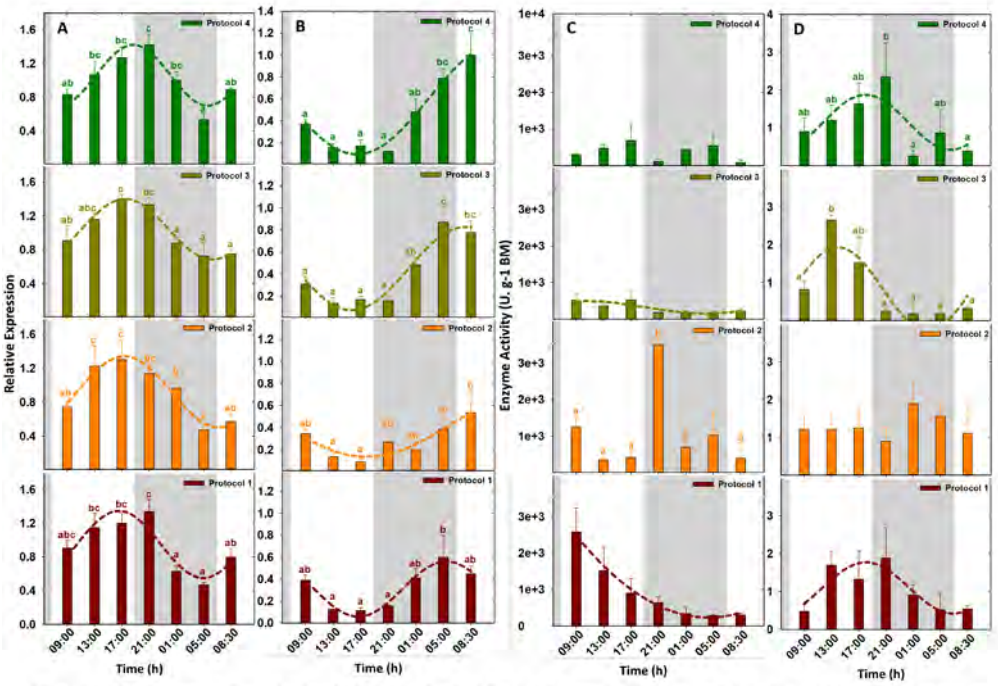

Fig. 1. Daily pattern of *clock* (A) and *slc15a1* (B) expression, and pepsin (C) and trypsin (D) activity in *S. aurata* with different feeding protocols. Different letters denote significant differences among sampling times within each protocol. Dashed lines exhibit significant circadian rhythms ($p < 0.05$). Grey areas indicate the dark period.

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protein homolog 3, *per3*; circadian locomotor output cycles protein kaput, *clock*) were quantified in the whole GIT tissue using the $\Delta\Delta C_T$ method. Pepsin and trypsin activity, as key proteolytic enzymes, were assessed using hemoglobin (pH 2.0) and BAPNA (pH 8.5) in the stomach and intestine, respectively. Besides two- and one-way ANOVAs, data were subjected to a cosinor analysis (EL TEMPS®) to test for circadian rhythms ($p < 0.05$).

Results

Significant influence of feeding protocol and/or sampling time was obtained in all the studied parameters. P2 and P1 had the lowest, while P4 and P3 had the highest *slc15a1* and *per3* expression levels. Nevertheless, P3 and P4 showed the lowest, while P2 and P1 showed the highest pepsin activity. In all protocols, expression of *clock* and *per3* had significant daily rhythms with almost opposite patterns. Except for *slc15a1*, other studied factors did not exhibit daily rhythms in all the feeding protocols (Fig. 1).

Discussion and conclusion

Rhythmicity of GIT expression of clock genes under all the tested daily feeding protocols, without major phase shift, indicated a strong light synchronization rather than food entrainment. It is speculated that entrainment of endogenous clocks by feeding-fasting cycles is mediated by hormones, metabolites, and other sensor molecules, through alterations in the family of *period* genes. Interestingly, in our study, *per3* was the only circadian gene that was significantly influenced by the feeding protocol. The strong correlation between *per3* and *slc15a1* expression and, to a lesser extent, between *clock* and *ghrl* expression in this work, supported the previously reported role of clock genes in daily organization of biological functions such as nutrient transport and appetite control, or could underline a putative interactive role of these parameters in entrainment of FEOs (Isorna et al., 2017). Activity of proteolytic enzymes, especially trypsin, showed rhythmic daily patterns that were mostly synchronized by food availability in GIT in most of the experimental protocols. Overall, higher incidence of daily rhythmic patterns in the studied parameters and a higher expression of *slc15a1*, as a marker of protein absorption function, may suggest a better digestive efficiency in P3 and P4. On the other hand, highest enzymatic activity in P2 might be a strategy to compensate the absence of daily rhythmicity in some of the studied parameters.

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Acknowledgments

Grants AGL2014-52888-R from MINECO (Spain) with FEDER/ERDF contribution, and WISEFEED funded by the European Union's H2020 programme (Marie Skłodowska-Curie grant No 691150). NG supported by BES-2015-071662 from MINECO.

INFLUENCE OF DIFFERENT DAILY FEEDING PROTOCOLS ON REGULATION OF CCK HORMONE AND PANCREATIC PROTEASES ACTIVITY IN SENEGALESE SOLE JUVENILES

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Introduction

Cholecystokinin (CCK) is a peptide hormone that plays an important role in intestinal phase of the digestion process, by being involved in the regulation of pancreatic enzyme secretion among others (Rønnestad et al., 2017). Peptides and free amino acids in the chyme act as signals that initiate the release of CCK, which in turn causes the release of pancreatic digestive enzymes into the gut lumen. Daily fluctuations of CCK and trypsin activity has been addressed especially in fish larvae. However, daily interaction of this hormone with pancreatic enzymes in juvenile fish under different feeding schedules has been scarcely studied. Therefore, the aim of the present work was to evaluate the influence of different feeding protocols, in terms of timing and frequency, on daily rhythm of CCK hormone and two pancreatic enzymes in juveniles of Senegalese sole (*Solea senegalensis*).

Materials and methods

Senegalese sole juveniles were randomly distributed into four groups with two replicates (for each group N = 118, initial body mass 51.61 ± 0.9 g), in 250-L tanks, with flow-through water system at 19.5 ± 1.0 °C, and a photoperiod of 11L/13D. All groups were fed a daily ration of 2 % body mass, with the same experimental diet but different daily feeding protocols (P): P1) one diurnal meal at 08:30 h (local time); P2) six diurnal meals at 08:30, 10:00, 12:00, 14:00, 16:00 and 18:00 h; P3) six nocturnal meals at 20:00, 22:00, 24:00, 02:00, 04:00 and 06:00 h; and P4) 12 meals during 24 h (at all times mentioned for P2 and P3). After two weeks, fish were sampled (four and five individuals for CCK and enzymatic activity measurements respectively) every 4 hours during a 24 h cycle.

CCK concentration was measured in epithelial tissue of the proximal intestine using the Fish CCK8 ELISA Kit (MyBioSource, Cat. No. MBS069488). Trypsin and chymotrypsin activity were assessed in the proximal intestine using BAPNA and GAPNA as substrates (pH 8.5), respectively. Besides One-way ANOVA, data were subjected to a cosinor analysis (EL TEMPS®) to test for circadian rhythms ($p < 0.05$).

Results

Fish with a single diurnal (P1) and 12 diurnal-nocturnal (P4) meals had maximum and minimum daily average CCK levels, respectively. While, P1 and fish with exclusively nocturnal meals (P3) showed the minimum and maximum daily average trypsin activity, respectively (Table I). Trypsin and chymotrypsin daily activity patterns were similar in all the experimental protocols. Only in P1, cosinor analysis showed significant daily rhythm for all the studied parameters, with acrophase at 13:33, 13:36, and 13:46 h, for CCK, trypsin and chymotrypsin, respectively. However, a general phase shift was obtained in the rest of the feeding protocols, with CCK peaks preceding the maximum enzymatic activity levels (Fig. 1).

Discussion and conclusion

The inverse relationship in total quantity and temporal displacement of daily CCK and digestive enzyme activity patterns supports the involvement of CCK in the pancreatic proteases secretion and the existence of a regulatory loop between these parameters. Moreover, this displacement depended on the feeding protocol; increasing the feeding frequency led to longer delay between CCK secretion and proteases activity peaks. In all the experimental protocols, expect for P1 with a single daily meal, two daily CCK and the subsequent protease activity peaks were obtained.

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Table 1. Daily average CCK concentration and trypsin and chymotrypsin activity in Senegalese sole juveniles under different daily feeding protocols.

	CCK (pg. mL $^{-1}$ Int.)	Trypsin (U. g $^{-1}$ BM)	Chymotrypsin (U. g $^{-1}$ BM)
	One-way ANOVA		
Protocol 1	71.6 ± 9.2 ^{b}	47.1 ± 2.2 ^{a}	36.2 ± 1.8
Protocol 2	46.3 ± 7.9 ^{a}	53.1 ± 2.2 ^{ab}	38.5 ± 1.7
Protocol 3	38.7 ± 5.8 ^{a}	56.7 ± 2.6 ^{b}	41.6 ± 2.0
Protocol 4	29.0 ± 6.6 ^{a}	54.5 ± 2.9 ^{ab}	37.4 ± 1.5

Values are in mean ± SEM. Different superscripts indicate differences among protocols ($p < 0.05$).

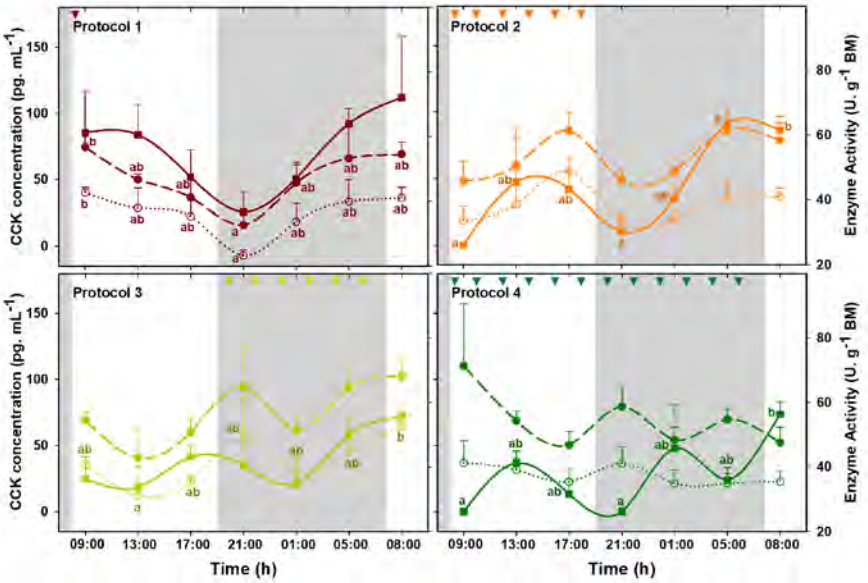

Fig. 1. Daily pattern of CCK concentration (solid line) and trypsin (dashed line) and chymotrypsin (dotted line) activity in Senegalese sole juveniles under different daily feeding protocols. Different letters denote significant differences among sampling times within each parameter ($p < 0.05$). Grey areas indicate the dark period and triangles on the topper margin of each plot represent the feed supply in the corresponding feeding protocol.

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Acknowledgments

Grants AGL2014-52888-R from MINECO (Spain) with FEDER/ERDF contribution, and WISEFEED funded by the European Union's H2020 programme (Marie Skłodowska-Curie grant No 691150). NG supported by BES-2015-071662 from MINECO (Spain).

GROWTH PERFORMANCE AND CIRCADIAN RHYTHM OF DIGESTIVE FUNCTION IN GILTHEAD SEABREAM JUVENILES WITH DIFFERENT DAILY FEEDING FREQUENCIES

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Introduction
Feeding efficiency requires an adequate nutrient composition and an efficient digestive process. Feed formulation has been the target of many studies; however, the actual digestive capacity of farmed fish, with pre- and post-prandial responses, is largely unknown. Besides, few studies have focused on designing optimized feeding schedules. Therefore, the aim of the present work was to evaluate the effect of two daily feeding frequencies on growth and daily fluctuations of parameters related to the circadian and digestive physiology in gilthead seabream (*Sparus aurata*) juveniles.

Materials and methods
Juveniles of *S. aurata* were randomly distributed into two groups in duplicate (each group N = 96, initial body mass 22.7 ± 0.6 g), in 500-L tanks, with flow-through water system at 19.5 ± 1.0 °C, and a photoperiod of 11L/13D. All groups were fed a daily ration of 2.5 % body mass, with the same experimental diet but different daily feeding protocols (P) during the daylight: P1) three meals at 08:30, 13:30, and 18:30 h, and P2) five meals at 08:30, 10:00, 13:30, 16:00, and 18:30 h. The experiment was carried out in a period of almost five months (139 days), over which the Specific Growth Rate (SGR), and Feed Efficiency (FE) were analyzed regularly. At the end of the experimental period, fish were sampled (eight individuals, four per tank) every 4 hours during a 24 h cycle. Expression of clock genes (*bmal1*, *clock*, *per3*, *cry1*) and genes related to appetite control and food intake (leptin, *lep*; ghrelin receptor, *ghsr1b*; glucocorticoid receptor, *nr3c1*) were quantified in the liver ($\Delta\Delta C_T$ method). Trypsin and chymotrypsin activity were analyzed in the proximal intestine (BAPNA and GAPNA as substrates, respectively). Besides t-test and ANOVAs, data were subjected to a cosinor analysis (EL TEMPS®) to test for circadian rhythms ($p < 0.05$).

Results
Final body mass, SGR, and FE were 123.7 ± 1.8 g, 1.22, and 1.23 in fish with three (P1) and 124.6 ± 2.1 g, 0.76, and 0.78 in fish with five daily meals (P2), respectively. No significant differences were obtained neither for final body weight nor for any other studied parameter between protocols. However, most of the variables demonstrated significant fluctuations along the daily cycle and in some cases, their patterns depended on the experimental protocol (Figs. 1 to 3).

Higher trypsin activity during light period in P2, coincides with the food ingestion and could lead to a more efficient alkaline protein digestion in this group (Fig. 1). Daily changes of the *lep* and *ghsr1b* are in accordance with their putative anorexigenic and orexigenic function, respectively. Circadian pattern of *lep* and *nr3c1*, with their peak at the transition of light-dark period, can imply their possible role in entrainment of peripheral oscillators through modification of clock genes expression (Fig. 3). Finally, due to the daily changes of the studied parameters, when estimating the fish digestive capacity or comparing the response to different diets or feeding protocols, decisions that are based on a single sampling time are incomplete and could lead to misleading conclusions.

Acknowledgments
Grants AGL2014-52888-R from MINECO (Spain) with FEDER/ERDF contribution, and WISEFEED funded by the European Union's H2020 programme (Marie Skłodocwska-Curie grant No 691150). NG supported by BES-2015-071662 from MINECO. This work is a part of GBR's Master Thesis.

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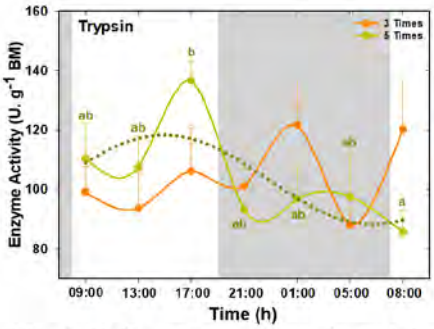

Fig. 1. Daily pattern of trypsin activity in *S. aurata* juveniles with different feeding protocols. Further details as described in legend of Fig. 2.

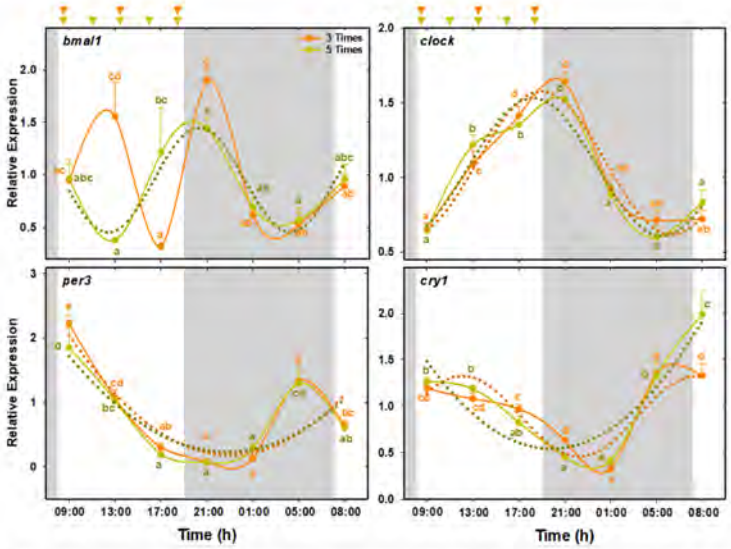

Fig. 2. Daily pattern of clock genes expression in *S. aurata* with different feeding protocols. Letters denote significant differences among sampling times within each protocol. Dotted lines exhibit significant circadian rhythms. Grey areas indicate the dark period and triangles on the topper margin represent the feed supply.

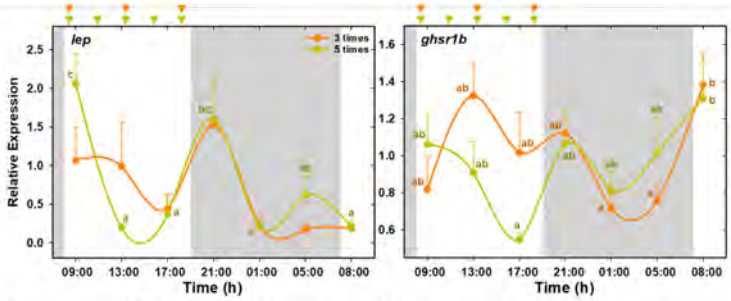

Fig. 3. Daily pattern of *lep*, *ghsr1b*, and *nr3c1* expression in *S. aurata* juveniles with different feeding protocols. Further details as described in legend of Fig. 2.

CRYPTIC INTROGRESSION: WILL PLASTICITY AND NATURAL SELECTION MASK THE EFFECTS OF GENE-FLOW FROM DOMESTICATED ESCAPEES ON NATURAL POPULATIONS?

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Farmed Atlantic salmon are domesticated, and where significant interbreeding with wild conspecifics occurs, this may lead to changes in fitness traits, and ultimatel , less productive wild populations (Reviewed by Glover *et al.* 2017).

Thus far, there are few data demonstrating direct evidence of life-history and fitness changes in native populations resulting from interbreeding with domesticated conspecifics. While the reasons behind this are many, including the fact that investigating native populations is logically demanding, in this talk we speculate whether plasticity, and natural selection, may combine to mask some of the life-history changes that could be expected in native populations following spawning intrusion of domesticated escapees.

The talk to a large degree bases itself on recently published work demonstrating the plasticity and selection can mask genetic changes in native populations (Glover *et al.* 2018), and the result of modelling work conducted via IBSEM (Castellani *et al.* 2018).

Background articles “open access”:

Castellani M., Heino M., Gilbey J., Araki H., Svåsand T. & Glover K.A. (2018) Modelling fitness changes in wild Atlantic salmon populations faced by spawning intrusion of domesticated escapees. *Evolutionary Applications*, Early online: DOI: 10.1111/eva.261.

Glover K.A., Solberg M.F., Besnier F. & Skaala O. (2018) Cryptic introgression: evidence that selection and plasticity mask the full phenotypic potential of domesticated Atlantic salmon in the wild. *Scientific Reports* **8**, 10.

Glover K.A., Solberg M.F., McGinnity P., Hindar K., Verspoor E., Coulson M.W., Hansen M.M., Araki H., Skaala Ø. & Svåsand T. (2017) Half a century of genetic interaction between farmed and wild Atlan tic salmon: status of knowledge and unanswered questions. *Fish and Fisheries* **18**, 890-927.

A FULLY INTEGRATED SIMULATION MODEL OF MULTI-LOOP AQUAPONICS: PUTTING THEORY INTO PRACTICE

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Introduction

Decoupled multi-loops aquaponics systems allows for co-cultivation of plants and fish in a circular system that can take advantage of nutrient, water and energy cycling. Sizing of systems is important so that wastes from the RAS component can be utilized effectively within the hydroponic (HP) system, based on parameters such as plant growth rates, evapotranspiration and species-specific plant nutritional needs. However, in order to create the optimal fit between RAS and HP, the temporal responses of the underlying processes need to be optimized so that water and nutrient flows are always synchronised. We have therefore created an aquaponics-sizing simulator based on deterministic mathematical models that are transferrable to various environmental conditions with simple parameterisation. Model outputs of this simulator allow us to further understand system dynamics under different environmental conditions, while also allowing us to perform parameter estimation, optimization and control for each location where output from the RAS systems can be estimated, greenhouse specifications can provide known growth rates for the desired species, and appropriate seasonal weather data is available.

Materials and methods

Two independent model systems, for (1) the greenhouse, incorporating HP and climate control biofeedback and (2) the aquaculture facility as illustrated in Fig. 1, were developed in the modelling and simulation environment MATLAB. The combined model system is a compilation of technical and biological sub-models, including greenhouse macro- and microclimate, energy consumption and crop growth that are thoroughly calibrated and validated (e.g. Körner et al., 2007).

Results

The simulation outcomes let us size the system and also determine the required flows in between the respective sub-systems (Goddek & Körner, 2019). The simulations, with the respective flow rates provide a good indication with respect to nutrient concentrations within the RAS system under different environmental conditions (Fig. 2).

The peaks in NO3-N concentration for tomatoes are a result of a lower crop-specific evapotranspiration rate of young plants during the winter (i.e. lower solar radiation). The uptake of NO3-N is linear related to transpiration rates of the crop, resulting in a higher accumulation of NO3-N in the RAS system in cooler regions. Unlike lettuce that is consecutively planted and harvested year-round (resulting in a homogeneous crop all the year), tomatoes are usually re-started with young plants once a year. Thus, season, geographical location and cropping practices alter the leaf-area, which in turn is the driving force for transpiration.

Discussion and Conclusion

Based on the results, the model makes it possible to gain a better understanding with respect to water and nutrient dynamics in an aquaponics system. More importantly, the simulation outputs also includ yield predictions for both plants and fish, energy (thermal and electricity) and water requirements for the system, thus allowing for economic feasibility calculations. The model uses small time-points of 5-min for the plant sub-modules, in order to simulating the faster dynamics within the HP system, while the slower RAS dynamics are simulated with daily time-points. We are currently applying this simulation model in both Namibia and Saudi Arabia, where different desert climates prevail (semi-hot and semi-humid, hot and dry). In order to fit RAS and HP to extreme climate conditions, where the choice of greenhouse construction and equipment is of particular importance.

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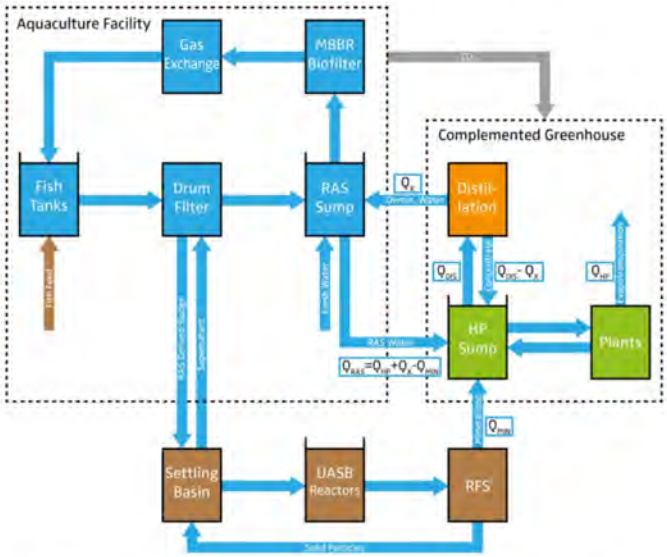

Fig 1. Water flow scheme of the multi-loop aquaponics system, incorporating a complemented greenhouse model, based on Goddek and Keesman (2018).

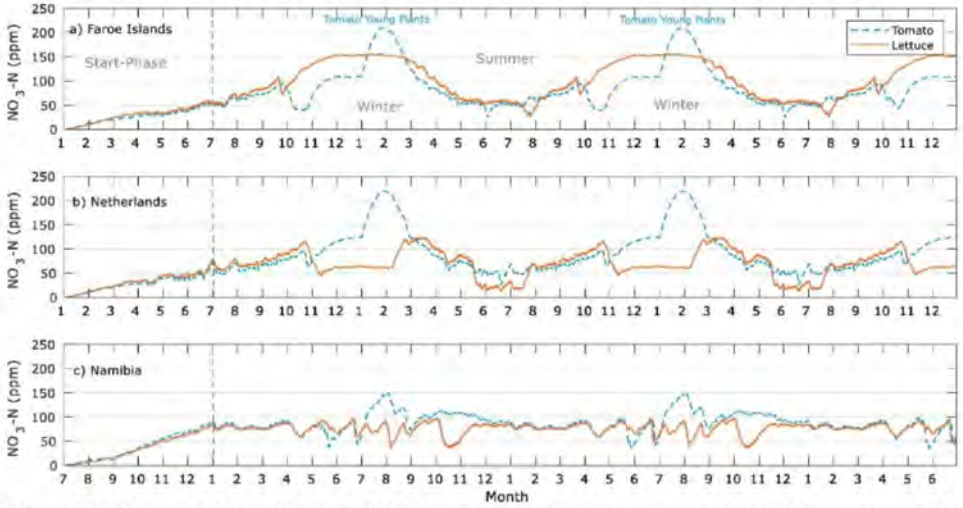

Fig. 2 NO_3-N in RAS combined with HP growing tomato or lettuce in three climate zones with adjusted area for HP in a 36 months simulation using local climate data and climate adjusted greenhouses as model input. See details for greenhouse equipment in Goddek & Körner, 2019).

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GENOMIC TOOLS FOR THE AQUACULTURE OF PIKEPERCH (*Sander lucioperca* L., 1758)

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Introduction

The pikeperch (*Sander lucioperca* L., 1758) belonging to the *Percidae* family is on its way to become an attractive aquaculture species in the western world. The aquaculture of this so far only rarely domesticated fish species confronts breeders with numerous challenges in breeding and keeping. The only poorly researched biology of pike-perch with regard to the behavior during and after periods of stress or the defense against infectious pathogens in aquaculture leads to high mortalities in the larval and juvenile stages of development, particularly during feed conversion. Multiparametric analyses with genome biological approaches in combination with classical stress-analysis methods and targeted fish monitoring contribute to the understanding of the biology of pike-perch with regard to aquaculture and to the development and optimization of breeding approaches. The project presented here on the genome analysis of pike-perch will boost the development of genomic tools for the aquaculture of pike-perch. Grants for this project (MV-II.1-LM-001) come from the European Maritime and Fisheries Fund (EMFF) and the Ministry of Agriculture and the Environment Mecklenburg-Vorpommern.

Material & methods

A male pikeperch was used for genome analysis. DNA sequencing was performed with different sequencing platforms. Long DNA fragments were sequenced with PacBio Sequel SMRT technology and short fragments with Illumina HiSeq 2500 (2 x 150 bp paired end fragment size). The ongoing genome analysis of another 394 pikeperches with the Illumina NovaSeq sequencing technology serves to identify and validate informative single nucleotide polymorphisms (SNP) and to create an initial linkage map for the pike-perch. For this, the software GATK (Genome Analysis Tool Kit) are used. Based on initial RT-qPCR gene expression analyses (Biomark HD system, Fluidigm 48.48 IFC chip or Roche LightCycler96, 96-well plates), key genes associated with growth, stress and immunity were validated with respect to animal welfare and performance under rising water temperatures, hypoxia or pathogen impact.

Results

PacBio sequencing of the pike-perch genome size was based on in average 20 kb DNA fragments and resulted in a 41-fold genome coverage whereas the paired end sequencing by Illumina resulted in a 373-fold coverage of the genome. The genome assembly analysis resulted in a first highly continued draft genome assembly for pike-perch. The assembled total length of the genome is 900 Mb covering 88% of the 1014 Mb estimated genome size. (More information can be found on our pikeperch genome poster.) We also sequenced 394 pike-perch by Illumina NovaSeq with a coverage between 25 and 30 per fish.

While the investigations on the ontogenesis of pike-perch and on some of the above mentioned animal-welfare-relevant characteristics are still ongoing, a network of interacting thermomarker genes has already been identified reflecting the response of pike-perch to rising water temperatures. Based on this, a molecular biochip for temperature stress analysis was developed. (Detail on these data are given in separate posters.)

Discussion and conclusion

The pike-perch genome sequence presented for the first time together with the genome data of nearly 400 individual animals provides an excellent basis for the further and more precise assembly of the pikeperch genome. Furthermore, it helps to identify the protein-coding and other gene sequences and provides the prerequisites for detailed molecular analyses in the five known sander species and future genome-based breeding approaches. The functional analyses revealed detailed information on optimized fish keeping with regard to essential basic parameters such as temperature and oxygen saturation.

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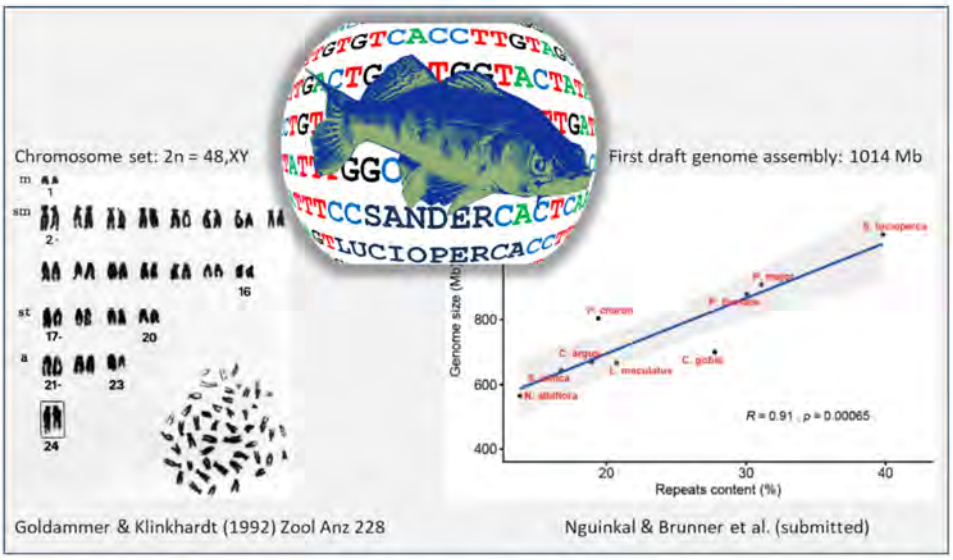

Fig. 1. Genome data for pike-perch: karyotype (left) logo of the running project from 2017-2020, (middle), genome size and repeat content of the phylogenetic young pikeperch (~5 million years, Haponski & Stepien (2013)) compared to earlier evolved perciforms (right).

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INHERITANCE AND EXPRESSION OF RED-EYE KOI MUTATION IN KOI (*Cyprinus carpio*) x GOLDFISH (*Carassius auratus*) HYBRIDS

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Introduction

Appearance of red eyes in fish is usually connected with the action of albino mutations. In contrast, Gomelsky et al. (2016) showed that appearance of red eyes in ornamental koi carp (*Cyprinus carpio*) is caused not by albino mutation but by another, dominant demelanization mutation (*R*), which has partially decreased the quantity of melanin in both the skin and eyes. The purpose of the present study was to investigate inheritance and expression of red-eye koi mutation in koi x goldfish *Carassius auratus*) hybrids.

Materials and methods

The general scheme of experiments included crossing of goldfish males with normal black eyes with koi females with either normal black or red eyes followed by analysis of segregation in progenies with regard to eye color and body pigmentation in swim-up larvae and 5-month-old juveniles. All progenies were obtained by artificial spawning; one male and one female were used for production of each progeny. Significance of difference between observed segregation and the 1:1 theoretical ratio was determined using a Chi-square test.

Results

A total of five progenies were obtained and analyzed (able I).

Table I. Segregation of fish in progenies obtained by crossing of goldfish (GF) males with koi females with normal black or red eyes. Different superscript letters indicate significant difference (P<0.05) between observed segregation and the 1:1 theoretical ratio.

Progeny no.	Parents		Segregation of larvae (n)		Segregation of juveniles with regard to body color (n)			
	Male	Female	Light body and lenses	Dark body and lenses	Yellow	Brown	Yellow + Brown	Wild Type
1	GF	Black-eyed koi	0	77	0	0	0	452
2	GF	Black-eyed koi	0	82	0	0	0	809
3	GF	Red-eyed koi	38[a]	33[a]	46	96	142[a]	148[a]
4	GF	Red-eyed koi	52[a]	43[a]	71	150	221[a]	271[b]
5	GF	Red-eyed koi	18[a]	25[a]	149	104	253[a]	326[b]

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Fig.1. Juveniles of koi x goldfish hybrids with wild-type, yellow, and brown colors (from top to bottom); bar = 1cm.

In progenies 1 and 2 obtained by crossing goldfish males with black-eyed koi females all larvae were dark and had normal black eye lenses while all juveniles had a wild-type color and black eyes. In progenies 3, 4 and 5 obtained by crossing goldfish males with red-eyed koi females, two types of larvae were observed: larvae with light body color and light eye lenses and larvae with dark bodies and black eye lenses; in all three progenies segregations of larvae did not differ significantly from the 1:1 Mendelian ratio. Three types of juveniles with regard to body color were observed in these progenies: wild-type, yellow, and brown. All juveniles had normal black eyes. As an illustration, juveniles of these three types from progeny 3 are shown in Figure 1. The cumulative proportion of colored (yellow + brown) juveniles varied in different progenies from 43.7 to 49% (Table I); in progeny 3 segregation of colored and wild-type juveniles did not differ from the 1:1 Mendelian ratio while in progenies 4 and 5 this difference was significant.

Discussion and conclusion

The results of the study show that the dominant mutation *R* causing appearance of red eyes in koi resulted in the occurrence of unpigmented larvae with light lenses in hybrids obtained by crossing goldfish males with red-eyed koi females. The 1:1 ratio of dark and light larvae in progenies indicated that goldfish males had genotype *rr* while red-eyed koi females were heterozygous (genotype *Rr*). At the juvenile stage, this mutation caused only partial demelanizanion of the body, which resulted in the appearance of yellow and brown fish with black eyes. A statistically significant deficit of colored juveniles in the two progenies was observed, apparantly because some hybrids with *Rr* genotype were indistinguishable from wild-type fish with genotype *rr*.

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IMPROVEMENT OF FAECES STABILITY THROUGH DIETARY MANIPULATION IN STARTER FEEDS FOR SEABASS (*D. labrax*) AND SEABREAM (*S. aurata*) REARED IN RECIRCULATION SYSTEMS

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Introduction

In the recent years there has been a tendency of seawater (SW) hatcheries to prolong the growth of the fry in land-based facilities before transfer to sea cages. Most of the new coming developments in this area will be done in recirculation systems (RAS) and due to water usage constraints, pathologies, stability of temperature and other rearing parameters a great majority of the existing pre-growing units will change into RAS as well. The first step in RAS is the solid removal. Depending on the stability of the faeces produced by the fish this step can be simple or quite complex. As a result, there is a market need for starter feeds designed for RAS with special emphasis on enhancing the faeces stability in the water.

This project aimed to develop a BioMar ORBIT starter feed product range for SW fish in the range within 0.2 – 15 g, especially for seabass (*D. labrax*; SBA) and seabream (*S. aurata*; SBE). Therefore, this development was focused on providing enhanced faeces stability without jeopardizing fish performance and gut health

Materials and methods

Four diets (D1, D2, D3 and D4) varying in what we named *BioMar ORBIT package* were tested in quadruplicate tanks (V=100-120 L) using 2 RAS systems placed in CTAQUA (Cádiz, Spain). System water renewal in each RAS was set at 5-10 % renewal per day after stabilization of the biofilte . The biofilter consisted of a submerged and a trickling filter with different plastic media. Temperature was 21 ± 1 ºC for SBA and 22 ± 1 ºC for SBE. Photoperiod was 12 L / 12 D (light/dark). Fingerlings had an initial body weight of 1.16 ± 0.02 g (SBA) and 0.90 ± 0.04 g (SBE).

Table 1. Difference (%) in the volume and area of the faecal particles released to the water by fish fed with the experimental diets D2, D3 and D4 compared to D1 (n=4)

	VOLUME OF FAECES (μl/L)							
Particle size (μm)	SBE				SBA			
% *vs.* D1	D1	D2	D3	D4	D1	D2	D3	D4
< 30	- (a)	-32.4 (b)	-38.3 (b)	-44.2 (b)	- (a)	-8.4 (b)	-14.5 (b)	-17.9 (b)
30 – 60	- (a)	-32.3 (b)	-44.3 (c)	-50.8 (c)	- (a)	-10.7 (b)	-20.8 (bc)	-28.2 (c)
> 60	- (a)	-27.9 (b)	-32.8 (b)	-37.5 (b)	- (a)	-14.9 (b)	-9.9 (b)	-13.1 (b)
TOTAL	- (a)	-29.7 (b)	-36.2 (b)	-41.5 (b)	- (a)	-14.9 (b)	-14.3 (b)	-16.3 (b)
	AREA (μm^2/L)							
< 30	- (a)	-23.3 (b)	-26.7 (b)	-32.5 (b)	- (a)	-7.7 (b)	-11.1 (b)	-16.1 (b)
30 – 60	- (a)	-23.5 (b)	-32.3 (bc)	-38.9 (c)	- (a)	-9.4 (b)	-15.8 (bc)	-21.2 (c)
> 60	- (a)	-20.0 (b)	-23.1 (b)	-29.0 (b)	- (a)	-12.2 (b)	-7.8 (b)	-10.3 (b)

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Faecal samples were collected using a modified Guelph system over a period of 17-18 h. Faecal particle size distribution analysis (PSD) was performed by means of a LISST-Portable|XR particle size distribution analyser (Sequoia Scientific Inc) based on Brinker *et al*. (2005). Analyses were carried out in duplicate per tank. Samples of liver and intestine were also taken and preserved in buffered formalin for further histological analyses. Samples of distal intestine were trimmed, processed and embedded in paraffin wax and the tissue blocks were cut at 5 µm and stained with hematoxylin and eosin (H&E), and Alcian blue, by Fish Vet Group (Inverness, UK). Each slide was digitalized at 40x magnification using a ScanScope AT Turbo slide scanner (Leica Biosystems, USA). The digital slides were examined using ImageScope Version 12.3.2 (Leica Biosystems, USA). Transverse sections of the intestine were scored using six parameters, and all parameters were scored on a scale of 1 to 4.

Results

At the end of the trials, we did not find any significant differences in the growth rates, performance, survival or the distal intestine architecture of fish fed with the experimental diets. However, the faecal assessment determined by measuring the particle size distribution did show statistically significant differences between the experimental diets in both species (Table 1).

Discussion and conclusion

In general, the addition of the *BioMar ORBIT package* in D2, D3 and D4 reduced the volume and area of faecal particles within each particle size range (<30 µm / 30-60 µm / >60 µm) when compared to D1, and this effect was more evident in SBE. Furthermore, D3 and D4 reduced the volume and area of the particles even more, in the range between 30-60 µm, when compared to D1. Therefore, with the purpose of improving the removal of solid waste as early as possible in the process, the results from this trial recommend the addition of the *BioMar ORBIT package* to starter feeds to be used in RAS systems in, at least, the levels of D3 in both SBE and SBA.

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TRACKING OF PLASTTIC EMISSIONS FROM AQUACULTURE INDUSTRY

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Introduction

There is a constant request to intensify the aquaculture production to fulfil the growing need of the market. In Norway farmed salmon has become a significan source of national income with the excess of 1 million tons produced every year (MOWI, 2018). The expansion of the industry and the diversity of materials used to build and maintain the aquaculture systems have paralleled the development of synthetic polymers over the last decades. Synthetic fibers ropes offer greater strength and durability than natural fiber ropes, they are cheaper, durable and easier to handle compared to their natural counterparts. All plastic material within an aquaculture site is maintained and controlled for chemical degradation, biofouling and corrosion, and are regularly inspected to ensure strength and stability. Lost gear, broken and fragmented equipment, release of debris because of intense use are sources of plastic emission from aquaculture at global and local level (Astudillo et al., 2009; Lusher et al., 2017;). However, the estimation of the level of contribution remains a knowledge gap that needs to be estimated (Haave et al., 2018; Gomiero et al., 2019). It is therefore crucial to look at the challenges and solutions related to the release of microplastics from the aquaculture industry. Therefore, the aim of the present project aims at : a) to identify the sources of emissions of plastic and micro-plastic in the sea from aquaculture facilities; b) to determine and quantify the contributions from aquaculture operations in the immediate vicinity of sea farms; c) Identify which processes within the seafood production is largely responsible for plastic discharge and suggest measures to reduce eventual emissions; d) encourage an active exchange of information and discussion between academia, industry and stakeholders for a common solution towards marine plastic pollution.

Materials and methods

To discriminate any production step-dependent contribution in the plastics enrichment of the final fish food product samples from raw materials (n=8) and from different steps in the fish food pellet production (n=4) have been collected together with the information on the products (origin, date of production, supplier, etc). As in the fish production phase produced pellets are high pressure transported to the cages by plastic pipes, such technique has been speculated to create a significant abrasion of the plastic thus possible environmental release of plastic particles of unknown size distribution. An ad hoc autosampler has been engineered to collect, quantify and characterize the plastic debris coming out from the hose of a standard feeding system before and during its operative phase. Furthermore, the occurrence of plastic fragments in different environmental compartments potentially affected by aquaculture production activities such as marine sediments, seawater, suspended matter and biota (farmed Atlantic salmon) at increasing distances from an active aquaculture facility is performed. Van Ween grab devices, multi-sieving collectors and sediment traps where used on 8 sites placed on two orthogonal sampling transects and compared to a control site not affected by the aquaculture production. 10 individuals of wild salmon (control) as well as farmed salmons were collected during the production phase and analysed for total content and polymers composition in the gut line and in the gills. The extraction and purification method trust in a combination of a multistep enzymatic-strong alkali-oriented purification followed a density-based separation to extract plastic fragments from digestates was performed. Extracted samples were size fractionated in four size classes (D1:500-300 µm; D2:300-150 µm; D3: 150-10 µm; D4: 10-1 µm; D5: 1 - 700 nm). D1 representing a fraction with large fragments was quantified by ATR-FTIR. The finer fractions D2 – D4 were analysed first by a µFTIR microscopy and finally by GCMS-pyrolysis technique. Eight among the most commonly used plastic polymers such as: polyethylene - PE, polypropylene - PP, polystyrene - PS, polyvinyl chloride - PVC, polyamide - PA66, polymethyl methacrylate - PMMA, Polycarbonate - PC and polyethylene terephthalate - PET of purity >99% were investigated.

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Results and discussion

Occurrence of microplastics in the fish food production: preliminary results indicate negligible levels of microplastics contamination in most of the analyzed raw materials. Limited amounts of plastics in the range of few µg/g of PE and PA66 in fish meal and PP in the wheat gluten source were observed. Further investigations on the wheat gluten production line helped to identify part of the industrial process responsible to the PP release.

Occurrence of microplastics in selected environmental matrices: PE, PP, PVC and PA66 were observed in all investigated matrices with highest levels, in range of tens of µg/g, for each of the addressed polymers, reported in sediments and in suspended matter. The site targeted as reference in the study showed a similarly represented pool of polymers but with different accumulation levels. The observed preliminary results point out the complexity of the polymers distribution interpretation hampered by the multiple input source in the aquatic environment.

Conclusions

The ongoing project has so far helped to identify some industrial process responsible to the release of small amounts of plastics. Some solutions were successfully placed to reduce the potential impact of microplastics on the environmental footprint of Atlantic salmon farming. Part of the analyses are still in and will be presented during the conference.

Acknowledgements. The project TRACKing of PLASTtic emissions from aquaculture industry (TrackPlast) has been granted by Fiskeri- og havbruksnæringens forskningsfinansiering (FhF) as part of the strategic plan aiming at evaluating the environmental and industrial impact of plastic litter.

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MOWI, 2018 Integrated Annual Report – Leading the Blue pp 145

NUTRITIONAL REQUIREMENTS OF EUROPEAN LOBSTER (*Homarus gammarus*, L.): EFFECT OF PROTEIN, LIPID, AND CARBOHYDRATE DIET CONTENT ON METABOLIC POSTPRANDIAL RESPONSE

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Introduction

The general decline of European lobster (*Homarus gammarus*) natural stocks has been counteracted with the release of hatchery-reared juveniles in the wild. However, growth and survival rates in lobster culture are still low. Moving away from live/frozen feed and embracing the use of dry formulated diets can support simpler and more reliable production considering their more stable storage and consistent nutritional quality (Powell *et al.*, 2017)which impact on larval survival, development and growth. For early-stage European lobster, Homarus gammarus larvae, feeding ecology and body composition are largely unknown. We initiated four progressive feeding experiments (novel feed types, feeding regime and feed size and cannibalism effects. The development of a balanced diet requires species-specific information on their nutritional requirements. Some nutritional studies have been conducted in *H. gammarus* (Glass and Stark, 1995, Powell *et al.*, 2017)"id":"ITEM-1","issue":"3","issued":{"date-parts":[["1995"]]},"page":"424-433","title":"Carbohydrate digestion in the European lobster Homarus gammarus (L. but appropriate requirement levels are still to be defined. The postprandial metabolic cost, commonly referred to as specific dynamic action (SDA) is dependent on several factors including animal size, meal composition and quantity. Therefore, SDA quantification is a useful metric of the overall nutritional performance of an animal (Secor, 2009)such as the mahi-mahi (Coryphaena hippurus. Understanding the species- and diet- specific postprandial metabolic costs can be used in the evaluation of formulated feeds. In an attempt to define appropriate requirement levels of protein, and the role of non-protein energy, the present work evaluated the metabolic response of lobster juveniles fed six experimental dry feeds, differing in macronutrient composition. SDA, feed intake, and growth performance were determined and their significance discussed

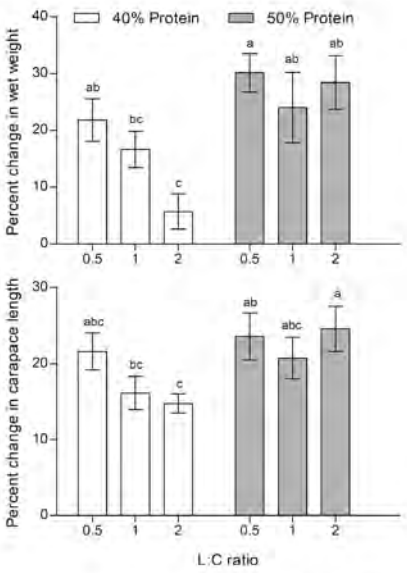

Fig. 1. Mean percent growth gain of E. lobster at the end of respiratory trials. Letters in superscript indicate significant differences between means.

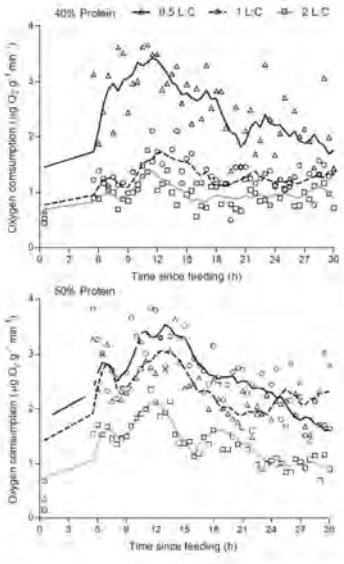

Fig. 2. Plots of postprandial metabolism over time in each diet.

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Materials and Methods

Lobster juveniles (1.0±0.1g) were individually acclimated in a recirculation system for 4 weeks to the experimental diets before respiratory trials (18±0.5˚C temperature, 34±1 psu, >7mg/L dissolved oxygen, <0.1mg/L NH_4), under a 8L:16D photoperiod. During acclimation they were fed their diets in excess each morning for 4h. Six formulated diets were evaluated in this experiment with two fixed protein levels (400 and 500 g/Kg) at 3 lipid (L) - carbohydrate (CHO) energy ratio (0.5, 1, and 2 L:CHO): Oxygen consumption rate (MO_2) was determined in individual metabolic chambers over a 96h period in 30min measurement loops. Standard metabolic rate (SMR) was determined from the first 48h of measurements on fastened individuals. After 48h, chambers were opened and 1 pellet offered to each lobster. After a 2h feeding period, remaining pellets were carefully removed and MO_2 postprandial measurements continued for 48h. Following each respiratory trial, each lobster was transferred to a 100 ml container and offered a pre-weighted pellet for 2h. The uneaten fraction was collected, filtered, and dried for feed intake estimation. Wet weight and carapace length of individual lobsters were measured at the end of the experiment.

Results:

Individuals were attracted to all experimental diets and actively fed. Protein content had a significant effect on the growth rate. In the group of animals fed 40P diets, a larger contribution of carbohydrate energy resulted in significantly better growth than when non-protein energy was mainly derived from lipid (Fig. 1). Higher protein levels in the diets prolonged the time to reach peak SDA, although no statistical differences were detected in the duration, peak, or magnitude of the SDA. There was a tendency for lower values in animals fed low protein and high lipid diets (Fig. 2), possibly as a result of significantly reduced feed intake in these groups

Discussion:

The positive effect of protein content on SDA response agrees with previous studies showing that SDA increases with the relative protein content of a meal (Whiteley *et al.*, 2002). Also, the smaller the size of the meal, the lower the assimilation and digestion metabolic costs (Secor, 2009)the metabolic response that accompanies meal digestion has been characterized, theorized, and experimentally studied. Historically labeled \"specific dynamic action\" or \"SDA\", this physiological phenomenon represents the energy expended on all activities of the body incidental to the ingestion, digestion, absorption, and assimilation of a meal. Specific dynamic action or a component of postprandial metabolism has been quantified for more than 250 invertebrate and vertebrate species. Characteristic among all of these species is a rapid postprandial increase in metabolic rate that upon peaking returns more slowly to prefeeding levels. The average maximum increase in metabolic rate stemming from digestion ranges from a modest 25% for humans to 136% for fishes, and to an impressive 687% for snakes. The type, size, composition, and temperature of the meal, as well as body size, body composition, and several environmental factors (e.g., ambient temperature and gas concentration. Therefore, feed intake observed for low protein - high L:CHO ratio diets would explain not only the lowest performance in terms of growth but also the smaller SDA response. Moreover, the decreased SDA response in 40P diets could be indicative of a decreased digestibility and assimilation of those diets. Growth and SDA results from this study corroborate the hypothesis that juvenile European lobster requires a minimum of 500 g/Kg protein content in their diet. This level agrees with the dry matter protein content reported by Powell *et al.* (2017) supporting the idea that diets should meet the organism biochemical composition. Our study showed that it is viable to grow E. lobster juveniles on formulated dry feeds without compromising their metabolism and growth. High protein is a requirement; however, its inclusion can be reduced when compensated with moderate carbohydrate levels. Reducing protein inclusion is particularly important taking into account the need for more sustainable formulated feeds.

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THE DEVELOPMENT OF AQUACULTURE IN THE CANARY ISLANDS: NEW PERSPECTIVES AFTER THE PUBLICATION OF THE "PROAC" AND THE "NEW CANARY AUTONOMY STATUTE"

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Aquaculture is an economic activity that is sustainable and respectful of the marine environment, since, in its exploitation, it favours regeneration, the development of fishery resources, the protection of marine protected species and human health (against plastic pollution that affects fish in the open sea).

In fact, thanks to aquaculture it is no longer necessary to catch or kill fish in the open sea, which may affect, even accidentally, to those others fish that are trapped in nets and are not suitable for food, especially those species that are protected. On the other hand, thanks to the cultivation of fish, aquaculture can satisfy the human needs of food (the average consumption of current marine products is 23.1 kg per person, of which 24% comes from aquaculture).

Despite the previous statement, an **orderly planning** is necessary because both the inadequate and lack of planning can generate a degeneration or imbalance of marine ecosystems motivated by the organic waste released from the cages and other facilities that are located in the sea, as well as, by the interference of human activity in the natural development of the marine environment. Because of that, it is essential to make an adequate aquaculture planning in the Canary Archipelago.

In the scope of the MarSP Project, indeed, an important part is reserved for the management of the aquaculture of the **Canary Sea**, with the aim of developing a respectful planning **of the principles and environmental limitations of the Directive n. 89/2014 (EU)** and, at the same time, **appropriate to the interests of entrepreneurs and researchers**.

In this line, **it is appreciable the effort of the Canary Islands Regions to renewal and adaptation to European demands**, thanks to regulatory changes of great interest such as the approval of **the new Statute of Autonomy of the Canary Islands** and the enactment, also, of the new **Regional Plan of Ordination of the Canary Islands Aquaculture** (PROAC). In this framework, the foundations are laid for a new scenario more current and adapted to the new reality.

In spite of this, however, the new ordination does not manage to solve with agility the problems encountered by entrepreneurs to access the aquaculture sector, basically because the new regulation is linked to the existing one, without clarifying its coherent application and clarity, and without the adequate responses to the new needs that arise in this sector. As it will be shown, indeed, the procedures are complex, long in time and above all, at times, contradictory to each other.

These problems will be the object of analysis in the present work, taking into account **the great opportunity that this sector supposes for the Canary Islands**, given its strategic geographical position and its vast marine resources, in order to be able to develop it in an increasingly constant and efficient way, so that this region can become a standard-bearer of this type of company internationally.

Legal Framework: Aquaculture in the Canary Islands

The Autonomous Community of the Canary Islands has such competence in accordance **with article 131, paragraph 3, of the Organic Law 1/2018, of November 5, of the reform of the Statute of Autonomy of the Canary Islands (hereinafter EAC),** according to which corresponds to the Autonomous Community of the Canary Islands competence in matters of activities in the maritime areas defined in Article 4 of the same Statute, which includes, in any case, the planning, management, management of shellfish and aquaculture, as well as the facilities destined to these activities. It is the responsibility of the Island Councils to perform executive functions in aquaculture and marine farming (Article 70.2 ñ of the EAC).

According to **article 131.4 of the EAC**, the Autonomous Community of the Canary Islands, without prejudice to those that may correspond to the State, has the exclusive competence in internal waters to delimit and declare protected areas of fishing interest, as well as to establish zones of special interest for shell-fishing, aquaculture and recreational, sports and ecotourism activities.

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In particular, with regard to aquaculture, the aforementioned **Law 17/2003 establishes that the Regional Plan for the Management of Canarian Aquaculture** (PROAC; *Plan Regional de Ordenación de la Acuicultura de Canarias*; original name) will be configured as an instrument for the management of aquaculture activity in the Autonomous Community of the Canary Islands, and will to be prepared by the Ministry of the Canary Islands Government responsible for fisheries

This norm was developed **by Decree 102/2018, of July 9,** which definitively approves the Regional **Plan for the Management of Aquaculture of the Canary Islands** (hereinafter, PROAC), norm that currently establishes the general management of aquaculture in the Autonomous Community of the Canary Islands and, specificall , the zones and species of interest for marine crops, the areas and prohibited species, as well as, the technical characteristics and the conditions of the exploitations.

Regarding the authorization procedures or concessions for the development of the aquaculture activity, it is necessary to take into account **the Decree 182/2004, of December 21,** which approves the Regulation of the Fisheries Law of the Canary Islands, as it is which continues to regulate the procedure for granting aquaculture concessions and the procedure to obtain the pertinent authorizations, the procedures can be activated by the stakeholders.

Therefore, and in response to what has been said, in practice the aquaculture activity in the Canary Islands is regulated in accordance with the following rules:

1) Regarding the authorization procedures or concessions for the development of the aquaculture activity, the **Decree 182/2004, of December 21, which approves the Regulations of the Fisheries Law of Canary Islands**, that continues regulating the procedure of granting aquaculture concessions and the procedure to obtain the pertinent authorizations.

With respect to the obtaining of the concession and/or authorization qualifying titles, the above-mentioned **Decree 182/2004, of December 21**, establishes, in its Title IV:

- The procedure for granting aquaculture concessions (Chapter I)
- The procedure for granting authorizations (Chapter II);
- The area of occupation (Chapter III);
- The constitution and composition of the Regional Aquaculture Commission (Chapter IV);
- The Register of Aquaculture Holdings (Chapter V).

2) Considering the location of the cages, the arable species and other technical measures specifically related to the aquaculture companies, it is necessary to take into account the **PROAC which, for its part, orders**:

- Areas and species of interest for marine crops;
- prohibited areas and species and technical characteristics;
- the conditions of the farms.

The PROAC regulates the areas and the keys species for the marine cultures, the areas and the prohibited species, and the techniques features, and the farms conditions.

According **to art. 22 of the PROAC**, in general, for the establishment of an establishment for aquaculture, will be mandatory:

* The delimitation of a Zone of Interest passes the Aquaculture (Z.I.A.);
- Ordering in detail the area by delimiting parcels. In addition, it will be necessary to specify the maximum productive capacity per parcels and the area of occupation of each of them, the species to be cultivated and the type of establishment, these two last determinations being of nature.
- However, for the cases of aquaculture authorizations for research/experimentation and/or training in the Z.I.A., they may be granted without the need for detailed management;
- Obtaining qualifying licenses and / or authorization, required by the Fisheries Law of the Canary Islands depending on the purpose intended; marketing, research / experimentation, training or repopulation.

Problems and possible solutions

The final part of our intervention will focus on identifying the main problems of the Canarian legislation thus conceived and proposing some possible solutions.

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In general, we can point out that the procedure required for entrepreneurs interested in investing in aquaculture in the Canary Sea seems well structured and updated (as far as the PROAC is concerned), but it is a bit complicated.

A. On the one hand, it is necessary to request authorizations or concessions according to legislation dating back to 2004. However, at the same time, in order to decide where the aquaculture companies can be installed and which species to grow, a compatibility *favourable report* between the project to be developed and the PROAC is indispensable.

We must also point out that within the PROAC, further authorizations or certificates are required (among them, the *environmental impact assessment*) for certain forms of aquaculture business, aggravating the procedure required of businessmen with the addition of many other complex formalities.

The regulatory options seem to be coherent, but, in any case, it would be desirable that the Canarian legislation be updated also with regard to the authorization procedures, to simplify the structured procedure and divided into different sectors, and as regards the new European targets set in the Directives enacted in recent years on biodiversity, the environment and the fight against climate change

B. It should also be noted that after the publication of the new **EAC, the boundaries of the Canary Sea were modified (Organic Law 1/2018, of November 5, article 4, second paragraph).** This means that it would be advisable to update the aforementioned Law 17/2003 and Decree 182/2004 on the basis of the new frontiers, especially in those articles that delimit the space dedicated to aquaculture, so that there is no confusion in the application phase current.
C. Another problem is about the difficult of introducing new cultivable species not included in the PROAC. It would be desirable to establish the formulas and procedures to evaluate the introduction of the cultivation of new species that are not included in the PROAC, provided, of course, that they do not generate any danger to the ecosystem, as demonstrated by the new scientific advances
D. Finally, we have received proposals from entrepreneurs interested in constructing aquaculture plants in the open sea, but it seems that the current legislation foresees this possibility, which is having a great result abroad.
E. Finally, we must insist on the effects of the environmental impact study required by **Law 9/2006, of April 28, modified and updated in 2013,** but in which the necessary coherence with this modification is not appreciated, due to the documents and annexes of the PROAC are calibrated in accordance with the 2006 law.

NOVEL INSIGHTS INTO SEX-RELATED GENES IN THE COMMON LITTORAL SHRIMP *Palaemon SERRATUS* BY GONADAL TRANSCRIPTOMIC ANALYSIS

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Introduction

The common litoral shrimp *Palaemon serratus* is an economically important decapod resource in some European communities (Fahy et al. 2006). Aquaculture practices prevent the genetic deterioration of wild stocks caused by overfishing and at the same time enhance the production. The biotechnological manipulation of sex-related genes has the proved potential to improve the aquaculture production (Ventura and Sagi 2012). However, since the lack of genomic data about *P. serratus* hinders the application of any genetic manipulation-based technique, a depth-understanding about the genetic factors underlying the sexual and reproductive development of this species is mandatory. The aim of this study was to reveal candidate genes featured in sex determination, sex differentiation and gonadal development processes using a high-throughput sequencing approach.

Material and methods

Gonads from three adult females and three adult males were removed and total RNA was extracted to prepare three ovary and three testis cDNA libraries, i.e. three biological replicates per sex. Libraries were sequenced using the HiSeq 4000 PE100 platform (Illumina). A reference gonadal transcriptome was obtained by *de novo* assembly both ovary and testis clean reads together. Functional annotation of the resulting transcriptome was conducted and differential expression analyses were performed between the ovary and testis samples to identify sex-related genes with sex-biased or sex-specific expression patterns.

Results

A total of 224.5 and 281.1 million paired-end reads were produced from ovary and testis samples, respectively. *De novo* assembly yielded a transcriptome with 39,186 transcripts. The 29,57% of the transcriptome retrieved at least one annotation and 11,087 differentially expressed genes (DEGs) were detected between ovary and testis replicates. 6,207 genes were up-regulated in ovaries meanwhile 4,880 genes were up-regulated in testes. Candidate genes to be involved in sex-determination, sex-differentiation/differentiation and gonadal development were found in the transcriptome. These sex-related genes were discussed taking into account whether they were up-regulated in ovary, up-regulated in testis or not DEGs between gonads and in the framework of previous findings in other crustacean species

Discussion and conclusion

This work encompasses the first large-scale RNA-Seq and comprehensive transcriptome analysis of *Palaemon serratus* gonads. This dataset will surely facilitate further research into the reproductive biology of this shrimp. Specificall , a wide inventory of candidate sex-related genes is discussed, being the first time that sex-related genes have been addressed in a *Palaemon* species. Relevant findings that might shed light about the evolution of sex-regulators in crustaceans are reported. Moreover, we highlight some genes that could be targets towards investigating future aquaculture applications for *P. serratus*, mainly doublesex- and mab-3-related transcription factor 1 (Dmrt1), insulin-like androgenic factor (Iag) or vitellogenesis genes such as vitellogenin (Vg) or vitellogenin receptor (VgR) among other candidates.

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DETERMINING THE IMPACT OF PHOTOPERIOD, FEEDING REGIME, AND PLOIDY ON ATLANTIC SALMON *Salmo salar* POST-SMOLT HEALTH, GROWTH PERFORMANCE, AND MATURATION IN FRESHWATER RECIRCULATION AQUACULTURE SYSTEMS

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Introduction

Early maturation of Atlantic salmon *Salmo salar* has been a major source of economic loss for farmers (Good and Davidson, 2016), as precociously developed fish often exhibit decreased growth and feed conversion efficiency (McClure et al., 2007), reduced product quality (Aksnes et al., 1986), and increased susceptibility to opportunistic microorganisms (St-Hilaire et al.,1998). The initiation of sexual maturation can be difficult to prevent, given the numerous factors, such as photoperiod, temperature, fish size, growth rate, nutritional status, and genetics, that can influence the onset of puberty (Taranger et al., 2010). Early sexual maturation has been particularly problematic in land-based, closed containment recirculation aquaculture system (RAS) facilities raising Atlantic salmon to market size (Good and Davidson, 2016), with reported early maturation ranging from 2% to 100% of the salmon populations raised at seven RAS facilities. With increased investment in land-based smolt and post-smolt production facilities, and with a recent focus on carrying out land-based salmon production in freshwater (versus brackish or seawater), it is critical to gain a solid understanding of the environmental influences of maturation in freshwater RAS

Materials and methods

In a series of three long-term studies, we investigated various photoperiod regimes, feeding rates, and ploidy (i.e., diploid vs. triploid) as Atlantic salmon were raised in freshwater systems from fry to 1,000 g in size and beyond. Briefl , in *Study 1* we investigated three photoperiod regimes – LD24:0 (i.e., 24 hours light, zero hours dark), LDN (simulated natural photoperiod, Bergen latitude), and LD12:12 beginning at 100 g. All fish were initially cultured in 12 replicate 0.5 m^3 tanks in a flow through system, prior to transfer to a partial reuse system consisting of three 10 m^3 circular dual-drain culture tanks, microscreen filtration and gas conditioning (stripping tower, low-head oxygenator) after fish achieved a mean weight of 100 g. Each culture tank received the aforementioned photoperiod treatments until fish reached a mean weight of 1,000 g, after which all fish were marked and stocked into a single semi-commercial scale RAS under LD24:0 and raised to a harvest size of 4 kg. In *Study 2*, we assessed LD24:0 and LDN photoperiods in factorial combination with 100% (full ration, FR) and 60% (half-ration, HR) feeding regimes. Treatments were applied in 12 replicated 0.5 m^3 tanks in a flow-through system, prior to marking and transfer of all fish at 500 g (mean weight of best performing group) to a single partial reuse system under LD24:0, FR conditions, where fish were raised to 1,000 g mean weight. In *Study 3*, we carried out a second factorial-design study investigating S_0 vs. no-S_0 winter photoperiods and ploidy (diploid vs. triploid) in all-female Atlantic salmon. Diploid and triploid salmon either received or did not receive a winter photoperiod (6 weeks LD12:12, followed by a return to LD24:0) while cultured in 12 replicated 0.5 m^3 tanks in a flow-through system. All fish were transferred to a partial reuse system and raised to 1,000 g, after which they were marked and transferred to a single semi-commercial scale RAS under LD24:0 photoperiod. At the time of abstract submission, these fish are approximately 3 kg mean weight, and will be raised to 4-5 kg harvest size.

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Results

At the time of abstract submission, results of numerous physiological analyses remain forthcoming. Major results available at this time include: *Study 1* LDN photoperiod was associated with reduced growth rate up to 600 g, and all photoperiods demonstrated high levels of maturation by harvest size, with the least maturation observed in the LD24:0 treatment group; *Study 2* FR groups demonstrated superior growth performance, with best condition factor and growth observed in the LD24:0/FR group and poor condition factor observed in both HR groups; and *Study 3* no maturation has so far been observed in triploid cohorts, while all-female diploid salmon are demonstrating approximately 20% maturation based on morphological signs (presence of ovipositor, ventral softness). A comprehensive assessment of maturation including gonadosomatic index quantification will be carried out at harvest. Full results for all three studies will be presented at Aquaculture Europe 2019.

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CARBON DIOXIDE STUNNING OF RAINBOW TROUT (*Oncorhynchus mykiss*): THE EFFECTS OF DIFFERENT SEASONAL TEMPERATURES AND THE RELIABILITY OF VISUAL INDICATORS OF CONSCIOUSNESS

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Introduction

Humane slaughter requires that the fish is stunned before being bled. The ideal stunning method before slaughter is one that induces instantaneous and long-lasting unconsciousness that allows staff sufficient time to kill the animal (Van de Vis et al. 2003). A fundamental limitation when assessing the effectiveness of different stunning methods used for fish is the difficulty of determining when consciousness is lost (Kestin et al., 1995; Lambooij et al., 2010). The overall objective of this study was to monitor how animal welfare is safeguarded during the stunning of rainbow trout (*Oncorhynchus mykiss*) at different acclimation temperatures using narcosis with carbon dioxide (CO_2). Specificall , we investigated if the time it takes for a fish to lose consciousness is affected by the seasonal temperature and how reliable visuals indicators are at identifying the moment when a fish loses consciousness

Material & Methods

For this, we visited a Swedish fish farm and recorded visual indicators of consciousness (*i.e.* loss of equilibrium, ventilation and eye-roll reflex) in rainbow trout during narcosis in a water bath fully saturated with CO_2. This was done on three different occasions over a year so that fish acclimated to 2, 8 and 14°C were included in the study. In addition, a complementary laboratory study was conducted to verify that the behavioral indicators in part 1 of the study did indeed function as reliable indicators of unconsciousness in rainbow trout. Here, we used a newly developed non-invasive technique that allows measurements of brain activity by electroencephalography (EEG) in rainbow trout with electrodes attached to the head of the fish with a silicone cup. EEG allowed us to monitor visual evoked responses (VERs) from light stimulation that are present while a fish is conscious and gradually dissipate as brain activity (*i.e.* consciousness) is lost (Kestin et al., 1995). By monitoring brain activity via VERs and behavioral indicators of consciousness simultaneously we investigated how accurate different visual indicators are at determining the loss of brain function in rainbow trout during CO_2 narcosis.

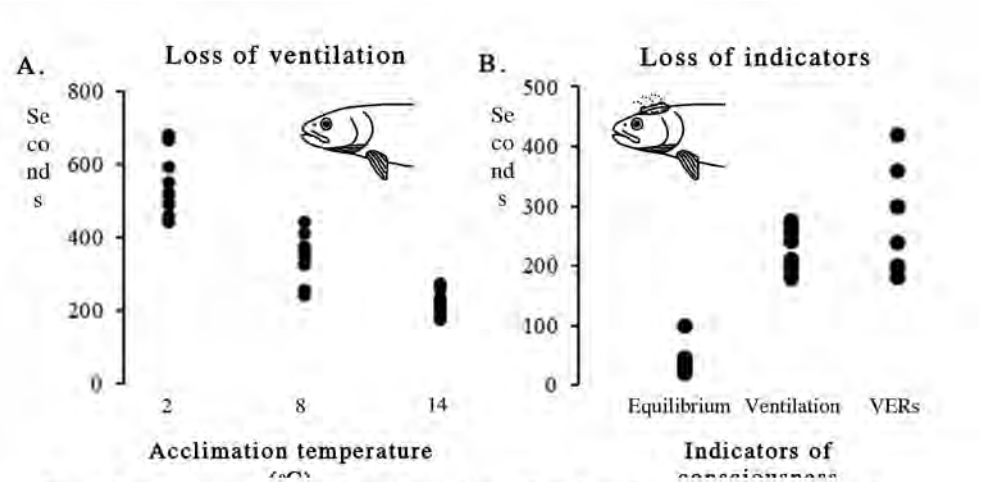

Fig. 1. Loss of consciousness during stunning with carbon dioxide. Times until ventilation is lost for individual fish at three different season temperatures (A). Times until the behavioral indicators (equilibrium and ventilation) and brain activity (Visually Evoked Responses on the EEG) are lost for individual fish (B).

Discussion & Conclusion

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Results

Low water temperatures significantly delayed the time it took for all visual indicators to be lost (figure 1A). When stunned with CO_2 during winter (2°C) it took up to 2.25, 6.0 and 11.3 min for the trout to lose equilibrium, eye-roll reflex and ventilation, respectively. While during summer (14°C) these times were shorter with only 1.4, 2.5 and 4.6 min required for loss of equilibrium, roll reflex and ventilation, respectively. Furthermore, our laboratory study, conducted at 10°C, showed a poor relationship between the visual indicators of consciousness and loss of brain function (VERs). The VERs were lost up to 6.5 and 3.5 min after the fish had lost equilibrium and ventilation, respectively (figure 1B). In both experiments, trout exhibited immediate aversive reactions after being submerged in the CO_2 water bath, including violent escape attempts and rapid swimming, which continued until equilibrium was lost.

Discussion & Conclusion

Our results show that CO_2 narcosis is not an ideal stunning method before slaughter as it does not induce instantaneous unconsciousness and causes aversive behaviours and that these problems are even further aggravated by low environmental temperatures. These results are not surprising considering that CO_2 narcosis was deemed unacceptable as a stunning method for fish by the European Food Safety Authority (EFSA) already in 2004. Yet, in Sweden, it is still the dominating method used for rainbow trout and other species. Furthermore, our results show that the visual indicators of consciousness that are currently used to assess if fish are unconscious are unreliable and if used risks that subsequent exsanguination and evisceration occurs in paralyzed but conscious animals. The results highlight that behavioral indicators are not sufficient to determine unconsciousness in trout during CO_2 narcosis. To safeguard the welfare of fish during stunning it is crucial to evaluate stunning methods using EEG to detect unconsciousness rather than relying on visual indicators alone.

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FISH FARM SENSORS AND THE INTERNET OF THINGS

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Precision aquaculture ((Føre et al 2018) involves a variety of sensors used to gain insight into the farm environment, make decisions which optimize fish health, growth and economic return, and reduce risk to the environment. This trend parallels developments in agriculture (Wolfert et al. 2017) where sensors and other observing technologies lead to enhanced insight into crop health as well as animal welfare. For aquaculture, this includes sensors specific to monitoring of oceanographic conditions as well as technology such as acoustic fish tags to detect fish movement and behaviour. Aquaculture has become part of the Internet of Things defined as interconnected sensors which store and serve data, interact with other sensors and devices, and provide decision support. Sensor networks produce huge volumes of information or Big Data. We have initiated extensive research in this area with multiple partners including fish farmers (Cooke Aquaculture; Cookeseafood.com) and the ocean technology company RealTime Aquaculture (rtaqua.com). In addition, a new research network DeepSense (deepsense.ca) has been established at Dalhousie in partnership with IBM to provide analytics to ocean industries (Fig. 1). We are also investigators in Horizon 2020 project GAIN (Green Aquaculture Intensification; unive.it/pag/33897), in which similar sensor deployments are underway in Spain, Scotland, and Norway. Hundreds of sensors have been deployed in Canada at multiple salmon farms. Sensors are primarily observing oxygen and temperature with data provided in real time to the cloud and to mobile devices. Additional data on fish position is provided by the CageEye acoustic system (cageeye.no) as well as individually tagged fish. Our research objectives include resolving (a) scales of variation in sensor deployment, (b) management of Big Data and use of data analytics to mine predictions related to fish growth and health, (c) development of additional sensors for understanding the aquaculture ecosystem, and (d) integration of data into coastal information portals and wider metrics of coastal health. Since we are in the initial stages, an overview of the ongoing research is presented as well as initial results of sensor outputs at Nova Scotia fish farms

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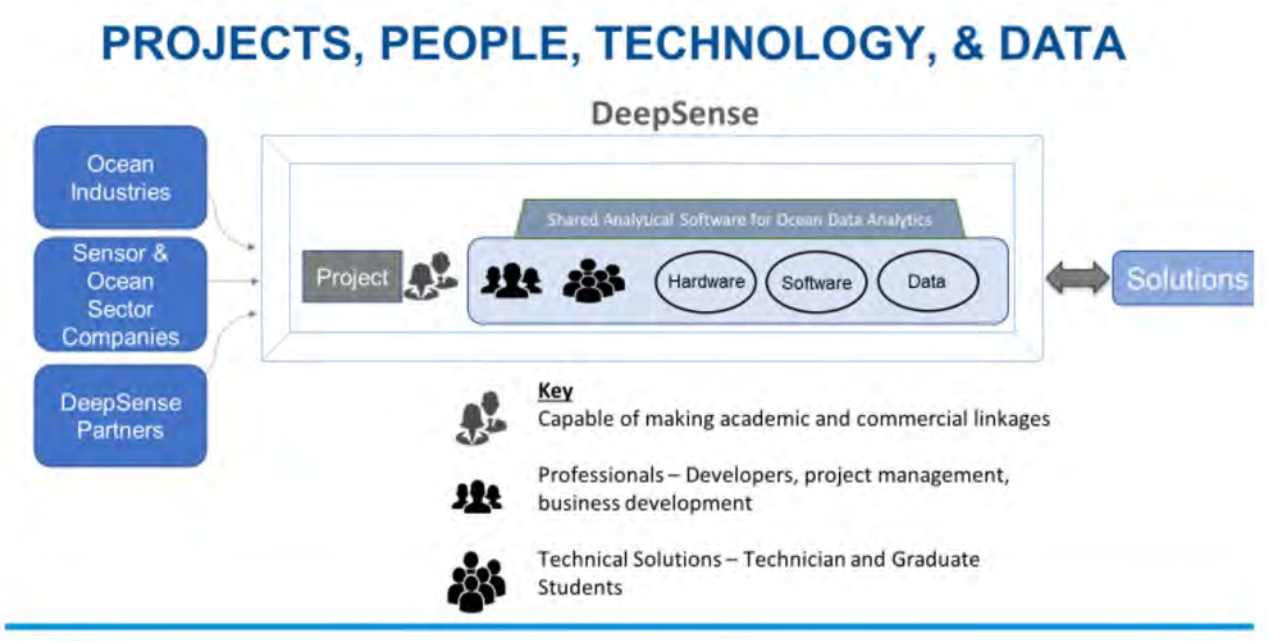

RISK ASSESSMENT OF NORWEGIAN SALMON FARMING – A NEW APPROACH

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Norwegian salmon aquaculture has since its pioneering days in the 1970s, grown into a significant industry. Of the total aquaculture production of nearly 1.3 million tons in 2017, Atlantic salmon counted for 94.5% and is one of the country's largest export industries by economic value.

A total of 1325 active licenses, including production of eggs and juveniles and open-cage production, is distributed along most of Norway's coastline. In 2018 the standing biomass in the salmon farms varied between about 600 000 and 800 000 tons during the yearly production cycle. Such substantial amount of fish represents on one hand a potential hazard to the environment but on the other hand constitute an efficient method for producing valuable seafood. Understanding the risk situation is essential for both local and national authorities to enable knowledge-based governance.

Annual risk assessments of the environmental impacts of Norwegian salmon farming have been conducted by the Institute of Marine Research and collaborators since 2010, and have evolved over time in parallel with increased knowledge of the risk factors. This includes effects of salmon lice and other diseases on wild salmonids, genetic introgression of farmed salmon in wild salmon populations, local and regional impacts of nutrients and organic loads, effects of contaminants and drugs, environmental impacts related to the use of cleaner fish in salmonid aquaculture, ecological impacts on wild fish populations and mortality as well as fish welfare of the farmed fis

Several different approaches have been suggested and discussed for risk analysis of marine ecosystems and marine aquaculture activities. Risk analysis, as a scientific field is young, not more than 30-40 years old. Risk assessment in the aquaculture industry is, however by comparison still in its infancy. The approaches adopted to analyse aquaculture-risk are for the most part driven by statistical analyses of available hard data where inclusion of expert knowledge and opinons are thought to introduce subjectivity and uncertainty to the end risk results. Lack of hard data is common and may thus lead to inability to perform a risk assessment on the subject at all. The objective of risk assessments is, however, not to accurately calculate risk, but to provide the best foundation for risk-based decisions.

A new risk assessment framework is needed that is tailor-made for risk-based governance of aquaculture and in line with the new perspectives on risk analysis. Documenting knowledge strength, evaluating assumptions and systematizing risk sources, consequences and associated uncertainties in hierarchical and intuitive structures contributes to risk understanding and improved decision making. Bayesian Networks allow combinations of hard data and expert knowledge on the input side and highlights and documents the results in terms of hierarchical structures of risk sources and consequences.

Contrary to some other methods such as deep learning or purely data-driven methods, Bayesian networks combines data and expert knowledge as well as support explanation of results. The graphical nature of the models is intuitive to the domain experts and other stakeholders and it is possible to explain why a specific result is produced by the model. Due to the modular construction of the model, it can be adjusted using local changes only.

In this talk we present the method used for the risk assessment of environmental impact of fish farming in Norway in 2019.

EXPLORATION OF THE QUALITY OF REARED VS. WILD GILTHEAD SEABREAM: PROFILING THE SENSORY DIFFERENCES OCCURING BETWEEN POPULATIONS

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Introduction

Gilthead seabream (*Sparus aurata*) is one of the main fish species cultivated in EU, accounting for approximately 14% volume of the total finfis aquaculture production. Due its great share in EU aquaculture, research has focused in exploring the quality features of reared counterparts, using as a reference for comparison wild ones, since consumers perceive wild fish as having a favourable quality to reared ones. While the research in this area is quite extensive, it mainly focuses in the nutritional quality while only scarce data exist in terms of the sensory differences of reared and wild gilthead seabream (Grigorakis, 2017). However, acquiring a full assessment of the sensory profile is considered crucial, since it has been shown that small changes in aroma and flavour modalities can have a higher impact in consumer preferences, than that of fish texture modality (Alexi, Byrne, Nanou, & Grigorakis, 2018). The aim of the current study was to explore the proximate composition, fatty acid (FA) profile and provide a full sensory profile, including aroma, taste, flavour and texture, of reared vs. wild gilthead sea bream.

Materials and Methods

Study design & sampling: The wild and reared gilthead seabream groups consisted of 12 specimens each. For each specimen, one fillet was kept for proximate composition and FA analyses (-20°C) and the other for sensory analysis (-80°C). The average weight of the wild and reared seabream fillets was 21g (±4.8) and 55.1g (±8.9), respectivel .

Analyses: Proximate composition analyses were performed according to the standard AOAC (2005) methods. FA composition was analysed after methylesterification by GC-FID. The oven-cooked fish samples were profiled via sensory generic descriptive analysis (DA). Details on the sample preparation and DA processes can be found in Alexi, Nanou, et al. (2018). Final evaluation of samples was performed in duplicates, in individual sensory booths by 7 expert panellists in fish evaluation, using a 15cm scale for the evaluation the attributes' intensities.

Results

The proximate and FA composition of the fillets is presented in Table I.
All proximate composition variables varied significantly (P <0.05), whereas significant variations were found in all FA groups with the exception of polyunsaturated FA (PUFA).

The odour, taste, flavour and texture profile of the reared and wild gilthead seabream specimens is presented in Figure 1. Specimens of different rearing origin were discriminated by their odour and texture, whereas the taste profiles were similar between wild and reared specimens. Some differentiations were identified between the flavour profiles of counterparts of different rearing origin, however the variations were not found significant

Discussion-Conclusion

Higher fat and protein content in reared seabream counterparts, compared to wild

- No differentiation in PUFA proportions between reared and wild counterparts. Higher ARA, EPA, DHA proportions and n-3/n-6 ratio in the wild specimens
- Distinctive shrimp odour in wild gilthead seabream specimens. Reared specimens characterized by boiled potato/ vegetable aroma and fish oil aroma as well as a juicier texture and oil coating sensation, when compared to wild

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Table I: Mean Values and standard deviation of fillet proximate composition and FA profile of wild and reared gilthead seabream. Variable significance is calculated by 1 way ANOVA performed with Tukey post-hoc test at 95% confidence level.

	P-value	Wild	Reared		P-value	Wild	Reared
Proximate composition (%)				***Fatty acid composition (%)***			
Moisture	<0.001	79.78a ±1.96	71.71b ±1.25	n-9	<0.001	23.19b ±4.82	38.16a ±4.31
Fat	<0.001	1.67b ±1.09	7.64a ±1.21	n-6	<0.001	11.13b ±1.91	18.70a ±2.39
Protein	<0.001	17.29b ±1.28	19.28a ±0.57	n-3	0.001	24.20a ±5.94	16.84b ±3.71
Ash	0.006	1.26b ±0.09	1.36a ±0.06	ARA	<0.001	7.62a ±1.52	0.85b ±0.41
Fatty acid composition (%)				EPA	<0.001	5.70a ±0.96	2.73b ±0.61
SFA	<0.001	33.03a ±2.19	22.69b ±1.08	DHA	0.008	13.59a ±5.31	8.27b ±3.31
MUFA	<0.001	29.10b ±5.15	40.96a ±4.61	n-3/n-6	<0.001	2.20a ±0.55	0.92b ±0.24
PUFA	ns	35.81 ±6.87	36.21 ±4.22				

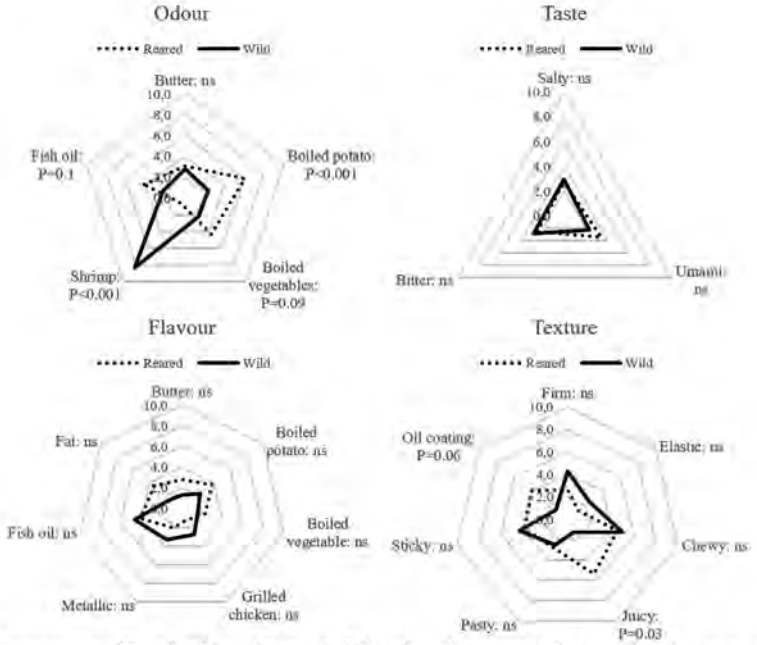

Figure 1: Sensory profile of wild and reared gilthead seabream specimens. Attributes measured on a 15cm scale and significance calculated by mixed model ANOVA; ns: non-significant

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POSTPRANDIAL PLASMA-FREE AMINO ACID RESPONSE TO BRANCHED-CHAIN AMINO ACID SUPPLEMENTATION IN ATLANTIC SALMON (*Salmo salar*)

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Introduction

Branched-chain amino acids (BCAAs), consisting of Valine (VAL), Leucine (LEU), and Isoleucine (ILE), are a group of dietary essential amino acids (EAA) (NRC, 2011). In humans, BCAAs have been linked to an increase in muscle growth and performance, and reduction in muscle loss (Holeček, 2018). The metabolism of BCAA in other vertebrates show they are metabolised differently to other EAA with the first step in catabolism occurring in extrahepatic tissue. This poster will be examining the digestibility of the experimental diets enhanced with BCAAs and the post-prandial plasma responses of free amino acids (fAAs) in *Salmo salar* (Atlantic salmon) over a 24h period.

Materials and methods:

Five extruded isoenergetic and isonitrogenous diets (4mm ø) were formulated and manufactured. The basal diet was supplemented with 20g kg^{-1} of each BCAA (LEU, ILE, VAL) in a crystalline form and yttrium oxide (2g kg^{-1}), as a marker for digestibility analysis. Thirty-five Atlantic salmon post-smolts, averaging 263.13g (±24.52), were stocked in each tank and allowed to acclimatise for one week on their allocated diets. Fish were fed to apparent satiation, with uneaten feed being collected to estimate the daily intake in each tank. Fish were starved for 24 hours prior to assessment of their postprandial responses. Two hours before feeding, three fish were euthanised to establish baseline. Feeding resumed on test diets at 1.25g fis $^{-1}$. Blood and tissue samples were then taken at 2h, 4h, 8h, and 24h postprandially from three fish per tank at each time point. In addition, at the 8hr time point, all but three fish were anaesthetised with MS222 to manually strip faeces for further assessment of diet digestibility. This entire procedure was conducted three times over a three-week period to obtain replication through a blocking approach, where fish were randomly reallocated amongst the five tanks

Blood samples were taken from the caudal vein and centrifuged, after which the plasma was removed and stored at -70°C. An adapted method was used in the analysis of the plasma fAAs. Samples were then derivitised according to the Waters AccQ•Tag derivitisation method. The AA composition in feeds was analysed using an adapted Waters feed hydrolysis method and run at two varying temperatures on a CEM Mars 6 microwave digester Results were statistically analysed using analysis of variance (ANOVA), followed by a Fishers LSD planned comparisons post-hoc test.

Results and Discussion

Initial AA analysis of the experimental feeds confirmed the expected levels of increased BCAAs specific to each diet. The basal diet values comprised of LEU 4.42%, VAL 2.43% ILE 2.22%. In contrast, the LEU diet contained 5.86% VAL diet 4.00% and ILE diet 3.68%. The mixed BCAA diet contained LEU 4.90% VAL 2.23% and ILE 1.98%. No significant differences in feed intake were observed between the different treatments during the acclimation periods. The preliminary plasma fAAs results demonstrated that the BCAAs had a post-prandial peak concentration in the plasma at the 4h sampling point before then subsiding to just above baseline by 24h. The treatment plasma fAA levels were compared against that of fish fed the basal diet, allowing for visualisation of the treatment specific fAA effects (Figure 1). The sudden and elevated peak times of plasma concentrations may be due to the inclusion of the crystalline AAs (cAAs) and rapid absorption times. Other studies have shown peak times of 12h with returns to baseline up to 48h post-feeding. As found in this study, supplementation of ILE in *Oncorhynchus mykiss* (rainbow trout) increased concentrations of VAL and LEU for up to 24hr post-feeding in other trials (Tantikitti & March 1995).

The levels of each of the added crystalline AAs peaked at 4 hours. This was similar to the peak time response seen in a study of the postprandial plasma fAA profile of *Lates calcarifer* (barramundi) when fed supplemental crystalline methionine (Poppi *et al.* 2019). However, in our study no significant variation in feed intake was observed among experimental diets, which contrasted observations of Comesaña *et al.* (2017) who reported significant variations in feed intake, with higher intake observed in VAL diets in comparison to a decrease in LEU diets.

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INTEGRATED MULTITROPHIC AQUACULTURE WITH THE SEA URCHIN *Paracentrotus lividus* AND THE SEA CUCUMBER *Holothuria tubulosa*: AN INNOVATIVE MODEL OF SUSTAINABLE AQUACULTURE

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Introduction

There is increasing focus on the effects of organic wastes produced by intensive aquaculture, and on solutions to reduce the pollutive impacts of marine farms worldwide. Integrated Multitrophic Aquaculture (IMTA) offers a natural means of converting the organic wastes of aquaculture activities into food inputs for the production of lower-trophic-level crops of commercial value, increasing both the environmental and economical sustainability of aquaculture (Troell et al., 2009).

The present study evaluated, for the first time, the potential co-culture of the sea cucumber, *Holothuria tubulosa,* and the sea urchin, *Paracentrotus lividus*. These species are good candidates for co-culture in IMTA systems, considering their feeding habits and high market value. Indeed, omnivorous animals, such as sea urchins, are traditionally considered to be relatively low-input and thus more sustainable market species. Instead, sea cucumbers are deposit feeders which are able to ingest sediment with organic matter produced by farm activities. *P. lividus* and *H. tubulosa* are two of the most commercially exploited echinoderms in Mediterranean regions and they are considered a delicious food with high market value. *P. lividus* gonads ("*roe*" or "*uni*") are luxury sea food, reaching prices of over 150 € kg^{-1}. *H. tubulosa*, as other sea cucumbers, is dried and exported to Asian countries where it is considered a culinary gourmet food reaching prices of above 100 € kg^{-1} (Purcell et al., 2012; Carboni et al., 2014). Therefore, this study was designed to evaluate an integrated mutitrophic aquaculture between these two species, directly feeding only the sea urchins a land-vegetable diet. The aim of this study was to demonstrate the possibility of developing a viable aquaculture of commercially important species of echinoderms in IMTA, with *P. lividus* as the primary species and *H. tubulosa* as an extractive species.

Materials and Methods

Thirty-six specimens of *P. lividus* and *H. tubulosa,* collected at S. Marinella in the central Tyrrhenian Sea, were employed in this experiment, which lasted three months (15 March-20 August 2018). The experiment was run in three aquaria of a RAS (Recirculating Aquaculture System). This rearing system, connected in tandem, guaranteed similar physio-chemical parameters: temperature (20±1°C); salinity (36±1‰); pH (8.20±0.12), and natural photoperiod. Each experimental aquariam contained 12 specimens of *P. lividus*, kept in holding baskets, under which we placed 12 specimens of *H. tubulosa* marked individually with micro tags. In this way, the sea cucumbers were free to ingest the sea urchin waste on the bottom of each aquaria, and we were able to monitor each specimen as a single statistic unit. During the experiment the same procedures were carried out on a daily and monthly basis. The daily routine consisted of feeding the sea urchins *ad libitum* with entire and raw land-based vegetables, such as soy, maize and carrots; however, before this feeding, we siphoned off

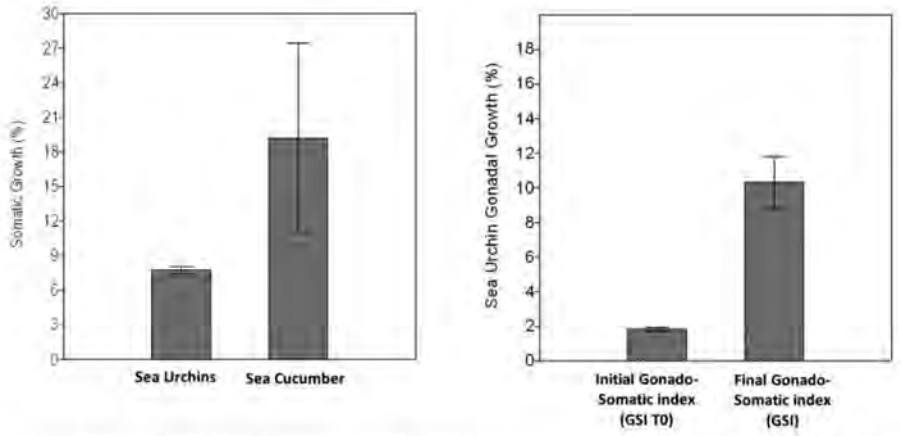

Figure. 1. Results of Gonadal Growth and Somatic Growth in *P.lividus* and *H. tubulosa*

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any leftover food and faeces produced the previous day. Every month, we measured the wet weight of each individual specimen of both species. At the end of the three month-long experiment, the efficiency of the diet in terms of **Somatic Growth Yield (SG%)**, for both sea urchins and sea cucumbers, and in terms of **Gonado-Somatic Index (GSI%)**, for sea urchins, were determined.

Results

During the three months of experiment, no mortality occurred in any aquaria and significant growth was obtained in the specimens of both species. In fact, positive somatic growths were achieved both for *H. tubulosa* and for *P. lividus*, at the end of the experiment (respectively 15.88% and 15.50%; Fig.1). Moreover, in *P. lividus,* the final wet weight of the gonads, calculated as GSI, was significantly higher (9.66%) than their initial wet weight (2.00%), estimated by a sub-sample at T0.

Discussion and Conclusion

Our study demonstrated that culturing the *P. lividus* sea urchin, using the *H. tubulosa* sea cucumber as an extractive-species in IMTA, is a promising avenue for developing a sustainable and profitable aquaculture. The results of this study highlighted that *H. tubulosa* is able to consume and assimilate sea urchin faeces waste when the latter were fed with vegetable foods. We obtained promising results in terms of survivorship and growth for both species, demonstrating the potential use of vegetables as an environmentally and economically viable alternative for co-culture of these species. The multitrophic integrated aquaculture among *P. lividus* and *H. tubulosa* showed, in this preliminary study, would have showed a potential for success in large scale co-culture of aquaculture applications. For example, the rearing of sea cucumbers and sea urchins in IMTA land-based systems could become cost effective due to for their high market price. In fact, the use of sea cucumbers could save reduce the treatment costs of sea urchin waste and, at same time, produce, at same time, high value sea food.

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EFFECTS OF DIFFERENT DIETS ON THREE SIZE CLASSES OF SEA URCHIN *Paracentrotus lividus* L

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Introduction

In the last few decades there has been increasing interest in the development of sea urchin aquaculture as a sustainable alternative to satisfy market demand and preserve natural populations worldwide. Although the sea urchin's life cycle is already controlled in captivity, echinoculture is still largely dependent on wild-caught sea urchins (Baião et al., 2019). The main limiting factor for these farming activities is the slow growth rate: it takes 4-5 years for reared sea urchins to reach commercially viable size (Sartori and Gaion, 2016). Therefore, the future success and sustainability of commercial sea urchin aquaculture strongly depends on the development of suitable and cost-effective diets, specifically designed to maximize growth rate at the various stages of sea urchin's life cycle (Fernandez and Boudouresque, 2000). In this study, we investigated the effects of land-based vegetable diets, enriched with different ratios of fish meal (0%; 20%; 40%) in order to evaluate the best performance of three size classes of sea urchin *Paracentrotus lividus.* The aim of the experiment was to define, for each size classes of sea urchin, the best form of food (whole or extruded) and the best aliquots of fish meal to add as a nutritional integrator in terms of somatic and gonadal growth.

Materials and Methods

144 specimens of *P. lividus* - 48 in each size class - were employed in this four-month experiment (15 May - 20 September 2018). Size classes were composed of sea urchins falling into three different test diameter ranges: 15 - 25mm (juveniles); 25 - 35mm (sub-adults) and 45 - 55mm (adults). The experiment was run in a RAS system made up of 12 aquaria of 30 l each connected in tandem (4 for each size class). This rearing system guaranteed a flow rate of 120 l h^{-1} and ensured similar physico-chemical parameters: temperature (20±1°C); salinity (36±1‰); pH (7.95±8.05), and natural photoperiod. Sea urchins were individually marked with micro tags, so that each specimen could be monitored as a single statistic unit. The 48 sea urchins of each size classes were divided in 4 groups of 12 specimens, and each group was fed daily *ad libitum* with one of the four experimental diets: DVI (100% whole) vegetables, DE-0 (100% extruded vegetables), DE-20 (80% extruded vegetables, 20% fish meal), DE-40 (60% extruded vegetables, 40% fish meal). In each condition the leftover food and the faeces produced were collected daily, whilst the wet weight and test diameters were measured monthly. Gonads were collected in a sub-sample at the start of experiment and at the end were harvested in all sea urchins in each condition. From the four months of experimentation, it was possible to determine the efficiency of each diet in terms of Somatic Growth (**Total Somatic Growth (SG))** and Gonad Production (**Gonad Somatic Index (GSI)).**

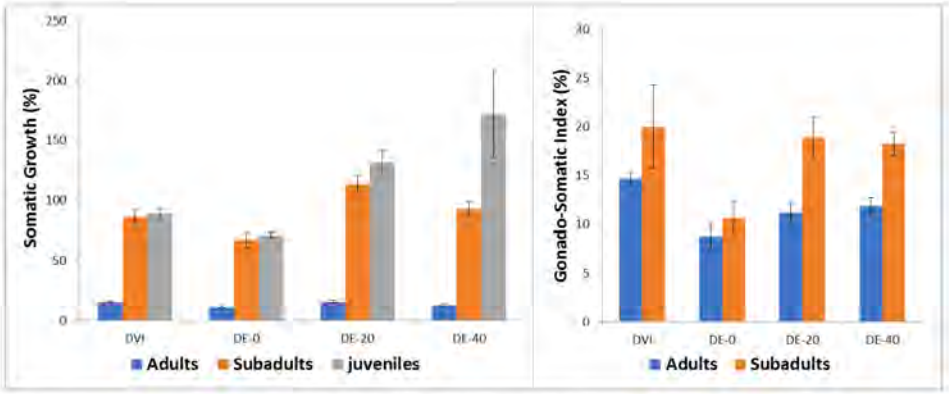

Figure. 1. Somatic and gonadal growth of the three size classes among the four different diets

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Results

Somatic and gonadic growth resulted in significant differences among the different diets and for the different size classes. Indeed, a significant difference in SG and GI was obtained between whole and completely extruded vegetable diets (DVI and DE-0) in all size classes. The highest somatic growth was obtained: in the juveniles with DE-40, and in both the sub-adults and the adults with DE-20 (Fig.1). Regardless of the size class, the somatic growth was lower with vegetable food than in the two fish meal-integrated diets. Conversely, the gonadal growth was largest in sea urchins fed on the vegetable (DVI) and low-fish diet (DE-20). In fact, the maximal gonad growth was obtained with DVI both in sub-adult and adult sea urchins (Fig.1).

Discussion and Conclusion

Terrestrial vegetables such as soy, corn and carrots proved to be a sustainable and economic basic food source for *P. lividus* aquaculture. Moreover, it was possible to estimate the optimal amount of fish meal necessary to achieve the best performance in terms of somatic and gonadic growth among the three size classes of *P. lividus*.

Sea urchin juveniles showed the best performance in both somatic and gonadic growth with the feed containing a high concentration of animal proteins (40%). This demonstrates that conditions with a high protein content are suitable for promoting a fast somatic growth during this stage. The sub-adult stage, where a repartition of somatic and gonadal growth energy is required, the best performance was found when using an intermediate level of fishmeal (between 20% and 40%). Finally, the adult sea urchins performed better when fed on whole vegetables food and low fish meal extruded food (20%).

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YEASTS B-GLUCANS IMPACT ON IMMUNE RESPONSE IN PLASMA AND SKIN MUCUS OF PACIFIC RED SNAPPER (*Lutjanus peru*) CHALLENGED WITH *Aeromonas hydrophila*

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Introduction

β-glucans are considered an immunostimulant that acts on fish non-specific defense mechanism. To date, several immunomodulatory influence of β-glucans has been documented on fish by many authors. However, very few studies have studied the effects of β-glucans administration on the fish skin mucosal immunity. As is known, skin of teleost fish represents one of the first barriers of defence against environmental factors like microorganisms, toxins and pollutants (Raeder et al., 2007) and since most of pathogens start the process of infection in the mucosal surfaces, the mucosal immune response plays an essential role in the course of the infection. In fact, the protective role of the skin mucus has been evaluated in several fish species with economic interest to aquaculture after bacterial challenge. However, few studies have reported the effect of β-glucans diets on the fish innate immune response after bacterial challenge. Therefore, the present *in vivo* study attempts to describe the immunostimulatory impact on several immune-related enzymes and bactericidal activity in plasma and skin mucus after bacterial challenge with *Aeromonas hydrophila* in Pacific red snapper fed diets supplements with glucans derived from *Yarrowia lipolytica* (N6), *Debaromyces hansenii* (Dh004) and *Saccharomyces cerevisiae* (Zymosan A as positive control) during 4 weeks.

Material and methods

Healthy Pacific red snapper (*Lutjanus peru*) (98 ± 18 g mean body weight and 16.3 ± 0.9 cm mean length) were obtained from hatched eggs and reared to the juvenile stage in the larviculture laboratory at CIBNOR. Fish were randomly distributed into twelve identical tanks (12 fish per tank) where the following groups were established in triplicate: 1) non-supplemented diet (control); 2) N6, diet supplemented with 500 mg Kg^{-1} of β-glucan N6 from *Yarrowia lipolytica*); 3) Dh004, diet supplemented with 500 mg Kg^{-1} of β-glucan Dh004 from *Debaromyces hansenii*; and 4) Zymosan A, diet supplemented with 500 mg Kg^{-1} of β-glucan Zymosan A from *Saccharomyces cerevisiae*. The fish were fed at a rate of 2% body weight day^{-1} during 4 weeks. Subsequently, the fish were challenged with a sublethal dose of *A. hydrophila* (1 x 10^8 cfu ml^{-1}). For that purpose, fish from each experimental group were anesthetized and intraperitoneally (i.p.) injected with 100 µl either PBS or *A. hydrophila*. Afterwards, injected specimens were reallocated and distributed into eight identical tanks (12 fish per tank) where four groups were established in duplicate (unchallenged and challenged) according to dietary treatments. Six fish were then sampled from each tank for blood (for plasma compilation) and skin mucus collection at 24 and 48 h after i.p. injection. The methodology of all immune-related activities is described in detail elsewhere (Guardiola et al. 2014; Reyes-Becerril et al., 2018).

Results

Our results revealed that the protease and antiprotease activities were unaffected by bacterial challenged in skin mucus of fish fed all experimental diets. Regarding SOD and CAT activities in skin mucus, the values of SOD activity decreased in challenged fish at 24 hours post-challenge respect to unchallenged ones whilst CAT activity not showed alterations between experimental groups. Interestingly, the bactericidal activity against *A. hydrophila* showed a decrease of challenged fish fed β-glucans supplemented diets respect to challenged fish fed control diet at 24 hours post-challenge. However, these alterations were reduced at 48 horas where any significant variations was observed. Skin mucus bactericidal activity against *Vibrio parahaemolyticus* not showed variations at both experimental times. However, the bactericidal activity against *Photobacterium damselae* exhibited a decrease in skin mucus of challenged fish fed β-glucans supplemented diets respect to control diet at 48 hours post-challenge.

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Regarding humoral immune-related enzymes, antiprotease activity not showed variations between experiments groups whilst protease activity decreased in challenged fish fed N6 and Zym A diets compared to unchallenged ones at 48 hours. Contrarily, peroxidase activity increased in challenged fish fed N6 and Zym A diets respect to the unchallenged ones at the same experimental time. Similarly, nitric oxide (NO) production increased in challenged fish fed N6 and Dh004 diets compared to the fish injected with PBS (unchallenged) at 48 hours post-challenge. The values of SOD activity decreased in challenged fish fed β-glucans supplemented diets respect to challenged fish fed control diet at 24 hours post-challenge whilst CAT activity not showed any variation between challenged and unchallenged groups. In the case of lysozyme activity, the values increased in the challenged fish fed control and N6 diets at 48 hours post-challenge respect to unchallenged ones. Finally, bactericidal activity against *A. hydrophila* and *Vibrio parahaemolyticus* increased in plasma from challenged fish fed all experimental diets at both sampling times.

Discussion and conclusions

These results confirm that β-glucans tested in this study, mainly the β-glucan N6 (from *Yarrowia lipolytica*), modulate positively the immune-related parameters measured in plasma and skin mucus of Pacific red snapper. Therefore, both β-glucans are beneficial for increasing the innate immune response and enhancing resistance against *A. hydrophila*, an opportunistic pathogen that severely restrict the development of aquaculture.

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PRESENCE OF CD8$^+$ LYMPHOCYTES IS MODIFIED IN EUROPEAN SEA BASS IMMUNE TISSUES UPON NODAVIRUS INFECTION

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Introduction

Nodavirus (NNV) causes the viral encephalopathy and retinopathy disease (VER) in fish and represents one of the most serious problems in the modern aquaculture, producing high mortalities in larvae and juvenile stages in several fish species and being maternally transmitted to the progeny (Munday et al., 2002). Among the immunological pathways involved in fish viral defence most studies have dealt with the interferon pathway and much less with the cell-mediated cytotoxic (CMC) activity. The innate CMC is played by the nonspecific cytotoxic (NCCs) and natural killer (NK) cells whilst the specific CMC is played by cytotoxic-T lymphocytes (CTL), which are characterized by the presence of the CD8 coreceptor (reviewed by Fisher et al., 2006). In the NNV-susceptible orange-spotted grouper (*Epinepheluscoioides*), the NNV infection resulted in increased *cd8* mRNA levels as well as the number of CD8$^+$ circulating lymphocytes and the specifi CMC activity against NNV-infected cells, in a process that was dependent on the MHCI (Chang et al., 2011). By contrast, the expression of T cell receptor (*tcr*) and *cd8* genes in European sea bass (*Dicentrarchus labrax*) and Atlantic halibut (*Hippoglossushippoglossus*) after NNV infection was unaltered, suggesting that this specific CMC activity is not generated in all the fish species (Scapigliati et al., 2010; Patel et al., 2008). Based on this lack of gene regulation upon NNV infection in the European sea bass, one of the most important fish species for Mediterranean aquaculture and highly susceptible to NNV, we aimed to study the implication of the CD8 cells in their defence. Thus, we have generated a polyclonal antibody against the European sea bass CD8a and, after validation of the antisera, we evaluated the localization, distribution and abundance of CD8a$^+$ cells in naïve sea bass tissues as well as after NNV infection.

Material and methods

Synthetic peptide of European sea bass CD8a was produced and used to immunize mice to obtain polyclonal antibodies. Sea bass tissues were used to validate the antiserum by both western blot and ELISA (Santana et al., 2013). Once validated, antiserum was used to localize CD8a$^+$ cells in thymus, head-kidney and spleen tissues from naïve specimens of European sea bass by routine indirect immunohistochemistry (IHC). Localization, distribution and abundance of CD8a$^+$ cells was also studied by IHC in the brain, the target NNV tissue, and the gonad from juveniles infected with NNV (Chaves-Pozo et al., 2012) and compared to mock-infected specimens. In addition, peripheral blood (PBL), head-kidney (HKL) and spleen leucocytes were isolated and the percentage of CD8a$^+$ cells evaluated by flow cytometry during a NN infection.

Results

Firstly, the western blot revealed a band of less than 25 kDa, very similar to the predicted 24 kDa of the mature sea bass CD8a. In addition, the ELISA also demonstrated that the signal was completely abrogated when the synthetic peptide was used to pre-adsorb the antiserum. These two techniques served to validate the specificity of the generated polyclonal antiserum. In addition, IHC revealed a strong staining in the cytoplasmatic periphery of discrete cells. As expected, the number of CD8a$^+$ cells in the thymus was very high, followed by the head-kidney and no detection was observed in the spleen.

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Focusing on NNV-infected European sea bass tissues, we firstly identified by IHC few scattered $CD8a^+$ cells in the brain, the main target tissue for NNV, and in the gonad, a tissue that is also colonized by NNV and used to be spread to the progeny. Upon infection, we observed an important increment in the number of $CD8a^+$ cells in both tissues. We also quantified $CD8a^+$ cells present in immune tissues through an infective process by flow cytometry. Interestingly, we found two distinct positive populations according to the staining intensity observed that depends on the presence of CD8a in the cells. Thus, populations were named $CD8a^{low}$ and $CD8a^{hi}$. Thus, NNV infection resulted in general decrease of $CD8a^+$ cells 3 days post-infection in the HKLs and PBLs but unaltered in the spleen. As the infection progressed, 7-15 days, the percentage of $CD8a^+$ cells significantly increased in all the tissues analyzed when compared to mock-infected specimens. Interestingly, the greatest increment was observed in the case of the $CD8a^{hi}$ population.

Discussion and conclusions

Our results showed the anti-CD8a serum as a valuable tool to characterize the $CD8^+$ lymphocytes in European sea bass and their role in immunity. This antibody is an important tool to investigate the fish acquired immunity at protein and cellular levels. The increment of $CD8a^+$ cells during infection in naïve and NNV-infected tissues reveals that sea bass are able to mount a rapid cellular acquired immunity against virus, as also demonstrated in grouper (Chang et al., 2011). However, further functional studies are needed to ascertain whether these lymphocytes are effective in the infected tissues to clear the virus depending on specimen size.

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ANTIVIRAL ROLE OF THE ANTIMICROBIAL PEPTIDE NK-LYSIN AND ITS POTENTIAL APPLICATION IN AQUACULTURE

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Introduction

The innate immune arm is an essential component of the teleost fish immunity due to their poikilothermic nature. Until the acquired immunity is achieved, fish fighting of pathogens is completely relegated on the innate immunity. Interestingly, many antimicrobial peptides (AMPs) have been isolated from fish and exert a powerful microbicidal activity against a wide range of pathogens. AMPs are gene encoded, constitutively expressed in most of the tissues studied to date, modulated upon infections and directly involved in microbicidal functions and immune regulators of the innate and acquired immune responses (Valero et al. 2018). Amongst them, some studies have pointed to the anti-bacterial, -viral and -parasitic role of NK-lysin (Nkl) in fish (Lama et al. 2018, Pereiro et al. 2017, Wang et al. 2018). Unfortunately, very little is known at functional level and nothing in the case of the European sea bass (*Dicentrarchus labrax*), one of the most important cultured fish species in the Mediterranean Sea. Thus, the aim of this work was to evaluate the antiviral function of synthetic sea bass Nkl peptides against the most important fish viruses *in vitro* as well as their potential protective *in vivo* actions in European sea bass juveniles upon nodavirus (NNV) infection.

Material and methods

Three different European sea bass Nkl peptides (Nkl1, Nkl2 and Nkl3) were synthetized and purified by high-pressure liquid chromatography (HPLC). For the *in vitro* antiviral activity, synthetic peptides were incubated with NNV, viral septicaemia haemorrhagic virus (VHSV), infectious pancreatic necrosis virus (IPNV) or spring viremia carp virus (SVCV) as major virus for European sea bass, salmonids and cyprinid fish species. Viral samples incubated with phosphate buffered solution (PBS) served as controls. Afterwards, viral suspensions were incubated for infectivity determination onto cultures of the E-11 cell line, in the case of NNV, and in the EPC cell line for VHSV, IPNV and SVCV at their respective optimal growing conditions. Cultures were evaluated by phase contrast microscopy for the presence of cytopathic effect (CPE) and virus titters determined (Reed and Müench, 1938). Viral infectivity was presented as inhibition of viral titter respect to the controls.

For the *in vivo* trial, European sea bass juveniles (15± 3 g mean body weight) were divided into groups and received a single intramuscular injection of 0.1 mL of PBS alone (control) or containing 1 µg Nkl peptides g^{-1} fish. After 3 days fish were intramuscularly injected with a pathogenic dosage of NNV and the mortality recorded during 1 month. The relative percent survival (RPS) was determined and expressed as %.

Results

Our results *in vitro* demonstrated that Nkl peptides have a clear antiviral role but it depends on the peptide sequence and the virus. Thus, treatment of NNV with Nkl1 reduced the viral infectivity 68.5%, respect to the controls or untreated viral stocks, while both Nkl2 and Nkl3 peptides did at 99%. In the case of VHSV, viral viability was equally reduced by the three peptides reaching a 97-98% of viral reduction. For IPNV, Nkl1 reduced the viral infectivity up to 90% while the other two peptides virtually cleared all the virus since the viability was lower than 0.01%. By contrast, SVCV infectivity was the less affected by the synthetic sea bass Nkl peptides. While Nkl1 and Nkl2 incubation reduced the infectivity 53% the Nkl3 failed to have any impact on viral CPE respect to the controls.

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In the *in vivo* exposure, European sea bass juveniles challenged with NNV resulted in 41.67% of mortality. Interestingly, when fish were pre-injected with the synthetic sea bass Nkl peptides the RPS for Nkl1-, NKl2- or Nkl3-treated sea bass juveniles was of 34, 21 or 40%, respectively.

Discussion and conclusions

Our results demonstrate that the European sea bass Nkl peptides have a potent antiviral activity both *in vitro* and *in vivo* as well as activity against different types of fish virus. Since they are able to reduce the viral infectivity by the direct contact with the virus it is reasonable to suggest that Nkl is able to produce holes or pores in the viral capsid, a fact that merits further evaluation to clearly determine the mechanism of action of Nkl peptides on virus. By contrast, during the *in vivo* administration, the fish pre-injected with Nkl peptides were more resistant to NNV infection suggesting that NKl peptides might trigger the immune response although further characterization of the different responses elicit are mandatory. Thus, the immunological mechanisms behind this finding are under investigation in our laboratory. Thus, Nkl peptides are presented as valuable tools for preventing viral diseases in fish and might be potential candidates for prophylactic measures in aquaculture.

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IN VITRO MICROBICIDAL AND IMMUNOSTIMULANT EFFECTS OF GUAVA LEAF (*Psidium guajava* L.) EXTRACTS

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Introduction

The indiscriminate use of antimicrobials has contributed to the emergence of resistant microorganisms, an alarming problem in public and animal health, since the speed of adaptation of microorganisms is much faster than the time of production of drugs to combat the diseases (WHO, 2018). In this sense, the study of natural substances that can be used as an alternative to chemical products has increased in recent decades. Of all these alternatives, which include micronutrients and probiotics, plants are considered the most reliable source of compounds to treat infections in both animals and humans (Dotta *et al*., 2018). Guava (*Psidium guajava* L), which is a plant that grows in tropical and subtropical areas of the world, has been used in medicine with reported antimicrobial, antihypertensive, anti-inflammatory and anti-neoplastic effects in both animals and humans (Gutiérrez *et al*., 2008; Gobi *et al*., 2016). Thus, in this work we have studied the effects of ethanolic and ethanolic-aqueous extracts of guava leaf (*P. guajava* L.) against several fish pathogenic bacteria, as well as their effects on the viability and innate immune activities on head-kidney (HK) leucocytes of gilthead seabream (*Sparus aurata*).

Material and methods

Guava leaves were collected in Santo Domingo (Dominican Republic) and afterwards were dried at room temperature and crushed. Ethanolic and ethanolic-aqueous extracts were obtained according to García-Beltran *et al.* (2018). In order to evaluate the antimicrobial power of the guava leaf, a bacterial sensitivity test was performed with each of the extracts by diffusion in agar against several fish pathogenic bacteria (*Vibrio harveyi, Vibrio anguillarum, Photobacterium damselae* subs. *piscida* and *Aeromonas salmonicida*). In addition, the effects of guava leaf extracts on viability and innate immune activities was analysed in HK leucocytes isolated from gilthead seabream exposed to 4 concentrations of each extract (0.01, 0.1, 0.5 and 1 mg mL^{-1}) for 24 hours. Viability was studied by flow cytometry using propidium iodide and respiratory burst, peroxidase and phagocytosis activities were determined according to the methods optimized by our research group (García-Beltran *et al.,* 2018).

Results

The guava leaf extracts inhibited the growth of *V. harveyi, V. anguillarum, P. damselae* and *A. salmonicida*. In other hand, the viability of HK leucocytes incubated with the ethanolic extract of *P. guajava* decreased only in those incubated with the highest concentrations (0.5 and 1 mg mL^{-1}) respect to untreated cells. However, in case of the ethanolic-aqueous extract the cellular viability decreased until it was significantly lower with the higher concentration of the extract (0.5 and 1 mg mL^{-1}). Regarding peroxidase activity, levels remained similar to the control with the lowest concentrations (0.01 and 0.1 mg mL^{-1}) when using the ethanolic-aqueous extract, while no effect was seen when using the ethanolic extract. In terms of phagocytic ability, high concentrations of ethanolic-aqueous extract (0.5 and 1 mg mL^{-1}) significantly decreased this activity. Finally, only the concentrations of 0.5 mg mL^{-1} of ethanolic-aqueous extract and 1mg mL^{-1} of ethanolic extract caused a significant decrease in the phagocytic capacity of gilthead seabream leucocytes.

Discussion and conclusions

According to our results we can conclude that *P. guajava* leaf has a powerful microbicidal effect against the pathogenic bacteria tested and low or moderate concentrations of aqueous and ethanolic extracts of this plant showed immunostimulant effects in HK gilthead seabream leucocytes, obtaining the best results when using the ethanolic extract.

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DIETARY ADMINISTRATION OF *Shewanella putrefaciens* SPPDP11 ALLEVIATES THE SKIN INFLAMMATION OF EXPERIMENTALLY WOUNDED GILTHEAD SEABREAM (*Sparus aurata* L.)

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Introduction

In teleost, the vertebrate integument (skin), with conserved organization consisting of the epidermis, dermis, and hypodermis, is the prominent mucosal surfaces and immune barriers bear the brunt of environmental stimulation (Esteban, 2012). Fish live in aquatic environments ubiquitous with pathogens and allergens, which may suppose a threat to the mucosal immunity (Gomez et al., 2013). On top of it all, higher density and intensification in aquaculture system can end into skin lesions, abrasions and ulcers, which are one of the major limiting factors for aquaculture production. To be safe, the skin injured is very rapidly repaired make the case for the defensive mechanism against the external environment (Costa and Power, 2018). On the other hand, *Shewanella putrefaciens* is a strain isolated from the skin of healthy specimens of gilthead seabream (*Sparus aurata*) (Chabrillón et al., 2005) and we previously have demonstrated its potential as an immunostimulant in gilthead seabream systemic immunity. Therefore, the aim of this work was to demonstrate if this bacterium has any positive effect on gilthead seabream skin inflammation.

Material and methods

A total of 48 gilthead seabream specimens from a local farm (San Pedro del Pinatar, Murcia, Spain) with a mean initial body weight of 21.81 g (0.87 g SEM) were randomly assigned into 4 re-circulating seawater aquaria (400 L), with continuous aeration and flow-through seawater at the rate of 900 L h^{-1} at 22 ± 2 °C and 24% salinity. The photoperiod was 12 h light: 12 h dark. A commercial diet (Skretting) was used as the basic feed. Saline buffer and bacterial suspension of equal volume were added to the basic feed, formulating the control and SpPdp11 (10^9 cfu g^{-1}) diets, respectively. Twice tanks of 12 fish were fed each of these two experimental diets at a rate of 2% body weight day^{-1}. After 30 days of trail, 8 fish per tank were selected randomly for periodical sampling, and then anesthetized with 20 mg L^{-1} of clove oil (Guinama®). For gene expression analysis, skin tissues were taken and frozen in liquid nitrogen immediately and stored at −80 °C till use. The rest of the fish were sedated as mentioned above, and then wounded by a metallic circular biopsy punch (Stiekel) with a diameter of 8 mm and 2 mm depth and were fed with the same diets. At the end of the experiment (1-week post-wounding), the acquisition of skin tissues was as indicated before.

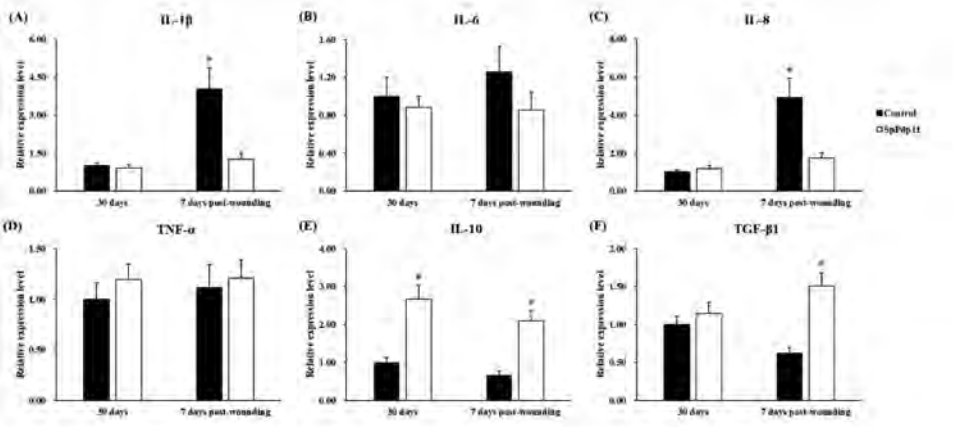

Figure 1. Relative expression level of *IL-1β*, *IL-6*, *IL-8*, *TNF-α*, *IL-10* and *TGF-β1* genes in skin samples of gilthead seabream of control and SpPdp11 group at 30 days of trial and 7 days post-wounding. Error bars of columns denote standard error of means (n = 8), asterisks denote significant differences between experimental groups ($P < 0.05$).

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The total RNA of the skin was isolated using TRIzol® reagent (Life Technologies). RNA purity and concentration were measured using Nano Drop® 2000 spectrophotometer (Thermo Fisher Scientific), and then treated with DNase I (Promega) to remove genomic DNA contamination. First-strand complementary DNA (cDNA) synthesis was reversely transcribed from 1 μg of total RNA using the SuperScriptIV reverse transcriptase (Life Technologies) with an oligo-dT18 primer (Life Technologies). All the real-time PCR analyses were performed using an ABI PRISM 7500 Instrument (Applied Biosystems) with using SYBR Green PCR Core Reagents (Applied Biosystems). The mRNA levels of these genes were normalized to the mRNA level of sea bream ef1α and the rps18, which were used as housekeeping gene. The gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method, and the relative expression level of gene in the Control after 30 days' sampling was used as a calibrator.

Results and discussion

Compared with the control group, seabream fed SpPdp11 diet showed significantly higher *IL-10* gene expression after 30 days' feeding ($P<0.05$). Besides this, 7 days post-wounding, the gene expression of *IL-1β* and *IL-8* were remarkably ($P<0.05$) declined in SpPdp11 group, while the gene expression of *IL-10* and *TGF-β1* was significantly ($P<0.05$) increased in SpPdp11 group compared to the expression level of the skin from fish belong to the control group (Figure 1)

The results revealed that gilthead seabream fed SpPdp11 diet showed a significantly reduced gene expression of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α), and significantly enhanced gene expression of anti-inflammatory cytokines (IL-10 and TGF-β1) 7 days post-wounding. Therefore, dietary administration of SpPdp11 could significantly alleviate the skin inflammation of experimentally wounded gilthead seabream.

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IMMUNE STATUS OF GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES FED DIETS WITH DIFFERENT PROTEIN AND FAT LEVELS AFTER *Tenacibaculm soleae* CHALLENGE

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Introduction

Tenacibaculosis (formerly called flexibacteriosis) is one of the most common bacterial pathogens in aquaculture and can affect a wide range of species, such as sole, turbot, European eel, salmon, European sea bass, gilthead seabream, etc. (Salati et al., 2005). In the case of fish studied, gilthead seabream is one of the most important fish crops in the Mediterranean area. Recently, the statistical data showed that the production of this species in 2018 was 246,839 tonnes in Europe with a percentage of 10.7 compared to the previous year's productions. However, the relation between nutrition and the pathogenicity of Tenacibaculosis have not been studied under a low proportion of daily feeding or with low protein diets. In fact, it is well known that proper nutrition in fish can protect against pathogenic and disease episodes (Oliva-Teles, 2012), but generally in marine cages, crops can be poorly fed by bad weather or inefficient distribution of granules. This can affect some fish by preventing them from feeding with the usual or recommended feeding ratios. Therefore, the aim of the present study was evaluated the immune response of gilthead seabream fed diets with different protein and fat levels and challenged with *Tenacibaculum soleae*.

Material and methods

One hundred twenty healthy gilthead seabream (*Sparus aurata*) (12,86 ± 0,28 g initial experimental mean body weight) were obtained from a local hatchery (Águilas, Murcia, Spain). Fish were randomly distributed and acclimated prior the experiment into eight identical tanks (15 fish per tank) where the following groups were established in duplicate: Diet A (Ti3- Skreeting for Tilapia); Diet B (Mar Perla- Skreeting as gilthead seabream Control diet); Diet C (Biothesan 40); Diet D (Biothesan 70). The diets were selected by their protein and fatty acid ratios: Diet A, crude protein (Cp): 33 %, and crude oils (Co): 6,5 %; Diet B, Cp: 56 %, Co: 15 %; Diets C and D, Cp: 28,6 %, Co 6,5 %. Diets C and D were isoproteic and isolipidic, and their main protein source used was spent brewery yeast at 55 % and 62,5% of wet weight kg^{-1} inclusion, respectively. Mainly the protein and oil source was different and whilst the Diet B (control treatment) had fish meal (FM) in a 48,40 %; Diet A has only 4,4 % of FM and Diet C and D have 25 % and 20 % of FM, respectively. The proteins ratios that fish meal used as raw material brings for commercial diets are impossible to know, but the feed formulated and designed as Diets C and D used low protein ratio FM (58 %) obtained from canning and fisheries by-products.

The fish were fed at 1,5 % of daily biomass body weight day^{-1} during 62 days. After that period, 5 fish of each treatment were anesthetizes and sampled whilst 5 of each treatment was intraperitoneally (i.p.) injected with 100 µl either PBS (unchallenged group) or *Tenacibaculm soleae* (10^6 ufc ml^{-1}, challenged group). The rest of the fish (5) were recovered. After 24 hours, the fish that were injected were anesthetizing and sampled. Intestine and skin were collected and then stored for the analysis of the expression of immune-related genes (*igt*, *tcr-β*, *il-1β*, *cox2*, *tnfα*, *hep*, *β-def*, *lyz* and *c3*) using Real-Time PCR (qPCR).

Results

Our results revealed that the expression of genes studied in gut and skin was unaffected by the different assayed diets at the end of trial (62 days). Interestingly, the expression of most genes was altered after the bacterial challenge. In the case of gut, the expression of *tcr-β* and *cox2* genes was down-regulated in the fish fed Diet D respect to values found in the fish fed Diet B. A significant down-regulation of *hep* gene expression was observed in specimens fed all diets compared to fish fed Diet B. Fish fed diet C showed a down-regulated of *c3* gene expression respect to fish fed Diets A and B. In the case of skin, the expression of the most genes tested (*igt*, *tcr-β*, *il-1β*, *cox2*, *tnfα*, *β-def*, *lyz* and *c3*) was down-regulated in the fish fed Diets B, C and D compared to the expression observed in the fish fed Diet A.

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Discussion and conclusions

These results suggest the diets used can modulate the susceptibility of fish to infectious diseases. Our results demonstrated that the expression of the most immune-related genes evaluated in the skin was up-regulated in the fish fed Diet A (Ti3-Skreeting for Tilapia) which is a deficit diet for the species under study. Interestingly, the expression of the genes tested not showed variations between Diet B (control, Cp: 56 %) compared to the values found in the fish fed Diet C and D (Cp: 28,6 %, mainly from spent brewery yeast). As is known, the adequate diet supplies the immune system with the amino acids, PUFA, enzyme co-factors and energy necessary to support lymphocyte proliferation and the synthesis of immune-effectors (Lall, 2000; Kiron et al., 1995). Therefore, the diets C and D, which contain a lower protein and fat proportion, seem supplies the essential requirements for cells of the immune system. This fact could mean lowering the costs involved in the formulation of diets for fish aquaculture

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EFFECTS OF EXPOSURE TO CYANOBACTERIUM *Microcystis aeruginosa* ON LIVER OF THE SOUTH AMERICAN FISH *Astyanax altiparanae*

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Introduction

Among disturbances of aquatic environments, the eutrophication of surface water seems to be accelerated throughout the world as a result of the release of nutrients under development anthropogenic activities. One of the consequences of eutrophication is a large proliferation of toxic cyanobacteria that may be present in the surface water area for a long period of time (Vieira et al., 2005). Frequently, as cyanobacteria dominate phytoplankton in temperate freshwater environments during the hotter periods of the year, and as global temperature elevations predicted from climate change models (Davie et al., 2009) suggest that Cyanobacteria will increase in frequency, duration and severity. The production of toxins by cyanobacteria such as Microcystis, which has temperature for growth and photosynthesis at or above 25 °C (Davis et al., 2009), and the frequency of blooms for the same cyanobacteria has increased in recent decades (Dziallas and Grossart, 2011).

Material and methods

Description of experimental groups. Group control: For the group, water from the treatment network of the city of Pirassununga (São Paulo) was used, which was oxygenated for 3 days to eliminate chlorine. Three aquariums are assembled, each of which is considered a replica. Treatment 1: for this treatment, dilutions of 5 ppb and 50 ppb of the extract of cyanobacteria of *M. aeruginosa* species, were used, for each concentration three aquariums were assembled, each of which was considered a replica. After the experimental periods of 48 and 96 hours, liver fragments were extracted from each of the 5 specimens collected in each aquarium (15 per group). These samples were fixed in buffered formaldehyde, dehydrated in alcohol and included historesin, for Hematoxylin-Eosin reactions, for the analysis of possible alterations in organ morphology in PAS to identify changes in glycogen accumulation and in Pearls solution for iron detection.

Results

The qualitative analysis showed that cytoplasmic vacuolations, sinusoidal capillary dilations and hyperemia. The dilatation of the sinusoidal vessels was only considered positive when the mean of its largest diameter in longitudinal sections was statistically higher than in the control group. The cases of hyperemia were identified and the percentage of individuals per group that had it. Apparently, the vacuolization present in the treatment groups is due to the accumulation of lipids, the deletion of glycogen or other substances, which do not lead to cell death since no evidence of cell death was found. The Pearls technique presented positive iron titers in the liver in the groups exposed to the toxic strain in the both concentrations tested, but only after 96h of exposure, and it was also possible to observe the accumulation of macrophages in the hepatic tissue.

Discussion and conclusions

Pham et al. (2015) and Woźny et al. (2016) who described that even exposure to a toxic strain tends to be reversed over time. However, these authors did not describe irreversible damage such as necrosis that has already been described by other authors, which leads them to believe that there would be a second hypothesis in our experiment if it were prolonged, just as in these authors the damages would tend to be diminished and reverted. This same pattern of reversal damage caused by microcystin was reported by Hou et al. (2015) in ovaries of zebrafish. This point leads us to highlight the importance of our work, since it not only focused on hepatocyte damage but also identified hyperemia and dilatation of sinusoid, hepatocyte nuclei, data that were not reversible and should be observed in experiments with microcystin a from this moment. In the work carried out by Preeti et al. (2016) we may observe hyperemia and dilatation of sinusoid vessels as in our work, however, these data were not reported by the authors..

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SINGLE AND COMBINED IMPACT OF MICROPLASTICS AND CADMIUM ON ANTIOXIDANT DEFENCE AND INNATE IMMUNITY IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Introduction

Plastics are a synthetic organic polymers, which are derived from the polymerization of monomers extracted from oil or gas that came out in 1909. The durability of plastics, one of the good factor for its use, it's also what makes them a potential material to harm different species and the environment, since it's highly resistant to degradation (Cole et al., 2011). Plastics can be divided in macro- (size superior to 5 nm), micro- (MPs) (size between 100 nm and 5 mm) and nanoplastics (NPs) (smaller than 100 nm) which can be found in the water and sediment of both marine and freshwater systems. In the case of MPs, several authors have reported that MPs are consumed by a great variety of aquatic organisms in multiple pathways including ingestion, adherence and trophic transfer (Wen et al., 2018). Therefore, the fish exposure to MPs can lead to multiple toxic effects in several organs and tissues, such as intestinal damage, oxidative stress, endocrine disruption, immune response and finally reduced growth and survival (Wen et al., 2018). In addition, the MPs might release chemicals that are part of their composition or that they were able to absorb when released to the environment such as metals and heavy metals, organic compounds or pharmaceutics allowing those products to enter in their bodies (Rochman et al., 2014). Therefore, the present *in vivo* study attempts to describe the single and combined effects of waterborne exposure to MPs and cadmium (Cd) on the antioxidant defence and innate immunity in European seabass (*Dicentrarchus labrax*).

Material and Methods

The bioassay was conducted in glass tanks (60 L capacity) to minimize plastic exposure, filled to 50 L. Water quality was maintained with aeration and daily water changes of 100%. The photoperiod was of 12 h light: 12 h dark and fish fed with a commercial pellet diet (Skretting, Spain) at a rate of 1.5% body weight day^{-1}. Ammonia levels in the water were measured every day using commercial kits (Palintest Ltd) and never exceeded 0.40 mg L^{-1}. During the trial, water temperature averaged 17.04 ± 0.5 ºC, salinity was maintained at 35.0 ± 1.0 g L^{-1}, and dissolved oxygen was kept near saturation (7.0 mg L^{-1}). The photoperiod was of 12 h light: 12 h dark and fish fed with a commercial pellet diet (Skretting, Spain) at a rate of 2% body weight day^{-1}.Eight specimens of European seabass (66.9 ± 13 g body weight) from a local fish farm (Cantabria, Spain) were divided into four tanks by duplicate and 4 experimental groups were established: *i)* control group (unexposed); *ii)* Cd group [exposed to Cd ($CdCl_2$; Sigma, 0.1 mg L^{-1})]; *iii)* MPs group [exposed to MPs (0.25 mg L^{-1}, red fluorescen MPs, Cospheric-Innovations in Microtechnology, USA)]; *iv)* MPs-Cd mixture group [exposed to Cd and MPs (0.1 mg L^{-1} and 0.25 mg L^{-1}, respectively)]. All experimental tank were exposed daily and therefore for each group, we had a cleaning tank that was used to transfer the fish while the experimental tanks were cleaned, allowing full renovation of the water, the addition of each contaminants and the decreasing as much as possible the decay of both contaminants (MPs and Cd). To reduce MPs adherence to plastic used on the tubes for aeration, a plastic pipette was used, since it is more inert compared to the plastic tubes. The concentrations used were ecologically relevant and the fish were allowed to acclimatise for 15 days before the start of the experimental trial. They were starved for 24 h prior to sampling and four fish per tank (8 per group) were sacrificed by an overdose of ethylene glycol monophenyl ether (Sigma-Aldrich, 1,000 ppm) for blood and liver sampling after 5 and 10 days of exposure.

Results

Our results revealed that the numbers of red blood cells (RBC) decreased in fish exposed to MPs respect to values found in the fish from Cd and Mix groups at the day 5. In the case of the number of circulating total WBC (white blood cells), a decrease was observed in all exposed fish compared to unexposed ones (control) at the day 10 of exposure. However, the haemoglobin concentration, hematocrit, MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin) and

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MCHC (mean corpuscular haemoglobin concentration) values remained unaltered. Regarding lipid peroxidation (LPO) and catalase (CAT) activity in liver, the values of LPO and CAT activity not showed alterations between experimental groups. Interestingly, the bactericidal activity against *Photobacterium damselae subsp. piscicida* (PP3 strain) showed a decrease in the plasma of fish exposed to MPs respect to values found in the plasma from fish unexposed and exposed to mixture (MPs-Cd) during 10 days. Similarly, the bactericidal activity against *Vibrio harveyi* decreased in fish exposed to MPs respect to unexposed fish at the same experimental time.

Discussion and conclusions

These results confirm that the combination of MPs with Cd not seem to increase the toxicity of this heavy metal. Interestingly, the exposure to MPs, Cd and MPs-Cd mixture caused a decrease in the number of WBC which could be related to the decrease observed in the bactericidal activity evaluated in the plasma. Regarding stress oxidative, no significant variations were recorded in LPO and CAT activity in liver of exposed fish. This fact could be related with the Cd and MPs concentrations tested (ecologically relevant) which was lower than that used in other studies (Miranda et al., 2019). Therefore, further studies should elucidate the potential risks of heavy metals and MPs combination for fish health to longer exposure times and whether this could contribute to significant e fects on the food chains by bioaccumulation.

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FUNCTIONAL AND PROTEOMIC CHARACTERISATION OF SALMONID COELOMIC FLUID

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Introduction

While in most fish species egg viability rapidly decreases after ovulation, Salmonids can hold their eggs in the body cavity for a week without any significant loss of egg developmental competence. In the body cavity, eggs are bathed in a biological fluid called ovarian fluid (OF) or coelomic fluid (CF). This fluid appears to be directly responsible for the good conservation of eggs. Indeed, eggs remain viable after several days of storage in this fluid, while their viability rapidly decreases when stored in artificial medium mimicking the mineral composition of CF. Understanding the biological properties of coelomic fluid and identifying the actors involved in this process would be of great interest for aquaculture and for further understanding the biological mechanisms preserving egg viability. It would particularly help improving egg storage conditions.

Results

We have used a proteomic approach to characterize rainbow trout (*Oncorhynchus mykiss*) coelomic fluid proteome. Proteins involved in immunity, part of the extracellular matrix, or involved in lipids metabolism and transport are the most highly present classes in this fluid. Comparison of this proteome with transcriptomic data of the PhyloFish database suggests the presence in the rainbow trout coelomic fluid of proteins specifically expressed in salmonid

To further characterize CF, rainbow trout fluid has been fractionated by HPLC on a gel filtration column. After 4 days of incubation in those fractions, trout eggs were fertilized. In some fractions, eggs exhibited a good developmental rate, even though lower than in non-fractionated coelomic fluid. Proteins present in those fractions are currently being identified. In parallel, proteomic comparison of salmonid coelomic fluids with non-salmonids ovarian fluids will allow us to identify proteins specifically present in salmonids coelomic fluid

Conclusion

Together, those experiments will help us identify potential protein candidates participating in preserving egg viability and unraveling pathways conferring this unique biological activity to salmonid coelomic fluid. The identification of those candidates could have direct applications in aquaculture and help improving the egg storage conditions.

IDENTIFICATION OF SNP MARKERS ASSOCIATED TO SOMATIC GROWTH IN THE FLATFISH SENEGALESE SOLE (*Solea senegalensis*)

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Introduction

The Senegalese sole is a species with high economical value whose aquaculture production has been consolidated in Southern Europe in recent years. One unexplored way to improve growth performance is the implementation of genetic breeding programs and the use of genomics to assist in the control of reproduction and to increase the accuracy of genetic estimates. With the rapid development of sequencing technologies, the analyses of single nucleotide polymorphisms (SNPs) has become an essential tool in genetic studies to identify quantitative traits loci (QTLs) and genes associated to growth as reported in other species such as turbot (Lyu et al. 2019), catfish (Zang et al. 2019), Asian seabass (Xia et al. 2013) or golden pompano (Zang et al. 2018), among others. The objective of this work has been the identification of SNP markers associated to growth in sole. For this purpose, a set of SNPs have been identified *in silico* using a genome draft of the sole, and tested in a mid-density DNA chip using wild animals. Later, the markers were studied in F1 families of soles with different growth rates (high and low) identifying 6 genes or chromosomal regions associated to growth.

Materials and methods

The set of SNPs used in this study were detected in silico by mapping transcriptome data onto a genome draft of a *S. senegalensis* (after sequencing with Nanopore and the correction of Illumina). The scaffolds were blasted onto the *Cynoglossus semilaevis* reference genome to positioned in the genome. The SNPs were used as target sequences to design specific probes compatible with the OpenArray DNA chip platform (Thermofisher). To validate the chips, a wild population captured in the Gulf of Cádiz (Spain) composed of 83 females and 81 males was used. Blood or fin samples were taken from each individual and high-quality DNA was isolated using the Qiagen DNA kit according to the manufacturer's recommendation. The quality and quantity of DNA was measured with a Nanodrop ND-8000 spectrophotometer and agarose electrophoresis. The chips were loaded with an Opencuray AccuFill ™ System and amplified in a real-time PCR (QuantStudio12KFlex model) according to the manufacturer's instructions. For the association analysis, four families were selected according to their growth rates at harvest. The slow-growing families LG1 and LG2 averaged 159.3g and 133.7g in weight, respectively. The fast-growing families FG1 and FG2 were 518.6g and 468.1g, respectively. The disequilibrium analysis was carried out using the JLIN (Carter et al., 2006) and the genome-wide association study (GWAS) with GWASpoly package in R (Rosyara et al., 2016) and Tassel 5 program (Bradbury et al., 2007). For the analysis, both the estimated breeding values (EBV) and a binary approach were used.

Results

The in silico analysis identifie a set of 110 potential SNPs that were used to design specific probes. A final set of 60 SNPs was considered that were located in the following chromosomes: 13 in chrW, 20 in chrZ, 14 in chr14 and 13 in other 10 chromosomes using the *C. semilaevis* genome as a reference. Genotyping analysis of a wild population showed 4 monomorphic assays, 7 with abnormal amplification profiles that did not allow a clear differentiation between heterozygotes and homozygotes and a total of 49 polymorphic SNPs in Hardy-Weinberg equilibrium. The association analysis using the GLM and MLM approaches revealed 6 markers with significant differences between the high and low growth families after Bonferroni correction. These markers followed the general model, 5 for the binary weight category (explaining a 61.44% of the total variation) and 2 for the EBV data (explaining a 15.85% of the total variation). One of the markers showed significant differences for the two variables. These markers, located 4 on the chr14, 1 on the chr17 and 1 on the chr22, were associated to the gem-associated protein 8, general transcription factor 3C polypeptide 4, inactive rhomboid protein, protein FAM222A-like, ephrin-A5-like and mitochondrial fission process protein 1

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Discussion and conclusion

In this work, we have tested a mid-density SNP chip in Senegalese sole. A set of 49 assays resulted optimal to association studies. The results identified a set of 6 markers associated to growth that may be linked to genes that participate directly or indirectly in the trait. In the absence of information about the function of some of these genes in fish, we see that in mammals, for example, that the inactive rhomboid protein regulates the secretion of several ligands of the epidermal growth factor receptor and indirectly activates the epidermal growth factor receptor signaling pathway and may regulate sleep, cell survival, proliferation and migration (GeneCards). This study is still ongoing to further delve into the function of these genes.

The results obtained are useful for the current genetic selection program ongoing that can benefit the aquaculture of this species in the medium / long term.

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BONE MINERALIZATION PATTERN DIFFERENTLY AFFECT PHOSPHORUS REQUIREMENT IN TAMBAQUI, *Colossoma macropomum* [a]

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Introduction

The phosphorus (P) requirement in animals diverges depending on the response parameters and the models used to estimate the requirement. For instance, requirement estimates based on weight gain are lower than those estimated based on bone mineralization. The use of P requirement estimates based on WG have led to several bone deformities in salmonids imposing a threat to the welfare of fish and the quality of the final product. This assumption has been used as a rule for most of the aquacultured fish species, at least for Nile tilapia and carp aquaculture. However, limited data on other fish species are available and further extrapolations should be made with care. Therefore, we designed a trial to determine the P requirement for tambaqui juveniles (from 15 to 150g) using different response parameters. Additionally, we provide evidence that the P requirement for bone mineralization might be similar to the estimate based on WG by using a CT-scanning method associated to the bone mineralization data.

Materials and methods

A total of 192 tambaqui juveniles of approximately 17 ± 0.85 g were randomly stocked into a set of 24 70L-aquaria connected to a water recirculation system. The experimental design consisted of six treatments (1.5, 3.0, 4.5, 6.0, 7.5 and 9.0 g kg^{-1} Pdisp.) and four replications in a randomized block design. Fish were fed the six semi-purified diets twice daily for 90 days. At the end of the trial, fish were euthanized and growth performance, whole-body, vertebrae and scales mineralization, CT-scans, as well as hematological and blood chemistry were analysed. Data were submitted to ANOVA and when significant differences were observed, the multiple comparisons test of Student Newman Kews - SNK or Duncan test were used to compare the treatments. Dietary P requirement was estimated using different regression models ($P < 0.05$). Models were selected based on the least sum of squared differences between the values of the observed and predicted values of the dependent variable, the P value and the R^2.

Results

No mortality or apparent signs of P deficiency were observed. Growth performance variables were significantly affected by dietary P levels. Based on weight gain, the P requirement was 6.33g kg^{-1} diet. For adequate bone mineralization, tambaqui seems to require 6.95 g kg^{-1} P. Hematology was not affected by dietary P levels, while blood chemistry parameters were affected by the P levels, except for serum calcium.

Discussion and conclusion

P requirement based on bone or whole-body mineralization are generally higher than the values estimated based on growth. However, our results with tambaqui surprisingly showed the contrary (6.95 versus 6.33 g kg^{-1} for bone mineralization and growth, respectively). We hypothesize that the P requirement based on growth is greatly affected by the amount of mineralized structures (such as scales, operculum and fins) of the species. Comparing tambaqui with Atlantic salmon, one of the most studied species in aquaculture, tambaqui seems to have a higher number of mineralized structures and bone density than salmonids and therefore this might affect the determination of the requirement. In fact, this hypothesis was supported by our findings that fish fed the deficient diet clearly showed a reduced number of mineralized structures and the total bone volume was 2.90 HU, while fish fed adequate P levels (6.3 g Kg^{-1}) showed a great increase on total bone volume (13.34 HU) (Fig. 1). Thus, the assumption that the requirement based on the whole-body mineralization is higher than those based on growth might not be a rule for all fish species

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Taking altogether, our study demonstrated that feeding tambaqui with 6.33 g kg^{-1} digestible P on the beginning of the growth stage is adequate based on several parameters of growth and welfare. Additionally, we have demonstrated for the first time that CT-scan might be an adequate and non-invasive method to assess the P requirement or bone health of fish. Further studies on the effect of P status on lipid metabolism and other blood chemistry parameters are warranted since a profound effect of P status on these parameters was observed in this study.

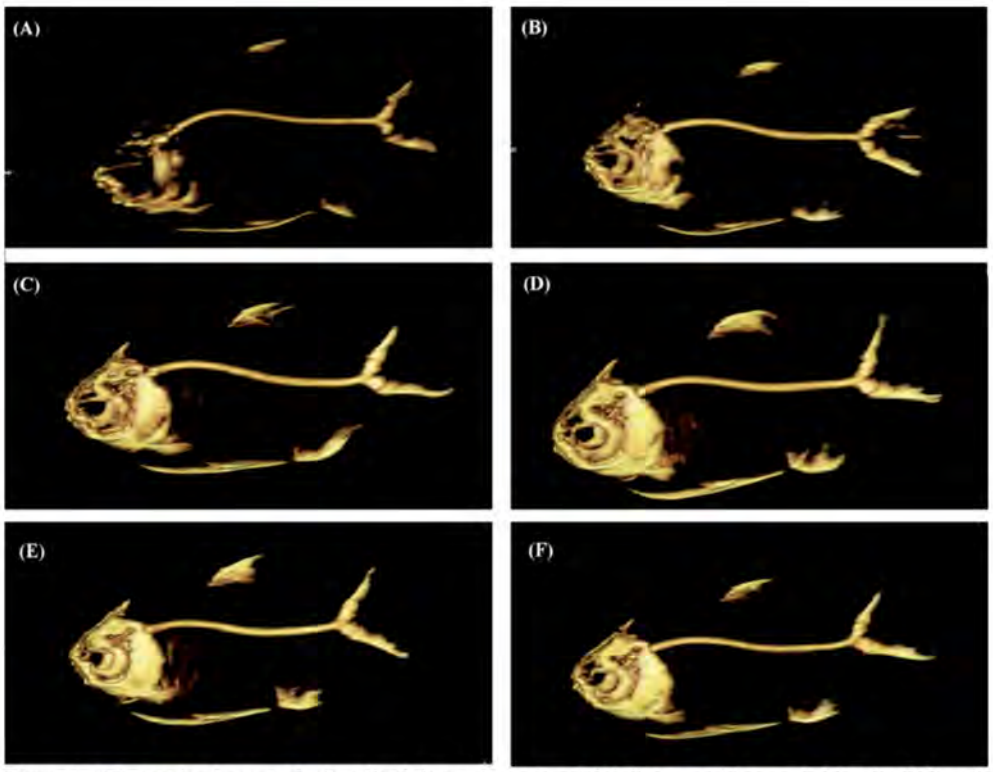

Figure 1. CT-scanning images of mineralized tissues in tambaqui fed diets containing graded levels of digestible phosphorus; Fishes fed basal diet (A), 2.5 g kg-1 dP diet (B), 4.8 g kg-1 dP diet (C), 6.3 g kg-1 dP diet (D), 7.8 g kg-1 dP diet (E), and 8.8 g kg-1 dP diet (F)

IDENTIFICATION OF FACTORS EXPLAINING THE GROW-OUT PERFORMANCE OF EUROPEAN SEABASS *Dicentrarchus labrax* AND GILTHEAD SEABREAM *Sparus aurata* IN THE MEDITERRANEAN

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Introduction
Despite a rapid growth in global aquaculture, growth of Mediterranean aquaculture of European seabass and gilthead seabream stagnates. The productivity and profitability of Mediterranean aquaculture are mainly determined by the zootechnical performance of the farms.

The objectives of the present study were to quantify variation in zootechnical performance and to identify variables that explains this variation.

Materials and Methods
Data were obtained from a survey performed in the EU-project MedAID. In short, a questionnaire was distributed among the grow-out producers across the Mediterranean. Survey participants were asked for detailed zootechnical performance for multiple recent batches and rearing practices applied. Thermal growth coefficient (TGC) ($g^{2/3}$.day $degree^{-1}$), survival rate (%) and deformity rate (%) were used as key performance indicators (KPI) of zootechnical performance from the available data. Information from 6 EU (Croatia, Cyprus, France, Greece, Italy, Spain) and 3 non-EU (Egypt, Tunisia, Turkey) countries was used in the analyses.

Variation in KPIs among batches of farms was quantified. To identify factors that explain the variation in KPIs, multiple regression models were used. Non-significant variables were excluded based on backwards elimination, where the criterion for model performance was adjusted R^2 values.

Results
Descriptive statistics of zootechnical performance in grow-out farms and the amount of data used for each variable are in Table I. On average, seabass had somewhat higher TGC and survival than seabream; however, deformity rates were also higher in seabass. Deformity rates had strong positive skew, which was apparent from higher-than-mean standard deviations. Between-farm variation was greater than within-farm variation for both species.

Multiple regression models that resulted in the highest adjusted-R^2 for each KPI included different variables (Table II). Although the origin of juveniles explained the most variation in simple regression models, it was excluded from four of the multiple regression models due to singularity issues. Presence of pathologies had consistently large negative effects on survival of both species. The effect of feed related variables was not consistent across the KPIs.

Discussion and Conclusion
This study identified several variables that, when they are manipulated by farm management, have the potential to improve the zootechnical performances. Significant associations between the KPIs and explanatory variables are observational rather than causal, hence these do not necessarily reflect the biological mechanisms. The effect of deformities and feed related variables on KPIs are not immediately clear either because their effects are not consistent in the two species or they were significant in one species but not in the other. Thus, further studies are required to confirm their influence on KPIs. Variation in explanatory variables indicates that practices differ among the farms. These results may help grow-out producers to evaluate their practices and allow for improvements to be made in specific management factors.

The origin of juveniles had very high influence on performances in simple regression models; however, relative importance of origin of juveniles could not be evaluated in multiple regression models because juveniles from certain origins were used by only one producer. This prevented to distinguish the effect of origin of juveniles from the farm effects. Multiple breeding programs for seabass and seabream are active in the Mediterranean (Chavanne et al., 2016)hindering an overall evaluation of their success. Here, we report on the results of an online survey of the major aquaculture breeding companies operating

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in Europe. Six main reared fish species were targeted. A total of 31 respondents contributed to the survey, representing 75 % of European breeding organizations. Family-based breeding schemes were predominant, but individual selection was more frequently applied in marine species. Artificial fertilization is the preferred means of reproduction; however, mass spawning is often used as a fallback method. The most frequently selected trait is growth performance, but the number of selected traits has been increasing over the years through the addition of traits such as disease resistance or product quality. The use of molecular tools is now common in all programs, mainly for pedigree traceability. An increasing number of programs use either genomic or marker-assisted selection. Results related to the seed production market confirmed that for Atlantic salmon there are a few dominant players at the European level, with 30-50 % market share. Only part of the European fish aquaculture industry today fully exploits selective breeding to the best advantage. A larger impact assessment still needs to be made by the remainder, particularly on the market share of fish seed (eggs, larvae or juveniles. Although the genetic levels of strains from these breeding programs will vary, strains from breeding programs may be expected to have a superior performance relative to unselected strains (Janssen et al., 2017).

Although controlling the diseases observed in farms is very difficult, there appears to be great benefits for grow-out producers to provide a disease-free environment since the presence of pathologies had very large negative effects on survival of both species.

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Table I. Descriptive statistics for grow-out performance of European seabass and gilthead seabream

KPI	SEABASS			SEABREAM		
	Mean (sd)	Farms	Batches	Mean (sd)	Farms	Batches
TGC	13.4 (2.5)	15	67	13.1 (3.9)	15	80
Survival	84.5 (9.8)	15	73	81.6 (8.7)	15	86
Deformity	2.6 (3.6)	16	69	1.2 (1.5)	14	75

Table II. Effects of variables on grow-out performances of European seabass and gilthead seabream

Explanatory variable	SEABASS			SEABREAM		
	TGC	Surv.	Deform.	TGC	Surv.	Deform.
Juvenile origin	-	-	-	-	-6.80 to 9.55	-5.01 to 0.49
Pathologies (0/1)	2.01	-22.1	2.11	-	-15.44	-
Deformities (0/1)	3.25	-	-	-4.17	-	-
Feed/Feeding						
Frequency (2–12)	3.38	1.92	2.22	1.59	4.46	1.48
To saturation (0/1)	-	25.01	-4.96	-11.09	-	-
Protein (%)	0.21	0.54	-0.25	-	-	-
Fat (%)	-	-	-	-0.32	-1.41	-
Digestible energy ($mJ.kg^{-1}$)	-	2.25	-0.57	-0.52	0.78	-0.72
Marine ingredients (%)	-0.15	-0.37	0.09	0.19	-	0.04

- (Not significant in the model for the respective KPI)

THE STRUCTURAL VARIATION LANDSCAPE IN ATLANTIC SALMON AND IT'S POTENTIAL CONTRIBUTION TO DISEASE RESISTANCE

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Introduction

Structural variants (SVs) are a major component of genetic variation additional to single nucleotide polymorphisms (SNPs). SVs include duplications, deletions and inversions, ranging in size from hundreds to millions of bp, and are known to impact gene expression dosage and function, contributing to commercially relevant phenotypic variation in livestock (e.g. Hou *et al*, 2012; Xu *et al*, 2014; Xu *et al*, 2019). However, there remains a major gap in our understanding and exploitation of SVs within the genomes of farmed aquatic species. Atlantic salmon (*Salmo salar*) is a commercially important salmonid contributing extensively towards global economic and food security. A reference genome (Lien *et al*, 2016), combined with sharply declining sequencing costs, will enable genome-wide studies of SVs at high resolution in this species, which may add significantly to current selective breeding programs

Methods

We have developed computational pipelines to detect SVs in Atlantic salmon genomes, and validated them both bioinformatically and experimentally. The basis of our approach involves SV detection usisng short-read paired-end sequencing data (10-20x coverage) aligned to the current reference genome assembly/annotation. The LUMPY framework, which incorporates multiple SV detection signals (Layer *et al*, 2014), is used for SV calling, with subsequent genotyping done using svtyper (Chiang *et al*, 2015). Our pipeline includes bioinformatic filtering steps to reduce false positive calls inherent to SV detection when using short-read data with complex genomes and imperfect reference assemblies. Critically, we use a tool called SV-plaudit (Belyeu *et al*. 2018) that allows efficient manual visual curation of SVs, enabling retention of only high-quality bioinformatic calls. We have also developed an amplicon-based long-read sequencing approach using the Oxford Nanopore MinION platform to validate SVs experimentally. Finally, we employ established tools to annotate the genomic location of SVs and their predicted impact on functional features including protein-coding genes.

Results

Our first in-depth SV analysis used n=9 resequenced Atlantic salmon individuals from distinct aquaculture strains, which led to ~32,000 highly-significant SV calls following initial filtering. Notably, only ~20% of these SV calls were retained as high confidence variants after SV-plaudit filtering, highlighting the inherent high false positive rate of SV detection using resequencing data, which is likely the case for many species. Among ~7,000 high confidence SVs 90% are deletions, 6% are duplications, and 4% are inversions. Around half the high-confidence SVs overlap with genes, and ~5% are predicted to strongly modify gene function or dosage. Notably, we identified a large number of immune genes affected by SV with predicted large effect. MinION sequencing of PCR-amplified genomic regions containing high confidence SV calls revealed that the true-positive call rate of our pipeline is ~95% (true genotyping call rate ~90%) for deletion variants, with lab validation work ongoing for duplications and inversions. We are currently using the same pipeline for SV detection in a broader set of resequenced Atlantic salmon, representing hundreds of individuals from diverse wild and farmed populations. We are using this dataset to document the SV landscape of Atlantic salmon as a species, including the impact an ancestral whole genome duplication event (Macqueen and Johnston, 2014; Lien *et al*, 2016) had on the probability of SV retention.

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Future work and perspectives

In a new project funded by the Biotechnology and Biological Sciences Research Council, we are focussing on exploitation of SV data in Atlantic salmon breeding. We will genotype high-confidence SVs across a pedigreed commercial population with natural variation in resistance a problematic disease in modern salmon aquaculture. The overall goal will be to assess the extent of heritability in resistance explained by SVs, ii) use association analysis to identify SV regions/genes underlying resistance, iii) establish the effect of incorporating SVs into genomic prediction for resistance, versus the predictive ability of SNP genotypes alone and iv) identify the impact of SVs on gene expression variation associated with resistance.

In summary, it is important to understand the SV landscape of farmed aquatic species, and to establish the extent of commercial trait variation explained by SVs as putative causative mutations. As sequencing technology improves and becomes cheaper, we predict it will become routinely possible to incorporate accurate SV data into breeding programmes in many aquaculture species.

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LINKING FRESH WATER QUALITY AND FISH WELFARE WITH SEA WATER FEEDING PERFORMANCE IN CHILEAN SALMON FARMING: A MODELLING TOOL BASED ON SMOLT PHYSIOLOGICAL STATUS

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Introduction

Salmon farming productivity strongly relies on smolt adaptability success on seawater (SW) environment after transference from the freshwater (FW) phase, especially during the first weeks after the arrival. According to current understanding, sub-optimal water quality (WQ) and physiological status at the end of FW stage are able to alter key smolt physiological traits (e.g. osmoregulation), which can be determinant for the further fish performance (growth and survival) (Brauner et al., 2000; Kroglund & Finstad, 2003; Damsgård et al., 2006; Emparanza, 2009; VKM, 2012; Filvestad et al., 2015). Even though there are advances on smolt welfare in land-based farming (VKM, 2012; Noble et al., 2018), there is a lack of quantitative tools to assess the link between fish welfare and physiological traits with subsequent SW smolt performance (e.g. feeding rate and growth). Also, recirculating aquaculture system (RAS) smolt production has increased over the years at a global scale (Emparanza, 2009; VKM, 2012). However, there are many uncertainties on smolt quality from RAS facilities which are known to be different from flow-through facilities (Gutierrez et al., 2018). Hence, we aim to understand the relationship between smolt physiological status and production systems for developing innovative predictive tools which can enhance salmon farming.

Materials and methods

The current study analyzed data from an ongoing smolt (*Salmo salar*) physiological monitoring program undertaken by NIVA Chile from both RAS and flow-through (FT) fish farms between 2015-2018 in Southern Chile. A total of 23 consecutive fish batches were examined days before the smolts are transferred to the sea. Our methodological approach is based on the integration of key WQ parameters at tank scale, as well as physiological blood parameters and metals concentration in target organs. These parameters were sampled at tank scale, with three tanks for each smolt batch. This information from FW farms was integrated with feeding and survival performance of transferred smolt during the initial of marine on-growing phase.

We categorized four types of components for the modelling: (1) FW farm-based information related to fish biomass and density, among others (FW-F); (2) water quality information from tank (WQ); (3) both blood parameters and heavy metal quantification were referred as fish welfare (W) and; (4) SW feeding and survival performance (SW-P). The four components (FW-F, WQ, W and SW-P) were analyzed by multivariate techniques such as Principal Component Analysis (PCA) and Path Modelling (PM). The first modelling stage consisted in an explorative analysis for identifying major variability from variables by using PCA. Afterward, we selected main variables by applying a relationship modelling by PM with the following criteria: unidimentionaly, loadings and communality, and cross-loadings. Variables selection was an iterative process based on mentioned criteria by including only those variables that better represent each component.

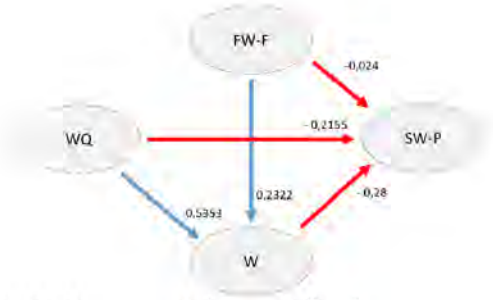

Figure 1: Path model which shows associations and directions among components. Arrow color indicates the sign of coefficients (blue=positive) which relate each component. All coefficients were significant ($p<0.05$), with the exception FW-F > SW-P.

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Results

Explorative analysis by PCA showed that most variability for each component depended on one to four variables. For the FW-F component, main variables selected were flow/load ratio, fish biomass, number of fish and tank density. In term of WQ, carbon dioxide (CO_2) was the main variable selected. Welfare blood traits such as bicarbonate (HCO_3) and partial CO_2 pressure (pCO_2) were selected. SW-P variables such as feeding intake at 2, 3 and 4 weeks after smolt transfer.

A first inner relationship by PM demonstrated that FW-F and WQ (predictors) have a causal and positive effect on W (response variable). In this case, both predictors were significant ($p<0.05$). A second inner model, using W and WQ as predictors to SW-P (response variable) showed a causal negative effect ($p<0.05$). The F-FW and SW-P relationship was no significant, demonstrating the lack of effect on tank traits on feeding and survival performance on SW phase. The following diagram shows direction and type of effect (negative/positive) among components (Figure 1).

Discussion and conclusion

Our modelling approach demonstrated that both WQ and tank traits can cause a positive impact on Atlantic salmon smolt welfare in terms of physiological blood traits such as acid-based parameters (HCO_3, pH and pCO_2). Importantly, we confirm that high levels of water CO_2 can alter acid-base equilibrium, increasing the risk of hypercapnia, as previously reported (Brauner et al., 2000; Fivelstad et al., 2015). This impaired smolt physiological condition was correlated with a poor feeding performance between 2 to 4 weeks after transfer to the sea farms. This revels the high importance of CO_2 for the physiological status of the smolt and its further initial feeding performance on sea water.

Outcomes from this study can serve as a guideline for decision making process to identify the risk of these variables over fish welfare and performance. The producer, for example, is able to prioritize and improve aspects of water quality conditions, which in turn, result in better fish physiological condition and further feeding performance.

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LINKING FRESH WATER QUALITY AND FISH WELFARE WITH SEA WATER FEEDING PERFORMANCE IN CHILEAN SALMON FARMING: A MODELLING TOOL BASED ON SMOLT PHYSIOLOGICAL STATUS

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Introduction

Salmon farming productivity strongly relies on smolt adaptability success on seawater (SW) environment after transference from the freshwater (FW) phase, especially during the first weeks after the arrival. According to current understanding, sub-optimal water quality (WQ) and physiological status at the end of FW stage are able to alter key smolt physiological traits (e.g. osmoregulation), which can be determinant for the further fish performance (growth and survival) (Brauner et al., 2000; Kroglund & Finstad, 2003; Damsgård et al., 2006; Emparanza, 2009; VKM, 2012; Filvestad et al., 2015). Even though there are advances on smolt welfare in land-based farming (VKM, 2012; Noble et al., 2018), there is a lack of quantitative tools to assess the link between fish welfare and physiological traits with subsequent SW smolt performance (e.g. feeding rate and growth). Also, recirculating aquaculture system (RAS) smolt production has increased over the years at a global scale (Emparanza, 2009; VKM, 2012). However, there are many uncertainties on smolt quality from RAS facilities which are known to be different from flow-through facilities (Gutierrez et al., 2018). Hence, we aim to understand the relationship between smolt physiological status and production systems for developing innovative predictive tools which can enhance salmon farming.

Materials and methods

The current study analyzed data from an ongoing smolt (*Salmo salar*) physiological monitoring program undertaken by NIVA Chile from both RAS and flow-through (FT) fish farms between 2015-2018 in Southern Chile. A total of 23 consecutive fish batches were examined days before the smolts are transferred to the sea. Our methodological approach is based on the integration of key WQ parameters at tank scale, as well as physiological blood parameters and metals concentration in target organs. These parameters were sampled at tank scale, with three tanks for each smolt batch. This information from FW farms was integrated with feeding and survival performance of transferred smolt during the initial of marine on-growing phase.

We categorized four types of components for the modelling: (1) FW farm-based information related to fish biomass and density, among others (FW-F); (2) water quality information from tank (WQ); (3) both blood parameters and heavy metal quantification were referred as fish welfare (W) and; (4) SW feeding and survival performance (SW-P). The four components (FW-F, WQ, W and SW-P) were analyzed by multivariate techniques such as Principal Component Analysis (PCA) and Path Modelling (PM). The first modelling stage consisted in an explorative analysis for identifying major variability from variables by using PCA. Afterward, we selected main variables by applying a relationship modelling by PM with the following criteria: unidimentionaly, loadings and communality, and cross-loadings. Variables selection was an iterative process based on mentioned criteria by including only those variables that better represent each component.

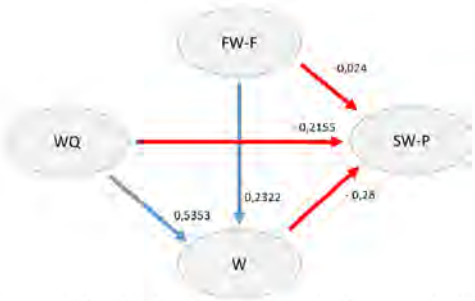

Figure 1: Path model which shows associations and directions among components. Arrow color indicates the sign of coefficients (blue=positive) which relate each component. All coefficients were significant ($p<0.05$), with the exception FW-F > SW-P.

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Results

Explorative analysis by PCA showed that most variability for each component depended on one to four variables. For the FW-F component, main variables selected were flow/load ratio, fish biomass, number of fish and tank density. In term of WQ, carbon dioxide (CO_2) was the main variable selected. Welfare blood traits such as bicarbonate (HCO_3) and partial CO_2 pressure (pCO_2) were selected. SW-P variables such as feeding intake at 2, 3 and 4 weeks after smolt transfer.

A first inner relationship by PM demonstrated that FW-F and WQ (predictors) have a causal and positive effect on W (response variable). In this case, both predictors were significant ($p<0.05$). A second inner model, using W and WQ as predictors to SW-P (response variable) showed a causal negative effect ($p<0.05$). The F-FW and SW-P relationship was no significant, demonstrating the lack of effect on tank traits on feeding and survival performance on SW phase. The following diagram shows direction and type of effect (negative/positive) among components (Figure 1).

Discussion and conclusion

Our modelling approach demonstrated that both WQ and tank traits can cause a positive impact on Atlantic salmon smolt welfare in terms of physiological blood traits such as acid-based parameters (HCO_3, pH and pCO_2). Importantly, we confirm that high levels of water CO_2 can alter acid-base equilibrium, increasing the risk of hypercapnia, as previously reported (Brauner et al., 2000; Fivelstad et al., 2015). This impaired smolt physiological condition was correlated with a poor feeding performance between 2 to 4 weeks after transfer to the sea farms. This revels the high importance of CO_2 for the physiological status of the smolt and its further initial feeding performance on sea water.

Outcomes from this study can serve as a guideline for decision making process to identify the risk of these variables over fish welfare and performance. The producer, for example, is able to prioritize and improve aspects of water quality conditions, which in turn, result in better fish physiological condition and further feeding performance.

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POTENTIAL OF GENOMIC SELECTION FOR THE IMPROVEMENT OF PACIFIC OYSTER RESISTANCE TO *Ostreid herpesvirus* (OSHV-1)

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Introduction

In genomic selection (GS), genome-wide SNP markers are used to generate genomic estimated breeding values (gEBVs) for selection candidates. The accuracy of the predictions obtained from GS for both livestock and aquaculture species has been shown to be higher than accuracy using traditional pedigree-based approaches. GS is particularly useful for the selection of polygenic traits and the benefits have been described for several aquaculture species (Zenger et al. 2019). The application of GS in shellfish also looks promising and has the potential to help in dealing with one of the main issues currently affecting Pacific oyster production worldwide, which is the so called "summer mortality syndrome". This causes periodic mass mortality in farms worldwide and has mainly been attributed to a specific variant of the *Ostreid herpesvirus* (OsHV-1-µvar) (Segarra et al. 2010). In the current study, we evaluated the potential of genomic selection for host resistance OsHV in Pacific oysters, and compared it to pedigree-based approaches.

Materials and methods

31 oyster families were produced in 2015 at the Cawthron Institute's hatchery in Nelson, New Zealand, as part of an ongoing selective breeding program (Camara and Symonds, 2014). Disease challenge was performed based on the preparation of a standardized, cryopreserved stock of the OsHV-1 virus and an immersion-based virus exposure treatment for oysters for seven days. Mortalities were recorded twice a day. DNA was extracted from survivors and mortalities, and 768 samples were genotyped using the medium density SNP array for oysters (Gutierrez, et al. 2017). GWAS was performed for the survival trait using a GBLUP approach in BLUPF90 software. Heritability and gEBV calculations to obtain genomic prediction accuracies were performed using the ASReml 4 software (Gilmour et al. 2015). Various different marker densities were tested to determine the number of SNPs needed to obtain high prediction accuracies. The first approach involved a progressive increase of the minor allele frequency (MAF) threshold, resulting in a progressive decrease in SNP numbers. The second involved choosing random subsets of SNPs for inclusion on low density panels.

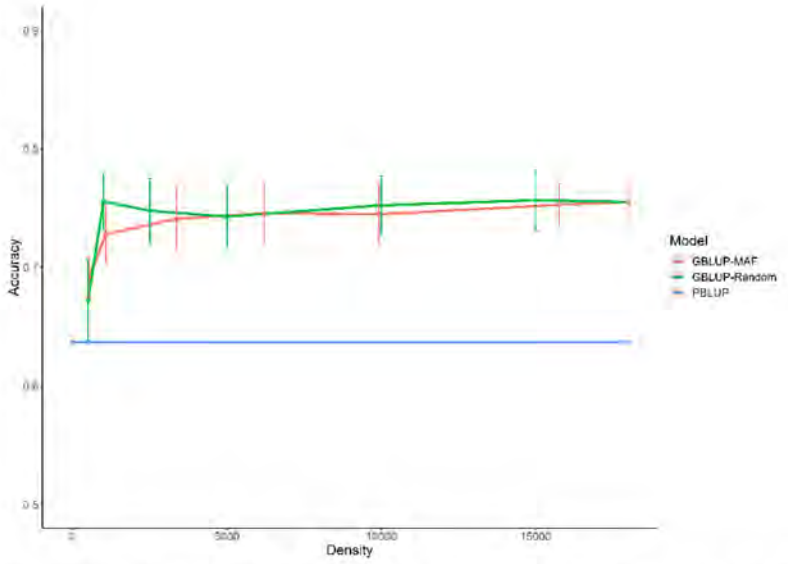

Figure 1. Genomic prediction results showing mean accuracies (±s.d) obtained from both pedigree A-matrix (PBLUP) and genomic matrix G-matrix (GBLUP).

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Results

No major QTL associated with OsHV-1 resistance were identified, although regions explaining moderate percentages of the genetic variance were located on LG1, LG7, LG8 and LG10. Heritability ranged from 0.25±0.05 to 0.37±0.05 (mean±s.e) based on pedigree and genomic information, respectively. Genomic prediction results showed that both SNP reduction approaches had little impact on prediction accuracy until marker densities dropped below ~1,000 SNPs, below which the accuracy tended to drop. With the MAF approach, the genomic prediction accuracies obtained using the lower density SNP panels ranged from 0.755 to 0.693 (MAF>0.475, 530 SNPs), while using the random subsets, accuracies ranged from 0.758 to 0.678 (500 SNPs), as shown in Figure 1.

Discussion

These results suggest that OsHV-1 resistance in this Pacific oyster population is polygenic. Under this scenario, the application of GS can still improve selection by taking into account the effect of all SNPs instead of only those linked to a particular QTL. Our results show that the accuracies obtained from GS are higher than pedigree-based values, even at low SNP densities. This demonstrates the potential for GS in Pacific oyster breeding programs and importantly, demonstrates that a low number of SNPs might suffice to obtain accurate gEBVs, thus potentially making the implementation of GS more cost effective.

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DEVELOPMENT AND VALIDATION OF OPERATIONAL WELFARE INDICATORS (OWI) FOR FARMED LUMPFISH *Cyclopterus lumpus* L.

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Introduction

Fish welfare is a growing area of research that has gained increasing public awareness over the last decade (Ashley 2007; Noble et al. 2012). A practical approach on how to measure welfare in farmed species is using Operational Welfare Indicators (OWIs) (Treasurer et al. 2018). Lumpfish (*Cyclopterus lumpus* L.) are widely used as biological alternatives to the use of medicines for sea lice control in the salmon farming industry (Powell et al., 2018a) but their survival is often challenged by poor husbandry practices and disease outbreaks (Brooker et al. 2018), both important causes of concern from a welfare perspective. Also, as a novel species, it is important to identify when their welfare is being compromised to maintain health and delousing efficienc . We have developed and validated a rapid Welfare Index based on visual scores of fin damage, external lesions, eye condition and suction disc deformities that can be easily implemented under farm conditions at both pre- and post-deployment stages.

Material and Methods

Juvenile lumpfish (n=95) of two different stages of development (pre-deployment, mean weight=37.78g±4.74; post-deployment, mean weight=53.54g±3.08) were measured, weighed and photographed to obtain information on (1) external condition, (2) fin damage (Hoyle et al. 2007), (3) eye condition, (4) eye darkening (Champneys et al. 2018; Freitas et al. 2014; Volpato et al. 2003) and (5) suction disc deformity, to develop a Scoring Index. Plasma cortisol and relative weight (Wr), obtained from the logged length-weight relationship, were used to validate the Index. Percentage of exact agreement and Cohen's weighted kappa coefficients were used to assess inte -rater and intra-rater reliability of the measured scores.

Results

Principal Component Analysis (PCA) indicated that the first component accounted for 39% of variation and was mostly associated with suction disc deformity (-0.56), eye darkening (0.47) and external condition (0.46). The second component explained 21% of variation and was mostly affected by eye condition (-0.79) and fin damage (-0.45), being also positively related to plasma cortisol levels (R^2=0.4, $p<0.01$). Welfare indicators were affected by age (Fig. 2), suggesting that welfare indicators should be tailor-made depending on life stage (pre- or post-deployment). The repeatability of welfare metrics was generally high, with quadratic weighted kappa values ranging from 0.74 to 1.00, for measurements between different observers (inter-rater) and the same observer and different sampling points (intra-rater).

Discussion and conclusions

Lumpfish (*Cyclopterus lumpus* L.) are increasingly being used as cleaner fish for sea lice control in salmon cages but knowledge on their welfare is poor. The development of reliable and easy-to-use Operational Welfare Indicators (OWI) for this species has been flagged as a priority by industry and will help with quality assurance. The OWIs we present here were based on the visual assessment of external condition, fin damage, eye condition, eye darkening and suction disc deformities. These proved to be repeatable and easy to implement in both hatcheries and sea farms and can serve as indicators of lumpfish welfare that depend on life stage. Fin damage and eye condition were the most important determinants of lumpfish welfare at post-deployment stage (sea cages), while suction disc deformities were more important at the hatchery stage. This could be explained because lumpfish producers select their fish for quality before deployment. Also, it was observed that fin damage and eye condition can deteriorate with time at sea cages as it is a more exposed and uncontrolled environment than in tanks on land, so the probability of suffering external physical lesions is quite higher. Eye condition, which included eye damage, cataracts and exophthalmia, is particularly important in sea cages as it can affect delousing efficienc , known to be highly dependent on having unimpaired vision (Jonassen et al. 2017). Our Welfare Score, which has been simplified into a Welfare Scoring Index based on the first results, is being tested currently in different hatcheries and sea farms to assess its practicality.

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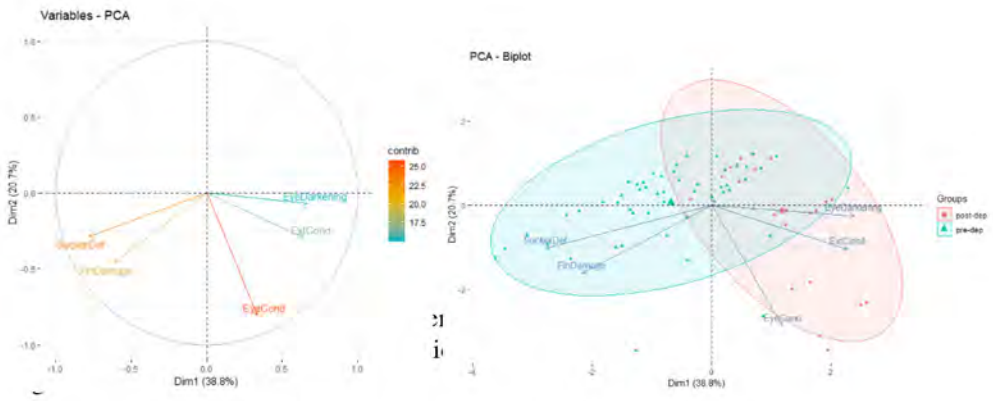

Figure 1 (left). Principal Component Analysis of variables.
Figure 2 (right). Principal Component Analysis of individuals and variables showing differences in life stages.

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IMPACT OF CLIMATE CHANGE ON CARP YIELDS IN HUNGARIAN POND AQUACULTURE

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INTRODUCTION

Pond culture is the dominating sub-sector in Hungarian aquaculture with an output of 14893t from 26065ha of fish ponds in 2017. Low-input farming technologies are dominant, characterized by reliance on natural pond food sources and high dependence on environmental conditions making the farming technology sensitive to climate change. In this paper, we describe a model that simulates the impact of climate change on carp yields.

MATERIALS AND METHODS

The model used for simulating yields is a food web based fish pond model implemented in Direct Computer Mapping (DCM) Programmable Structures. The model describes and simulates: i) physical processes (e.g. air-water temperature exchange, evaporation, air-water oxygen transmission); ii) nutrient flows among the elements of pond food web; and iii) managerial interventions (stocking, feeding, manuring, water management). Both climatic and managerial stressors were introduced into the model in order to simulate the impact of different climate regimes and several pond farming technologies on carp yields. NORESM 4.5 and 8.5 climate scenarios were used for forecasting the impact of climate change on production. Two geographical locations were considered representing the two main production regions. The model was validated by industrial production data.

RESULTS

Figures 1 present the forecasted gross common carp yield as function of technological settings in the Southern Great Plain simulation locations. Simulated results show that there is a monotonic increase of yields over time. Yields in 2046-55 will be 3-8% higher than in the reference period taking into account the most typical technological options. There is minor difference between climate scenarios. In the mid run (2026-35), climate change impacts are higher under RCP8.5 than under RCP4.5. In the long run (2046-55), there is no any significant di ference between RCP scenarios.

However, there is large difference in climate change impacts between different management scenarios:

- Higher feeding rates are associated with bigger growth in yields (A -> D subgraphs). This is because warming temperature positively influences the anabolic activity of carp and its capability to take up food. Thus, more intensive feeding is required to meet increased nutrient demand and to exploit growth potential.
- Increment in yields is higher for strategies with lower stocking densities. Enhanced plankton productivity can be fully exploited with more extensive technologies, when predation pressure on zooplankton fauna is lower.

CONCLUSION

Simulation results show that global warming may generally increase carp yields. However, different farming technologies will unequally benefit from climate change: farming technologies, applying low stocking densities and intensive feeding will be impacted more beneficially than the other technological options

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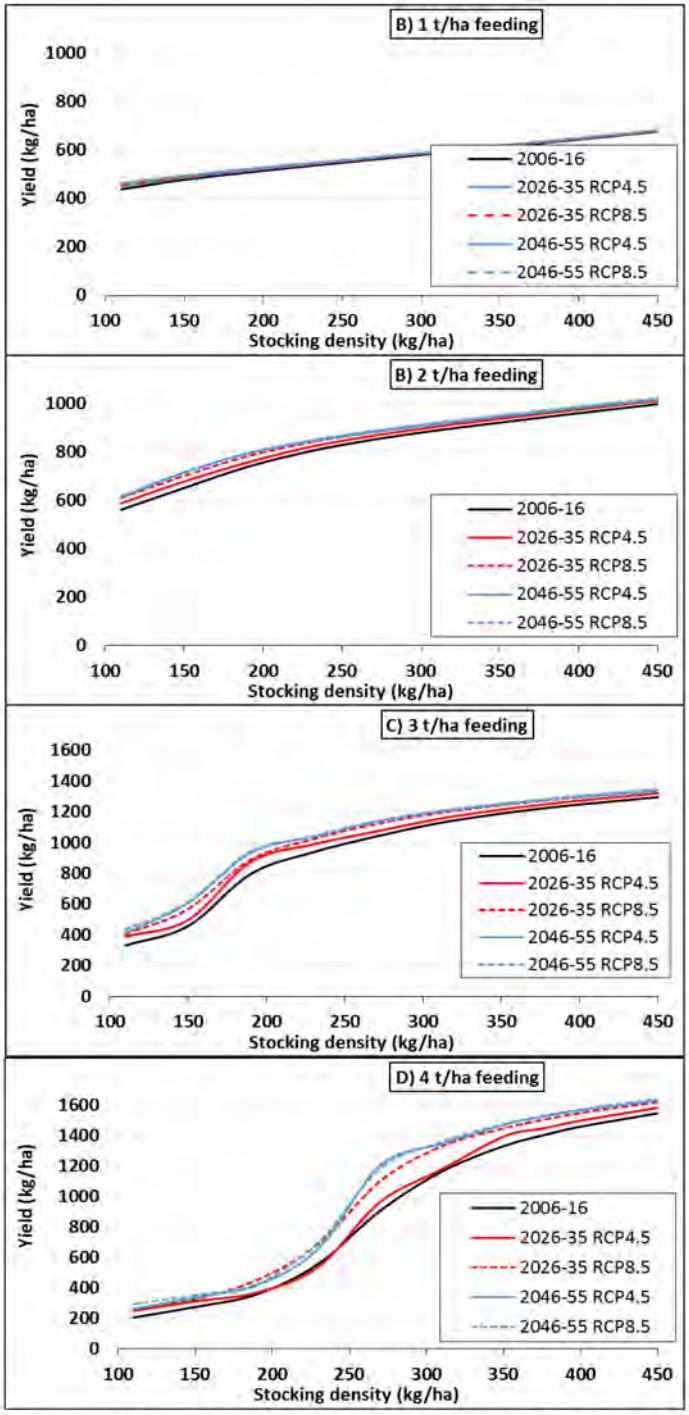

Fig 1. Forecasted gross yields of Common carp as function of stocking density, under different time horizons and climate scenarios. A-D subgraphs represent different feeding strategies. Southern Great Plain simulation location.

CULTIVATION OF ARTEMIA SP. ON SOME AGRICULTURE BY-PRODUCTS AND THEIR UTILIZATION AS FOOD FOR THE FRESHWATER PRAWN, *Macrobrachium rosenbergii* (DE MAN, 1879)

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Two laboratory experiments were conducted to determine (1) the influence of a cheap and widely available agriculture by-products (soybean, rice bran and cotton seed meal on survival , growth and fecundity of *Artemia sp.* beside fresh algae as control diet for 25 days. (2) Effect of dried adult *Artemia* as sole food, mixed with formulated diet (50:50) and formulated diet as control on growth performance and feed utilization of the freshwater prawn, *Macrobrachium rosenbergii* . *Artemia* were fed a concentration of 60 mg per day up to 10 days and 80 mg per day up to 25 days was found to be the best regime for culturing *Artemia* in all cases. Growth, fecundity and naupliar production differed significantly ($P<0.05$) for *Artemia* fed on soybean than other test diets.

The proximate and minerals composition of *Artemia* fed on optimum concentration (80 mg/day) of different diets were determined.

M. rosenbergii fed on mixed diet (dried adult *Artemia* and formulated diet) showed the best significant ($P<0.05$) growth performance, survival and feed utilization. It can be suggested that dried *Artemia* can be mixed with artificial diets for larvae of the freshwater prawn *M. rosenbergii.*

MOLTING CAN BE A MAJOR CAUSE OF MORTALITY IN INTENSIVE SYSTEMS (RAS) FOR SHRIMP (*L. vannamei*) CULTURE

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1. Introduction

Culture of shrimps in controlled systems, such as RAS, seems by many to be a sustainable alternative to the traditional, outdoor culture systems of shrimps; the controlled systems can be bio secured, not depending in environmental conditions and can have low environmental impact.

Culture of shrimp in RAS requires intensification of the shrimps. Substantial loss of shrimps along the culture period is one of the major bottle necks in developing this technology.

In a bio-secured facility, when the stocked animals are Specific Pathogen Free (SPF), no outbreak of acute shrimp diseases are expected. Yet, previous observations carried out in the experimental RAS facility of Maof Hanegev Ltd., revealed that in high densities (>1000 shrimps/m^3) there is a significant loss of shrimps during culture time; the cause of mortality of these animals is unknown as the dead shrimps do not show any clinical signs and their mortality cannot be related to any known environmental factor.

The data that has been collected until now in Maof Hanegev support the assumption that this apparent asymptomatic mortality might be focused to negative processes occurring during molting time of the cultured shrimps.

The aim of the present research was to make histological and microbiome analyses in shrimps that were collected (dead/alive) from intensive RAS and to estimate the primary cause of mortality in these culture conditions. This research is an important step for developing the required technology to reduce mortality of shrimps cultured in high densities and by that contributing to optimization of shrimp culture in RAS.

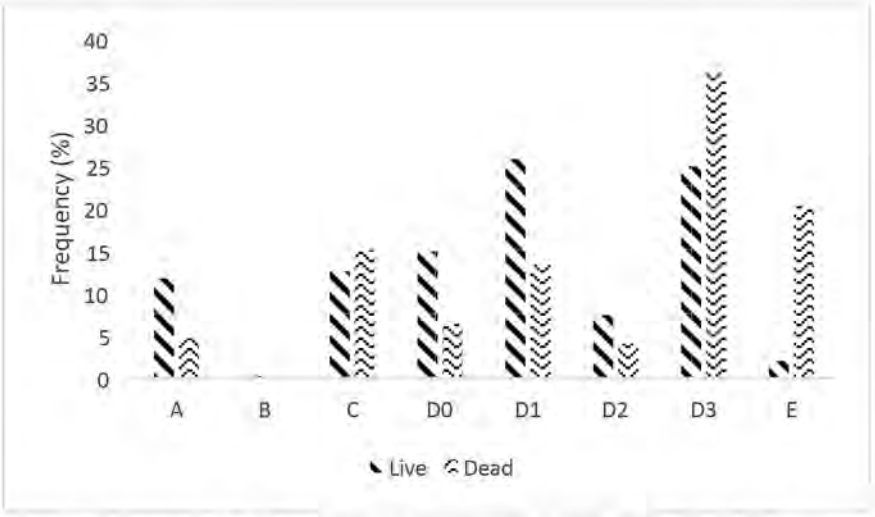

Figure 1: frequency of molting phase of *L. vannamei* cultured in intensive tanks. live refers to shrimp that were sampled alive from the culture tanks (n=348); Dead means shrimp that were collected from the tank as freshly dead animals (n=172).

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2. Materials and methods

The research work had been carried out in the experimental RAS facility of Maof Hanegev Ltd in Kibutz Revivim, Israel. The experiment included 3 culture tanks, 5m^3 each, stocked with 5500 shrimps (L. vannamei) per tank, at an average size of 5gr. Dead shrimps were collected continuously from the experimental tanks and were frozen or fixed immediately; this sampling method enabled the analysis of as fresh as possible dead animals. As a reference, normal animals were randomly pooled from the tanks and were stored and analyzed at the same way as the dead shrimps. To determine the molting stage, the physiological condition and the microbiome of the shrimps, the following tests were carried out:

2.1. The molting stage of each sampled animal (dead/alive) was tested by microscopical observation of the uropod.

2.2. Animals (dead/alive) were fixed for histological examination of the different tissues and organs to observe tissue abnormalities.

2.3. Microbiome analysis of the sampled shrimps, by sequencing specific fragment of microbial 16S

3. Results

Shrimps were cultured in the experimental tanks up to size of 16gr. Analysis of the molting stage profile (figure 1) shows that the profile of the dead shrimp is significantly different from the molting profile of the live shrimp population in the culture tanks. In the live population in the tank only 27% of the animals were in late pre-molt and molt phases (D3&E), compared to 57% of the dead shrimps that were found to be in those phases.

The microbiome analyses and the histological tests do not support any conclusion that the dead shrimps were affected from any acute pathogenic infection.

4. Conclusions

The present research shows that a bio-secured RAS can keep shrimps in high densities without any outbreak of acute bacterial diseases. The data collected in this research support the hypothesis that most of the mortality of shrimp in RAS can be related to the molting phase. It is yet unknown if mortality is due to the molting process itself or due to aggressive interactions among the shrimp population in the tank - predation of soft shell, defenseless shrimps.

BIOREMEDIATION OF AQUACULTURE AND BIOGAS SIDE STREAMS USING POLYCHAETES (*Hediste diversicolor*, O.F. MÜLLER, 1776). PART I: GROWTH & MORTALITY

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Introduction

"*In a world with growing pressures on resources and the environment, the EU has no choice but to go for the transition to a resource-efficient and ultimately regenerative circular economy*" (EREP, 2014). Bio industries are especially well suited to spearhead circular principles, as biological processes *per se* rely on recycling and reuse of organic and inorganic compounds. Circular approaches can help making aquaculture operation greener, e.g. by producing high quality feed components rather than harvesting them. Polychaetes are a natural part of the diet of many aquatic animals and contains high levels of sought after biochemicals, such as omega-3-fatty acids and proteins. Further, they are detritivores, making them perfectly suited as recyclers of organic side streams. Sludge from RAS aquaculture is an obvious feed source for aquatic vermiculture, and has been shown to be promising (Bischoff et al., 2009; Pajand et al., 2017). As an alternative, biogas production is on the rise, and the solid digestate after methanogenesis might also pose a promising substrate for worm faming.

Material & Methods

We reared the polychaete *Hediste diversicolor* along a gradient spanning from pure sludge from salmon smolt production to pure solid biogas digestate in 33% steps for 30 days. A group receiving fish feeds served as the positive control. Worms reared on these five different diets were analysed for growth, survival and biochemical composition (cf. part two of this study; Malzahn et al).

Results

The polychaetes accepted all diets and displayed positive growth. The worms fed FF showed a significantly higher SGR compared to worms produced on the other diets (0.018 ± 0.003 d^{-1}; $p < 0.05$), whereas there were no significant differences in SGRs between the other treatments (Fig. 1). Previous studies have shown negative growth for *H. diversicolor* when starved (-0.02 ± 0.007 d^{-1}). The survival rate ranged from 82 - 92% between the treatments, and there were no significant dietary effects on mortality (Fig. 2).

Discussion & Conclusion

H. diversicolor is a promising candidate to produce high quality feed components from to-date-considered wastes. However, current regulations on an EU level do not allow to use animal products produced on aquaculture and biogas side streams as feed ingredients. Clearly, regulations must be reviewed to unleash the full potential of circular bio production approaches.

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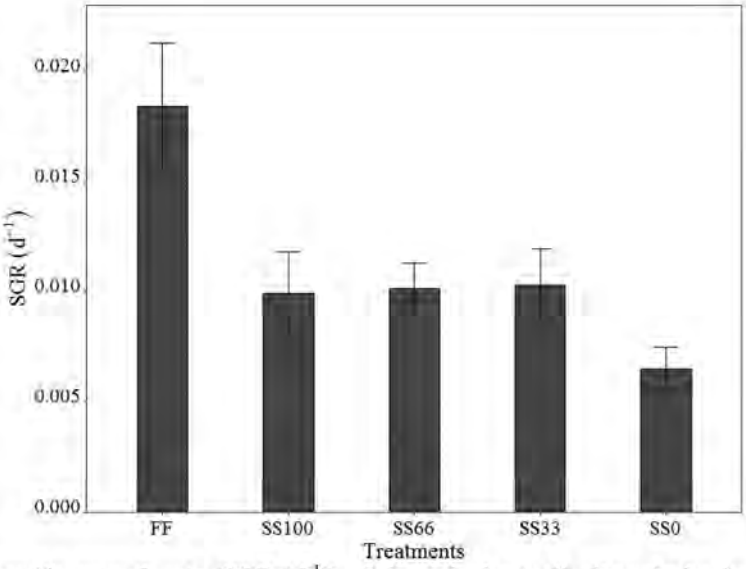

Fig. 1 Specific growth rate (SGR (d^{-1})) of the polychaete *H. diversicolor* in a 30-day cultivation experiment when fed on fish feed (FF), solid biogas digestate (SS0), smolt sludge (SS100) or a 2:1 and 1:2 mixed ratio of smolt sludge and solid biogas digestate (SS66 and SS33, respectively).

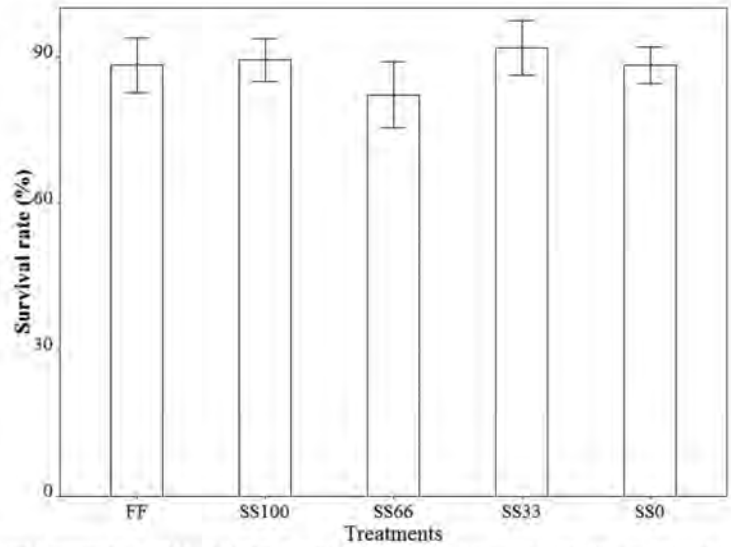

Fig. 2 Final survival (%) of the polychaete *H. diversicolor* in a 30-day cultivation experiment when fed on fish feed (FF), solid biogas digestate (SS0), smolt sludge (SS100) or a 2:1 and 1:2 mixed ratio of smolt sludge and solid biogas digestate (SS66 and SS33, respectively).

NUTRIENTS INVOLVED IN DIGESTION AND TRANSPORT OF LIPID ACROSS THE INTESTINAL MUCOSA OF ATLANTIC SALMON (*Salmo salar* L).
PART 4: EFFECTS OF DIETARY PHOSPHATIDYLCHOLINE AND CHOLINE ON INTESTINAL LIPID METABOLISM

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See abstract of Krogdahl et al.: Nutrients involved in digestion and transport of lipid across the intestinal mucosa of Atlantic salmon (*Salmo salar* L). Part 1: An overview.

INCREASED TAURINE CONTENT IN START-FEEDING DIET BOOSTS GROWTH IN FARMED ATLANTIC COD (*Gadus morhua*)

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Atlantic cod from a selected broodstock (Norwegian National breeding program for cod) were hand stripped to obtain a genetically uniform starting point for start feeding experiment. Effects on survival and growth of taurine content in start-feeding diet for farmed Atlantic cod larvae were measured. Fish fed diets with high and low level of taurine were compared to fish fed a control diet. Dry diet was introduced at 14 days' post hatch and a co-feeding strategy with rotifers and artemia nauplia were used. Treatment ended at day 120 post hatch and all groups were fed standard diet until end of experiment at day 170 post hatch. Fish fed high level of taurine were significantly longer at day 71 and 85 post hatch. Fish fed high content of taurine was significantly heavier than fish from control group at day 94 to end of experiment at 170 days' post hatch. At 170 days' post hatch fish fed high taurine diet were 47 % heavier than fish from control group. There were no significant differences in survival between treatments. The experiment is repeated and data (deformity and histological) from ongoing experiment will be added.

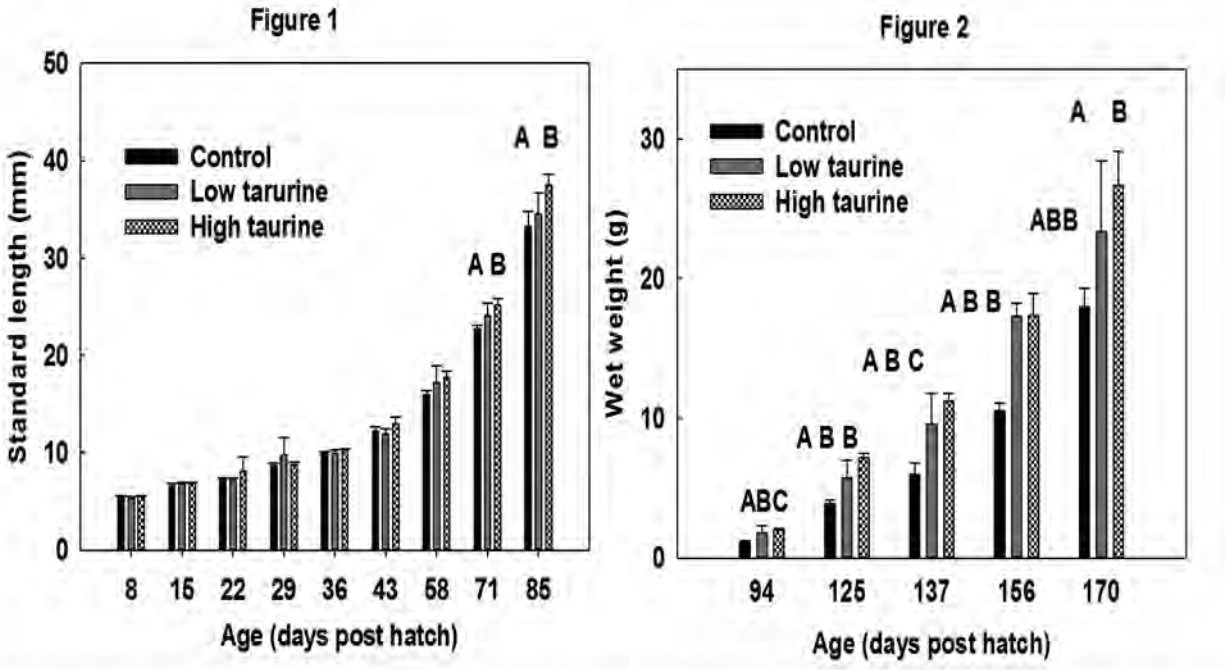

Figure 1: Fish fed high content of taurine were significantly longer than fish from control group at 71 and 85 days' post hatch

DEVELOPMENT OF A HYDRODYNAMIC PARTICLE TRANSPORT MODEL TO STUDY JELLYFISH INTERACTIONS WITH FISH FARMS

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Introduction

Fish kill events or gill disorders in marine-farmed fish due to jellyfish sting toxins are a global problem (Rodger et al., 2011; Purcell et al., 2007) with numerous fish-kill events reported in Europe, Asia, North America, and Australia. The economic consequences are significant with annual losses of 68–205 million USD (Kim et al., 2012) and 10 million USD previously estimated for Korea and the Gulf of Mexico (Graham et al., 2003), respectively. In Ireland, in 2017, swarms of jelly wiped out about 80 percent of the salmon stock from several farms in Killary Harbour (Fig 1) and some 10,000 fishes from adjacent waters along the west coast (O'Sullivan, 2017). The present research aims to develop a hydrodynamic coastal model to simulate the transport and fate of jellyfish which can be used to mitigate / prevent adverse interactions of jellyfish with fish farms. Due to its recent problems, Killary Harbour, a fjord on the Irish west coast, was chosen as the case study site for the research.

Materials and methods

A 3D baroclinic model of Killary Harbour has been developed using the Environmental Fluid Dynamics Code (EFDC). The model domain extends 20 km East-West and 16 km N-S and is resolved horizontally at 64 m giving a grid of 320×100 computational cells. Average water depths in the harbour are 20 m and the model uses 20 sigma layers in the vertical direction. The model has been validated against ADCP-measured (446.7 E 5939.7 N) current velocities and water levels (Fig 2) for which good agreement has been achieved. Activities of jellyfish within Killary were recorded in August-September 2015 by releasing tagged jellies and tracking their movement with 8 stationary receivers distributed along the harbour. In this first phase of the research, the jellyfish were modelled as passive drifters using EFDC's particle transport module and the outputs were compared with the measured data. Particles were released in the surface and mid-depth layers at the identical locations and times that tagged jellyfish were released.

Results

In Figure 3, the measured movements of one of the tagged jellyfish is shown by the multi-colored rings. The numbers represent sequential times of detection by receivers (each receiver range was approximately 500m) and the thickness of the rings is representative of the time spent within a detector's range. The figure also shows the positions of modelled particles at two time instances of detection (1 and 9), the first being just a few hours after the release time (indicated by time 0 on the water level insert graph) and the second being approximately 125 hours (ten tidal cycles) after the release. It can be seen that there are particles present at the detection locations (S4 and N1) at the times of detection even though the receivers are located approximately 5 km apart. The plot at time 9 shows a significant difference in the long-term transport of particles released in the middle of the water column compared to those released at the surface. Wind-induced surface currents result in many surface particles being transported west of the release point towards the sea, whereas the sub-surface particles are all transported eastward of the release point.

Discussion and conclusion

Analysis of the jellyfish tracking data has indicated a strong tidal advection influence on jellyfish movements. This agrees with the literature (e.g. Berline et al. (2013) and Yin et al. (2019)) which reports ocean and wind-driven currents to be the main drivers of jellyfish transportation. Simulating the jellyfish in Killary harbour as passive drifters has shown that tidal and wind driven currents alone can indeed cause particles to be transported in a similar manner to the tagged jellyfish which further corroborates the literature. However, further statistical analysis of model results must be conducted. Killary Harbour is home to both salmon and mussel farms and the next phase of the research will involve including jellyfish behaviours and the salmon/mussel farms in the model so that potential jellyfish interactions with farms and their means of prevention can be investigated.

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Fig 1. Killary Harbour, Ireland

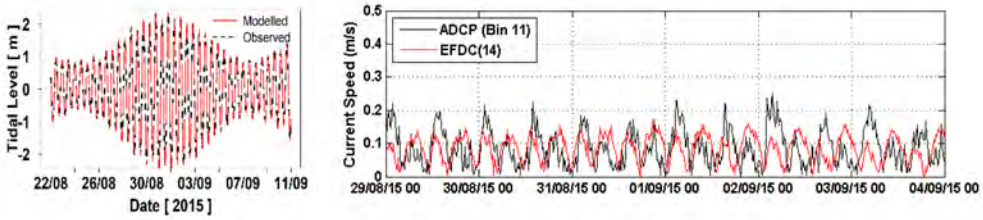

Fig 2. Comparison of modelled (red) with measured (black) hydrodynamics in Killary.

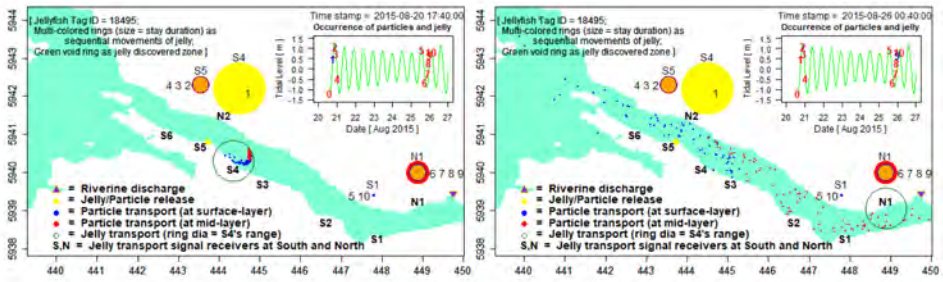

Fig 3. Comparison of Lagrangian particle with observed jellyfish transportation in Killary

Acknowledgements

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COPING WITH THE INVASIVE JAPANESE OYSTER DRILL (*Ocinebrellus inornatus*) ON OYSTER CULTURE PLOTS

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Oyster farmers are struggling with large losses due to predation on the oysters by the invasive Japanese oyster drill (*Ocinebrellus inornatus*). By drilling a hole through the shell this marine predatory snail can access and feed on the meat. In the Netherlands, oyster production has steeply declined over the past few years as a consequence of oyster drill predation. Without additional measures the production of oysters will continue to decline and this will have a huge impact on Dutch oyster cultivation, potentially leading to the complete closure of oyster farming companies.

The Japanese oyster drill was first discovered in the Oosterschelde, the Netherlands, in 2007 where it most likely arrived via international oyster transport.

The population of Japanese oyster drills has increased rapidly over time due to the fact that they do not have a natural predator in the area, and have a very high fecundity. The distribution of the Japanese oyster drill is also expanding, and now include Lake Grevelingen and Lake Veere, possibly due to transport of oysters between different cultivation areas.

In 2018 the project ‘Learning to cope with the Japanese oyster drill’ was initiated. In this project Dutch oyster farmers and researchers cooperate to provide oyster farmers with validated methods in order to maintain the oyster cultivation, and increase the survival of oysters on culture plots in the presence of the Japanese oyster drill.

Because little information is available on how the Japanese oyster drill behaves in Dutch waters, research on the biology and behavior of the drill is conducted. This knowledge can be used by oyster farmers to develop effective breeding measures to increase oyster survival.

The current focus within the “Learning to cope with the Japanese oyster drill” project is predation behavior as well as gaining knowledge about the general mobility - a combination of monitoring and in situ manipulative experiments in enclosures.

To explore the feeding behavior of the Japanese oyster drill, the effects of oyster species, morphology and size on predation rate are being investigated.

The general mobility of the oyster drill is also tested, along with the effect of wind, current and prey location on the direction of movement.

The monitoring and experiments are still ongoing and the results will be discussed.

NUTRITIONAL QUALITY OF DIFFERENTLY PROCESSED *Moringa oleifera* SEED BEFORE AND AFTER OIL EXTRACTION FOR INCLUSION IN *Clarias gariepinus* BASED DIETS

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Introduction

Previous investigations reported on a range of different fish species fed relatively high inclusions of plant proteins, including African catfish (Goda *et al.,* 2007). The inclusion of plant protein sources in the ration of fish requires investigation on proper processing for effective utilization (Francis *et al.,* 2001). Presence of certain limiting factors in plant ingredients such as high crude fibre content and anti-nutritional factors have been demonstrated (Alegbeleye *et al.*, 2005). The objective of this study was to evaluate the effect of different processing methods on the nutrient and anti-nutrient profiles of *Moringa oleifera* seed.

Materials and Methods

The experiment was conducted in Sokoto, Nigeria on latitude 13^0 07' 47.6''N and longitude 05^0 12'11.3''E in accordance with the University's Research rules and guidelines. Processing and analyses were conducted using standard procedures. For each of the twelve (12) different processing methods two hundred grams (200g) of the seeds were used for proximate analysis, amino acid assay and anti-nutrient determinations.

Results

The anti-nutrients detected in the raw Moringa seeds were Oxalate, saponins, alkaloids, phytic acid, tannin, cyanide and phytate with the following contents; 0.86±0.13, 1.50±0.06, 2.32±0.06, 269.84±1.62, 1.21±0.14, 0.56±0.04 and 69.82±0.86, respectively (Table 1). It was only B90mins/S72hrs that significantly ($p<0.05$) reduced phytic acid to FAO (2009) permissible limit of ≤5mg/kg amongst all the processing methods. As shown in Table 2, treat Moringa had the highest crude protein content of 55.05±0.16.

Discussion and Conclusion

Reduction trend in the anti-nutrients was observed in all the treatments with increasing processing periods in this study. This is in agreement with the finding of Siddhuraju and Becker (2003), who reported reduction in anti-nutritional factors in mucuna seeds with different processing techniques. The results suggest that boiling for 90mins and soaking for 72hrs was the most effective processing method that significantly ($p<0.05$) reduced the phytic acid content. Boiling and soaking treatments observed in this study revealed no significant increase in the crude protein contents of the treated seed. This is contrary to the findings of Mbah *et al.* (2012) who reported increase in protein. The three toasting treatments in this study significantly ($p<0.05$) increased the crude protein contents of the Moringa seed compared with the other treatments. The fat content of the untreated moringa was lower than the value (42%) reported by Ogunsina *et al.* (2011) and higher than 30.36-35.20% observed by Anwar *et al.* (2006) for moringa seed meal. The fat content was also higher than the values reported for other oil seeds, melon seeds (17.36-25.06%) (Ebuchi and Avwobobe 2006).

The significant ($p<0.05$) increase in the protein content in the treated and defatted seed meal recorded in this study could be as a result of the displacement of oil from the seed thereby increasing other parameters. Robinson *et al*. (2001) reported that feed ingredients with crude protein greater than 20% are considered as protein source which qualifies *Moringa oleifera* meal as an alternative protein source. The result further indicates that oil extraction had significant ($p<0.05$) influence on crude protein yield of *Moringa oleifera* seed meal. The crude protein content in the treated and defatted seed meal was relatively higher than the value reported for Moringa cakes after oil extraction (Govardham *et al*., 2013). There was a significant reduction in the fat content of seed meal after oil extraction and this is in tandem with the finding of Govardham *et al*. (2013). This study shows that boiling for 90min and soaking for 72hrs effectively reduce the anti-nutrients to safe levels and revealed the high nutrient profile of *Moringa* seed meal.

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WSSV RISK FACTORS OF SHRIMP FARMING IN BANGLADESH: STATISTICAL MODEL-BASED ASSESSMENT

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Background
White Spot Syndrome Virus (WSSV), a deadly disease constitutes the main cause of massive mortality in shrimp farming of Bangladesh resulting in huge economic loss every year. Disease prevalence as opposed to virus presence is however uneven, and may be partially dependent on a range of farm management, physical pond parameters and other factors. In this study of 233 farms in South-Western Bangladesh where there range of pond types, culture techniques and farming practices exist in relative close proximity, were surveyed to generate a dataset on relevant variables. A group of potential factors contributing to WSSV prevalence was first analyzed using a univariate logistic regression analysis to identify key variables and then reanalyzed using a multivariate logistic regression analysis following a maximum model to establish incremental risk factors.

Methods
Four categories of data (site/farm characteristics, environmental variables, disease history, and management variables) with associated risk factors were selected following a conceptual framework and participatory rural appraisal tool. Association of the selected factors with WSSV prevalence was examined using log likelihood ratio tests for a univariate analysis and a maximum model following the continuous removal for a multivariate analysis.

Findings
From the surveyed category, extensive farms (65.57%) were more prone to WSSV than that of the semi-extensive (23.81%) one. In both categories of the farm a wide range of variables from site/gher characteristics to management practices act to prevalence and/or transmit the disease within or between the farms. Rice and other cropland converted (63.31%) to large shrimp farms with the possession of loamy soil (71.33%) were more vulnerable to WSSV. WSSV prevalence was also common irrespective of the farm operation because of indiscriminate use of mixed fertilizers (79.41%) and chemical for pond preparation (95%) as well as water treatment. The maximum farmers were found not interested in using aerator, drying the gher, sludge removal, and proper dike repairement after each crop to minimize the production cost. However, these all accelerate the WSSV outbreak in the amalgamated and interconnected shrimp farms. Under the domain of 'water and culture management' different impecunious practices like perilous source of water, frequent water exchange within and between the farms, absent of reservoir, introducing water via other farms and vague source of atrocious PL were also responsible for unexpected occurrence of WSSV. In some farms, WSSV was linked with the usage of adulterated feed and poor biosecurity system. Farms operated by the tenant worker (p: 0.03), mixed use of fertilizer (p: 0.009), indigent water source (p: 0.001), absent of reservoir for water purification (p: <0.001), and frequent exchange of water between a single crop culture (p: <0.001) were significantly associated with either WSSV prevalence index in the multivariate analysis.

Interpretation
Maintaining good biosecurity, feed management, good culture practice, and managing the farm characteristics will not be sufficient on their own to control WSSV prevalence. Improving some features of site/farm management variables, safe source of water, controlled exchange of water and maintaining constant salinity are necessary to reduce WSSV prevalence.

IRRADIATION USING MIDDLE- TO LONG-WAVELENGTH LIGHT INDUCES OVEREXPRESSION OF *GSDF* AND MALE-SPECIFIC GONADAL DIFFERENTIATION IN GENETIC FEMALE MEDAKA (*Oryzias latipes*)

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Introduction

Environmental factors such as high temperature, low pH, and rearing density affect gonadal differentiation in fish (Devlin and Nagahama, 2002). Lighting conditions also play an important role in regulating physiology in fish. While the effects of irradiation with specific wavelengths on the growth and reproduction in fish have been demonstrated, their effects on sex differentiation have not been demonstrated. Our previous study demonstrated that green light induces female-to-male sex reversal in medaka (Hayasaka et al., 2019). In the present study, we developed the method further by selecting green light-mediated sex-reversed fish at an early developmental stage and evaluated how light wavelength affected their gonadal differentiation.

Materials and methods

Strain d-rR-Tg (*olvas*-GFP) (Strain ID: TG141) of the medaka (*Oryzias latipes*), supplied by NBRP Medaka (https://shigen.nig.ac.jp/medaka/), was used in this study. Newly hatched medaka larva were irradiated with light at 450nm (white), 462nm (blue), 518nm (green), or 623nm (red), from 0 to 15 days post hatching (dph). The phenotypic sex of the fish was determined by gonad size via GFP fluorescence. Genotypic sex was determined by presence/absence of the sex determination gene *DM domain gene on the Y chromosome* (*dmy*) in genomic PCR. Total RNA was extracted from the phenotypically and genotypically identified fish and performed to real-time PCR for quantification of *gonadal soma derived factor* (*gsdf*).

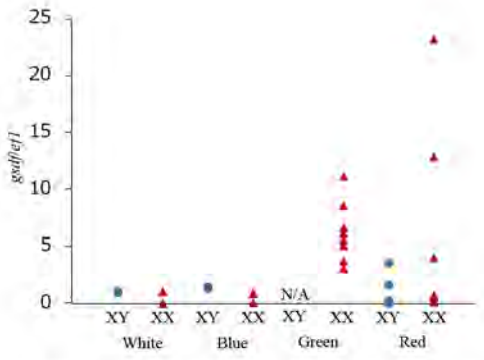

Figure 1. Expression of *gsdf* in fish at 15dph from each LED treatment. XY/XX indicates genetic sex. Each treatment used 3–10 samples.

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Results

Gonadal fluorescence clearly demonstrated sexual dysmorphia in the fish at 15dph. The appearance rate of neo-males (male-specific-gonad $dmy^{-/-}$) was higher under green (39%) and red (38%) light treatments than under white (9%) and blue (16%). Moreover, the expression of *gsdf* was high under green and red light treatments (Fig. 1). Notably, XX fish showed higher levels of *gsdf* expression than XY fish under green and red light treatments, especially in genotypic XX fish (Fig. 1).

Discussion and conclusion

The present study showed that sex-reversed fish was successfully detected at 15dph. The rearing trial demonstrated that middle- to long-wavelength (green and red) light induced high expression of *gsdf* and increased the appearance rate of sex-reversed fish. We concluded that irradiation using middle- to long-wavelength light induces masculinization of medaka in the early developmental stage, with fish at 15dph undergoing sex reversal as shown by gene expression and gonadal differentiation. Further research is necessary to clarify the significance of cortisol in the sex-reversal.

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USING FARMERS EXPERIENCES TO STUDY THE EFFECT OF POND PRACTICE ON DISEASE IN POLYCULTURE OF NILE TILAPIA IN BANGLADESH

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Introduction

Increasing intensification of aquaculture has produced higher yields but also higher incidences of disease (Garza et al, 2019), a problem that may be linked to overuse of antibiotics (Watts et al, 2017). In this paper we use questionnaire data from farmers in Bangladesh who are cultivating *Oreochromis niloticus* in ponds using a polyculture method. We examine farming practices to attempt to understand how they may influence disease presence in the ponds

Methods

To gather information about farming practices a questionnaire was performed in the north-central region of Bangladesh in 2018. Data cleaning and data analysis was performed on the results using R and R Studio (RStudio Team, 2016).

Results

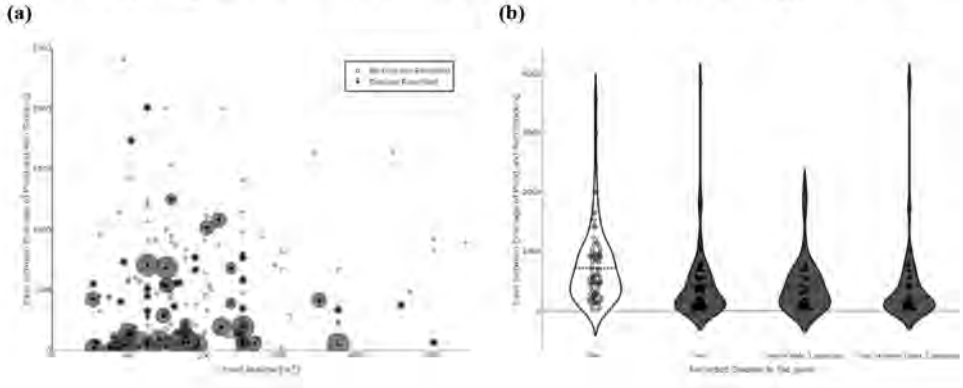
Overview of disease in ponds. From the survey the ponds performing polyculture of *O. niloticus* in Bangladesh had a mean surface area of 3000m^2 and an overall mean stocking density of 6.8 fish per m^2 (Table I). 43% reported signs of disease during production and of these 43% reported disease in tilapia, 54% in pangasius and 44% in carp. Disease was reported in more than 1 species of fish in 20% of the ponds, nearly half (48%) of those ponds that reported disease presence.

Analysis of stocking densities in ponds with recorded diseases. There was no significant difference in mean pond surface area, mean pond volume or overall stocking density (6.95 fish. $^{-2}$ in ponds with disease versus 6.54 fish. $^{-2}$) for those ponds that recorded disease and those that did not. However, the mean stocking density of tilapia was significantly lower in ponds with recorded disease (2.70 fish. $^{-2}$) than those with no recorded disease (3.38 fish. $^{-2}$; $p<0.05$, Kruskal-Wallis rank sum test (KWT)).

Table I *Pond details from survey. Values are mean values ± std.*

Surface area (m^2)	Water depth (m)	Stocking Density (Fish m^{-2})		Volume per fish (m^3)	Fingerling weight (g)		Ponds reporting disease (% of total)	
		Total	Tilapia		All	Tilapia	Any species	Tilapia
3000	1.51	6.72	3.08	0.29	53.5	16.1	43%	19%
±1877	±0.38	±4.28	±1.90	±0.15	±33.1	±19.5		

Figure 1.(a) Effect of recency of pond sludge removal on disease being recorded during production. Here the size of the bubble reflects the number of diseases recorded; (b) Violin plots of the recency of sludge removal and whether the pond had recorded disease during production.



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More recently drained ponds were more likely to report disease presence. How recent the pond had been drained and the sludge removed was determined by counting the number of days between the recorded last drainage/sludge removal date and the date that the main stocking of the pond occurred. Figure 1a shows no significant correlation between pond volume and recency of sludge removal ($r = 0.13$). However, this figure and the violin plots in Figure 1b reveal an association between recency of sludge removal and the recorded presence of disease. Ponds that recorded disease during the production cycle had significantly less days since sludge removal than those that did not record disease ($p<0.01$; KWT) and there was no significant difference between ponds recording disease in 1 species only and those recording disease in more than 1 species ($p=0.36$; KWT).

Association between the disease history of the pond and recency of sludge removal. Farmers reported how often disease was observed in their ponds. The responses were stratified into "Frequent" for yearly or more often; "Infrequent" for every 2 to 3 years; or "Rarely" for every 4 to 5 years. Ponds with a frequent occurrence of disease had sludge removed more recently than those that rarely encountered disease (455 versus 734 days) and the incidence of disease in these ponds was higher (84% versus 24%). Furthermore, those ponds that have regular water replacement (at least weekly) appear to have a higher incidence of disease than those where the water is exchanged monthly (56% versus 38%; $p<0.05$; $\square^2$ test); ponds that rarely had disease and where the water was replaced monthly had the lowest proportion of recorded disease and a mean recency of drainage of 880 days.

Discussion

Disease is a long-standing issue in aquaculture and may become a barrier to realizing aquaculture's potential (FAO, 2018). Our evidence suggests that the current overall stocking densities used in polyculture of tilapia in Bangladesh do not increase the likelihood of disease and there may be an opportunity to increase the stocking density of tilapia as this appears to be linked to lower disease prevalence at present.

Analysis of the farmers responses indicates a link between the recency of pond sludge removal and disease. This association may be due to sludge disturbance increasing the possibility of disease, or that ponds that suffer an outbreak of disease are more likely to have sludge removed before restocking, or a combination of both. There is evidence in our data for both processes. Ponds with a high disease incidence were drained more recently prior to stocking than those that rarely encountered disease, suggesting that after disease farmers drain ponds and then restock. However, ponds that rarely encounter disease, but reported disease during production, had sludge removed more recently. This suggests that the action of sludge removal may be linked to disease.

Overall our data suggests that removal of pond sludge does not act as an effective means of reducing disease. In the survey, only 9 ponds reported antibiotic use during production. With increased antibiotic use in fish farming (Watts et al, 2017) this may be due to underreporting, slow uptake or abandonment due to publicised antimicrobial resistance.

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USING FARMERS EXPERIENCES TO COMPARE TILAPIA FARMING IN BANGLADESH AND MALAWI: UNDERSTANDING GROWTH POTENTIAL

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Introduction

Increasing food production from the aquaculture sector has been promoted to deal with declining capture fisheries production (FAO, 2018). For a LEDC increased aquaculture also improves food security and provides an additional income for local farmers. In some countries, such as Bangladesh, the sector has grown considerably resulting in intensification and higher yields but also higher incidences of disease (Garza et al, 2019). In this paper we use questionnaire data to compare aquaculture of tilapia in a country intensively farming Nile tilapia (*Oreochromis niloticus* in Bangladesh) with one subsistence farming red-breasted tilapia (*Tilapia rendalli* in Malawi). From this data insights are made about improving yields without increasing disease prevalence.

Methods

To gather information about farming practices a questionnaire was performed in the north-central region of Bangladesh and in 6 districts in Malawi in 2018. Data cleaning and data analysis was performed on the results using R and R Studio (RStudio Team, 2016).

Results

Bangladesh ponds have a larger surface area and a higher stocking density. Ponds culturing tilapia in Bangladesh have significantly larger surface areas than those in Malawi (Table I; 773% larger; $p < 0.01$; Kruskal-Wallis rank sum test (KWT)), have significantly greater densities of fish per m^2 (63% greater; $p<0.01$; KWT) but have a similar water depth ($p=0.35$; KWT). These results compare favourably with those of other studies (Alam et al 2012; Limuwa et al. 2018). Stocking density differences were not the result of polyculture with smaller fish as overall fingerling weight was larger in Bangladeshi ponds ($p<0.01$; KWT) with no difference in tilapia fingerling weights in the 2 countries ($p = 0.48$; KWT)

Table I *Aquaculture practices for tilapia. Unless indicated values are mean ± std.*

Country	Number of Ponds (monoculture)	Surface area (m^2)	Water depth (m)	Stocking Density (Fish m^{-2})	Volume per fish (m^3)	Fingerling weight (g)	
						All	Tilapia
Bangladesh	189(1)	2995 ±1873	1.51 ±0.37	6.79 ±4.67	0.290 ±0.158	53.2 ±33.2	16.1 ±19.5
Malawi	68(17)	343 ±224	1.65 ±0.41	4.16 ±1.89	0.471 ±0.230	10.0 ±6.51	8.94 ±4.64

Table II. *Harvest size and WLR of tilapia in Bangladesh and Malawi. Values are mean ± std (N).*

Harvest Parameter	Bangladesh	Malawi
Mean fish weight (g)	353±147 (189)	245±162 (64)
Mean fish length (cm)	16.6±2.6 (189)	18.6±6.0 (64)
WLR (gcm^{-1})	20.8±7.3 (189)	12.5±7.5 (64)
> median length	22.7±8.4 (95)	21.2±5.8 (33)
< median length	18.8±5.3 (94)	20.5±8.1 (31)
Farmer feed used	23.4±8.4 (16)	(as all tilapia)
Commercial feed	21.4±7.6 (112)	None
Antibiotics used	23.0±10.2 (9)	None
No antibiotics used	20.7±7.1 (179)	12.5±7.5 (68)
Mortality level 0-10%	19.3±6.0 (127)	12.5±7.5 (68)
Mortality level 10-20%	20.8±7.0 (33)	None
Mortality level >20%	27.0±9.4 (29)	None

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Nile tilapia show a higher weight to length ratio. Comparison of the weight-to-length ratio (WLR) of tilapia revealed a significantly larger mean WLR in Bangladesh compared to Malawi ($p<0.01$; Student's t-test (STT)) suggesting smaller and heavier harvested fish (Table II). Tilapia above median length in Bangladesh had a higher mean WLR than the smaller fish ($p<0.01$ STT) whereas in Malawi there was no significant difference. Analysis of food input in Bangladesh showed no significant difference in WLR between commercial and own food sources (Table II) and all the ponds in Malawi were fed using food from the farmer. As the amount or type of feed was not reported these factors cannot be completely ruled out. Further, use of antibiotics during production showed no significant increase in the WLR in Bangladesh (Table II; $p = 0.52$, STT).

A higher WLR may be associated with higher pond mortality. All ponds in Malawi reported a mortality level of 0-10% during production whereas in Bangladesh the overall level was 0-10% (67%), 10-20% (17%) and over 20% (15%) indicating a higher level of disease. The WLR for tilapia was found to be higher in those ponds in Bangladesh showing over 20% mortality ($p < 0.05$; STT) indicating that a higher WLR is associated with increased disease prevalence. In Malawi 52% of the ponds observed the presence of disease or parasites during production compared to just 43% in Bangladesh. Considering the increased level of mortality in these ponds this may indicate under-reporting of disease.

Discussion

A survey on tilapia pond aquaculture has indicated a more intensive practice in Bangladesh with larger pond surface areas, higher stocking densities and the almost universal use of polyculture; resulting in ponds with larger volumes of fish in each pond. By contrast, in Malawi ponds are smaller and form part of an integrated agricultural approach where nutrient recycling from farm wastes occurs (Brummett and Jamu, 2011). Fingerlings are sourced locally from farmers (only 4% from hatchery or NGO) and fed using pond primary productivity and added crop wastes. The different growth patterns observed may reflect the different species of tilapia being cultured although previous studies have indicated a higher growth rate in *T.rendalli* (Nyirenda, 2017) not seen in our results. Using commercial fish feeds, increasing stocking densities or using faster growing fish to increase yields should be treated with caution according to our results. These did not affect the harvest weight to length ratio (WLR) of either species and a higher WLR was linked to higher mortality levels in *O.niloticus*. At present aquaculture in Malawi uses little antibiotics (none reported in our survey) and with problems of antibiotic overuse in aquaculture care must be taken when driving intensification of aquaculture

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THE INFLUENCE OF STRUCTURE ORIENTATION TO WATER CURRENTS AND WAVES AND THE EFFECT OF WATER DEPTH ON STRUCTURAL STRESS AND SHELLFISH RETENTION IN EXPOSED OCEAN ENVIRONMENTS

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Exposed ocean farming is developing around the world. In New Zealand there is over 10 000 ha of exposed ocean space permitted for shellfish and seaweed farming. The structures which allow for the efficient farming of these exposed sites are developing but generally are in their early stages. The Cawthron Institute in New Zealand has a government funded (Ministry of Business, Innovation and Employment) program designing new shellfish and seaweed structures for exposed ocean sites. This program is assessing several innovative structures by numerically modelling them (using SolidWorks and OrcaFlex), testing scaled down models of them in wave basins and deploying full scale prototypes in the ocean.

Two features that have become very obvious during the assessments of structures with multiple moorings are: (1) the importance of the structures' orientation to waves and water currents and (2) the consequence of the water depth in which they are deployed.

Using a generic structure, the influence and importance of these two factors will be shown and discussed for surfaced and submerged systems in both deeper (50 m) and shallow (20 m) sites.

THE EFFECT OF LIGHT COLOUR ON PERFORMNACE PARAMETER OF JUVENILE PIKEPERCH *Sander lucioperca*

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Introduction

Light influences the entire life cycle of many fishes from embryonic development to sexual maturity (Downing and Litvak, 2001). Several studies have been carried out to investigate fish cultivation under different light intensities (Luchiari et al., 2006, Kozlowski et al., 2010). In recent years, wavelength (light colour) has also increasingly become the focus of research (Luchiari et al., 2009). Previous studies showed that pikeperch prefer low light intensities and grow better under such conditions (Luchiari et al., 2006, Kozlowski et al., 2010). Luchiari et al. (2009) demonstrated that individually reared juvenile pikeperch showed better growth performances under red light compared to fish reared under other light colours (i.e. white, blue, green, yellow). The aim of the present study is to examine the effect of light colour on the performance parameters of pikeperch under production conditions.

Material and methods

A study series with three trials is currently conducted. In the first trial, the light colours red (650nm) and white (4400K) were compared and results are presented for this trial. A second trial during which the colours blue (450nm) and white are examined is currently in progress. In a third trial, all three light colours will be compared in a single study to verify the previous results. Trial two and three will be completed until summer. In the first trial, each four tanks per light colour were stocked at a mean biomass±SD of 31.00±0.26kg. Individual mean weight±SD of pikeperch was 401.32±28.70g. Light intensity was kept at 45 µmol/m²/s. Fish were reared for 5 weeks. After this period, 10 individually marked fish from each tank were sacrificed, measured for total length and weight and the total biomass in each tank was assessed to compare biomass gain, specific growth rate (SGR), absolute growth rate (AGR), Fulton's condition factor (K), coefficient of variation (CV), the gonadosomatic index (GSI), the viscerosomatic index (VSI) and the hepatosomatic index (HSI) among treatments.

Results

Growth was similar between fish reared under red and white light conditions as indicated by similar SGR and AGR (Table 1). Likewise, there was no difference in growth homogeneity between the two light colours as indicated by similar CV (Table 1). Furthermore morpho-anatomical indices (i.e. VSI and HIS) and the gonadal development (i.e. GSI) were not significant di ferent between fish reared under red and white light conditions (able 1).

Table 1: Specific growth rate (SGR), absolute growth rate (AGR), coefficient of variation (CV), Fulton's condition factor (K), gonadosomatic index (GSI), viscerosomatic index (VSI) and hepatosomatic index (HSI) of fishes reared 5 weeks under red and white lighting conditions.

Colour	Red	White	F	df	p
SGR [% d^{-1}]	0.45±0.11	0.51±0.14	0.124	6	0.520
AGR [g d^{-1}]	2.03±0.57	2.31±0.68	0.045	6	0.552
CV	9.40±1.55	9.44±3.60	2.112	6	0.981
Fulton's K	0.78±0.02	0.79±0.04	1.599	6	0.870
HSI [%]	0.87±0.14	0.97±0.05	4.366	6	0.520
GSI [%]	0.75±0.55	0.39±0.16	1.976	6	0.214
VSI [%]	3.07±0.54	3.46±0.75	0.072	6	0.463

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Discussion and conclusion

No difference between red and white light on pikeperch performance under production conditions was observed. Small-scale studies showed that red light improves the growth of juvenile pikeperch (Luchiari et al., 2009, Baekelandt et al., 2018). Possibly, the contradictory results are an indication of social hierarchy effects under production conditions (Luchiari et al., 2009).

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MICROALGAE CELL WALL RUPTURE WHEN INCORPORATED INTO FISH FEED: EFFECT ON SEA BASS *Dicentrarchus labrax* PERFORMANCE

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Introduction

The incorporation of microalgae as a substitute for fishmeal or fish oil in fish feed has been studied for decades, since they can improve the nutritional value of fish feed thanks to its chemical composition and the large number of bio-active compounds they contain. The results obtained to date are not clear in terms of growth and intake, and the latter has even been reduced by a supposed problem of palatability (Walker & Berlinsky, 2011). Despite the abundant research on the subject, many of these studies do not address the effect of the different microalgae processing systems prior to their incorporation into diets. In this sense, the rupture of the cell wall of microalgae has attracted the interest of researchers and companies, in view of the studies and the existing patents on it (Prabakaran and Ravindran, 2011; US9358553B2, 2016), having the majority of them the main objective of extracting their oils for industrial purposes. The effect of cell breakage on the digestibility of a great variety of nutrients has been studied in salmon (Tibbetts et al., 2017). However, its effect on performance parameters such as growth or feed conversion has not been studied, despite the predictability of greater bioavailability of nutrients. More specifical y, there is no information on this in mediterranean marine fish, so the objective of this study is to evaluate the effect of cell wall rupture on the main productive performance parameters of seabass *Dicentrarchus labrax*.

Materials and methods

525 juvenile seabass (*Dicentrarchus labrax*) were acclimated at the IMIDA Marine Aquaculture Station, with an initial mean weight of 28.0 ± 4.1g. They were homogeneously distributed in 15 tanks of 612 l that form part of a closed recirculating seawater system, in groups of 35 animals. Five isoproteic and isoenergetic diets were tested in triplicate: Diet 1 (basal diet); Diet 2 (basal diet with 10% of unaltered *Tetraselmis chuii*); Diet 3 (basal diet with 10% *T. chuii* with broken walls); Diet 4 (basal diet with 10% unaltered *Nannochloropsis gaditana*); Diet 5 (basal diet with 10% *N. gaditana* with broken walls). The water temperature was maintained at 23 ± 1 ° C, the photoperiod at 12:12 (L: D), and the dissolved oxygen above 80%. Fish were fed twice a day to apparent satiety. The intake of the animals was monitored daily and the weight of the animals was measured at the beginning and at the end of the experiment. The specific growth rate (SGR), the specific feeding rate (SFR) and the feed conversion rate (FCR) were calculated from these data. The data were compared by means of a one-way ANOVA and the significant di ferences between the means were analysed by the Tukey test.

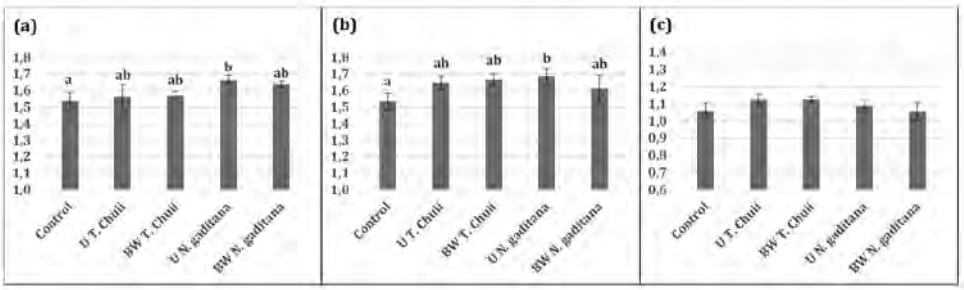

Figure 1. Specific growth rate (SGR), specific feeding rate (SFR) and feed conversion rate (FCR) of seabass (*Dicentrarchus labrax*) fed with experimental diets: Control (basal diet), U *T. chuii* (diet with unaltered *T. chuii*), BW *T. chuii* (diet with *T. chuii* with broken walls), U *N. gaditana* (diet with unaltered *N. gaditana*), BW *N. gaditana* (diet with *N. gaditana* with broken walls).

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Results

All diets were well accepted by the fish, being the specific feeding rate (SFR) between 1.53 and 1.69. The group fed with the diet containing unaltered *N. gaditana* showed a higher growth, being the weight gain (WG) (data not shown) and the specific growth rate (SGR) significantly higher than in the control group. The rest of the groups fed with diets containing microalgae also showed a tendency to improve growth although without significant differences. This improvement in growth was associated with higher relative feeding rates (SFR), so the conversion rate was similar, not observing significant differences in it (Figure 1).

Discussion and conclusion

The results contributed by this study can help to design a strategy for the incorporation of microalgae in commercial diets for mediterranean fish, since there is no information on the effect of this type of pre-treatment on these fish species. The results suggest that the treatment to break the cell wall is not able to improve performance parameters of seabass. However, greater bioavailability of nutrients has been demonstrated, including omega-3 long chain polyunsaturated fatty acids after cell breakage (Lemahieu et al., 2016). In addition, cell wall rupture maintains nutrient digestibility to higher levels of inclusion (30%) (Tibbetts et al., 2017).

In conclusion, the results obtained in this study suggest an improvement in the performance of seabass due to the incorporation of these microalgae species, but which does not depend on cell wall rupture. The effect of cell wall rupture on aspects of fish nutrition has been studied with *Chlorella vulgaris* but not with *T. chuii* or with *N. gaditana*, so these results are important for the design of diets containing these microalgae as ingredients. However, it would be interesting to study how this treatment affects the digestibility of the main nutrients in Mediterranean fish diets and other aspects such as the oxidative status or the immune system of fish

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VALORIZATION OF AGRI-FOOD INDUSTRY BY-PRODUCTS THROUGH THE PRODUCTION OF INSECT MEAL: "VALORAGRIN" PROJECT

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VALORAGRIN project started in January 2018, being the execution period of 48 months. It is a coordinated project funded by the European Union and regional authorities through RIS3 strategy, with the participation of research institutions, agri-food industry companies, and universities, forming a broad consortium. The general objective of the project is the evaluation of a new process of transformation of by-products and agri-food residues that allows its valorization through the production of insect meal or its high added value by-products. To this end, pilot experiments will be carried out with by-products of agro-food industries as substrates for the cultivation of insects, new knowledge will be acquired for the application of insect meal and its derivatives as raw material in aquaculture feed and, finall , new applications will be designed for the fertilizer generated during the insect culture. This whole project is based on the principles of sustainability, process optimization, revaluation and recycling.

Justification

The inclusion of insect meal in animal diets has already been the subject of research and a great investment worldwide for more than a decade. It is easy to verify the continuous growth of publications in this regard and the involvement of entities of great importance such as the European Commission. The latter recently drafted a regulation in this regard (Commission Regulation 2017/893 of May 24, 2017) which establishes the conditions for the use of insect meal in animal feed. In addition to this, important companies in the fish feed sector worldwide have issued on insect meal utilization in aquaculture, describing them as a "feasible alternative with numerous environmental, health and nutritional benefits" (Alex Obach, Skretting ARC). Despite promising results a priori for its exceptional nutritional profiles, its ability to supply and reuse organic waste, and the apparent influence on the immune system of fish, in-depth research is necessary to accurately determine the viability of these insect meals as a substitute for fishmeal

At the same time, other industries are focusing their attention on the cultivation of insects, both because of their potential as food and because of their ability to recycle by-products and waste. The agri-food industry is of great importance in the south of Europe due to the climate and the availability of land. However, the great development of this industry leads to an increasing production of by-products that are difficult to recycle and could be re-valorised when used as substrates for the cultivation of insects. Therefore, the cooperation of the agri-food and aquaculture industries can provide synergistic solutions promoting the circular economy and environmental sustainability.

Objectives

The main objective of the Project is the evaluation of a new process of transformation of by-products and agri-food residues that allows its valorization through the production of insect meal or its high added value by-products. Partial objectives are as follows: 1. Design the appropriate pretreatments to transform the by-products and residues of the agri-food industry into substrates intended for the cultivation of insects. 2. Acquire new knowledge for the application of insect meal and its derivatives as raw materials in aquaculture feed. 3. Design new specifications and applications for the fertilizer generated during the cultivation of insects.

Work Plan and Consortium

The work plan is divided in 3 work packages divided in several tasks and sub-tasks, WP2 and WP3 will be carried out simultaneously once the insect system culture is designed.

WP1. Insect culture design at pilot scale.

Task 1.1. Design of the insect cultivation system to be tested.

Task 1.2. Evaluation of by-products and waste destined for the cultivation of insects.

Task 1.3.Determination of the necessary pre-treatments for the recovery of by-products and residues through the cultivation of insects.

WP2. Design of an insect meal from by-products of vegetable origin intended for aquaculture.

Task 2.1. Design of the composition of a substrate formed by different vegetable by-products that improves the performance of insect production.

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Task 2.2. Determination of the effect of the inclusion of insect meal produced from vegetable substrates in the diet of fish
Task 2.3. Life cycle analysis of the production of insect meal for aquaculture.
WP3. Valorization of substrates of non-vegetable origin through the production of insects.
Task 3.1. Analysis of the viability of the production of insect meal from substrates of non-vegetable origin destined for the foreign market.
Task 3.2. Demonstration of fertilizer production from substrates of non-vegetable origin.
Partners:
Alimentos del Mediterráneo, S.Coop. (ALIMER): plant by-products and fertilizer tests.
Grupo Alimentario De Lorca, S.L. (LACOMARCA): Animal by-products. Insects fed with these by-products will not be used in feeding of fish (EU Regulation)
Estrella de Levante Fábrica de Cerveza, S.A. (ESTRELLA): by-products of beer production.
Juan Jiménez García, S.A.U. (JISAP): slurry and manure from pork production.
Catholic University of Murcia (UCAM): experimental tasks with insects and substrates.
Entomo Agroindustrial (Subcontracting by UCAM): pilot plant and technology transfer.
IMIDA (Agrarian and Food Research and Development Institute of Murcia): The Aquaculture Research Group is dedicated to applied research in aquaculture and will carry out fish nutrition experiments

Acknowledgment
VALORAGRIN project is funded by the regional government of Murcia through the Regional Research and Innovation Strategies for Intelligent Specialization (RIS3), promoted by the European Commission.

EFFECT OF INCREASING DOSES OF *Nannochloropsis gaditana* ON THE IMMUNE SYSTEM OF GILTHEAD SEABREAM *Sparus aurata*

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Introduction

The incorporation of microalgae in fish feeds has been studied in recent years with several objectives: mainly as an alternative source of protein and omega-3 long chain polyunsaturated fatty acids but also as a probiotic or as a source of immunostimulant compounds. Despite the great quantity and quality of nutrients and immunostimulant compounds they contain, the results in nutrition have not been definitive and the immunostimulating effects depend on the species used. Some species such as *Tetraselmis chuii*, *Phaeodactylum tricornutum*, *Navicula* sp. or *Chlorella* sp. have been shown to improve the immunological status of fish (Cerezuela et al., 2012; Reyes-Becerril et al., 2013; Zhang et al., 2014).

The microalga *Nannochloropsis gaditana* has shown positive effects on the immune system of gilthead seabream in doses of up to 10%. Despite of this, it has not been studied as an immunostimulant at higher doses that may be viable in more sustainable feeds with high levels of fishmeal and fish oil substitution. Based on the above, the objective of this work was to study the effect of the incorporation of increasing levels of *N. gaditana* in gilthead seabream diet, on its humoral and cellular immunity.

Materials and methods

450 gilthead seabream (*Sparus aurata*) were randomly distributed on fifteen tanks and fed with five diets (three replicates by diet): standard diet (control), or standard diet supplemented with microalgae *N. gaditana* at 2.5, 5, 10 and 15%. At the end of the experiment fish were killed and blood and head kidney samples were taken for immunological analysis. The blood samples were left to clot and later the serum was collected after centrifugation. Leucocytes were isolated from head kidney samples and used to determine the respiratory burst, peroxidase and phagocytic activity of leucocytes. Serum samples were used to evaluate the peroxidase activity and IgM levels. Samples of liver and intestine were processed for light microscopy analysis. The data were compared by means of a one-way ANOVA and the significant differences between the means were analysed by the Tukey test.

Results

In terms of cellular immunity, a general increase in the percentage of phagocytic cells was observed in fish fed with microalgae diets, being significant in the group fed with 15% microalgae (Figure 1-A). However, no statistically significant differences were observed in phagocytic capacity (Figure 1-B). Leukocyte respiratory burst activity also showed no significant differences (Figure 1-C). Peroxidase activity was significantly higher in leukocytes from fish fed the diet with 10% microalgae (Figure 1-D).

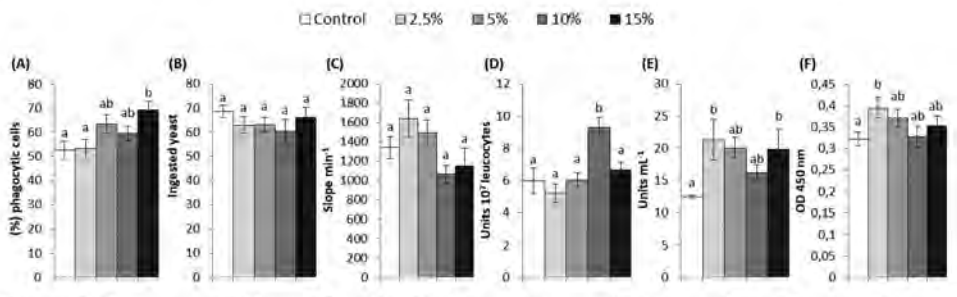

Figure 1. Percentage of phagocytic cells (A), phagocytic capacity (B), respiratory burst activity (C), leucocyte peroxidase activity (D), serum peroxidase activity (E), serum IgM level –450 nm absorvance–(F) in *Sparus aurata* fed with diets containing increasing doses of *N. gaditana* (colour legend).

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Regarding humoral immunity serum peroxidase activity was significantly higher in fish fed diets containing 5 and 10% microalgae (Figure 1-E). The immunoglobulin M (IgM) levels in serum increased significantly in the serum of the fish fed with 2.5% microalga, showing a tendency to increase with the rest of the microalgae diets (Figure 1-F). All histology slides from gilthead seabream liver and intestine showed a normal histological pattern from a healthy situation with any of the treatments.

Discussion and conclusions

These results suggest that the incorporation of *N. gaditana* in the diet of sea bream has a certain immunostimulating effect. Other authors have also observed an immunostimulant effect at a dose of 5 and 10% (Cerezuela et al., 2012), which in our case is only the optimal one regarding peroxidase activity in leukocytes -not previously studied-. In general, the main immunostimulant effect at the cellular level can be observed in the percentage of phagocytic cells at high doses (15%) and at the humoral level in the peroxidase activity and in the level of serum IgM. Based on these results, it would be interesting to perform viral, bacterial or parasitic challenges to get a more definitive approach

In conclusion, the incorporation of the microalga *N. gaditana* promotes certain immune activity both at the cellular level and at the humoral level, with the optimal dose being different depending on the parameter studied.

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DIFFERENTIAL IN VITRO HYDROLYSIS OF ALGAE PROTEIN BY THE DIGESTIVE ENZYMES OF JUVENILE TURBOT, *Psetta maxima*

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Introduction

Algae constitute a heterogeneous protein source, taking into consideration the vast group of species available, and hence, the actual utilization of the protein contained in a given algae biomass can't be predicted easily. Indeed, great differences in algae cell wall composition, thickness, and structure have been reported, and thus, reasonable doubts might arise related to their digestibility and amino acid bioavailability owing to such disparity. In the last years numerous studies have assessed their potential as dietary protein ingredient in practical diets for different fish species (Vizcaíno et al. 2019). However, to our knowledge, little research has been reported about the way digestive enzymes of fish hydrolyzes algal protein (Tibbetts et al. 2017), and particularly to our knowledge, scarce research has been reported about the rate and extent of the hydrolysis of algae by digestive enzymes of turbot. The aim of this work was to assess the *in vitro* hydrolysis of *Arthrospira platensis* (cyanobacteria), *Chlorella vulgaris* (microalgae), and *Ulva compressa* and *Ulva rigida* (macroalgae) by the digestive proteases of juvenile turbot (*Psetta maxima*).

Materials and methods

The *in vitro* hydrolysis of the algae biomass was carried out in 10mL-jacketed reaction vessels connected to a circulating water bath maintained at a constant temperature of 25°C, under continuous agitation by a magnetic stirrer. For each assay, a known amount of each alga species, providing 80mg crude protein, was suspended in 50mM Tris HCl buffer pH 9.0. After 15min stirring, the protein hydrolysis was initiated by the addition of a previously calculated volume of the turbot digestive enzymatic extract comprising 600U of total alkaline proteolytic activity. The susceptibility of algae protein to be hydrolysed by fish digestive enzymes has been assessed by sequential characterization of the hydrolysis products using sodium dodecyl sulphate polyacrylamide gel electrophoresis (Vízcaíno et al. 2019). Total released amino acids at each sampling time were quantified spectrophotometrically at 340 nm using L-leucine as standard. In addition, total reducing sugars released from microalgae were evaluated using the dinitrosalicylic acid (DNS) method.

Results and discussion

Results obtained evidenced that turbot digestive proteases hydrolyse algae protein (figure 1). Overall, turbot proteases are able to release from 28 to 50% of the total amino acids contained in algae protein after 120min of *in vitro* hydrolysis (figure 2). *Arthrospira* and *Chlorella* showed the highest accumulative values of free amino acids released, 50 and 44%, respectively. The lowest values were obtained in macroalgae, 27 and 31% for *U. compressa* and *U. rigida*, respectively. In this case, the lower protein content and the complex cell wall structure in macroalgae might reduce the accessibility to cleave peptide bonds by turbot digestive proteases. However, these values were similar to those obtained for fishmeal, plant proteins, and even microalgae using similar *in vitro* assays with fish digestive enzymes (Sáenz de Rodrigáñez et al. 2011; Vizcaíno et al. 2019). The amount of glucose equivalents released during the digestive simulation never reached more than 4 g of glucose per 100 g of algae biomass. It was evidenced a negligible contribution of fish digestive carbohydrases but values obtained for macroalgae were lower compared to *Arthrospira* and *Chlorella.*

Conclusions

The results obtained confirmed that algae are potential ingredients for feeding turbot, although the suitability of these algae as feed ingredient in this fish species should be tested by means of *in vivo* feeding trials.

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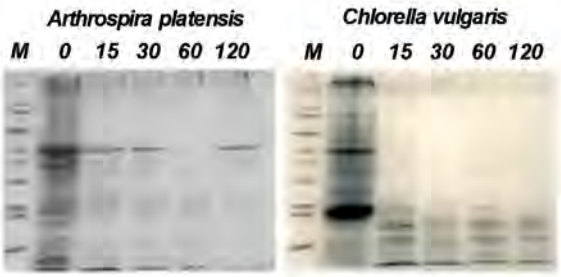

Fig. 1. Time-course *in vitro* proteolysis of *A. platensis* and *C. vulgaris* by *P. maxima* intestinal proteases. Photographs show SDS-PAGE separation of protein fractions withdrawn at different sampling times (0, 15, 30, 60 and 120min). M: molecular weight marker.

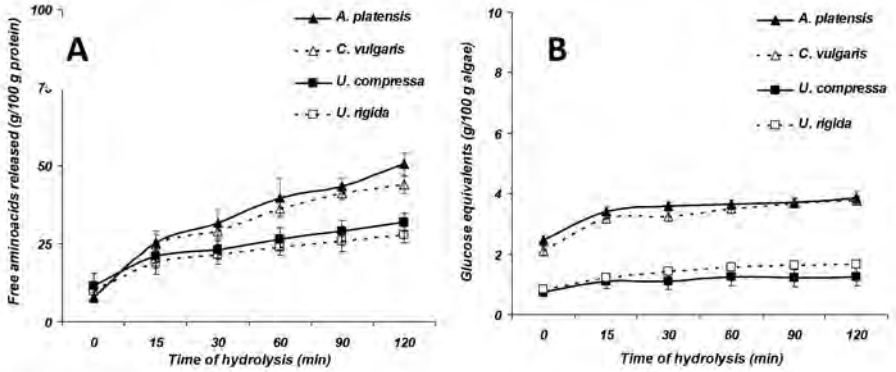

Fig. 2. Concentration of free amino acids released (A) and glucose equivalents released (B) during the *in vitro* hydrolysis of algae by *Psetta maxima* intestinal enzymes.

Acknowledgements
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INSECTS IN AQUACULTURE FEEDS: CURRENT STATUS AND SWOT ANALYSIS

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Introduction:
Since the beginning of the so-called ¨blue growth¨, the increase in the use of fish oil and meal has become a concern. Oceans have shown not to be an inexhaustible source of fishery resources, as a result, its bio-availability is lowest day by day and its costs also increases, so, for aquaculture industry represents a challenge in terms of its sustainability and their public image. In this sense, efforts in the search for alternative sources have increased, especially the use of insects. From industrialization to its use in feed for aquaculture have shown its benefits, but also their difficulties able 1).

Overview:
Interest on the use of insects for aquaculture feeding was described more than 50 years ago, it was concluded that silkworm might be an important component in diets of carp in Japan and China. Later, in the 1980s, were carried out studies with silkworm in complete diets and substitution of fishmeal for Hybrid Carp (*catla-rohu*) and the use of *Hermetia illucens* in the diet of *Ictalurus punctuatus* and *Oreochromis sp*. (Henry et al., 2015).

Even though these trials based on unprocessed larvae, these proved to be suitable for feeding fish, other nutrition features (e.g. amino acid profile, fatty acid profile), or technologies (e.g. extruded, among others feed use) were not referred to, or, in the case of aquatic species under investigation, digestibility test, utilization of nutrients or their nutritional composition or sensory analysis.

The use of insects as feed in aquaculture has taken more interest from 2010, studied in species of commercial inclination as *Carassius auratus, Clarias anguillaris, Clarias gariepinus, Dicentrarchus labrax, Ictalurus punctatus, Orcorhychus mykiss, Oreochromis niloticus, Pagellus bogaraveo, Psetta maximum, Salmo salar and Sparus aurata* in nutrition, molecular biology, immunology, histology and economics trials (Makkar et al., 2014; Henry et al., 2015; Gasco et al., 2018; Halloran et al., 2018).

Table 1. SWOT analysis of the use of insects for feeding in aquaculture.

Weaknesses. -Its high lipid levels make it difficult for feed formulation, pellet stability and storage when using the full insect or non-defatted meal. -Anti-nutritional factors: chitin. -High inclusion levels affect the sensory quality and decrease PUFAs omega 3 and 6 in fish fillet (Gasco et al., 2018).	**Threats.** -Recent interest may be subject to legislative and policy changes and can affect its sustainability and generate an uncertain scenary. -Currently, insect flour prices ($ 5-10/kg) are higher than the fish (2.37 €/ kg) (www.allaboutfeed.net.).
Strengths. -It requires little land and water for cultivation. -Possibility of using agricultural, urban, or industrial by-products as substrates for the breeding of insects. -Easy management of organic waste, transforming 3 t into 150 kg crop / dry matter. -Its protein and lipid level is high, varies according to insect species, biological stage and substrate. -Insects are part of natural diet in carnivorous fish, (Diener et al., 2009; Gasco et al., 2018)	**Opportunities.** -Regulation EU 2017/893, allows the use of insects and animal feed that contains them for aquaculture. -Sets to black soldier fly (*Hermetia illucens*), common fly (*Musca domestica*), mealworm (*Tenebrio molitor*), bed beetle (*Alphitobius diaperinus*), domestic cricket (*Acheta domesticus)*, striped cricket (*Gryllodes sigillatus*) and bicolor cricket (*Gryllus assimilis*) as suitable for feed in aquaculture species.

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What strategies can be applied to reduce these difficulties?

The combination of insect meal with by-products of other animal products such as meat, bone, or blood meal, besides to the combination in diets or use as substrate for cuts, rendered and fish offal have shown comparable results to the diets with fish meal or in some cases higher in terms of growth performance and nutrients utilization in the fish On the other hand, technology for the transformation of insects like deffated meal, protein extract, oils, chitin and Chitosan has shown to fix feed quality and nutritional aspects.

Also, adequate finishing-diets based on fish meal and fish oil for short periods of time in insect meal-fed fish, has demonstrated to promote a rapid return on their levels of essential fatty acids. These strategies must be validated by identifying if there are differences or not in terms of sensory quality.

It was found that chitin reduces digestibility and nutrients utilization from fish fed with insects. However, also has been observed that carnivorous fish have the ability to degrade by digestive enzymes (chitinases). On the other hand, their inclusion has demonstrated immuno-stimulants effects, so it is of interest for the development of functional feed.

Widely variability in nutritional composition was found in insects and new species are investigated to known if can satisfy nutritional requirements of fish around the world. Is the case of silkworm, which has one of the highest levels of protein, amino acids and fatty acids, depending on the substrate that is given to them (e.g. fish offal), compared to other species of insects. Studies on fish have shown good results of digestibility, growth and nutrients utilization in diets to replacement levels from 38% to 100% fishmea (Makkar et al., 2014).

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MICROALGAE CELL WALL RUPTURE WHEN INCORPORATED INTO FISH FEED: EFFECT ON SEA BASS *Dicentrarchus labrax* PERFORMANCE

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Sea bass (*Dicentrarchus labrax*) were fed for 8 weeks in a seawater recirculation circuit. Five experimental diets in triplicate were used: Diet 1 (basal diet); Diet 2 (basal diet with 10% of *Tetraselmis chuii* complete); Diet 3 (basal diet with 10% *T. chuii* with broken walls); Diet 4 (basal diet with 10% complete *Nannochloropsis gaditana*); Diet 5 (basal diet with 10% *N. gaditana* with broken walls). The intake of the animals was monitored daily and the weight of the animals was measured at the beginning and at the end of the experiment. The rates of growth, intake and feed conversion were calculated. The results obtained showed a greater growth of the animals fed with microalgae compared to the control. Among the groups fed with supplemented diets the growth was higher with *N. gaditana* (with walls and without walls). Higher feeding rates were also observed with diets containing microalgae. Despite this, no differences were observed between the same type of microalgae treated or not to break its wall. In conclusion, although the microalgae studied have a positive effect on growth and intake, the rupture of the cell wall of *T. chuii* and *N. gaditana* before its incorporation into the 10% sea bass feed has no effect on its performance.

MINIMIZING THE IMPACT OF MARINE LITTER AND GEAR IN THE AQUACULTURE INDUSTRY GLOBALLY

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Introduction:

Every year 300'000.000 MT of plastic are produced, Our oceans are nearing tipping point and plastic waste is one of the greatest threats to them. Large numbers of plastic bottles and bags float around the earth but there is another, lesser-known, man-made killer plastic lurking in our oceans. Fishing gear is designed to catch and kill marine life, and 'ghost gear' – abandoned, lost or discarded fishing gear - is the most harmful form of marine debris for animals

Each year at least 640,000 tonnes of this 'ghost gear' is lost or left in our oceans. The enormous impacts of ghost gear spell out the need for urgent attention: if this deadly threat to our oceans, marine animals and ecosystems is not addressed, there is a great risk that that ghost gear will interact and combine with other current oceanic threats to create what the UN termed "a destructive cycle of degradation." Ultimately this could mean our oceans simply stop providing for humans in the many ways we now rely on them. Worryingly, the level of ghost gear has increased in recent years and it is likely to grow further as fishing efforts intensify all over the world. Effective solutions are being found locally and nationally, yet I believe only a global approach can enable us to monitor and fight this threat. (aken from Global Ghost Gear Initiative - GGGI)

In 2018, Aquaculture Stewardship Council (ASC) approached to GGGI and assesses how the Aquaculture sector can minimize the impact of using plastic tools in the farms operations. ASC joined GGGI officially in 2018 to support the initiative and developed a criterion to be request to the aquaculture industry globally based on FAO code of conduct for fisheries and in the 3 principles of GGGI

The technologies of the aquaculture are evolving at rapid pace and therefore, the application of the research and development in other fields is welcome to the sector. One of such development is the plastic application in aquaculture. The use of plastics in agriculture / aquaculture in countries have increased rapidly, and have acquired substantial significance in the conversion of material and energy, contributing greatly to the increased production of aquaculture.

The plastics are perhaps the most versatile of the materials known. They being synthetic can be tailor made to meet the very specific performance requirements of the enduse. By virtue of their versatility, plastics are fast replacing the conventional materials such as wood, glass, metals, papers, etc. in vary varied segments like agriculture, irrigation, water management, aquaculture, packaging, etc.

The plastics most commonly used in aquaculture are Low Density Polyethylene
(LDPE), High Density Polyethylene (HDPE), Polyvinyl Chloride (PVC), Polypropylene (PP), Polystyrene (PS), Polyamide (nylon), Polycarbonet (PC), Acrylic (PMMA), Fibre Reinforced Plastics (FRP), etc.

The research in application of the plastics for aquaculture operation certified by ASC has produced list per sector that have been evaluated as farmers fields for their applicability. At present, many of these gadgets are being used in the Aquaculture industry. The overview of these applications will be presented. This policy is affecting and minimizing the discarding of marine litter globally in farms that are producing: Tilapia, Pangasius, Shirmp, Troutfish, Salmon, Seriola, Seabass/ Seabream, Oysters, Abalone and Bivalves.

EFFECTS OF COMBINED ELEVATED TEMPERATURE AND SALINITY ON PROTEIN, ENERGY REQUIREMENTS FOR MAINTENANCE AND FEED UTILIZATION OF RED TILAPIA (*Oreochromis* sp.)

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Introduction

Climate change is predicted to affect the biodiversity in Vietnam, particularly in the Mekong Delta. The predictions include an increase in the environmental temperature and rising of sea level, which lead to increased estuary salinity and salinity intrusion into the fresh water bodies. Many studies have been conducted to evaluate the effect of salinity and temperature on the growth, digestibility and feed utilization of different fish species. Other studies on interaction between temperature and salinity in tilapia or red tilapia were conducted in order to investigate the changes in growth performance (Rodriguez *et al*., 2015), feed utilization (Hassan *et al*., 2014), growth (Barreto-Curiel and Durazo, 2015), adaptation and nutrition requirements of tilapia in tropical areas where climate change is being observed apparently. However, the interaction between elevated temperature and salinity has not been investigated on the tilapia, especially in red tilapia (*Oreochromis* sp.). Therefore, this study aimed to determine the interaction between elevated temperature and salinity on protein and energy requirements for maintenance and growth of red tilapia, *Oreochromis* sp.

Materials and methods

Two-factorial experiment was randomly allocated, consisting of 12 treatments. The first factor was the combined temperature and salinity including normal temperature (28°C) and 0ppt; and high temperature (34°C) and 12ppt. The second factor was the three levels of protein in the formulated diets (25, 30 and 35%) combined with the two levels of lipid in the diets (6 and 9%). Each treatment was randomly triplicated. Fish (7.1±0.03g; 30 fish) were stocked in 200 L tanks. Fish was acclimated with salinity and temperature for two weeks before starting the experiment. Fish was fed to satiation two times a day (8AM and 4PM). Temperature and salinity were measured and adjusted daily for each treatment. At the end of experiment, fish was weighed and counted to calculate the growth performance and survival rate (SR). Feed conversion ratio (FCR) and specific growth rate (SGR %) were calculated. Statistical difference in mean of investigated parameters were analyzed by the General linear model followed by the Duncan test at a significance leve of 0.05, using SPSS program 16.0.

Results

Growth performance, there was interaction between feed composition and salinity-temperature on specific growth rate ($p<0.05$), while there was no interaction between feed composition and salinity-temperature on final weight and daily weight gain ($p>0.05$). At the same protein proportion, growth rate of fish were better at dietary 9% lipid level than that in dietary 6% lipid level, especially in 30% protein with 9% lipid the growth performance of red tilapia was the best. Besides, in 12ppt -34^{o}C treatments, the growth performance of red tilapia was better than in freshwater treatments for all diets. The growth performance (Wf, and SGR) was highest in fish fed with feed (9% lipid and 30% protein) in 12ppt-34^{o}C.

Feed efficac , feed composition affects directly to feed utilization efficiency ($p<0.05$). Interestingly, only in feed intake did both feed composition and salinity-temperature influence the amount of feed consumed per day. With the same type of feed, the experimental fish which were reared in higher temperature and salinity consumed higher amount. Besides, with the same level of protein in feed, the fish consumed more feed which have lower lipid level (6%) than the feed that contains higher lipid level (9%).

Fish composition, there was interaction between salinity-temperature and protein composition of diets on fish composition. In 0ppt-28^{o}C, the total moisture in fish body composition is higher than in 12ppt-34^{o}C treatments. The highest total moisture was 77.88±0.96% which was showed in (salinity 0ppt-28^{o}C with diet 6% lipid and 30% protein), and there were interaction between temperature-salinity and protein proportion; protein and lipid in diets ($p<0.05$). In all remain parameters (crude protein, crude lipid and total ash), the same trend was seen, in which the figure was in the same parameter in the same diets in 12ppt-34^{o}C treatments were all higher than salinity 0ppt-28^{o}C treatments.

Discussion and conclusion

In 0ppt salinity-normal temperature condition, the best growth performance of red tilapia was shown by using the diet with lipid 6% and protein 25%.

In 12ppt salinity-34^{o}C condition, the best growth performance of red tilapia was shown by using the diet with lipid 9% and protein 30%.

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Table 1. The growth parameters of red tilapia fed experimental diets for 25 days. W_i: initial mean weight, W_f: final mean weight, SGR: specific growth rate, PER: protein efficiency ratio, FCR: feed conversion ratio, SR: survival rates

S-T	Diets	Wi (g)	Wf (g)	SR (%)	SGR (%/day)	FCR
0ppt-28°C	L6P25	7.10±0.00	13.5±0.83^{Ab}	95.7±5.13^{a}	2.60±0.26^{Ac}	1.83±0.17^{b}
	L6P30	7.03±0.06	13.3±0.25^{Ab}	91.3±7.50^{a}	2.53±0.06^{Abc}	1.61±0.17^{ab}
	L6P35	7.07±0.06	12.3±0.48^{Ab}	95.3±4.04^{a}	2.23±0.21^{Abc}	1.70±0.13^{ab}
	L9P25	7.00±0.00	10.2±0.24^{Aa}	98.0±1.73^{a}	1.50±0.10^{Aa}	2.36±0.05^{c}
	L9P30	7.00±0.00	12.1±0.42^{Ab}	94.7±6.80^{a}	2.17±0.15^{Abc}	1.72±0.02^{ab}
	L9P35	7.03±0.06	12.4±0.97^{Aab}	98.0±1.73^{a}	2.23±0.29^{Aab}	1.59±0.26^{a}
12ppt-34°C	L6P25	7.10±0.00	13.9±0.50^{Bb}	99.0±1.73^{b}	2.70±0.10^{Bc}	1.89±0.13^{b}
	L6P30	7.07±0.06	13.2±0.72^{Bb}	99.0±1.73^{b}	2.50±0.20^{Bbc}	1.74±0.17^{ab}
	L6P35	7.10±0.00	13.8±2.02^{Bb}	98.0±1.73^{b}	2.63±0.55^{Bbc}	1.65±0.25^{ab}
	L9P25	7.10±0.00	13.1±1.14^{Ba}	100.0±0.00^{b}	2.43±0.32^{Ba}	2.14±0.32^{c}
	L9P30	7.03±0.06	14.2±1.81^{Bb}	98.0±1.73^{b}	2.77±0.50^{Bbc}	1.68±0.21^{ab}
	L9P35	7.10±0.00	12.5±0.50^{Bab}	97.7±4.04^{b}	2.23±0.15^{Bab}	1.58±0.17^{a}
P (S-T)			*0.003*	*0.024*	*0.002*	*0.339*
P (Feed)			*0.022*	*0.630*	*0.006*	*0.001*
P (S-TxD)			*0.084*	*0.640*	*0.043*	*0.252*

Value are means of treatment and presented as mean±SD
In the same column, different capitalized letters presented significant difference affected by salinity-temperature factor; whereas different small letters presented significant difference affected by diets (p<0.05).

Table 2. Fish composition variation in different diet treatments

S-T	Diets	Total moisture	Crude Protein	Crude lipid	Total ash
0ppt-28°C	L6P25	76.6±0.88^{Ba}	12.9±0.36^{Abc}	4.82±0.21^{Aab}	3.91±0.12^{Aa}
	L6P30	77.9±0.96^{Bc}	13.3±0.69^{Abc}	3.79±0.32^{Aa}	4.28±0.23^{Aab}
	L6P35	77.5±0.23^{Bbc}	12.9±1.06^{Aab}	3.64±0.98^{Aa}	4.40±0.29^{Ab}
	L9P25	77.6±0.64^{Babc}	12.2±0.34^{Aa}	4.85±0.27^{Ac}	4.08±0.21^{Aa}
	L9P30	76.2±1.06^{Bab}	13.0±0.62^{Aab}	4.43±0.52^{Ab}	4.60±0.07^{Ab}
	L9P35	77.7±1.42^{Bbc}	12.9±0.72^{Ac}	3.66±0.98^{Aa}	4.56±0.10^{Ab}
12ppt-34°C	L6P25	73.6±0.54^{Aa}	14.4±0.39^{Bbc}	6.01±0.89^{Bab}	4.57±0.24^{Ba}
	L6P30	76.1±0.42^{Ae}	13.9±0.60^{Bbc}	4.47±0.44^{Ba}	4.65±0.24^{Bab}
	L6P35	75.4±0.94^{Abc}	13.4±0.15^{Bab}	4.61±0.33^{Ba}	4.84±0.13^{Bb}
	L9P25	75.1±1.33^{Aabc}	13.1±0.56^{Ba}	6.55±0.41^{Bc}	4.41±0.16^{Ba}
	L9P30	75.2±1.43^{Aab}	13.2±0.91^{Bab}	5.37±0.65^{Bb}	4.68±0.25^{Bb}
	L9P35	75.6±0.51^{Abc}	15.2±0.27^{Bc}	4.61±0.33^{Ba}	4.70±0.04^{Bb}
P (S-T)		*0.000*	*0.000*	*0.000*	*0.000*
P (Diets)		*0.027*	*0.007*	*0.000*	*0.001*
P (S-T x D)		*0.539*	*0.062*	*0.657*	*0.154*

Value are means of treatment and presented as mean±SD
In the same column, different capitalized letters presented significant difference affected by salinity-temperature factor; whereas different small letters presented significant difference affected by diets (p<0.05).

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SNAKEHEAD FISH FEED DIET INNOVATIONS USING ALTERNATE SOURCES OF FISH MEAL

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Introduction

Aquaculture of freshwater carnivorous fish in Vietnam, Cambodia, and the broader Mekong Delta region has been highly dependent on inland fisheries of low value fish for sourcing key dietary nutrient inputs. Hien et al. (2017) at Can Tho University (CTU) found that successful weaning of snakehead larvae using a formulated feed enables farmers to domesticate snakehead culture in Vietnam and Cambodia and eliminate reliance on fish captured from the wild as both seed and feed. The CTU feed formulation was shared with feed mills in the Mekong Delta by CTU researchers, along with further outreach and demonstration projects for farmers in An Giang and Dong Thap provinces. This led to widespread adoption of the formulated pelleted diets by snakehead farmers in Vietnam (Hien et al., 2018) and some early successes. However, the development of the snakehead (*Channa striata*) aquaculture industry in Vietnam and Cambodia depends on additional innovations in supplemental diets. Pangasius fish meal (PFM) made from the by-products of striped catfish is a new fish meal resource for making fish feeds. This study evaluated the possibility of using PFM as alternative feed ingredient for snakehead.

Materials and methods

The study included two experiments: (1) evaluation the digestibility of PFM and marine fish meal (MFM) as feed ingredients for snakehead; and (2) replacement of marine fish meal protein by PFM. In the first experiment, the test ingredients were a reference diet, PFM and MFM. Two diets were formulated using 70% reference diet and 30% of each of the test ingredients. The snakehead reference diet is made by (Hien et al., 2016). Chromic oxide (Cr_2O_3) was added to all diets at a level of 1%. Feces were collected daily, dried at 60°C, and analyzed for crude protein, crude lipid, moisture, and ash. Apparent digestibility coefficient (ADC ingredients) of protein and lipid were analyzed and calculated. In the second experiment, the protein of MFM was replaced by the protein of PFM at levels of 0%, 25%, 50%, 75% and 100%. Each treatment was randomly triplicated. Fish (6g; 30 fish) were stocked in 250 L tanks. Fish were fed to satiation two times per day (8AM and 4PM). At the end of the experiment, fish were weighed and counted to calculate growth performance, feed conversion ratio (FCR), and specific growth rate (SGR%). Statistical difference in the means of investigated parameters were analyzed by the General linear model followed by the Duncan test at a significa ce level of 0.05, using SPSS program 16.0.

Results

Experiment 1:

Apparent digestibility coefficient (ADC) of protein and lipid of PFM were significantly higher than those of MFM. Table 1 shows that ADC of protein in MFM (86.2%) was lower than that of PFM (93.4%). Similarly, lipid digestibility of PFM was higher than that of MFM.

Experiment 2:

Results showed that replacement of MFM by PFM did not affect snakehead survival rate. The highest specific growth rate (SGR) was found in fish fed 100% PFM (3.67%/day). Feed intake (FI) was not significantly different among treatments. There was no significant difference of feed conversion ratio (FCR) and protein efficiency ratio (PER) between treatments using 75% and 100% PFM ($p>0.05$).

Discussion and conclusion

Apparent digestibility coefficie ts of protein and lipid of PFM were significantly higher than those of MFM. Replacement of 100% MFM by PFM showed the highest fish growth performance and feed efficien .

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Table 1. Apparent digestibility of MFM and PFM (%)

Feed ingredients	Dry matter (%)	Protein (%)	Lipid (%)
MFM	68.4±0.21[a]	86.2±0.80[a]	77.1±0.90[a]
PFM	69.3±0.70[b]	93.4±0.60[b]	90.6±0.62[b]

Values are means of three replicate group's ± SD. Within a column, value with the same letters are not significantly different (P>0.05).

Table 2. Growth parameters, survival rate and nutrient utilization in snakehead fed experimental diets for 60 days. Wi : initial mean weight, Wf : final mean weight, Wg: mean weight gain, DWG: daily weight gain, SGR: specific growth rate

Treatment	Wi (g)	Wf (g)	Wg (g)	DWG (g/day)	SGR (%/day)
0% PFM	6.02±0.10[a]	35.0±4.27[a]	29.0±4.22[a]	0.52±0.08[a]	3.13±0.20[a]
25% PFM	6.04±0.11[a]	38.1±5.77[a]	32.0±5.66[a]	0.57±0.10[a]	3.27±0.23[a]
50% PFM	5.96±0.06[a]	45.6±3.44[b]	39.7±3.49[b]	0.71±0.06[b]	3.63±0.15[b]
75% PFM	6.06±0.13[a]	46.7±4.53[b]	40.7±4.53[b]	0.73±0.08[b]	3.64±0.18[b]
100% PFM	5.91±0.11[a]	46.2±2.72[b]	40.3±2.66[b]	0.72±0.05[b]	3.67±0.09[b]

Values are means of three replicate group's ± SD. Within a column, values with the same letters are not significantly different (P>0.05).

Table 3. Feed efficiency, FI: feed intake, FCR: feed conversion ratio, PER: protein efficiency ratio, NPU: Net protein utilization

Treatment	FI (%/fish/day)	FCR	PER	NPU (%)
0% PFM	4.59±0.30[a]	1.13±0.03[c]	1.91±0.06[a]	29.3±0.25[a]
25% PFM	4.60±0.41[a]	1.05±0.01[b]	2.08±0.03[b]	32.7±2.00[b]
50% PFM	4.72±0.28[a]	1.00±0.01[a]	2.18±0.03[c]	33.7±1.49[b]
75% PFM	4.90±0.28[a]	1.01±0.03[ab]	2.15±0.08[bc]	34.5±2.92[b]
100% PFM	4.91±0.21[a]	1.01±0.03[ab]	2.14±0.07[bc]	32.2±2.33[ab]

Values are means of three replicate group's ± SD. Within a column, values with the same letters are not significantly different (P>0.05).

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QUANTIFYING GENETIC AND ECOLOGICAL EFFECTS OF ESCAPED FARMED SALMON ON WILD SALMON

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Introduction

The impact of escaped farmed Atlantic salmon (*Salmo salar*) on conspecific wild populations has been a matter of discussion since the 1980s. In order to attain a facts-based discussion on this topic, the Research Council of Norway in 2011 announced a call for proposals to establish a knowledge platform that included research institutions from environmental, fisheries and aquaculture science to study farmed-wild genetic and ecological interactions. Four Norwegian institutions won the competition for a knowledge platform, QuantEscape, which was established in 2012. By end of 2019, the knowledge platform has run for two 4-yr periods. This talk gives an overview of what has been accomplished and of the type of knowledge that remains to be established.

Methods

QuantEscape uses a mix of different methodologies and approaches to study how escaped farmed salmon and their offspring affect wild populations. First, a method for distinguishing between wild and farmed salmon has been developed by finding a set of Single Nucleotide Polymorphisms and a novel, statistical analytical approach to separate the two groups. This method has been used to quantify the level of genetic introgression in a large number of wild populations reaching 225 populations and more than 90% of the wild Norwegian salmon resource by 2019. Moreover, the method has been used to study how introgression at the individual level affects the life history and body size of wild Atlantic salmon.

Experiments have been used to establish how farmed and farmed-by-wild offspring compete with wild salmon offspring in hatchery, semi-natural and natural environments. Also, the experimental protocol allows testing of naturalised farmed salmon in competition with wild salmon, and how a predator (brown trout, *Salmo trutta*) affects the outcome of the competition.

Modelling has been used to accompany the empirical and experimental studies, both by group-based demographic models, by quantitative genetic models and by individual-based eco-genetic models.

Results

QuantEscape has established that genetic introgression occurs in the majority of wild Atlantic salmon populations in Norway and in a large proportion of the wild Atlantic salmon resource. QuantEscape has further established that at the individual level, genetic introgression leads to changes in growth rate, age at smoltification, age at maturity, behaviour and phenology of migration.

Experiments show that offspring of farmed salmon, by having been selected for fast growth, dominate wild offspring under favourable environmental conditions, and that this also holds true for farmed-by-wild hybrids competing with wild offspring. In the presence of a predator, however, the advantage of farmed offspring is counteracted by their higher vulnerability to the predator. These results point to possible mechanisms for the variable fitness of farmed offspring in nature, which is seen as asymmetries in competitive advantage during different life stages.

Models show that genetic introgression leads to lower average fitness of the wild population, and that intermediate differences between farmed and wild salmon may lead to more dramatic effects than either small or very large differences. An area for further research is whether or not wild populations retain their average fitness if escapes are controlled

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Discussion and Implications

The genetic integrity of wild Atlantic salmon populations is threatened by escaped farmed salmon. The enormous success of salmon-based aquaculture industry in Norway has led to problems for wild fish, not only because of interbreeding between escaped farmed and wild salmon, but also because of the associated population growth of disease agents, such as sea lice.

These phenomena have resulted in a large increase of research money to understand and find solutions to aquaculture-related environmental problems, and as a side-track, has also contributed to world-leading new knowledge of salmon biology and more generally, sex-based dominance as a way of maintaining genetic variation in major loci (Barson et al. 2015). Moreover, the knowledge platform was a major contributor to the documentation and advice given by an ICES expert group on the effects of salmon aquaculture of wild salmonids (ICES 2016).

The detailed knowledge acquired by QuantEscape, and its collaboration and dialogue-based structure, points a way to understand and tackle complex environmental problems where vital conservation values and economic development are at stake.

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HEART RATE LOGGERS AS A TOOL TO IDENTIFY AND QUANTIFY DETRIMENTAL STRESSORS IN AQUACULTURE

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Introduction

European Whitefish (*Coregonus lavaretus)* has been suggested as a novel candidate for further expansion of aquaculture in northern Europe. However, whitefish are highly sensitive to stress. Farmers report that it is difficult to translate farm practices from other farmed salmonids to whitefish as they rapidly respond to stressful events with reduced feed intake and even loss of equilibrium. The stress response result in increased circulating levels of e.g. corticosteroids and catecholamines which elicit adaptive, secondary responses in target tissues. When fish are not allowed sufficient recovery time, maladaptive tertiary responses to repeated stress is a potential threat to homeostasis and thus fish health and welfare (Barton and Iwama, 1991; Wendelaar Bonga, 1997). Measurements of secondary physiological stress responses (e.g. heart rate) can therefore be used as a tool for welfare assessment in fish (Ashley, 2007). To ensure economic sustainability and improved welfare for the fish, it is important to identify and minimize stressors during farming conditions. The use of implantable heart rate loggers for continuous long-term recording of heart rate, in combination with measurements of plasma cortisol concentration, have previously been shown to be a useful tool to detect stressful events in aquaculture for rainbow trout (Brijs et al., 2018). The aim of this study was to assess the effects of anthropogenic stressors on whitefish associated with harvest by measuring heart rate and cortisol levels. This information can facilitate proper farm management of whitefish and safeguard the welfare of the fish.

Materials and methods

The study took place at a commercial fish farm where 20 whitefish were surgically instrumented with heart rate loggers (STAR-Oddi DST milli-HRT) before releasing them back into a sea cage together with 3000 conspecifics. Thereafter, heart rate were recorded and we kept a logbook of all husbandry events and environmental conditions that occurred at the farm during a period of 3 weeks. During the last 2 days the fish were exposed to a series of farming practises related to the harvest of the fish (e.g. crowding, brailing, well-boat transport and CO_2 narcosis). To validate the stress responses during these days, a sub-sample of 100 fish were sampled for haemoglobin, haematocrit and circulating levels of plasma cortisol during each event and compared to a group of “undisturbed” fish prior to harvest.

Results

The results showed that the repeated stress induced by subsequent farming practises had a cumulative effect as heart rate roughly doubled and peaked at ~35 beats min^{-1} above resting heart rate following the combination of crowding, brailing and transportation that commenced the harvest at day 19. After the initial increase in heart rate it remained elevated until they fish where slaughtered. Additionally, haematocrit was significantly increased when fish were exposed to crowding before, and also during, transport in the well boat. At the last stages of harvest, where fish were crowded and brailed and later stunned, mean corpuscular haemoglobin concentration (MCHC) decreased significantl . To our surprise, we also found a clear increase in heart rate 18 days after being released back into the sea cages which was before the harvest had begun. Cortisol data are unavailable at time of abstract deadline, but will be presented at the conference.

Discussion and conclusion

Based on the present results, we conclude that repeated stress has a cumulative effect on whitefish causing an increase in heart rate that remained elevated during a 14 hour recovery-period after transport. We also found a drop in MCHC indicating adrenergically-induced red blood cell swelling. The unexpected rise in heart rate that preceded the handling of the fish indicated a stressful event. From the logbook we could see that a second net cage containing rainbow trout was transported into the bay at this time, which presence may have caused this response. With rainbow trout in their vicinity the heart rate of the whitefish never recovered but remained significantly elevated, 10-15 beats min^{-1} above resting hear rate. These findings are important for management purposes, but also validate how heart rate can be used to monitor how fish perceive events occurring in aquaculture.

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EFFECT OF VARIOUS COMMERCIAL FEEDS ON GROWTH RATE AND VERTEBRAL COLUMN ANOMALIES IN EURASIAN PERCH (*Perca fluviatilis* L.) JUVENILES

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Introduction

Skeletal anomalies in farmed teleosts are a world-wide problem in aquaculture as the high rate of malformed fish often results in downgraded product and impacts economic profit (Boglione et al., 2013a). Although many factors have been implicated in skeletal deformities in fish larvae/fry reared in intensive production systems. Recent data provides evidence of the critical role several dietary components (such as crude protein, crude fat, polyunsaturated fatty acids – PUFA etc.) play in the early development of fish (Cahu et al., 2003). The goal of this study was to assess the effects of three different commercial diets (different composition of nutritional ingredients and level of PUFA) on Eurasian perch juveniles growth, survival and frequency of anomalies in their vertebral column.

Materials and methods

The four-week experiment was carried out on 32-day perch post-larvae (average body weight of 0.048±0.027g and average body length of 16.5±2.4mm) fed with three commercial feeds with varied nutritional components: Aller Futura EX (group AF); Perla Eel Protec A (group PE); Perla Larva Proactive 4.0 (group PP). The fish were reared at 20±0.5°C, with a photoperiod of 12L:12D. After the experiment, fish were anaesthetised (MS-222, Sigma-Aldrich Ltd., Poznan, Poland), measured and radiographed using a X-ray apparatus (FAXITRON MX-20, DOTmed.com Inc., New York, USA). The pictures were digitized and the vertebral column of each fish was thoroughly examined. Additionally, analysis of the basic chemical composition (dry matter, crude protein, crude fat and crude ash) of feeds and fish filets were examined according to the procedures proposed by AOAC (2012). The fatty acids profiles of fish feed and muscles were determined by gas chromatography (Christie, 1993).

Results

The study showed the effect of the varied feeds on the growth indexes of the perch juveniles. At the end of the experiment mean body weight (B_w; g) and mean total length (B_L; cm) were significant greater ($P<0.05$) in PE (1.13±0.36 and 4.3±0.5, for B_w and B_L respectively) than AF (0.72±0.24 and 3.9±0.4, for B_w and B_L respectively) and PP (0.72±0.22 and 3.9±0.5, for B_w and B_L respectively). Similarly to B_w and B_L, specific growth rate (SGR; % day^{-1}) was significant higher ($P<0.05$) in PE (11.30±0.26) than AF (9.65±0.25) and PP (9.73±0.06). The survival of fish was comparable in all groups and varied between 42.1% (group AF) to 58.2% (group PE). At the end of the experiment, the overall rate of deformities was at 20.9% (59 individuals). The vertebral column deformities determined as dislocation (Fig. 1A), ankylosis and compression (Fig. 1B) of vertebrae were found in all experimental groups: AF (22.5%; n=9), PE (17.5%; n=21) and PP (23.8%; n=29). Significant differences ($P\leq0.05$) in levels of crude fat (highest in PE group 8.25%) and polyunsaturated fatty acids, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)(highest in AF group 5.04% and 6.14%, respectively) in fish muscles, were recorded among groups.

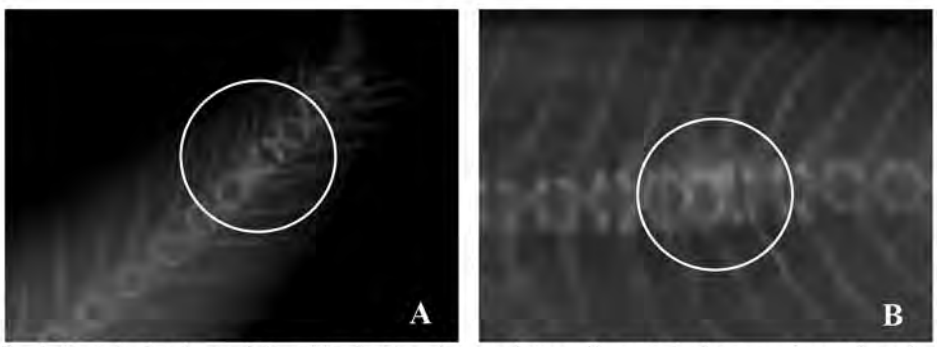

Fig. 1. Vertebral column deformities in Eurasian perch juveniles noted after experimental rearing. Lateral radiographs showing dislocation (A), ankylosis and compression of vertebrae (B).

(Continued on next page)

Discussion

Existing literature indicates that finfis species important to European aquaculture industry could be divided into two groups based on the type, severity and frequency of vertebral anomalies. The first group includes gilthead seabream, European seabass, flatfis and most of the candidate new species like Eurasian perch, whereas the second group includes salmonids. In the non-salmonid group, vertebral axis anomalies are quite frequent in the same lot or species, and concern almost all the recorded types to date (Boglione et al., 2013b). At the same time vertebral deformities are one of the most important causes of losses in the culture of Atlantic salmon (Fjelldal et al., 2007). Our data suggest that differences in level of polyunsaturated fatty acids in tested commercial diets does not affect the prevalence of skeletal malformation in perch juveniles. The percentage of fish with anomalies does not vary greatly among experimental groups though significant differences in growth rate were observed.

High prevalence of vertebral column anomalies noted in our study (~ 20%) suggests the need to improve the knowledge about factors leading to skeletal anomalies under percids intensive culture conditions.

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INNOVATIVE TECHNOLOGIES TO PROMOTE SUSTAINABLE AQUACULTURE IN EASTERN-AFRICA

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Fisheries is the most vital industry in the Lake Victoria Basin, being a major source of income for the population and an important part of the national economies in the region. However, the lake is under pressure due to overfishing and severe pollution. One solution to the problem of overfishing is aquaculture. Recirculating Aquaculture Systems (RAS) incorporates water treatment and reuses 90-95% of the water. RAS offers a variety of important advantages compared to open pond culture, such as reduced water and land requirements, environmental control, year-round operation, waste management control, and food safety benefits.

VicInAqua, a project under the EU Horizon 2020 programme, has developed a tilapia hatchery in Kisumu, Kenya using RAS adapted to local conditions. The hatchery is designed as a flexible, scalable and modular system. An online monitoring system enables the farmers to access farm data also for the energy system in real time and act quickly when something is out of the ordinary. The hatchery runs predominantly on solar energy making use of a 14.3kWp PV system including 30kWh Li-battery storage.

The purpose of the project is to demonstrate the possible technologies and present a financially feasible upscaled design, ready for the African market. The hatchery is used by the the Department of Livestock, Agriculture and Fisheries as a training and demonstration facility to promote the aquaculture sector and increase awareness, knowledge and skills for fish farmers.

Acknowledgement
This research has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement number 689427 for the project VicInAqua.

ANTI-INFLAMMATORY FEEDS AND TRYPTOPHAN METABOLIC PATHWAYS TO STRESS RESILIENCE IN FISH

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Diets of salmon in aquaculture have been changing due to a range of factors including constraints to marine resources, generally resulting in reduced levels of the anti-inflammatory n-3 fatty acid, eicosapentaenoic acid and increased pro-inflammatory n-6 fatty acids. In human medicine there is a surging interest in the beneficial effects of anti-inflammatory feeds on mental health and stress coping ability. This has giving rise to a neuro-immune framework where high n-3 diets are shown to increase immune function and stress resistance by suppressing pro-inflammatory eicosanoids and shunting catabolism of the essential amino acid tryptophan towards neuroprotective serotonin production, Fig 1.

Anti- and pro-inflammatory fatty acids and tryptophan have widespread and evolutionary conserved roles. However, to what degree the emerging mammalian neuro-immune framework is present and activated in teleost fish is currently unknown.

Here, this potential interplay between dietary fatty- and amino- acids compositions affect inflammatory processes, TRP catabolic pathways and brain signaling systems involved in stress coping and resilience in farmed salmon will be presented. Moreover, its applications in aquaculture will be discussed, with focus on stress mitigating aquafeeds targeting critical periods in the production cycle, such as vaccination, transport and seawater transfer.

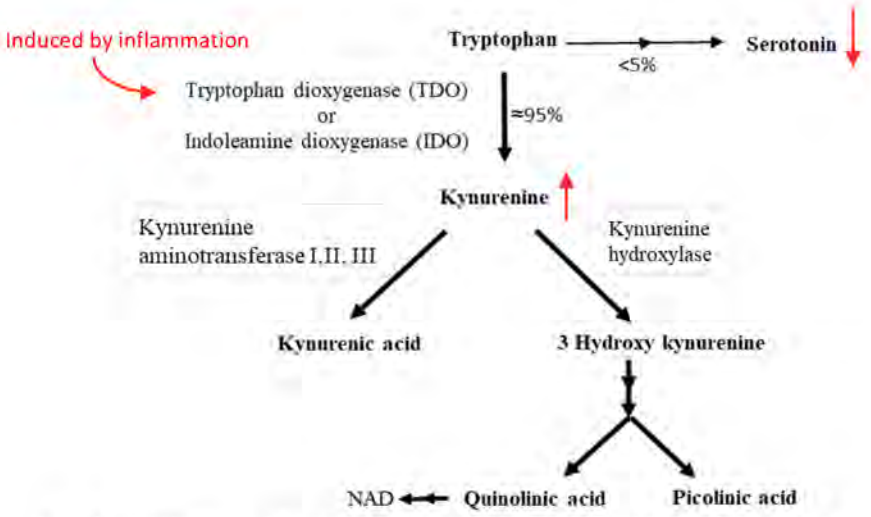

Fig 1. Interaction between the immune system and production of serotonin. A neurotransmitter/modulator having neuroprotective functions.

COMPARISON BETWEEN THE RESULTS FROM THE EU H2020 CERES AND CLIMEFISH PROJECTS ON THE POTENTIAL EFFECTS OF CLIMATE CHANGE FOR AQUACULTURE OF MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*)

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Mediterranean mussel (*Mytilus galloprovincialis*) culture is the top shellfish farming activity in Europe, with an annual production of over 300.000 tons. Given the socioeconomic importance of this culture, the Mediterranean mussel is a target species of the EU H2020 projects “Climate change and European aquatic resource” (CERES) and “Co-creating a decision support framework to ensure sustainable fish production in Europe under climate change” (ClimeFish). The overall aim of CERES and ClimeFish is to forecast the effects of and propose adaption measures to climate change on European fisheries and aquaculture. Particularly, CERES studied Mediterranean mussel farming in SW Portugal and ClimeFish in NW Spain and the Northern Adriatic Sea. The NW Spain and SW Portugal sites belong to the coastal upwelling system of the Western Iberian Peninsula, under the influence of intermittent upwelling-favourable northerly winds during the spring and summer. Coastal upwelling events regulate sea surface temperature and chlorophyll levels. Conversely, the Northern Adriatic Sea is a shallow semi-enclosed basin that receives cold water from many alpine rivers. Although CERES and ClimeFish focus on contrasting sites using different approaches, they are working together for the sustainable production of Mediterranean mussels along the European coast under climate change.

Oral presentations based on Abstracts 930 and 420 in this conference will provide details from the respective studies on Mediterranean mussel in CERES and ClimeFish. This poster will compare the similarities and differences in the results from these two projects.

GOODFISH – A PROJECT FOR THE DEVELOPMENT OF POND AQUACULTURE IN HUNGARY

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Introduction

Hungarian aquaculture is primarily a cyprinid-centered pond aquaculture which is characterized by seasonal production mainly for the Christmas market, low production intensity and sale of raw material (live fish) instead of processed products. A consortium of four leading Hungarian institutions of higher education and research has joined their resources to address this problem. The consortim is led by Szent István University and its objective is to improve and intensify the production of common carp (*Cyprinus carpio*), pikeperch (*Sander lucioperca*) and wels catfish *Silurus glanis*) in Hungary.

Materials and Methods

In the wels catfish and pikeperch, a cryopreserved gene bank has been developed of various populations of these species along with population genetic studies of both species. In addition a two-year production technology along with a specific breed of wels catfish is developed for intensive production of the species. In the pikeperch, a complex commercial-scale larviculture and fingerling culture technology is developed to ensure the supply of healthy juveniles for grow-out. Finally in the common carp, production is intensified by developing a two-year production cycle along with a specific carp breed and cost-benefit analyses, development of monosex all-female carp stocks and the development of a veterinary e-tool for the early detection of pathogens and diseases.

Results

A cryopreserved gene bank of wels catfish and pikeperch was developed with more than 1000 straws from catfish over 70 straws in the pikeperch originating from Hungarian and foreign populations. Three panmictic spawning events were conducted in the wels catfish and parents of the best performing progeny were selected based on DNA tests. The technology of broodstock handling, off-season spawning and larviculture of pikeperch is continuously developed. Regarding the two-year carp production, an intensive fingerling rearing technology is under continuous development, experimental feeds are also developed targeting intensification. Monosex carp broodstock is developed to improve production using meiotic gynogenesis as well as sex reversal of the progeny.

Discussion

The consortium is making progress in developing intensive technologies for three commercially important fish species for Hungarian aquaculture. We hope that intensified production will improve the productivity of the Hungarian aquaculture sector.

Acknowledgements

The work was supported by the GINOP-2.3.2.-15-2016-00025 and EFOP-3.6.3-VEKOP-16-2017-00008 project co-financed by the European Union, the European Social Fund and the Government of Hungar .

NITROGEN REMOVAL FROM A SALT-WATER RECIRCULATING AQUACULTURE SYSTEM (RAS) USING A STEP FED BATCH REACTOR (SFBR) OPERATED UNDER EXTERNAL AND INTERNAL CARBON SOURCES

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Introduction

Following the Marine Strategy Framework Directive (MSFD) to achieve Good Environmental Status by 2020 and Water Framework Directive (WFD), the Baltic Marine Environment Protection Commission (HELCOM) in 2013 agreed upon Country Allocated Reduction Targets (CARTs) to limit the allowable nutrient inputs in Baltic Sea sub-basins. Therefore, the development of recirculating aquaculture systems (RAS) in Baltic area depends on a more sustainable and cost-effective solution. Denitrification as an end-of-pipe treatment technology to reduce nitrogen discharge, gains increasingly more attention in particular for brackish and salt-water RAS (Hamlin et al., 2008; Suhr et al., 2013; van Rijn, 2013). Both external (commercially obtained) and internal (produced within the system) carbon sources can be used by denitrifying bacteria (Letelier-Gordo et al., 2017). Methanol and ethanol are frequently applied in commercial denitrification, but as flammable alcohols they are complicated in transport, storage and usage. In comparison, acetate and propionate as nonalcohol carbon sources can be better options. On the other hand, internal carbon sources originating from fish faeces can also be used for end-of-pipe denitrification in order to save operational costs in RAS (Arbiv and van Rijn, 1995; Suhr et al., 2013).

Materials and Methods

As a potential solution in RAS, the following study evaluated the denitrification capacity of a small scale SFBR working under different operational cycles (2h, 4h and 6h) with acetate, and under 4h operational cycle using external (acetate, propionate and ethanol) and internal (fresh and fermented fish organic waste (FOW & FFOW)) carbon sources from a salt-water RAS.

Results and Discussion

The results (Figure 1) showed that among different carbon sources denitrification with acetate had the best nitrate removal rates (57.64±6.55mg N/h/g bacteria) in SFBR, followed by propionate and ethanol. The differences can be explained by their feasibilities in the tricarboxylic acid cycle (TCA) (Yatong, 1996). The coexistence of fermentation and denitrification described by Sun et al. (2016) were observed in both FOW and FFOW groups, while the latter had higher efficienc . Only internal carbon groups had fluctuant total ammonia nitrogen production along denitrification process. Total hydrogen sulfide production was under control in all groups. Denitrificati n rates with acetate increased as SFBR operational cycle time decreased, might due to the selective pressure (Liu and Tay, 2007; Yatong, 1996). SFBR cycle of 2h with acetate reached a denitrification rate of 99mg N/h/g bacteria and a denitrification capacity of 600g N/d/m^3 working volume. A commercial case study indicated that SFBR using acetate as carbon source had a great potential in denitrification efficienc , reliable operation and required system volume compared to traditional biomedia-based systems.

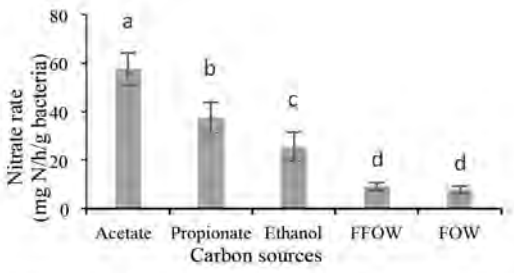

Figure 1. Nitrate rates with different carbon sources (acetate, FOW, FFOW, ethanol and propionate) under 4h SFBR cycle. n=4

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OPTIMISING LEVELS OF DIETARY FATTY ACIDS AND LIPIDS OF ATLANTIC SALMON UNDER NORMOXIC AND HYPOXIC CONDITIONS

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Introduction

Long-chain polyunsaturated fatty acids (LC-PUFA), such as DHA and EPA, are important for growth and health of farmed fish. However, the required level of LC-PUFA and their proportion to dietary lipid or absolute intake levels are not well understood. A previous study on rainbow trout found that more PUFA (i.e. alpha-Linolenic acid) was required when feeding higher levels of lipids, which suggests PUFA requirements are proportional to lipid (or energy) intake (Watanabe, 1982). Furthermore, the demand for LC-PUFA may be influenced by environmental factors, such as low oxygen (hypoxia), that are relatively common in commercial aquaculture systems. Hypoxia has been found to reduce feed intake by rainbow trout while protein and energy utilisation efficiency remained unchanged (Glencross, 2009). Minor changes in nutrient uptake and steroid synthesis into the blood may explain interactions between diet, stressors and fish performance. The objective of this study was to determine the effect of dietary lipid, LC-PUFA and dissolved oxygen on the growth performance, feed digestibility, nutrient retention, haematology, plasma biochemistry and steroid synthesis of juvenile Atlantic salmon.

Materials and methods

Atlantic salmon post-smolts of 184 g initial weight were reared in 24 circular tanks (32 fish/tank) for 138 days (20 weeks) at the University of Stirling's Marine Environmental Research Lab (Machrihanish, UK). In triplicate, each tank of fish was fed one of four extruded diets that were isoenergetic and isonitrogenous, where diets A and C had a higher total lipid level (230 compared with 180 g/kg) and diets C and D had a higher LC-PUFA level (14 compared with 7 g/kg). Protein was also varied to ensure diets were isoenergetic. Yttrium oxide was included as a digestibility marker in the diets. In half the tanks, the flow rate was decreased to lower the dissolved oxygen from approximately 90% to 70% saturation in order to subject the fish to a chronic hypoxic stress. Fish were sedated with MS222 and weighed at day 0, 56 and 138. Faeces were stripped from all fish, pooled and frozen on dry ice. Four fish per tank were euthanised and caudal blood and whole carcass were frozen on dry ice. Proximate, fatty acid and mineral composition of the diets, carcasses and faeces were performed using Kjeldahl, Folch, GC of FAMEs and ICP-MS methods at the Institute of Aquaculture (Stirling, UK). Haematology and plasma biochemistry were analysed at Scotland's Rural College (Edinburgh, UK) and plasma steroids were analysed at University of Edinburgh. For statistical analyses, normality tests and linear mixed effects model (LME) were performed using R software.

Results

Weight gain was significantly reduced for fish reared in low dissolved oxygen and fish fed high levels of lipids and LC-PUFA had the highest weight gain after 138 days (Fig. 1). FCR was not significantly affected by lipid, LC-PUFA or oxygen levels, whereas protein intake was affected by all three factors. In high oxygen, protein digestibility increased when fed high lipid or LC-PUFA whereas the opposite was found for lipid digestibility. Fish fed high lipid diets had higher protein retention while lipid retention was unaffected. For blood and plasma, differences in lipid, LC-PUFA and/or oxygen resulted in significant changes to levels of creatinine, total protein, cholesterol and mean corpuscular haemoglobin (MCHC), although fewer effects were found at day 138 compared to day 56 (Table 1). At day 56, both androstenedione and its derivative were affected by lipid and oxygen level, but PC-PUFA had no effects on any steroid synthesis.

Discussion and conclusion

The results indicate that hypoxia was the most influential of the three factors as it reduced feed intake, weight gain, lipid digestibility, androstenedione synthesis and elevated plasma creatinine. In both hypoxia and normoxia, fish fed diets with high lipid and high LC-PUFA levels had the highest weight gain (Fig. 1) and only fish fed the low lipid diet under hypoxia resulted in significantl lower weight gain. This result is in agreement with previous research (Watanabe, 1982)

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and provides further evidence that the level of LC-PUFA required by Atlantic salmon is proportional/relative to the total lipid level rather than the absolute level in the diet. This effect seems to be enhanced when fish are exposed to a chronic stressor, where both high lipid and LC-PUFA can improve growth. In contrast to rainbow trout, hypoxia significantly affected protein and lipid digestibility coefficients of Atlantic salmon in the present study (Glencross, 2009). Fish fed high lipid and LC-PUFA diets consistently had higher levels of total protein, cholesterol, MCHC and androstenedione in the blood, which may explain higher weight gain. In conclusion, these results indicate that LC-PUFA requirements for Atlantic salmon should be considered as proportional to total lipid in the diet and this demand can be influenced by hypoxia

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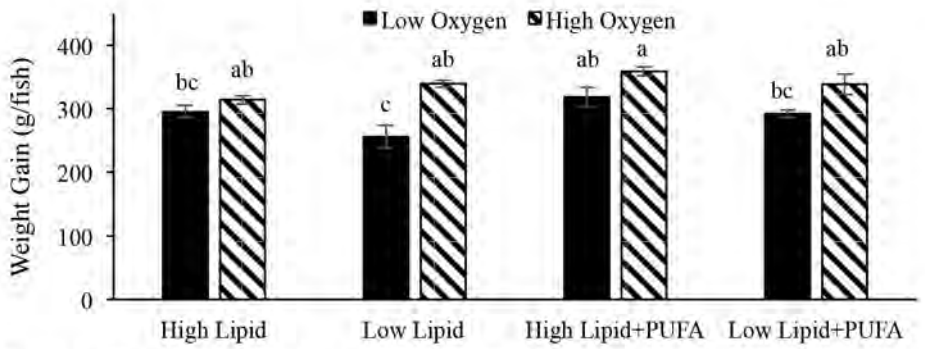

Figure 1. Weight gain of Atlantic salmon fed diets with high or low levels of lipids and LC-PUFA and exposed to dissolved oxygen of 70 (black) or 90% (diagonal). Differing letters indicate significant differences ($p<0.05$).

Table 1. Factors significantly affected by plasma biochemistry and haematology.

	Day 56			Day 138		
	Lipid	PUFA	Oxygen	Lipid	PUFA	Oxygen
Creatinine (μmol/L)	*	*	***	ns	ns	***
Total Protein (g/L)	*	ns	ns	ns	ns	**
Cholesterol (mmol/L)	***	ns	*	**	ns	ns
MCHC (g/dL)	ns	ns	*	*	ns	ns

Symbols ***, **, * and ns refer to p-values <0.001, <0.01, <0.05 and not significant.

RECENT DEVELOPMENTS OF DIETARY RESEARCH IN FARMED CLEANERFISH – LUMPFISH (*Cyclopterus lumpus*)

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The use of cleaner fish, such as lumpfish (*Cyclopterus lumpus*), is common practice in the salmon industry to control sea lice in seacages. They are an effective biological control compared to chemical treatments which sea lice have largely become resistant to. They are of particular relevance in organic farming of salmon, where there are limitations on the number of chemical treatments permitted. Despite their widespread use in the industry, there are limited studies on their nutritional requirements or the effect that commercial formulated diets have on lumpsucker. To further understand this, two feeding trials were carried out to determine the protein requirements and the potential for alternative protein sources in the diets of juvenile lumpfish.

The first feeding trial was carried out on juvenile lumpfish to investigate optimal dietary protein levels. Four protein levels were tested, with three replicates per treatment. The second feeding trial focused on the use of mealworm and black soldier fly larvae as an alternative source of protein. Similar to the first trail there four feed treatments with three replicates of each. The feed treatments consisted of control, mealworm, black solder fl , and a mix of both mealworm and black solder fly diets.

This paper is an overview of some of the nutrition work being carried out by ANARU, with a focus on experiments on alternate protein sources (insect meals) and optimal levels of dietary protein required for juvenile lumpfish

POTENTIAL EFFECTS OF CLIMATE¨ CHANGE ON OFFSHORE AQUACULTURE OF MEDITERRANEAN MUSSEL (*mytilus galloprovincialis*) ON THE SW COAST OF PORTUGAL

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Introduction

There is increasing interest in the development of offshore aquaculture in Europe. In the case of Portugal, *Mytilus galloprovincialis* , the Mediterranean mussel (Med.mussel), is an endemic species and is well adapted to the local environment; the seed can be collected naturally from this environment with suspended ropes as collectors, and it grows relatively quickly with limited handling (O'Hagan et al., 2017). Although Med. Mussel is probably not critically vulnerable to climate change, their capacity as a farmed species will be sensitive to warming under scenarios of future climate change. For SW Portugal, the primary production is regulated by the occurrence of upwelling which brings cold and nutrient rich water promoting increased chlorophyll (proxy for primary production). During periods of upwelling relaxation, there is an increase in temperature and a reduction in temperature; extended periods of these conditions is reflected by a loss in mussel condition and an increased susceptibility to disease. Furthermore, fluctuations in environmental conditions will also modify the patterns related to Harmful Algal Blooms (HAB) and the recruitment of mussel seed.

Development of the Med.mussel 'story line' from the EU CERES project

As part of the EU project Climate change and European Aquatic RESource (CERES) to forecast and anticipate the effects of climate change (CC) on fisheries and aquaculture, the offshore aquaculture of Med.mussel in SW Portugal has been developed as one of the 'story lines' to explore how this industry might be affected by the effects of future CC. Essentially, two scenarios have been selected for the future Representative Concentration Pathway (RCP) of carbon emissions: with RCP 8.5, emissions create concentrations of carbon up to 1370 ppm (causing a radiative forcing higher than 8.5Wm^{-2}); and with RCP 4.5, the total radiative forcing and carbon concentrations are stabilised at 4.5Wm^{-2} and ~650 ppm, respectively, shortly after 2100 due to more rapid reduction of carbon emissions (IPCC 2001, Kay *et al.*, 2018). The 'story line' starts with a review of the species background and the current economic activity related to the culture of Med.mussel. Using this current information on expected projections under CC, potential future socio-economic scenarios are analysed such as 'world market', 'local stewardship, and 'global sustainability' (Pinnegar *et al.* 2018). Using the review of the literature and some specific CERES research on future CC effects, individual and local-scale models have been validated against the current culture practices for offshore aquaculture of Med.mussel in Portugal (Ferreira *et al.*, 2019)

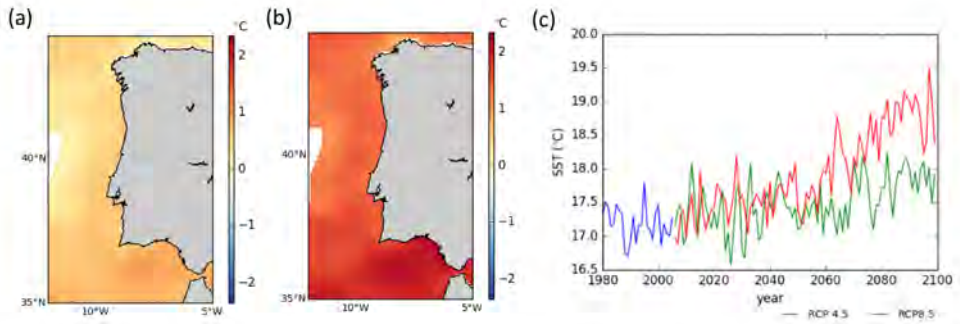

Fig 1. Projected change in sea surface temperature for the Portugal region. (a,b) Difference in 20-year mean temperatures for 2080-2099 compared to 2000-2019 under (a) RCP 4.5 and (b) RCP 8.5. (c) Annual mean for the same region (with thanks to Susan Kay).

(Continued on next page)

The sensitivity of the Med.mussel aquaculture to future CC is considered further with an analysis of the adaptive capacity in the system to accommodate change, using different approaches including, quantifying the resultant and residual vulnerability to decrease future potential problems from CC. The bottom-up approach using Bow tie analysis (Cormier et al 2019) is a risk analysis technique adopted from IEC/ISO 31010 that uses local stakeholders to define, in the case of Med,mussel, what are the principle issues affecting losses and mortality from the offshore aquaculture in Portugal. Also, the Bayesian Belief Network approach has been implemented; this is an innovative meta-analytical approach using Bayesian hierarchical models to use "data-rich" information to infer responses about "data-poor" information. The information from all these techniques is the core of the CERES project, as the data defines and addresses the risks from future CC that allows for the development of practical and operational solutions in collaboration with the aquaculture industry.

Some of the outcomes from the Med.mussel 'story line' in the EU CERES project

Due to both shortage of space for the Abstract and time for the talk, only two examples of the results from the Med. mussel 'story line' are presented here, but acknowledgement through the Authors list are given to all the contributors to this extensive work that will be presented in a future Deliverable of the project .

CERES climate change projections suggest that waters around the Portuguese coast will warm by up to 2°C by the end of the century under RCP 8.5, with the largest increases in the south (Fig.1). Increases under RCP 4.5 are lower, up to 1°C. Whilst projected changes in net primary production (PP), using chlorophyll as a proxy, are less distinct between the RCP scenarios, with a general trend of slightly increasing production under both RCP 4.5 and RCP 8.5. Historical *in-situ* measurements in SW Portugal indicate a decrease in PP with increasing temperature and, therefore, the future projections of changes in PP should be treated as uncertain (Kay *et al*. 2018).

Physiological models including the AquaShell model modified for individual Med.musssels and incorporated into the local scale Farm Aquaculture Resource Management (FARM) model that has been used to examine direct climate driven responses on harvest and environment effects (Ferreira *et al.* 2019). Three time slices have been selected: present (2000-2019), near-future (2040-2059), and far-future (2080-2099). The time slices are related to the two emission scenarios: the more conservative RCP 4.5 and the more severe RCP 8.5. The individual growth model has been run for 20 years within each time slice, and the years which yield the minimum and maximum individual biomass have been selected to give an idea of the statistical spread. Mussel weight at harvest is very similar under both emission scenarios. Similarly, production yield and profit show no significant differences between RCP 4.5 and 8.5 at any time slice, although the average values are greater under the 8.5. The better growth performance under the high-emission scenarios is reflected in the greater consumption of food and lower energy expenditure of these mussels and can be mainly explained by greater chlorophyll concentrations. Slightly better average yields and profits have been obtained from the mid-term time slice (Ferreira *et al*, 2019)

Conclusion

The Med.mussel 'story line' has helped to define the current and future risks for offshore aquaculture and is providing practical and operational solutions in collaboration with the aquaculture industry to reduce future losses to the Blue Economy in SW Portugal.

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REAL TIME MONITORING OF ENVIRONMENTAL CONDITIONS FOR THE MANAGEMENT OF OFFSHORE AQUACULTURE AT SAGRES, SW PORTUGAL

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Introduction

There is concern that aquaculture is not developing at the same pace in Europe as other regions of the world (FAO,2018). With this situation in mind, the EU has funded projects to encourage expansion of aquaculture in Europe including the Horizon 2020 project GAIN (Green Aquaculture Intensificatio in Europe). GAIN is designed to support the ecological intensification of aquaculture in the European Union, with the dual objectives of increasing production and competitiveness of the industry, while ensuring sustainability and compliance with EU regulations on food safety and environment. This poster focuses on how to improve the management of shellfish farms in SW Portugal using environmental data from *in situ* sensors that can be integrated with appropriate data from Earth Observation. The ultimate objective is to implement a Management System for the farms that can use large volumes of information (Big Data) processed in real time.

Methods

Fig 1 (left panel) shows the location of the offshore concessions for mussel aquaculture licensed to Finisterra L[da]. Using one of Finisterra's signal bouys moored within in the aquaculture concession, Sagremarisco L[da] SGM has installed a solar powered data logger with sensors for solar irradiance above the sea surface, and sensors for temperature, chlorophyll fluorescence, turbidity (transmissometer), and optical conditions below the sea surface (Fig.1, right panel). Data from these sensors are transmitted every two hours to be incorporate in a common cloud-based data management and services platform. The overall objective is to offer unified access to information which not only includes the *in situ* sensor data, satellite data, relevant public data, model hindcasts, nowcasts, and forecasts, as well operational data from the farm.

SGM has also set up an experiment that monitors a cohort of mussel spat collected in June 2019 from the shoreline facing the concession (Fig 1, left panel) that simulates Finisterra's current production protocol for mussel production on long-lines. The experimental mussels are grown on one of the long-lines adjacent to the commercial production where temperature and salinity is also recorded every 30 minutes HOBO sensors. Mortality and growth from sub-samples of the experimental cohort of mussels is monitored monthly. As the submerged sensors need to be cleaned regularly, a CTD (conductivity, temperature and depth) is deployed at each visit to obtain temperature and salinity profiles in the water column and water samples are collected for estimates of chlorophyll, total suspended matter, nutrients and identification f phytoplankton.

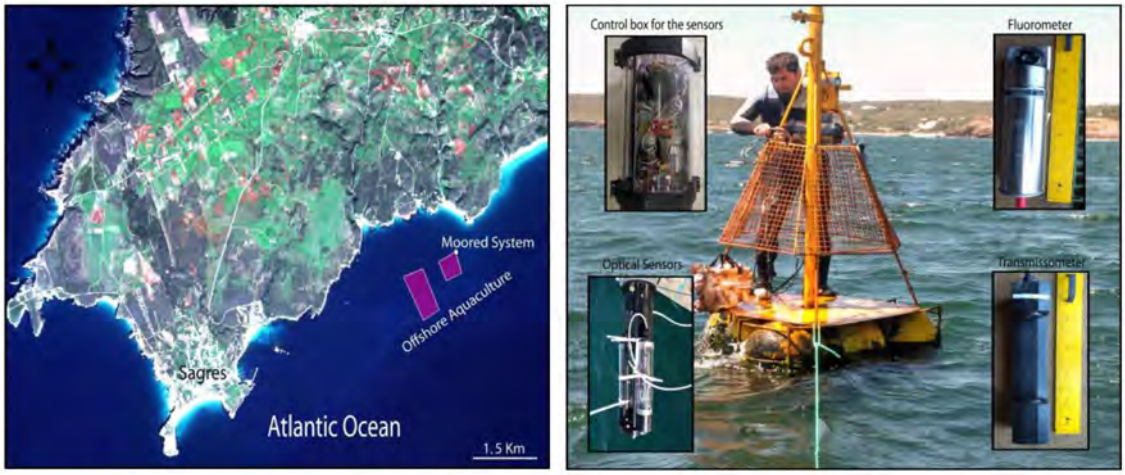

Fig.1 Left panel: geographical location of offshore aquaculture at Sagres (Sentinel-2 Level 1 satellite image provided by Copernicus). Right panel: moored signal bouy providing real time data from a range of sensors powered with a solar control box.

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Results and Discussion

Fig 2 shows an example of the environmental *in situ* data that is being recorded at the site and uploaded to the cloud platform.

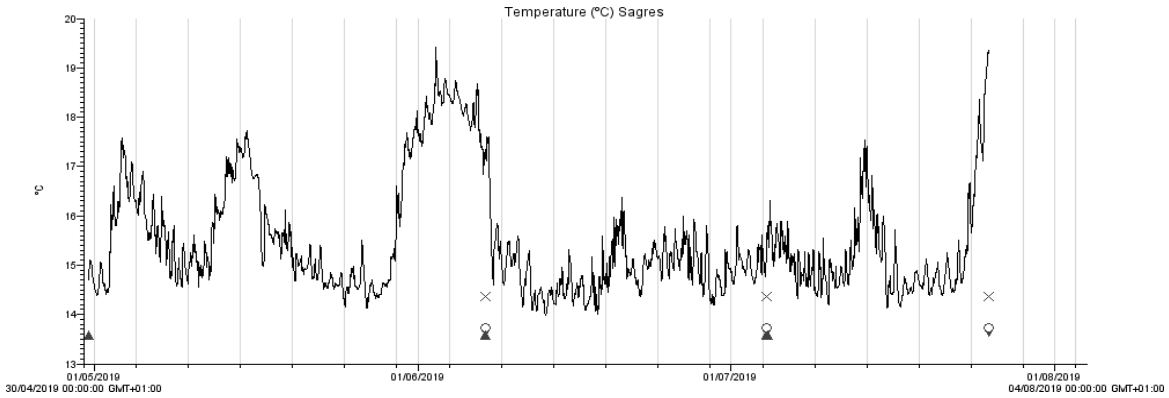

Fig 2 Temperature trace at 5 metres depth from a HOBO sensor deployed with the growth and mortality experiment for mussel initiated in June 2019. X shows when mussels where sampled for mortality and growth.

The *in situ* data set will be complemented with remotely sensed environmental data harvested from publicly available portals, such as the Copernicus Marine Service; these additional data will be relevant in particular for shellfish culture, as some products are a good proxy of food availability, and therefore of shellfish potential growth rates. Also, there are other important issues such as Harmful Algal Blooms (HAB), storm conditions and mussel spat recruitment that might be understood and predicted by integrating large volumes of data from these heterogeneous sources. There is already a substantial body of published Earth Observation data obtained at the Finisterra concession (e.g Icely et al. 2013; Goela et al, 2015,2016; Cristina et al. 2016).

In conclusion, the work at Sagres contributes to one of the objectives of the GAIN project, namely, to implement a cloud based Information Management System (IMS) by combining sensors, key performance indicators, Big Data analysis, and predictive mathematical models for production and environmental effects. The IMS should enable the daily management of precision aquaculture at shellfish farms (GAIN project https://cordis.europa.eu/project/rcn/216474/en)

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WASTE FROM DISCARDS: SOCIO-ECONOMIC CHALLENGES OF ARTISANAL FISH PRODUCTION IN NIGERIA

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Introduction

Fish is an important source of protein to the teeming population in Nigeria. According to Adekoya (2004), Fish represents about 55% of the protein sources intake of Nigerians. In spite of her enormous oil wealth, Nigeria is confronted with a number of developmental challenges especially in the areas of reducing poverty and meeting adequate nutritive requirement of its 200 million people (Business Day, 2016). The major compelling factor for the development of the sector is the huge domestic market with an existing demand of about 1.5 million metric tonnes per annum. Incidentally, domestic fish catch which comes mostly from artisanal sources with its dwindling fortunes (Ojo and Fagbenro, 2004) is just 511,700 metric tonnes leaving a wide gap of 988,300 metric tons that could possibly be bridged through fish farming. Discards are the waste incurred from incidental capture of non-targeted fish and this part of the catch is usually unregulated. The capture of by-catch may pose a threat to species diversity and ecosystem health (Eayrs, 2007). Discards mostly consists of juvenile food-fish species and is therefore a threat to food security and sustainable fisheries production. There is an awareness that waste from discards is a global problem that must be addressed therefore the need to examine the economic implication of waste reduction, acknowledging the need for general awareness on the need to conserve fisheries resources to ensure food security.

Research Methodology

The study was carried out in river Igbokoda which is one of the longest water body in the country. Primary data source was only employed for the study. The data were collected with the aid of structured questionnaire administered by snowballing. Hundred (100) questionnaires were prepared but nine six (96) were valid and distributed to the targeted respondents. Samples and Data were collected for six months starting from March to August 2017. The data on the socio-economic characteristics of the respondents, marketing costs returns, processing cost and data on fishing operations; as well as constraints militating against fish marketing in the study area, were collected. Data was analyzed using SPSS (14.0) package Descriptive statistics such as measures of central tendency and dispersion and frequency distribution were used to analyze respondents and perceived awareness of bycatch and its reduction devices. The data gathered were fitted to different regression models then the lead equation with the best fit was then chosen.

Table I. Awareness characteristics of waste from discards among artisanal fishers in the study area

Variable	Dominant Indicator
Awareness of discards	10.26% very much aware, 58.33% aware, 31.41% not aware
Occurrence of discards	32.69% always, 67.31% sometimes.
Common discarded species	Snake fish, Crabs, Shrimp/prawn, fin fish, sea turtle, Crayfish, Oyster, electric fish, Periwinkle, black snail, Water snail, white fish, Turtle, crocodile, Cockle, Whelk, Squid, Octopus,
What they do with discards	78.85% sell as additional income, 21.15% cook for household
Value realized from discards	₦18, 583.33 peak season, ₦11, 076.19 moderate, ₦4963.10 low
Reasons for getting discards	Small quantity of harvest, storage and preservation, sorting and preparation, low market, low demand
Fishing gear used	44.23% cast net, 32.69% gill net, 12.9% Dragnet, 12.18% hook and line, traps and basket.

Source: Field survey (2017)

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Results

Table l showed the awareness characteristics of discards among artisanal fishers in the study area. It is important to note that these were the only options that were selected by respondents, other options such as 'return them to the waters after catch,' 'discard them but not to the waters,' 'sort and prepare them as foods for animals' and 'sort and give them away to community people' were not selected.

Discussion and Conclusion

In the study of characteristics of discards as shown above, the fisher folks were using fishing gears that make reduction of waste from discards difficult. However, when asked about the knowledge and use of bycatch reduction devices, most of the respondents linked reduction devices to commercial trawlers. Majority (95%) also revealed that the introduction of a bycatch reduction device to artisanal fishers will raise the unit cost of effort and this cost increase will induce a reduction in the profit maximizing level of effort and also reduces profit. This is in consonance with (Eayrs, 2007) that the fishermen are strongly urged to use appropriate bycatch reduction measures to help maintain the productivity of the fishery and the long-term prosperity of the fishing industry. He stated further that by appropriately, fishermen could help to protect the marine environment and assist global food security both now and in the future. While this problem of cost increment with the use of bycatch reduction devices should not be ignored, there is an urgent need to sensitize the fishermen on the implication of non-discard reduction device. The economic implication of the findings is that in the long run, the fishing households in the study area may become food insecure. Therefore, there is a crucial need for interventions that will reduce bycatch and discarding, improve selectivity and therefore lead to better managed fisheries while not neglecting sustainable food security of the fishing households and consumers and alternative means of livelihoods for those that are mainly discards and bycatch fisherfolks

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EFFECT OF FEEDING STRATEGY WITH ROTIFERS *(Brachionus plicatilis)* ON PIKEPERCH (*Sander lucioperca)* LARVAL PERFOMANCE

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Introduction

Rotifers are commonly used in marine larval culture (Girin 1975; Nash and Kuo 1975; Yufera, Pascual et al. 1993; Howell 1997) and recently reintroduced for pikeperch larviculture. Their smaller size and higher level of DHA and LA values compared to *Artemia* proved them as a suitable first diet for pikeperch larvae (Yanes-Roca et al. 2018). However, larval feeding with rotifers is expensive and labor demanding. Therefore, the aim of the present study was to optimize the duration of pikeperch larval first feeding with rotifers under the controlled conditions.

Materials and methods

Experimental culture of pikeperch larvae with rotifers *B. plicatilis* was performed in the Experimental Fish Facility of the Faculty of Fisheries and Protection of Waters (FFPW), University of South Bohemia. The rotifer (mean length 280 µm) were cultured in flat-bottomed, polyethylene tanks of 50-l capacity using a batch culture protocol fed with microalgae paste of *Nannochloropsis* sp. (Nanno 3600, ReedMariculture Inc., USA) at a rate of 1 ml of paste per liter of culture twice a day. Pikeperch larvae (TL= 5.69 ± 0.26 mm, BW=6.68±0.42 mg, 4 DPH) were stocked randomly into each 2-l rearing tank at initial density 100 larvae L^{-1}. The larvae were divided into five experimental groups (Table 1), each in four replicates and reared until 17 DPH. Microalgae *Nannochloropsis sp*. was added twice per day throughout the experiment. The larvae were fed with rotifers *B. plicatilis* or/and *Artemia* nauplii three times a day. Light regime was set at 13L: 11D. Light intensity on the water surface ranged from 90 to 100 lux. The tanks were cleaned twice daily by siphoning to remove excrements, dead larvae and uneaten food. The water temperature, pH and the concentration of dissolved oxygen were measured before each feeding with an oximeter (OxyGuard International A/S, Farum, Denmark) and pH tester (HI98129, Hanna Combo) and the average values were: temperature (17.86±0.63°C), pH (7.38 ± 0.17) and DO (88.55 ± 9.50%). Ammonium concentration was below 0.1 mg L^{-1} and nitrite concentration was below 0.01 mg L^{-1}. Survival, SGR_{TL} and morphometric parameters were measured and calculated. All data were statistically analyzed using RStudio while differences were considered significant at $p<0.05$ using one-way ANOVA followed by Tukey post hoc test.

Results

The highest survival rates mean (±SD) of larvae at 17 DPH was in groups D (68±5.51%) and C (53±5.43%) and significantly different compared to larvae in groups B (50±7.2%), A (36±9.2%) and K (33±6.9%). The highest TL of pikeperch larvae at the end of experiment was in group D (8.57±0.57 mm) and significantly different compared to larvae in groups C (8.23±0.50 mm), A (8.22±0.52 mm), B (8.22±0.46 mm) and K (8.21±0.56 mm). The highest SGR_{TL} at 17 DPH was in groups D (20.76±4.61 % d^{-1}), C (18.28±3.74 % d^{-1}), B (18.26±3.66 % d^{-1}) and A (18.22±4.49% d^{-1}) and significantly differed compared to larvae in group K (18.18± 4.20% d^{-1}).

Table 1. Feeding regime of experimental groups

	5-7 DPH	8-10 DPH	11-13 DPH	14-16 DPH
Group A	rotifers	rotifers	rotifers	Artemia
Group B	rotifers	rotifers	Artemia	Artemia
Group C	rotifers	Artemia	Artemia	Artemia
Group D	rotifers	rotifers+ Artemia	rotifers+ Artemia	rotifers+ Artemia
Group K	rotifers	rotifers	rotifers	rotifers

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Discussion

The success in larval production generally depends on the first feeding (Ostaszewska, 2005). The study shows that pikeperch larvae fed with rotifers during first three days and then replacing with *Artemia* (group C) or combination with *Artemia* (group D) resulted in the best performance for fish survival and growth. Similar results were obtained in a study of Yanes-Roca et al. (2018) where larvae had mixed diet of rotifers and *Artemia*. This study can conclude that feeding pikeperch with rotifers during first three days (from 5 DPH till 8 DPH) and afterwards replacing with *Artemia* is proper feeding regime that supports larval development with minimal investments in live feed and labor.

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EFFECT OF VISUAL ENVIRONMENTAL ENRICHMENT ON THE WELFARE OF FARMED RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

In conjunction with an increasing significance of animal welfare in our society, research on how to improve husbandry conditions in commercial fish farms is also gaining importance. One way of contributing to this is to study various forms of environmental enrichment. While structural enrichment can have several benefits on fish welfare and health, it also reduces tank hygiene with an associated increase in infectious diseases, increases the risk of injuries and is an overall practicability impediment for fish farmers (Näslund and Johnsson, 2016). Therefore, the present study tests the implementation of a purely visual form of enrichment and its effects on welfare, health and condition on farmed rainbow trout. While eliminating several drawbacks, this method maintains a complex visual environment. An improved sensory and cognitive stimulation is believed to play a key role in the neuroendocrine development in fish (Salvanes et al., 2013), which regulates important functions such as immunocompetence and stress tolerance. In consequence, enhancement of these functions would achieve an overall increase in fish welfare

Material and methods

Eight tanks, holding 1m^3 of water each, were stocked with 12kg of rainbow trout (mean weight 20.5g). Previously, four of the tanks have been equipped with surfaces imitating the look of a natural riverbed, while the remaining four control tanks have been lined with a structureless monochromatic green motive. After the acclimation phase, stocking density was kept at 30kg/m^3. Regular samplings of 10 fish per replicate were perf rmed in three-week intervals over a period of 5 months.

As indicators of fish stress, plasma cortisol and glucose levels as well as a differential blood cell count were determined. The fin damage index according to Hoyle et al. (2007) served as additional parameter to quantify fish welfare. Fish health was evaluated by necropsy, including parasitological and bacteriological examinations. Growth rate, condition factor and bacterial load of tank water were determined. Complementary, fish behaviour was examined by video observations

For a second phase of our study, the trout of four tanks were marked with fluorescent pigment (Siebenthal et al., 2017) and were transferred to a commercial fish farm. The remaining trout stayed in their respective tanks at their initial location. On the fish farm, trout of an enriched tank were pooled with trout of a control tank, resulting in two mixed groups. Each group was put in a custom-built cage in the farms raceway, one cage exhibiting the same kind of enrichment as the tanks in the previous phase and the other one featuring a monochromatic green motive serving as a control. After the transfer, sampling continued in both locations for another 5 months.

Results

First results will be presented at the Aquaculture Europe 2019 conference in Berlin.

Discussion and conclusion

Findings of this study will show if purely visual environmental enrichment in farmed rainbow trout has any discernible benefits concerning fish welfare, health or condition. If this is the case, an easy to apply visual modification of fish tanks or raceways would be a valid alternative to other forms of enrichment, without compromising hygiene. Additionally, by mixing fish exposed to the different treatments, the influence of visual enrichment on the ability to cope with neophobia could be evaluated.

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AQUAVITAE: NEW SPECIES, PROCESSES AND PRODUCTS CONTRIBUTING TO INCREASED PRODUCTION AND IMPROVED SUSTAINABILITY IN EMERGING LOW TROPHIC, AND EXISTING LOW AND HIGH TROPHIC AQUACULTURE VALUE CHAINS IN THE ATLANTIC; H2020 BG-08-2019 [Part C]

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Introduction

AquaVitae is a new four year H2020 project with the overall objective of increasing aquaculture production in and around the Atlantic Ocean in a sustainable way by developing new and emerging low trophic species and by optimising production in existing aquaculture value chains. AquaVitae will conduct research and facilitate innovation in five value chains (VCs) selected with regard to their potential for contributing to improved, sustainable food production and their lasting, significant impacts, specifically

VC I: Macroalgal production; New species, offshore production, and post-harvest processes
VC II: Integrated Multi-Trophic Aquaculture (IMTA); land-based and sea-based, new species and systems
VC III: New echinoderm species: Sea urchins and sea cucumbers
VC IV: Existing shellfish species: Oysters and mussels
VC V: Optimised production of selected existing finfish species; freshwater and mari

A series of cross-cutting Work Packages (WPs) will include research on biosensors, Internet of Things (IoT), product characteristics, consumer attitudes, market potential, sustainability, environmental monitoring, risk assessment, analysis of value chains, profitabilit , and other socioeconomic aspects.

AquaVitae will contribute to various policy dialogues and produce briefs on policy and governance issues. The AquaVitae consortium consists of 36 full partners from Europe and countries bordering the Atlantic Ocean, in addition to an Industry Reference group, a Policy Advice Group, and an External Advisory Group. AquaVitae supports extensive communication and outreach activities, employs a multi-actor approach to ensure stakeholder engagement in all phases of the project, and will set up a durable aquaculture industry and research network around the Atlantic Ocean. Industry partners are present in all case studies, and they have a special responsibility for exploitation and commercialization of the project research results and outcomes. AquaVitae will have a lasting impact on society through the introduction of new species, and through the development of new processes and products based on a circular economy / zero waste approach with improved sustainability. AquaVitae will produce Good Practice standards, facilitate industry apprenticeship and student exchange, support extensive training programs for industry, academia, and the public, and contribute to the implementation of the EU-Brazil-South Africa Belém Statement. This presentation will give an outline of how the project will be conducted and will highlight examples from the Case Studies within the project.

JOURNEY FROM INTRODUCTION TILL LOCAL PRODUCTION OF SOY-BASED FLOATING EXTRUDED AQUAFEED IN PAKISTAN; A REVOLUTION IN AQUACULTURE INDUSTRY

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In Pakistan, Aquaculture is a rather recent activity and is in a phase of transformatioin from extensive fish farming to semi-intenstive fish farming; nevertheless there is an immense potential for development of this sector. Major challenge faced by this industry hindering progress of this sector had been lack of commercial floating fish feed. Soy-based extruded floating aquafeed was introduced for the first time in Pakistan by United States Department of Agriculture (USDA) in 2012. The imported feed was trialled at various commercial farms by FEEEDING Pakistan Program of American Soybean Association (ASA)/WISHH funded by USDA to convince farmers about nutritional and commercial value of this feed for multiple species.

Amongst these several research trials, FEEDING Pakistan Program carried out a trial (June – November, 2017) to study the effects of locally produced extruded floating pelleted soybean meal (SBM) feed on the growth of genetically improved farmed tilapia (GIFT) *Oreochromis niloticus* at two commercial earthen ponds (n= 4,560 each pond), average stocking weight = 110 g. During this trial, fish were fed with floating soy based feed containing 32% of crude protein (CP) for the first 90 days and 28% CP for next 90 days. Control group was fed with conventional mixture of mash feed; 32% CP for first 90 days and 28% CP for next 90 days without SBM. Thirty days after commencement of trial, a significant increase of 93% was observed in total body weight of fish in treatment group as compared to those in control group. At end of the trial, an increase of 55% was observed in total body weight of treatment group as compared to control group. Feed Convesion ration (FCR) of 1.2 was observed in treatment group and 4.10 in control group. High success of extruded soy-based floating feed in improvement of fish growth was a breakthrough for local aquaculture industry develpment. After strenuous hard efforts of two years, FEEEDING Pakistan Program successfully collaborated with local industry to establish the first floating soy-based aquafeeed mills in 2013. Number of soy based floating aquafeed mills has been increased up to five by 2018. Total production of soy based aquafeed was recorded to be 11,000 metric tonnes in 2017 which has increased up to 21,750 metric tonnes by the end of 2018.

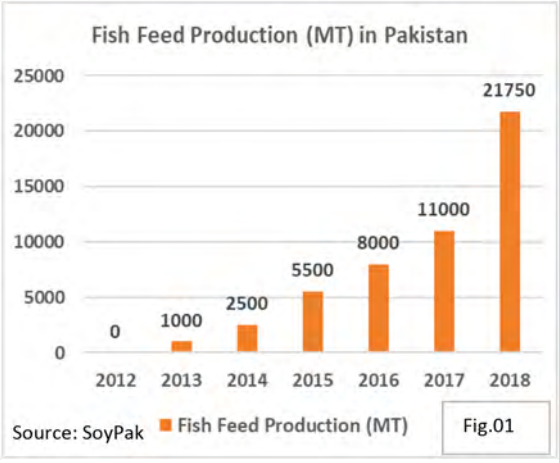

Fig.01

MICROBIOTA-RELATED INDICATORS OF GUT HEALTH IN ATLANTIC SALMON (*Salmo salar*)

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Introduction

An increasing number of studies have been published on the composition of the bacterial microbiota in Atlantic salmon. However, few studies have been made to discriminate between optimal, well-performing communities and suboptimal, "unbalanced" bacterial communities in the intestine. Identificati n of such dysbiosis markers in the salmon gut microbiota, as well as markers of optimal gut community function, would be a vitally important tool in the development of new functional feeds targeting gut health.

The GutBioM project is an initiative that aims to identify and use quantifiable microbiota-related markers as indicators of gut health and intestinal function for the development of functional feeds. The project will increase our knowledge on host-microbiota interactions in fish, by identifying and describing microbiota-related markers such as single bacterial groups, metabolites in blood or feces or specific host responses associated with normal or abnormal health status.

Material and Methods

The first part of the project involved a long-term sea water feeding trial with Atlantic salmon to detect quantifiable microbiota-related markers for gut health and intestinal function. To meet this aim, 28 healthy fish were fed on a standard commercial grower feed diet. These fish were then sampled from the same net pen and divided into two groups based on weight, i.e. fourteen large (High performers, average weight 3.709 kg) and fourteen small (Low performers, average weight 2.111 kg) based on growth performance. The second part of the project focuses on the interplay among functional feed ingredients, gut microbiota, intestinal health, and fish performance, and will assess whether functional feeds can improve the re-establishment of a healthy gut environment after dysbiosis events caused by chemotherapeutic agents such as antibiotics.

The variables chosen for identification of microbiota-related markers of intestinal health and evaluate host response were fish performance, intestinal bacterial microbiota profiling using 16S rRNA gene sequencing, targeted and untargeted metabolomics and short chain fatty acid profiling of fecal and blood plasma samples, targeted and untargeted gene expression profiling and histopathological evaluation of the intestine. Data from the different endpoints will be integrated to identify microbiota-related markers of gut health and function.

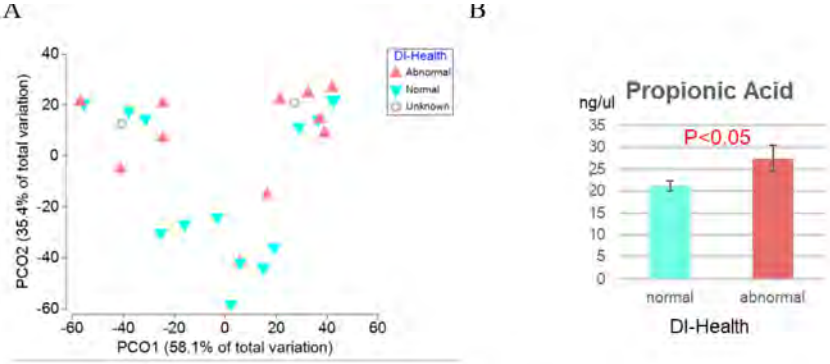

Figure 1. Differences in digesta-associated microbiota according to beta diversity PcoA (Bray-Curtis) (A) and blood plasma propionic acid levels (B) between fish with normal and abnormal distal intestine (DI) histological score.

(Continued on next page)

Results and Discussion

Results from the trial conducted to evaluate intestinal markers of large and small fish indicated that despite that the sampling was conducted in apparently healthy individuals a large number (46%) of the fish showed changes that ranged from mild to marked inflammation in the distal intestine. However, these changes were not associated with fish size. Metabolic differentiation between the two fish groups (large vs small) appeared to be pronounced within the blood plasma, but not in fecal samples. These differences in the plasma were mainly due to changes in metabolites related to glucose utilization and lipid and creatinine metabolism. Short chain fatty acid profiles between large and small fish were similar in fecal and plasma samples except for acetic acid that was significantly higher in the fecal content of large fish. Microbiota profiling showed high dominance of *Aliivibrio*, *Photobacterium* and unidentified member family *mycoplasmataceae* in both the mucosa and digesta. No significant differences in the diversity and overall bacterial structure were observed between large and small fish. Nonetheless, fish with signs of inflammation in the distal intestine had a different digesta-associated microbiota than the fish with a healthy intestine (Figure 1A). Fish with no sign of enteritis had also lower levels of propionic acid in the plasma (Figure 1B). Analyses of samples from the trials conducted in the second part of the project aiming to evaluate functional feeds are on-going and will be presented at the conference.

Conclusion

Signs of suboptimal gut health were apparent in both large and small fish. There were a number of differences in the metabolic profiles between the two groups of fish, possibly reflecting differences in growth rates and energy utilization. Gut bacterial communities were similar for the two groups of fish. Fish with signs of intestinal inflammation showed different bacterial communities, gene expression levels, and short chain fatty acid profiles compared to healthy individuals. The study has provided novel information on intestinal microbiota and metabolic profiles in Atlantic salmon, and identified certain metabolites and bacterial groups associated with high growth and intestinal inflammation that should be targeted in future studies.

EFFECTS OF DIETARY PHOSPHOLIPIDS ON EARLY STAGE ATLANTIC SALMON (*Salmo salar*) PERFORMANCE: A COMPARISON AMONGST PHOSPHOLIPID SOURCES

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Introduction

The present study aimed to confirm that additional phospholipids are needed in Atlantic Salmon (*Salmo salar*) fry diet for optimal growth. Furthermore, comparisons were made between two marine-based phospholipids- krill oil and marine phospholipids extracted from fishmeal. Marine-based ingredients are sustainable, but limited; therefore, it is important that many numerous, effective ingredient sources are available for the aquaculture industry.

Materials and Methods

First feeding fish were fed a fishmeal-based diet with 54% protein and 19% fat. Phospholipid (PL) levels in diets were adjusted by using fishmeals with differing levels of PL content and adding either krill oil or fish-based PL oil to levels of 1.3% (low PL control diet), 1.9% (control diet) and 3.5% (diets with added PL). The diets were balanced with regards to astaxanthin and EPA/DHA levels, so the focus was on PL source and level.

Atlantic Salmon fry (0.14g) were distributed in four 60x60cm tanks per diet, and mean water temperature was 13°C during the experiment. Diets were randomly distributed amongst tanks. Fish were sampled after 30 days and 60 days via individual weighing, while number of dead fish was recorded daily. At the termination of the trial, fish were preserved in order to perform intestine histology.

Results and Discussion

Growth of fry fed with diets with added krill oil and fish-based PL oil (3,5% PL) were significantly higher than both control diets with fry fed the lowest PL diet showing the lowest SGR. Final weight of fry fed marine-based PL were more than 12% (fish based PL) and 9% (krill oil) heavier than the control diet (Figure 1). Overall survival was high (more than 93%) and was significantly correlated with PL concentration. Previous research also shows Atlantic Salmon fish larvae (up to 2.5g) fed diets with 2.6, 3.2, 3.6, and 4.2% PL (added krill oil) had higher growth rates and lower mortality than the un-supplemented baseline diet of 1.5% PL (Taylor et al., 2015). Taylor et al. (2015) also found intestinal steatosis in early stage Atlantic Salmon fry when fed the baseline diet and no steatosis in any of the krill oil supplemented diets. Intestinal steatosis analysed via histology in the present study will also be discussed. The results verify that Atlantic salmon have a dietary requirement of PL in their early stage. These results correspond with previous work on multiple species of fish larvae and juveniles (Couteau et al., 1997; Tocher et al., 2008; Dapra et al., 2011; Taylor et al., 2015). Most importantly, growth of these fry increased with addition of marine-based PL, regardless of the marine-based source.

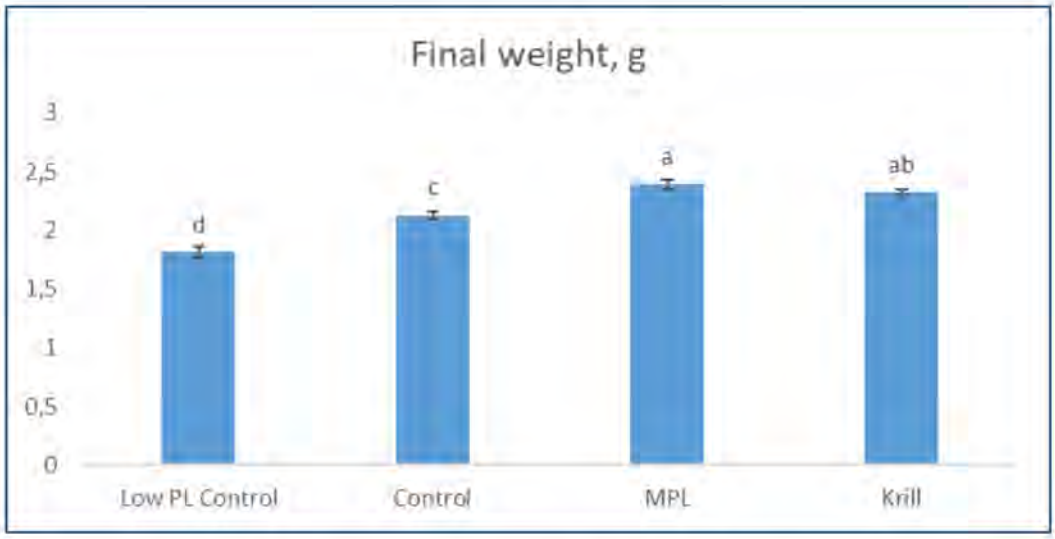

Fig. 1. Final weight of Atlantic Salmon first feeding fry (0.14g) after 60 days at 13°C. Diets were in quadruplicate and fish were individually weighed

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INTEGRATION OF FISHERIES AND AQUACULTURE INTO A MORE HOLISTIC SEAFOOD PRODUCTION SYSTEM

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Background

Food security has been highlighted as one of the major global challenges to 2050 with fisheries and aquaculture identified as key contributors to this challenge. Sustainable aquaculture needs to fit with and supplement sustainable fisheries. An increasingly large share of fish entering global markets derives from aquaculture; the world's fastest growing food production sector for more than four decades, making it roughly equal to global capture fishery production. The two production systems have important complementary roles in meeting rising demand for fish and other products (such as animal feed and fish oil) and enhancing incomes and nutrition. However, policies associated with the two are not always complementary. The aim of our two-year project was to strengthen Cefas knowledge in future integration of fisheries and aquaculture.

Key Objectives

1) Have literature of what is happening across Europe and perhaps globally with respect to fishermen diversifying/ integrating into aquaculture;
2) Have the results of a series of workshops with UK fishermen
3) Have information on any social and economic barriers to this happening;
4) Write at least two papers on results.

Methods

Year 1 An internal workshop including fisheries, aquaculture and socio-economist personnel was followed by a snowball literature search and subsequently a systematic literature for agreed search terms. The results were then sorted, selected and placed into Mendeley software before being allocated and read by project members.

In addition, a parallel grey literature search contacted key personnel involved in both aquaculture and fisheries and asked them for case studies where fishermen had
1. Switched from fishing into aquacultur
2. Combined fishery activities with aquaculture
3. Used aquaculture technology for their benefit within the capture fishe .

The case study results from the grey literature search were then tabulated by type of aquaculture and region.

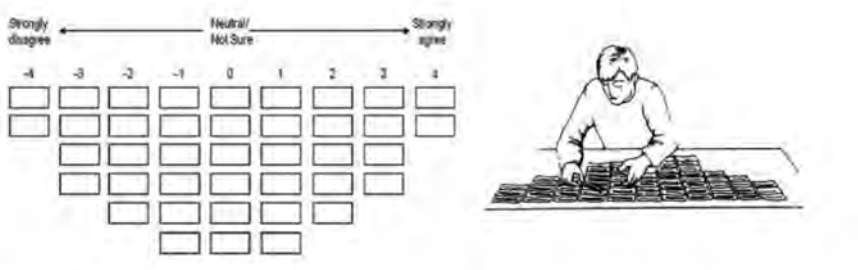

Figure 1 Q-Sort methodology.

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Year 2 The summarised findings from both literature searches were used within workshops with fishermen to canvas their views and identify any social, technical, financial, economic or other barriers that may affect greater integration of the two sectors. The workshops (which focussed on the UK inshore fleet) were organised within fishing ports with differing types of local fisher .

- Hastings (sole, plaice, skate, cod, bass)
- Mevagissey (mackerel, pollock, mullet, bass, pilchards, bream, flatfish, herrin
- Kingsbridge (shellfish

A fourth workshop in Bridlington resulted in no show due to a spell of calm weather.

The approach during the workshops was use of the Q-method. The fishermen were presented with 30 statements obtained from the literature reviews and preliminary interviews with 5 fishermen. The statements were grouped into five themes: Social, Economic, Training/skills, Regulatory/policy & Environmental. They were then asked to sort them into those they either strongly agreed or disagreed with.

The 30 statements were sorted into distribution (shaped as a normal distribution curve.) so that we could conduct principal component analysis that allowed us to identify common groupings of responses amongst the participants (that we call factors).

Results / Outcome

The year 1 literature search provided some emerging themes such as economies of scale, complementarity with marine livelihoods, social acceptance and individual skills and attributes. The grey literature search provided some excellent examples for all three categories from many parts of the UK coastline showing that to some extent the diversification and movement towards a more holistic seafood chain has already happened or is in progress. The year 2 workshops with fishermen demonstrated the benefit of using the Q methodology via successful engagement of the fishermen and the resulting data sets for analysis. Our analysis identified four factors within the inshore fishing communi .

Factor 1 (The professional fisherman Contra
Occupational pride and family tradition.
Factor 2 (The worrier) Pro
Concerned about economic aspects and having the relevant equipment.
Factor 3 (The thrill seeker) Contra
Value the enjoyment they receive from fishing – which they feel would not be the same with aquaculture (less "hunting" more "farming").
Factor 4 (The inexperienced) Pro
Have heard success stories but would need more information/training and skills to start up an aquaculture business.

Summary

To enable closer integration of fisheries and aquaculture into a more holistic seafood chain, policy teams and funding bodies could consider providing financial incentives for fishermen within factor 2 and training and information for those within factor 4. The project has identified socio-economic barriers and provided valuable background information for helping facilitate closer integration of fisheries and aquaculture. Two papers are in draft as a result of this project.

Acknowledgements

Thanks to Cefas for funding and providing resources for this project, to the aquaculture industry experts who contributed to the grey literature search and all the fishermen and their representatives in Mevagissey, Kingsbridge, Hastings and Bridlington that contributed to the workshops.

DETERMINING THE HERITABILITY OF GROWTH TRAITS IN CULTURED EUROPEAN LOBSTERS USING GENOMICS

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Since the early 2000s, there has been a steep rise in aquaculture production, a trend driven in part by technological advances and socio-economic factors such as food security and the stagnation or overexploitation of many wild fisheries. This has led to a common goal among aquaculture managers, scientists and governments, namely, to enhance productivity and maximise yield in species targeted for aquaculture. Growth is an economically important trait in aquaculture, and while it has been studied extensively in agriculture and some pioneering aquatic species (e.g. salmon) for selective breeding, the heritability of growth traits in species emerging as appealing aquaculture candidates, such as lobsters, is generally not well understood. The European lobster (*Homarus gammarus*) is a prized seafood product in the UK, where it is one of the highest value export species by weight (£14.06kg^{-1} on average in 2017 – more than triple that of cod), making it an extremely attractive option for aquaculture. However, although molecular techniques have been applied in the species (i.e. to elucidate genetic structure and paternity ecology in *H. gammarus* populations), the heritability of growth traits in this species remains unknown. A recently completed project (Lobster Grower 2) revealed that hatchery-reared lobsters cultured at sea in passive systems from an early post-larval stage show considerable variation in growth over the same time period (Fig. 1). In this study, therefore, we are conducting a genome-wide association study (GWAS) on these cultured lobsters, with the aim of identifying loci (if any) that are associated with growth traits. In particular, a genotyping-by-sequencing approach will be implemented on ~180 individuals to screen for single nucleotide polymorphisms (SNPs) that significantly associate with growth measurements. Furthermore, because the life history of each animal is known, this means that variation as a result of rearing batch or environmental conditions can be controlled for in the GWAS. The results of this study will provide novel information about the heritability of growth traits in the European lobster and will potentially provide a valuable tool to inform future development of aquaculture and selective breeding programmes in this species.

DOMESTICATION-ASSOCIATED LIPID METABOLISM REGULATION IN ATLANTIC SALMON

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Introduction

Atlantic salmon (*salmo salar*) has been domesticated in Norway since 1971 and has been selected for better growth and performance for 11 generations. This has resulted in a farmed strain with larger size and growth rate, but genetically and morphologically diverged from the wild populations (Fleming, Einum, 1997).

Long chain polyunsaturated fatty acids (LC-PUFA) such as docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6) are especially important for salmon. Farmed salmon are getting less LC-PUFA in the past 15 years since they are fed more vegetable oil (VO) rather than traditional fish oil (FO) in diets. The present strains of farmed salmon should be better adapted to VO diet compared to earlier generations, but whether this includes higher LC-PUFA synthesis abilities is unclear. Dietary phospholipids (PL) are more efficient at delivering LC-PUFA into fish body rather than triacylglycerols (Olsen, et al., 2014). The efficiency of utilizing dietary PL could be different between farmed and wild salmon.

The present study has compared the whole transcriptomic and fatty acid differences between farmed and wild salmon feeding either FO, VO or PL diets, aiming to identify 1) differences in transcriptomic state between farmed and wild salmon and 2) domestication associate changes in lipid metabolism regulation when feeding contrasting diets rich in either FO, VO or PL.

Materials and methods

The farmed and wild salmon were separated into 12 tanks (2 fish strains x 3 diet treatments x 2 replicate tanks) and started feeding at the same time. The fish was given three contrasting diets from start feeding up to 94 days, either a FO diet high in LC-PUFA, or a VO diet low in LC-PUFA, or a PL diet with high LC-PUFA in PL. Fish was sampled at day 0 (before start-feeding), day 65 and day 94 after first feeding. The pyloric caeca and liver tissues were sampled and used for RNAseq and fatty acid analysis. Differential expressed analysis was applied between wild and farmed salmon under any contrast of developmental stages and dietary treatments. The cut-off of differential expressed genes (DEGs) was q<0.05 and log2 fold change >1 or <-1 at any contrasts group.

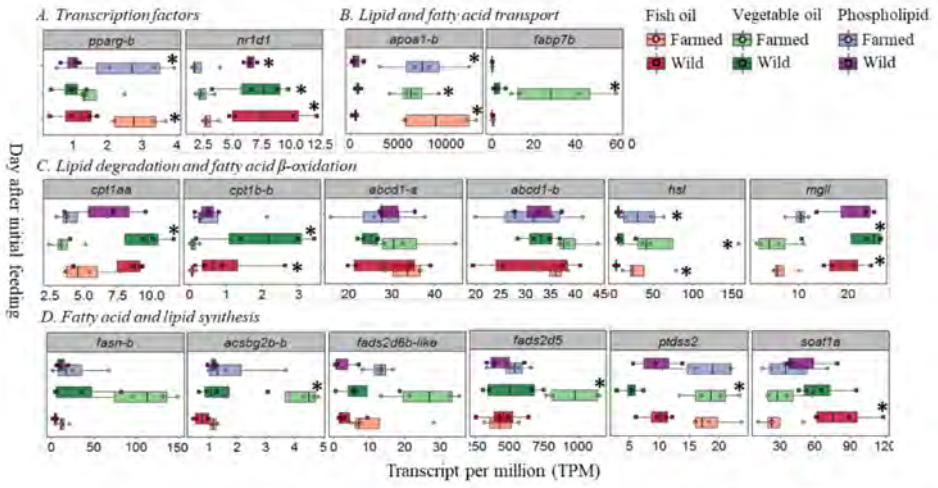

Figure 1 Expression of genes involved in lipid metabolism in liver of wild and farmed salmon at day 94 after feeding FO, VO and PL. Gene expression was shown as transcript per million (TPM) which was normalized by library size and mRNA length. Asterisk indicates DEGs between farmed and wild salmon under each strain and dietary treatment.

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Results and Discussions

After 93 days of feeding, the farmed salmon reached 4.5g, while wild salmon was 2.6g. No difference was found on the weight of farmed salmon when fed FO, VO and PL diets. However, the growth of wild fish seemed more sensitive to dietary lipids, with PL-fed fish having the best growth followed by FO-fed fish and then VO-fed fish. We found 4418 DEGs in pyloric caeca and 2137 DEGs in liver across all dietary treatments. DEGs with higher expression in farmed salmon were enriched energy metabolism, amino acid and lipid synthesis pathways, suggesting more efficient energy conversion via protein and lipids synthesis.

Several genes of lipid metabolism were differentially expressed between liver of farmed and wild salmon (Figure 1). Gene *apoa1-b* and *hsl* was always higher expressed in farmed salmon, suggesting that they have higher ability of lipid transport, lipid turnover and phosphatidylserine synthesis. Genes of transcriptional regulation (*srebp1a* and *pparg-b*), peroxisome oxidation (*abcd1a* and *abcd1b*), fatty acid transport (*fabp7b*) and fatty acids synthesis (*fasn-b*, *acsbg2b-b*, *fads2d6b-like* and *fads2d5*) was only differentially expressed between farmed and wild salmon when fed VO diet. This shows that farmed salmon is able to modify its fatty acid metabolism to compensate for the shortage of essential LC-PUFA in the diet, while such ability is very low in wild salmon. Meanwhile, higher content of DHA, ARA and EPA and lower content of 18:3n3 and 18:2n6 was found in phospholipid of liver in farmed salmon than wild only when they were given VO diet. This was likely an effect of *fads2d5* and *fads2d6b-like* genes (Monroig, et al., 2010).

Conclusion

The higher growth and development of farmed salmon was likely due to combination of various genetic advantages including better uptake, transport and biosynthesis of nutrients. Moreover, farmed salmon had higher regulatory plasticity when given a VO diet with less essential fatty acids, while such ability is very low in wild salmon.

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THE EFFECT OF WAVES ON VERTICAL DISTRIBUTION OF ATLANTIC SALMON (*Salmo salar*) IN AN EXPOSED SALMON CAGE

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Introduction

As salmon aquaculture expands into more exposed locations, the environmental conditions become more extreme with stronger currents and larger waves. Atlantic Salmon (*Salmo salar*) are well equipped to cope with both currents and waves, but the nature of salmon cages can compromise their ability to cope in poor conditions. This study considers to what extent salmon are able to use behavioural coping mechanisms when exposed to large waves.

Materials and methods

One salmon cage was monitored for the duration of two months in a wave exposed salmon farm on the Faroe Islands (Miðvágur farm location). Observations were timed to allow for monitoring during poor and good weather conditions (from late February to early April) and the chosen cage was located such that it had maximal exposure to waves. The salmon were approximately 2kg in size and night lights were located in the cage to prevent sexual maturation.

Two current profilers outside of the farm and a single point current meter inside the cage were used to monitor water conditions. Two echo sounders located underneath the cage and looking up were used to monitor vertical distribution as well as horizontal movement of salmon within the cage. Both echo sounders were located approximately half way between the centre and the edge of the cage, one at the side where waves entered the cage ("front") and one at the side where the waves exited the cage ("back"). The echo sounders looked through approximately 15 metres from the bottom of the cage to the water surface in a cone shaped beam and at the surface, they covered an area of approximately 11m Ø.

Four video cameras were in place to monitor detailed behaviour, such as shoaling and swimming effort.

Results

Wave data indicated that wave intensity or power decreased rapidly with depth with much lower water movement speeds at 10m depth than at the surface. The data coincided fairly well with the data from the single point current metre within the cage, so the wave data are fairly representative of conditions within the cage. At wind directions from 90 to 180 degrees, wave height correlated with wind speed, whereas there was no correlation at other wind directions. This allowed us to use wind as a proxy for wave height at the relevant directions.

Salmon had a general tendency to seek further down when waves grew taller. This effect was countered by wave period, with a longer period being associated with salmon moving higher up in the water column. This indicates that the large waves caused by bad weather (which typically have sort periods) are those that affect salmon most. This is corroborated by the fact that there is a similar response in vertical distribution of salmon when wind is used as a proxy for wave height while vertical distribution is differently, but not unaffected by wind speed at the other wind directions.

As relevant wind speed increased, tail beats per minute decreased and became less regular. Video footage indicates that the salmon switched from using their tails for propulsion to instead balancing within the waves and moving forward very slowly.

Salmon also showed a tendency to switch swimming direction as wind speed increased.

Discussion

The cage depth was only 15m at the deepest monitored point. Salmon were often seen at this depth, and it is unclear if they would have chosen to move further down if they had the option. Salmon behaviour differed at the front and back echo sounders. The cause of this difference is unclear, but if it is due to restriction due to cage deformation, then consideration of the effect of short period, large waves must be taken into account when designing cages for exposed locations.

ECOINTENSIFICATION OF AQUACULTURE: CAPTURE AND VALORISATION OF SLUDGE FROM AQUACULTURE WASTEWATER

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Aquaculture is expected to contribute to fill the foreseen gap in food supply towards 2050 and beyond. Hurdles to overcome to allow for an industrial growth of relevant scale relates to economic, environmental and social sustainability, and the solutions sought must be correspondingly multi-disciplinary.

GAIN (Green Aquaculture Intensification in Europe), a recently awarded Horizon 2020 project, aspire to deliver services and technologies to market within the project period to contribute to the ecointensification of European aquaculture production. Resource efficienc , reduced environmental impact, increased precision and valorisation throughout the production chain are all key elements in the approach to improve seafood self-proficiency and regional stabilit .

As intensificati n of aquaculture warrants reduced environmental impact, parts of our efforts are focused on capturing dissolved and particulate matter in wastewater from landbased aquaculture. Valorisation of the resulting products provides incentive for the industry to invest in environmental technologies, augmenting resource efficiency and facilitating circular economy.

A GAIN demonstration facility has been established at a Norwegian Atlantic salmon smolt producer, for evaluation, documentation and professional training on relevant substrate in industrial scale. Results from the characterisation of wastewater and sludge, and evaluation of different valorisation pathways will be presented. Technologies to be demonstrated are identified as having potential for superior performance, cost- and energy-efficienc , autonomous and resilient towards fluctuations in o ganic loading.

We will present some encountered obstacles for circularity and the most promising value chain perspectives.

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INTEGRATED MULTITROPHIC AQUACULTURE SYSTEM PAVES SUSTAINABLE WAY TO MITIGATE THE BIOEFFLUENT

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Introduction

Combination of fed aquaculture with inorganic extractive (and organic extractive aquiculture to generate balanced systems for environment remediation, economic stability and social acceptability is called Integrated Multitrophic Aquaculture System (IMTA). For example, farmers can combine fed species, like salmon, with seaweeds, suspension feeders, such as scallops and mussels, and or organic deposit feeders such as sea-cucumbers to increase production efficiency and decrease waste.

Fed aquaculture system:

Feed cost covers maximum input to the operation cost. But, Integrated Multitrophic Aquaculture System containing fish can provide dissolved and particulate nutrients to one to another species. Hence, combined species can effectively utilize the nutrients without lose. The quantity and form of these nutrients is depends on species size and feed formulation among other factors. Feed formulation provides perhaps the most obvious route for fish effluent modification for the extractive computes conversely other trades in the aqua feeds industry may impact fish effluent quality for an I A system.

Pros and Cons of Integrated Multitrophic Aquaculture System

Proper species selection to this system can mitigate the bio effluents through absorption of suspended and dissolved nutrients which minimize the nutrient discharge and feed cost. By adopting this technology can promote the economy and generate better profit. One system can produce three species with low feed input. Product diversification may office financial protection and decrease economic risks when price fluctuations occur if one of the alp is lost to dies or increment weather. Prevention or reduction of disease among farmed fish can be provided by certain seaweeds due to their antibacterial activity again fish pathogenic benefits. Potential for differentiation of the IMTA product through eco labelling or organic certification program

Higher investment is required to establish this technology. Integrated farming in open sea request a higher level of technological and engineering sophisticated and up forty investment. Difficulty in coordination, if practiced by means of different working in concern, it would require cools collaboration and coordination of management and production activities.

Integrated Multitrophic Aquaculture System is an emerging research carried out by several central institute of India. It will enhance the production without affecting environment. Recently this technology operated in freshwater which is called Freshwater Integrated Multitrophic Aquaculture System (FIMTA).

SALMON LICE (*Lepeophtheirus salmonis*) INDUCED MORTALITY OF ATLANTIC SALMON (*Salmo salar*) SURING POST-SMOLT MIGRATION IN NORWAY

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Introduction

Marine aquaculture in open-cages has resulted in diverse environmental challenges. In Norway, the sustainability of aquaculture is evaluated in 13 management areas along the coastline. The environmental impact with respect to salmon lice is evaluated in the "Traffic Light System", whereby a management area is determined as "green" if lice are estimated to cause < 10% mortality of wild salmonids, "yellow" if estimated mortality is 10-30%, and "red" if estimated mortality is > 30%. These colours refer to that the status: permit further expansion, no change, or production reduction, respectively (St. Meld. 16, 2014-2015).

For wild Atlantic salmon post-smolts that migrate from their rivers to the ocean, the first phase of their journey, in fjords and the coastal zone where aquaculture occurs, is considered as the critical and potentially population-regulatory phase with respect to exposure to lice (Torrisen et al.; 2013; Taranger et al.. 2015; Glover et al., 2017; Forseth et al., 2017). Therefore, to estimate lice-induced mortality of wild salmon post-smolts in Norway, we developed a novel model system predicting the lice infestation pressure level and mortality for over 400 rivers covering all of the Norwegian coastline. Thereafter, the modelled lice infestation pressure was used to estimate the mortality of Atlantic salmon post-smolt. These estimated mortalities were used to evaluate the sustainability of the aquaculture production in 13 management areas along the coastline, considering the influence of salmon lice

Material and methods

To estimate salmon lice induced mortality of wild salmon post-smolts migrating towards the ocean, we built a virtual post-smolt (VPS) migration model using knowledge of migration speed and timing from empirical observations. To include the lice infestation on the VPS, we combined data from a fine-scal distribution of lice in time and space from a previously published lice dispersion model (Johnsen et al., 2016; Sandvik et al., 2016, Myksvoll et al., 2018). The infestation level in the model was calibrated using measurements of lice infestation levels of wild salmon post-smolts that had been captured in the sea in 2015-2017. The river of origin for the wild post-smolts captured by trawling was determined by genetic assignment to provide the optimal dataset for model calibration (Harvey et al., submitted). Further, observations from trawl-captured post-smolts in 2018 was used to validate the model outputs.

Finally, by assuming tolerance of lice as given by Taranger et al. (2015), lice induced mortality was estimated for all salmon rivers along the Norwegian coastline using the model.

Results

The lice level on modelled post-smolts, using the tuned infestation level, was strongly correlated (spearman rank order correlation coefficient = 0.8, $p>0.01$) to the lice level observed on captured wild post-smolts. Mortality for the 401 salmon rivers was calculated and ranged from 0 to 83% among rivers in 2018. The highest mortality estimates were found for the rivers situated in the western part of the country and in particular in the inner part of the long fjords. The estimated mortality categorized in three classes with low (<10%), medium (10-30%) and high (>30%) mortality is presented in green, yellow and red respectively in Figure 1. Of the 401 rivers, 299 of them were found to be in the low category, 64 medium and 38 at high mortality levels for 2018.

Concluding remarks

Salmon lice induced mortality has a negative effect on wild Atlantic salmon post-smolts in areas of intensive salmonid aquaculture. The effect is now well documented (Skilbrei et al., 2013; Vollset et al.,2016), but can be hard to evaluate for all rivers in Norway and on an annual basis. Here, we developed a unique virtual post-smolt (VPS) model that estimates the lice induced mortality of out-migrating salmon post-smolts. The VPS model estimates presented here provides the regulatory authorities with an objective measure of the influence salmon lice have on wild fish in a time period, and in areas that the post-smolts are likely to migrate through.

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FJORD BASIN WATER EXCHANGE IN SILL NORWEGIAN FJORDS

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Introduction

The global change in climate is expected to have large ecological implications, although knowledge of such changes in fjords and coastal waters is sparse. A fjord is characterised by the presence of a shallow sill at its mouth that cuts off basin water below the sill depth from direct communication with the continental shelf and the ocean water.

The exchange of the basin water will only occur when the oceanic water at sill depth is denser than the fjord basin water. Between exchange episodes the fjord basin water density is continuously decreasing due to vertical mixing processes. The time intervals between exchanges of deep fjord water depends on several factors, like sill depth, conditions of water mass both inside and outside the sill, freshwater supply, tides and wind conditions (Inall et al., 2010). Generally, the fjord basin exchange occurs less frequently in fjords with shallow sills, and more frequently in northern Norway (annually) compared to southern Norway (8-9 years in between) (Sælen 1950, 1967, 1976, Gade 1973, Aure et al. 2007).

A shift in the environmental conditions provide beneficial conditions for some species, for other species it can degrade previously suitable habitats (Pörtner & Peck, 2010).To increase the knowledge of climatic alterations in the fjord basins, we have investigated how the environment is altered through warming, increased stratification and reduced water exchange resulting in less frequent re-oxygenation of the deep fjord basins in the last 50 years. This knowledge is essential for studying variability in and the potential changes to these ecosystems, and to evaluate the sustainability of aquaculture activity in sill fjords to obtain a predictable environmentally sustainable production that is demanded (St. Meld. 16, 2015).

Material and method

Information of the water column at the coast was analysed from the IMRs coastal stations observing salinity and temperature weekly going back to the late 1930s. Additional information of the coastal environment will was provided by analysis of the wedge between coastal and Atlantic water, mapped from the sections marked in blue in Figure 1, mapped 4-6 times a year. Alterations in the structure of the water masses during the latter 50 years was analysed. The wind close to surface was analysed for temporal trends and set in context to the vertical depth of the interface between Atlantic and Coastal water.
Fjord basin exchange episodes was recognised in time series of salinity and oxygen from the historical fjord surveys containing observations of temperature, salinity, oxygen and more from 139 stations, in 32 fjords from south to north from the time period 1975 – 1997. The monitoring of fjords continued less systematically after this at the western part of Norway. However, in Northern Norway the fjords have been monitored for the latter 100 years (Mankettikkara R. 2013). This long term the dataset made it possible to evaluate the long-term levels, trends, and fluctuations in temperature, salinity, and oxygen the fjords basins.

Results

The coastal station observations showed that there has been an environmental change since the 1990s. The temperature in the upper 200 m has increased in the latter 30 years at all stations, with the increase largest close to the surface at the southern stations (Figure 1). At Utsira (59.5°N) the average temperature at 20 m depth from 2010-2015 was 10°C compared to under 8°C during the 1985-1990 period. The observations from the coastal stations clearly show that the water column has a stronger vertical temperature gradient in the southern station (Utsira) compared to the northern station (Ingøy, 71°N) (Figure 1).

At the southern station (Utsira) the upper water salinity has decreased with increasing temperatures, resulting in less dense water (not shown). During the last 30 years, the density at 20 m depth at Utsira has decreased one sigma unit, increasing the stability of the water column. In contrast, the density at Ingøy has stayed relatively constant.

The alterations in the coastal environment was reflected in the fjord basins with respect to temperature (not shown). It is hypothesised that the increased stratification at the coast will impact the fjord basin exchange rate in sill fjords, however this remains undocumented.

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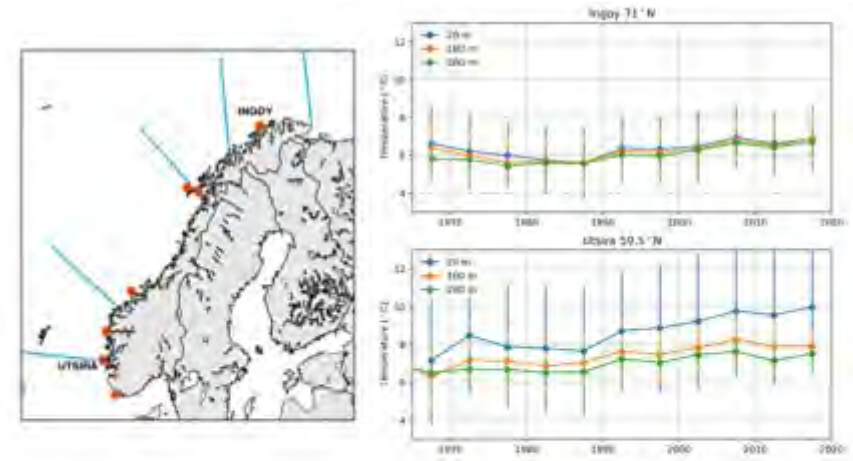

Figure 1: Map of Norway with its 25 000 km long coastline (left panel). The hydrodynamic properties are monitored in sections along the coastline marked by blue line and fixed stations marked by orange dots.
5-year averaged temperature +/- standard deviation from 20, 100 and 200 m depth from Ingøy 71 °N (upper right panel) and Utsira 59.5 °N (lower right panel).

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SHELTERFISH
NEW TOOLS TO IMPROVE FISH HEALTH AND ENVIRONMENT IN ORGANIC AQUACULTURE

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Rainbow trout farming and in particular organic rainbow trout farms are critically challenged by the relatively high prevalence of skin/gill infections caused by various pathogens, especially the parasite Costia (*Ichthyobodo necator*) and amoebae, which are ultimately lethal for fry/smaller fish. In addition, a *Midichloria*-like bacterium causes the non-lethal skin disease Red Mark Syndrome (RMS), which results in downgrading/rejection of up to 30% of the fish when placed on the market. Treatment by use of antibiotics/parasitics/ auxiliary compounds is only possible to a limited extent in organic trout production. Hence, solutions to prevent and/or treat costia, amoebae and RMS are urgently needed, not only to secure production of organic rainbow trout in Denmark, but also enable a larger and more cost efficient production with high animal welfare and minimal environmental impact.

ShelterFish will focus on solutions addressing the interactions between fish - pathogens – farming environment and water quality; including 1) Test of artificial shelters (shade) to enrich environmental conditions and lower stress; 2) Test of biological herb extracts and a new bacterial surfactant to minimize gill/skin parasite infections; 3) Test of induced immunity to Red Mark Syndrome (RMS) by early exposure; and 4) Test of tools to reduce organic matter load in organic trout farms and hereby improve water quality, fish health/welfare

TRANSPORT STRESS IN BALLAN WRASSE (*Labrus bergylta*) AND LUMPFISH (*Cyclopterus lumpus*)

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Introduction

Ballan wrasse and lumpfish produced in land-based systems are used in sea cages to reduce sea lice infestation. The transport to sea by truck or boat and physical disturbances associated with loading, transport, and discharge will cause stress of varying degree, possibly leading to long-term health impairment. Hence, it is important to understand the physiological response to transport to ensure acceptable fish welfare and the fish s ability to adapt to the new environment in salmon cages.

Materials and methods

Primary stress response (plasma cortisol) from four commercial transports of Ballan wrasse and lumpfish, was analyzed and discussed in relation to transport conditions. For each species, two transports were by truck and two by boat. Blood from five fish in each transport groups were sampled before transport and after primary transport. For two groups of Ballan wrasse with a two-step transport (truck + boat) fish were also sampled after the secondary transport. In addition, five fish from each groups were sampled 7-10 days after transfer to sea cages. Good water quality condition (89-124 % oxygen and pH 8,8 – 7,0) was reported for all transports. A two-way nested ANOVA (group nested in time) followed by SNK-test ($\alpha = 0,05$) was used to test changes of plasma cortisol over time. A one-way ANOVA was used to test differences between groups at each sampling time.

Results

Lumpfish showed a significant increase in plasm cortisol after transport for all groups ($p<0,001$), with the highest increase in fish transported by boat (T3 and T4, transport time 6 and 5 h) compared to truck (T1 and T2, transport time 6 and 5 h). 7-10 days after transfer to sea T2 and T3 returned to levels slightly higher than before transport, while T1 and T4 returned to stress levels significantly lower than before transport ($p<0,05$)

With the exception of T1, there was an increase in plasma cortisol after primary transport compared to pre-transport levels and a stabilization (T4) or slight decrease (T3) after the secondary transport. T1 had the highest cortisol level before transport compared to after transport and 7-10 days in sea. For the other groups (T2-T4), the fish did not regulate plasma cortisol to pre-transport levels after 7-10 days in sea. T2 showed an exceptionally high plasma cortisol in sea cages.

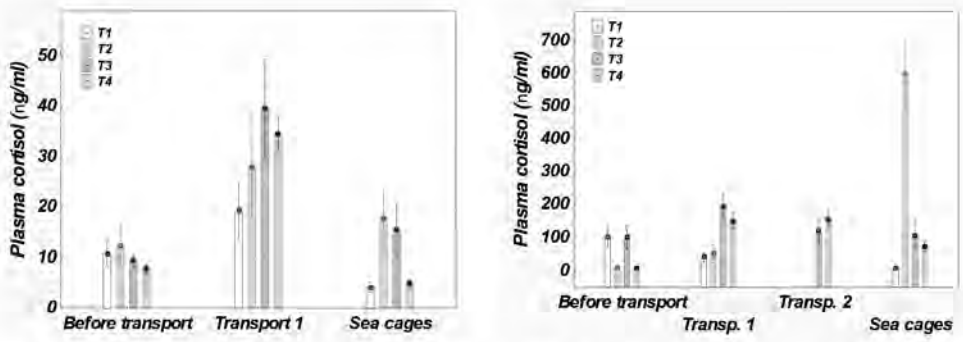

Fig. 1. Development of plasma cortisol (ng/ml) in four transport groups (T1 – T4) of lumpfish (left) and Ballan wrasse (right) before and after transport, and 7-10 days after discharge to sea cages. Vertical lines on bars indicate standard error of mean (SEM). Transport 1 = primary transport, Transport 2 = secondary transport.

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Discussion and conclusion

In general, the level of transport stress in lumpfish was lower than previous reported from 15 commercial transports of lumpfish, mainly truck transports (Remen et al. 2017), indicating recent improvements in transport practice and conditions. The tendency of higher stress after boat transport of lumpfish compared to truck transport was most likely a result of the shorter transportation time by boat, as increased transport time under good conditions increase time for recovery after handling-stress (Iversen et al. 2005). Independent of transport conditions the seawater conditions or site characteristics seemed to influence the acclimation of the lumpfish after discharged to sea cages. The higher stress levels in T2 and T3 7-10 days after transport compared to T1 and T4 may be associated with the higher water current at the sites for release.
This is the first recordings of stress during transport of Ballan wrasse. The low pre-transport levels are similar to (T4) or slightly higher (T3) than resting levels previously measured in tank experiments with Ballan wrasse (Epmark et al. 2017). The cortisol levels in T3 and T4 after primary and secondary transport are similar to 1-hour pre-stress levels after exposing Ballan wrasse for 20 min. tank draining (Iversen 2016). Based on the elevated levels of plasma cortisol in T3 and T4 compared to T1 and T2 after the primary transport, truck transport seemed more stressful to Ballan wrasse compared to boat transport, but was reduced following a 16 hour secondary transport by boat (T3). The pre-transport sampling from T1 was, unlike the other groups, done after the water level in the tank was drained (lowered) to prepare for pumping fis to transport tanks. Hence, the elevated stress level was probably a response to crowding, indicating that a significant part of the stress associated with transport of Ballan wrasse is related to pre transport procedures. The exceptionally high stress level in T2 after transferred to sea, was followed by an accumulated mortality of 19% after 7 days in sea. This suggests that high seawater temperatures (16-18 ˚C) under stressful conditions caused the elevated mortality.

This investigation of stress response of lumpfish and Ballan wrasse to different transport conditions indicate generally good welfare during transport, but that special considerations should be payed to pre-transport conditions and handling, and reduced environmental challenges after transfer to sea cages.

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DOSE DEPENDENT EFFECTS OF CARP PITUITARY EXTRACT ON INDUCTION OF OVARIAN DEVELOPMENT IN EUROPEAN EEL, *Anguilla anguilla*

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Introduction

The aquaculture production of European eel has declined due to stock depletion and low abundance of juvenile glass eels used as fry in eel farming. Captive breeding and larval rearing to the glass eel stage would overcome this obstacle. Yet, eels do not reproduce in captivity due to a dopaminergic inhibition of the follicle stimulating hormone (FSH) and luteinizing hormone (LH) at the brain-pituitary level, preventing sexual maturation (Vidal et al., 2004). However, weekly hormonal treatment with pituitary extract from salmon (SPE) or carp (CPE) to stimulate ovarian development can overcome these maturational barriers (Mylonas et al., 2010). Such breeding protocols for anguillid species were established using different treatment schemes for SPE and CPE (Bezdenezhnykh & Prokhorchik, 1988; Ohta et al., 1997). Thus for European eel, breeding protocols generally use a constant dose scheme for SPE, while treatment with CPE often use a stepwise increasing scheme starting at a low dose. Nevertheless, the efficacy of these treatment schemes needs to be validated. In the present study, we investigated differences in egg quality and reproduction success when using a constant CPE dose versus an increasing dose, while considering the underlying molecular mechanisms involved in the progression of vitellogenesis.

Materials and methods

CPE was administrated weekly to farmed broodstock females (n=87) to artificially induce maturation. Two treatments were compared: 1) Constant dose of 20 mg kg^{-1} of CPE until ovulation (n=38) and 2) Increasing dose of CPE (i.e., 5, 10, and 15 mg kg^{-1} for nine weeks, three weeks per dose, followed by 20mg kg^{-1} until ovulation; n=41). Morphometry (i.e., gonado-somatic index (GSI) and liver index (LI)) and histology was used to follow the development and growth of ovaries and livers for the two treatments. Females were dissected at day 0 (n=8), and after 6, 9, and 12 weeks eels (n=8 per treatment) as well as after spawning (remaining females). Organs were weighed and tissue samples were obtained for histology and molecular analyses. Ovarian tissues were stained with periodic acid Schiff's (PAS) hematoxylin and metanil yellow (Quintero-Hunter, Grier & Muscato, 1991). Oocytes were categorized into developmental stages within the phases pre-vitellogenesis (LV3 and LV4), vitellogenesis (VT1-3), and final maturation and hydration (HYD) (da Silva et al. 2016). Evaluation of ovarian maturation was based on the most advanced oocyte stage present in the sample. In addition, expression of ovarian gonadotropin receptors (*fshr, lhr1* and *lhr2*), estrogen receptors (*erα, erβ1, erβ2, gper1* and *gper2*), androgen receptors (*aa-ara* and *ara-arb*), and vitellogenin genes (*vtg1* and *vtg2*) in ovaries and livers was analyzed through real-time polymerase chain reaction (qPCR) and compared between treatments and over time. The remaining females (n=31) were spawned and ovulated eggs were fertilized. Spawning success (i.e., successful ovulation of eggs), fertilization-, hatch-, and larval deformity rate were calculated for the treatments to evaluate egg quality.

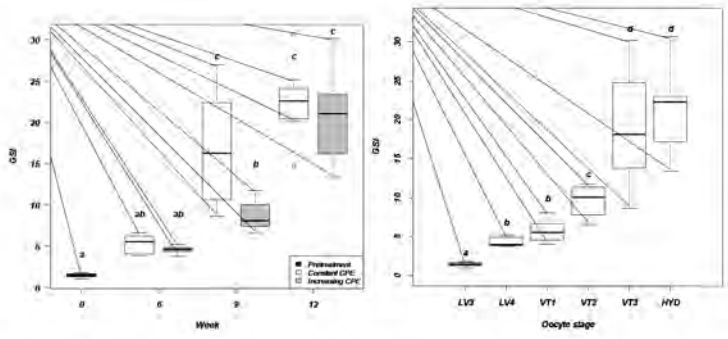

Fig. 1. Boxplots of gonado-somatic index (GSI) according to treatment and sampling week (left) and oocyte developmental stage (right). The top and bottom edges of the box represents 75th and 25th percentile data; line within the box medians; whiskers 90th and 10th percentile data; circles outliers. Boxes labelled with the same letter are not significantly different (P>0.05).

(Continued on next page)

Results and discussion

The progression of ovarian development differed between treatments with a significant difference in GSI at week 9 (Fig. 1). The constant dose group had accelerated ovarian growth compared to the increasing dose group. Nevertheless, the latter caught up to the former when the full dose of CPE was administered at 10 week, eliminating any detectable difference between the treatments at week 12, when all fish had reached final maturation. Aligning ovarian development independent of treatment revealed that development based on the furthest advanced oocyte stage was similar (Fig. 1) with stages being significantly different between treatments only at week 9. Here, the constant dose group was at the end of vitellogenesis approaching final maturation, while the increasing dose group was still in early vitellogenesis. Nevertheless, both groups were in the final maturation phase at week 12. Results of the gene expression analysis support these results and substantiate our understanding of mechanisms involved. Lastly, spawning success and egg quality was similar among treatments.

Conclusion

The results indicate the hormones FSH and LH in the pituitary extract do not correspond to the requirements at the specific developmental phases and may either be in excess or demand resulting in a difference in the speed of ovarian development for the two treatments. The spawning results, on the other hand, did not show any difference between the two treatments regarding spawning success and egg quality.

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MICROALGAL MIXOTROPHY AS A SOURCE OF FEED FOR *Mytilus edulis* LARVAE

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Mytilus edulis aquaculture represents an important section of the UK and EU economies. Current production mechanisms rely on the supply of wild larvae to be settled upon longlines and subsequently on-grown. This leaves larval collect and production of adult blue mussels reliant upon larval supply and so is one of a number of limiting factors in upscaling production. In Scotland 7000 tonnes of mussels were produced in 2015 and mussel growers aim to double production by 2020. To do so, the limiting factor of larval supply must be overcome. One method of doing this is the creation of a *M. edulis* larval hatchery. The costs associated with microalgal culture, for use as feed, in a larval hatchery are currently around 40% of total costs. As a result the creation of a larval hatchery is not economically viable (Hickman, 1992). This is due to the requirement for live microalgae, mussel larvae do not feed on dead cells. This study aims to address this limiting factor through use of mixotrophically cultured microalgae as a feed source.

Microalgae are classically considered to be photoautotrophic and are cultured in the light, in seawater with additional nitrate and phosphate. Mixotrophy is the ability of a microalga to uptake organic nutrients, such as carbon or nitrogen, in addition to, or as an alternative to, photoautotrophic fixation of carbon. This ability to be cultured mixotrophically is considered to be ubiquitous in the marine environment (Borowitzka, 2013) and has a number of advantages. Mixotrophic culture often results in greater cell numbers and dry weight, as well as an increased culture stability (Morales-Sánchez & Martinez-Rodriguez, 2015). Furthermore, dependent upon the method of culture and the carbon source utilised, the biochemical profile can be tailored to create a "designer" feed.

Over the course of three feeding trials this study takes current "industry standard" microalgal species and compares larval growth and survival to mixotrophic "designer" microalgal feeds, then subsequently optimises the diet used. The "designer" feed can be considered to be as effective as the standard benchmark feed and subsequent optimisation shows that a diet that is tailored towards the development performs the most effectively in terms of larval growth. Mixotrophic culture requires an additional organic carbon source which has a significant cost implication. Therefore using first order modelling, the economics of use of mixotrophy as an alternative to photoautotrophic culture reveals a more complex picture in terms of the optimal methods of culture. Culture optimization, a move to semi-continuous culture and alternative, low cost carbon sources potentially reveal a way forwards.

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SEASONAL VARIATIONS IN THE ABUNDANCE OF *Vibrio* sp. IN THE MARINE CAGE FARM AREA OF MALI STON BAY, EASTERN ADRIATIC

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Introduction

Vibriosis is one of the most prevalent bacterial fish diseases in the Adriatic region (Thompson et al., 2003). It is caused by certain species and strains of heterotrophic bacteria from the genus *Vibrio.* These bacteria are indigenous to the marine environment, but different environmental factors regulate their growth and distribution (Asplund, 2013). The aim of this study was to investigate seasonal variations of *Vibrio* sp. in marine cage areas of the Bay of Mali Ston, and to determine the influence of di ferent environmental parameters on their growth.

Materials and Methods

The sampling was carried out at the fish cage farm located in the Kabli, outer part of the Mali Ston Bay (Eastern Adriatic) and at the control site situated 2 km away from the farm in Mali Ston Channel. Samples were collected quarterly during 2017. Temperature, dissolved oxygen, pH, salinity and total dissolved solids were recorded at each sampling site by Metler probe in situ, while water samples for microbiology, total nitrogen, phosphorus and particulate matter analyses were collected using 8L Niskin bottle at four depths (0.5m, 5m and 10m, and 0.5m above the sea bottom). Water samples were further processed and analyzed in the laboratory. For *Vibrio* enumeration, serial dilutions of the water were applied onto selective medium Difco™ TCBS (BD) agar and incubated at 22°C for 3-5 days. Bacterial isolates were further processed on MALDI TOF MS for identification. The results of the *Vibrio* counts were expressed as colony forming units (cfu)/mL. Seston quantity parameters (total-TPM, organic - POM and inorganic particulate matter -PIM) were analyzed according to the method described by Peterson et al. (2003). Samples for total phosphorus and nitrogen determination were digested using a Hach DRB200 reactor. Total phosphorus was determined on Hach dr 6000 spectrophotometer by using Hach kits (PhosVer 3 Acigd Persulfate Digestion procedure), while total nitrogen was determined on a Hach colorimeter DR/870 by using a Hach kit (TNT Persulfate Digestion method). Person's correlation coefficients were calculated to evaluate the relationship between the *Vibrio* sp. count and each environmental variable.

Results and Discussion

The highest mean *Vibrio* sp. counts in the water column were found at both sites in September (170 cfu/mL at the fish farm, and 412 cfu/mL at control site), while the lowest counts were in samples from June (Table 1). The abundance of these bacteria showed the strongest positive correlation with total nitrogen, TPM and salinity (Table 2). According to MALDI TOF MS identification, *Vibrio pomeroyi* and *Aliivibrio* (*Vibrio*) *fischeri* were the dominant *Vibrio* species in the water column at fish farm site, while at control site *Vibrio pomeroyi*, *Vibrio harveyi* and *Vibrio fortis* prevailed. Comparing the results from both sampling sites, it seems that the fish farm is not the main source of *Vibrio* sp., and does not present the main source of nutrients promoting their growth.

Acknowledgements

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Table 1. Mean *Vibrio* count (cfu/mL) and measured water quality parameters per each sampling at two sampling sites during 2017

	Vibrio sp. (22°C) CFU/ml	TPM (g/L)	POM (g/L)	Temperature (°C)	Salinity (ppt)	TDS (mg/L)	pH	O_2 (%)	N (mg/L)	P (mg/L)
FISH FARM										
Feb 2017	60,5	0,00468	0,0014	12,01	33,75	26,05	7,83	101,63	0,60	0,01
Jun 2017	13,25	0,00641	0,0026	21,30	33,68	25,70	7,92	104,65	0,68	0,05
Sept 2017	170	0,00868	0,0016	21,35	34,65	26,33	7,93	100,85	0,83	0,02
Nov 2017	35	0,00376	0,0016	15,25	34,53	26,58	7,79	98,03	0,68	0,07
CONTROL										
Feb 2017	26,5	0,0035	0,0014	12,25	34,28	26,48	8,09	101,63	0,30	0,01
Jun 2017	14,75	0,0051	0,0017	22,25	33,40	25,50	7,83	105,83	0,60	0,03
Sept 2017	412,75	0,0060	0,0017	21,53	34,85	26,48	7,91	102,90	1,00	0,02
Nov 2017	75	0,0050	0,0013	15,70	34,18	26,20	7,95	97,53	0,58	0,06

Table 2. Pearson's correlation coefficient (r) between measured environmental parameters and *Vibrio* sp. count at two sampling sites during 2017

Environmental variable	Pearsons' corelation coefficient (r)	
	Fish farm	Control site
TPM	0,74	0,72
POM	-0,50	0,39
Temperature	0,31	0,45
Salinity	0,64	0,80
TDS	0,37	0,50
pH	0,44	-0,20
O_2	-0,22	0,05
N	0,80	0,89
P	-0,52	-0,14

QRT-PCR ANALYSIS OF *Parvicapsula sp.* IN DIFFERENT ORGANS FROM EMACIATED OLIVE FLOUNDER, *Paralichthys olivaceus* IN KOREA

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Introduction

In Korea, the emaciation disease of olive flounder (*Paralichthys olivaceus*) are causing serious economic losses on the aquaculture industry every year. Fishes that were infected by this illness showed symptoms such as darkening body colors, decreases in body weight, and bleeding. The causative agent of this disease was identified as *Parvicapsula* sp. (Kim et al., 2015). In this study, we analyzed the copy number of pathogens in different organs using the real-time PCR.

Materials and methods

Olive flounders with emaciation symptoms, such as the darkening of the body color, the emaciation of the abdomen and the loss of weight (length 19.8±2 cm, weight 70±5 g) from 2 farms in Jeju Island of Korea were selected as the experimental group, while healthy olive flounders (length 20±3 cm, weight 90±10 g) from 1 farm without any history of emaciation disease were selected as the control group. The kidney, intestine, spleen, brain and liver of each fish were extracted, and signs of infection were checked using PCR (Kim et al., 2015). Real-time qPCR was performed using a LightCycler^{R} Nano Instrument for real-time PCR (Roche, USA) and the marker used was SYBR Green I dye (Faststart Essential DNA Green Master, Roche, USA). PCR reactions were performed in 20 ↑ volumes consisting of 0.5 ↑ of each RTEM-F(5'-GGATACATGTTGGTCGAC-3'), RTEM-R(5'-CGAATCGCATTAATTATC-3'), 10 ↑ of SYBR Green I master mix, 2 ↑ of DNA and 7 ↑ of Deionized sterile distilled water.

Results and discussion

Real-time PCR was performed using DNA extracted directly from the internal organs (kidney, intestine, spleen, brain and liver) of emaciated fishes (farm 1 and 2) and normal fish (farm 3). The highest DNA copy number (1.7×10^{7} copies/mg tissue) was detected in kidney of the emaciated olive flounder of farm 2, while the DNA copy number was below detection limit in all the organs of the olive flounder of farm 1. There was not positive result in all organs isolated from olive flounder of farm 3. PCR and histopathological analysis were also performed using the same specimen and showed same results as those by real-time PCR.

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DEVELOPMENT OF ANTIBIOTIC RESISTANCES IN BACTERIA ISOLATED FROM ORNAMENTAL FISH AND FISH FOR FOOD PRODUCTION BETWEEN 2005 AND 2017

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Introduction

Antibiotic resistance is one of the biggest threats to human and animal health. Antibiotic resistances can occur naturally, but misuse of antibiotics is accelerating the process. As resistances against antibiotics occur in bacteria isolated from fish as well, it is recommendable to perform a sensitivity test for the detected bacteria before treatment of fish.

Materials and methods

All bacteria isolated from diagnostic samples between 2005 and 2017 at the Fish Disease Research Unit were analysed for resistance against antibiotic substances. In total 19 substances were tested, whereas some substances were tested during the whole period of 13 years and others were tested only for two to 12 years.

The antimicrobial susceptibilities of bacterial isolates were determined by the use of the disk diffusion method. Bacterial isolates were inoculated on blood agar plates. Antibiotic disks containing amoxicillin (10 µg), ampicillin (10 µg) chloramphenicol (30 µg), chlortetracycline (30 µg), colistin (50 µg), doxycyclin (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), florfenicol (30 µg), flumequine (30 µg), furazolidone (100 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (10 µg), oxolinic acid (10 µg), oxytetracycline (30 µg), trimethoprim/sulfonamide (25 µg) tulathromycin (30 µg), or tylosin (30 µg) were used according to the manufacturer's instructions. Inhibition zone diameters were measured and evaluated inspired by CSLI if possible. According to the diameter of the inhibition zone, the results were given in resistant (R), intermediate (I) and sensitive (S).

Results and discussion

The results show that the detected bacteria showed mainly resistances against amoxicillin, ampicillin, neomycin, oxolinic acid and tylosin. Yet, over the last 13 years, resistances of bacteria against antibiotic agents were decreasing in total and for most substances the resistance situation improved. Only for single substances, like trimethoprim/sulphonamide, the number of resistant bacteria increased.

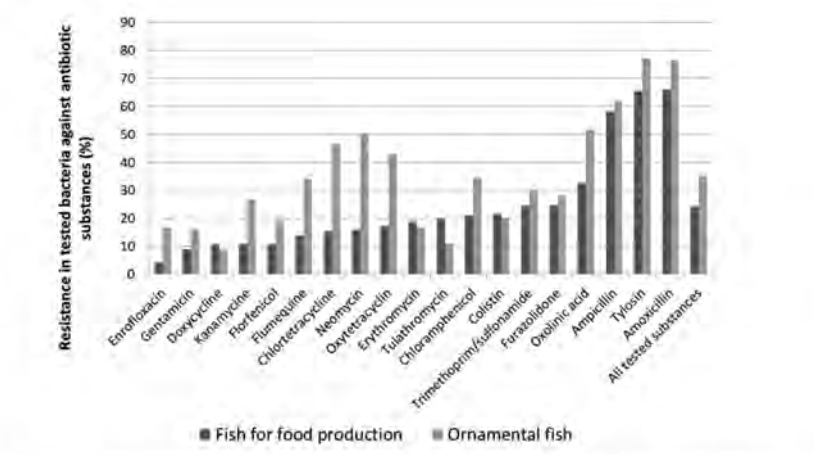

Fig 1. Resistances in tested bacteria isolated from fish for food production and from ornamental fish against 19 antibiotic substances.

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Differences were seen in bacteria isolated from fish from different keeping units. Especially in bacteria isolated from ornamental fish at wholesaler facilities more resistances were detected, whereas in bacteria isolated from fish for human consumption fewer resistances were found. Differences were also detected in the resistances of specific bacterial species. Especially Flavobacteria, some species of motile Aeromonads and Pseudomonads showed frequently resistances against a number of antibiotic substances.

Conclusion

Between 2005 and 2017 resistances against antibiotic substances of bacteria isolated from fish were decreasing in general. Only for single substances, like trimethoprim / sulfonamide, the number of resistant bacteria increased Bacteria isolated from ornamental fish showed more often resistances against antibiotic substances compared to bacteria isolated from fish for food production.

INFLUENCE OF A NANOFILTRATION - REACTOR ON THE BACTERIAL MICROFLORA AND ON *Ichthyophthirius multifiliis* THERONTS IN RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

Recirculating aquaculture systems offer the opportunity to keep high numbers of fish without the need of high amounts of fresh water due to recirculation and filtration of tank water. Problems can occur if the amount of nitrate, bacteria or parasites in the water increases. To maintain a good water quality, nanofiltration of the water is described as one method to reduce the amount of bacteria in the water and to keep the chemical water parameters in an optimal range.

Materials and methods

We tested nanofiltration reactors with integrated denitrificatio membranes in four different recirculation aquaculture facilities. One system in each facility was run with a membrane denitrification reactor (MDR) and as control identical systems without reactor were used. The aquaculture facilities were stocked either with carp, sturgeons, golden orfes or rainbow trout and the systems were run at a water temperature between 20 and 25°C. In three facilities the bacterial microflora was analysed in tank water, biofilms of tanks and partly also of the filters and on skin and gills of fish kept in the systems. In one of the systems cortisol measurements in the water and in the blood of fish were performed to determine the stress level of the animals in the system. In the fourth system fish were examined for infection with the parasite *Ichthyophthirius multifiliis* and the effectivity of nanofiltration against the theronts of this ciliate was determined.

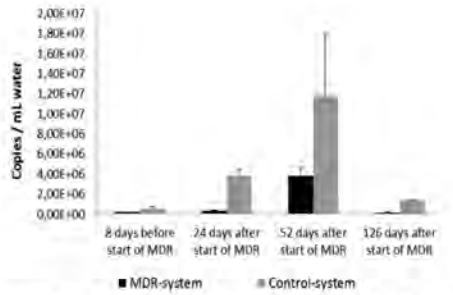

Fig 1. Total number of bacteria in the water of RAS with and without the MDR. Shown are the total bacteria numbers measured by the copy number of the 16S rDNA using quantitative PCR Shown are the mean values and standard deviations from two sampling points per system and time-point.

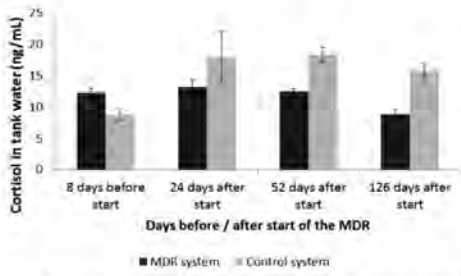

Fig 2. Amount of cortisol (ng/mL) in the tank water from the MDR system and the control system. Shown are the mean values and standard deviations from three measurements per time-point and system.

(Continued on next page)

Results and discussion

Overall it could be shown that the reactor with a filtrating membrane could decrease the total amount of bacteria in the tank water of a recirculating aquaculture system (Jung-Schroers et al, 2019). Also the amount of bacteria on the gills of fish was decreased in the systems with installed reactor.

The diversity of bacteria was higher in the systems with installed reactor and the fish in this system seemed to have less stress.

A reduction of stages of *Ichthyophthirius multifiliis* could also be detected in a system with installed reactor. One challenge was the increasing water temperature in systems with installed reactor and the operation of the reactor itself is time consuming.

Conclusion

The usage of a reactor with filtrating-membrane can have a positive influence on fish health and welfare (Boley et al 2017, Jung-Schroers et al. 2019).

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RECOMMENDATIONS FOR STUNNING AND KILLING OF COMMON CARP (*Cyprinus carpio*) AND RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

In Germany stunning of animals in general is regulated in a directive and special requirements for stunning of fish are given. For all fish species these regulations prescribe stunning by percussion or electric current. Only for salmonids, additionally stunning by CO_2 exposure in a water bath is authorised. Precise instructions on how these methods should be used are not available. It is known that stunning of special fish species, like common carp, is difficult by using the authorised methods.

Materials and methods

In total 24 fish farms throughout Germany were visited and the whole process of stunning and slaughtering was evaluated. Some of these farms were slaughtering carp and trout; others were slaughtering only one of these species. Therefore the process of stunning and killing was documented 22 times in aquaculture farms for trout and 17 times in farms for carp. If possible catching of fish from the ponds was documented. In all farms, keeping fish in special tanks before slaughter and the transport of fish from these tanks to the stunning site was evaluated. Also in all farms stunning and killing of fish was documented.
An evaluation score with 93 points was established which includes all measured parameters and data about the process. Different evaluation factors were multiplied with scores assessing their importance. By combining the scores from different aspects of the harvesting process, an overall evaluation score was calculated. With the overall evaluation score a gradual classification and an assessment of different techniques and methods for stunning and killing of rainbow trout and carp was possible.

Results and discussion

Most of the rainbow trout were stunned by electric current, followed by percussion. In two farms trout were stunned by a combination of both and in one farm CO_2 was used for stunning. In contrast, in most of the documented cases carp were stunned by a combination of electric current and percussion. Stunning by percussion was used in 3 cases and stunning by electric current was uses in four cases. Most of the rainbow trout were successfully stunned by all evaluated methods. Only around 60% of carp were successfully stunned by electric current and around 80% of carp were successfully stunned by percussion (Retter et al, 2018). Only a combination of both methods was leading to successfully stunned carp. With the collected data it could be shown, that for stunning by electric current, the conductibility of the water, the stunning time and the size and shape of the stunning tank can have an important influence of the success of stunning. Short stunning times of less than 2 minutes and water conductibility lower than 500µS/cm or higher than 1000µS/cm were leading to problems with stunning of especially carp.

Conclusion

In conclusion, a combination of stunning by electric current with adequate conductibility and adequate stunning time and by percussion seems to be the best method for stunning of rainbow trout and carp.

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EPIDEMIOLOGICAL STUDY ON THE OCCURRENCE AND THE PATHOGENICITY OF THE CARP EDEMA VIRUS (CEV) IN FISH IN GERMANY

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Introduction

Infections with the carp edema virus, a pox virus, are known from Japanese koi populations since 1974. Lethargy is one characteristic clinical sign associated with the infection is and therefore the disease is called “koi sleepy disease”. For a long period of time, disease outbreaks seemed to be restricted to Japan. During the last years clinical outbreaks of koi sleepy disease also occurred in many European countries (Jung-Schroers et al, 2015, Way et al, 2017). Koi sleepy disease seems to pose a potential risk to carp aquaculture and koi trade also in Europe.

Materials and methods

During the years 2015 and 2016 an epidemiological study on the occurrence of CEV in fish in Germany was performed. In total 421 gill samples were analyzed. Most of these samples were taken from common carp or koi carp, only a few samples were taken from additional fish species that were kept together with carp.

Results and discussion

In 194 samples CEV genome fragments were detected. Most detections, in total 179, were made in samples from koi carp (*Cyprinus carpio*), in 61 samples of common carp (*Cyprinus carpio*) CEV was detected and in 1-2 samples each of *Ctenopharyngodon idella*, *Esox lucius*, *Gymnocephalus cernua*, *Perca fluviatilis*, *Sander lucioperca* genome fragments of CEV were found in low amounts (1.10E+00 – 1.19E+03). Highest amounts of viral DNA were detected in samples of koi carp (1.00E+00 – 4.82E+06) and common carp (1.00E+00 – 4.03E+06). Sequencing of the DNA fragments revealed that there are at least two different genogroups of the virus are present and that almost all isolates detected in common carp are belonging to genogroup 1 whereas almost all isolates detected in koi carp are belonging to genogroup IIa.

Characteristic symptoms for an infection with CEV were enophthalmus, anorexia, gill necrosis, gill swelling and lethargic behavior. Mostly in spring, between May and July, CEV was detected. In koi carp disease outbreaks due to CEV were mostly seen when the water temperature was between 17-18°C, whereas in common carp at water temperatures between 9-13°C CEV was detected most frequently.

In 46.66% of samples taken from clinically healthy koi or carp from retailers, CEV was detected. Taken all samples from clinically healthy koi and carp, CEV could also be detected, but only in 26.32% of all examined fish. Therefore purchasing new fish from retailers might be one risk factor for the introduction of CEV in a pond. In common carp more frequently diseases signs and mortalities were recorded compared to koi carp. The probability of losses of more than 50% in a system was around 5 times higher in common carp aquaculture than in facilities for koi carp.

Conclusion

Fish health services should therefore be aware of the presence of CEV which may result in high losses in carp aquaculture and testing of koi and carp for CEV should become part of fish disease surveillance programs of national and regional fish disease laboratories.

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STUNNING OF AFRICAN CATFISH (*Clarias gariepinus*) BY A PENETRATIVE CAPTIVE BOLT DEVICE

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Introduction

African catfish (*Clarias gariepinus*) are particularly suitable for keeping in recirculating aquaculture systems because the not utilised heat from biogas plants can be used to heat the tank water. However, with the innovation of "exotic" fish for food production, new challenges are facing aquaculture farms and the regulatory authorities. Especially the development of an ethical method of stunning and slaughtering of African catfish is needed. In Germany, stunning of animals in general is regulated in a directive and special requirements for stunning of fish are given. For all fish species these regulations prescribe stunning by percussion or electric current. Due to anatomical and physiological peculiarities, both these methods are problematic for the stunning of African catfish. For example, the skull anatomy of this species hinders rapid percussion stunning. Anaesthesia is achieved with this procedure only if the impact is made with considerable force on a precise localization. If the blow does not hit the skull at the correct location, it will not cause anaesthesia, but respiratory tract damage. The situation is similar with electrical stunning. From practice and the scientific literature (Sattari, Lambooij et al., 2010) it is known that satisfactory stunning cannot be achieved with conventional electric stunning devices and that high current densities are required, which pose a threat to occupational safety.

Materials and methods

A penetrative captive bolt device was used to stun 14 African catfish with a mean body length of 55.5 ± 4.9cm and a mean weight of 1900.4 ± 610.3g. Previous investigations of African catfish showed that on the skull externally visible adhesion zones of skull plates were recognizable. The positional relationship of these structures to the brain could be visualized during dissection of slaughtered catfish and by using three-dimensional computer models of the skulls. The anterior fusion zone, which was covered with connective tissue, was located rostrally in front of the brain, while the posterior fusion zone was caudodorsal to the brain. For the localization of the brain, a line in the median of the skull was used for orientation on the basis of the present anatomical findings. This line was drawn in conjunction with the two congenital zones of the skull, which are usually not ossified. It runs from rostral to caudal in the median over the skull and divides it into two equal halves. In the rear third between these zones the brain was located. This point was used for placing the captive bolt device. After shooting, the skulls of the catfish were x-rayed to control the correct localization of the bolt.

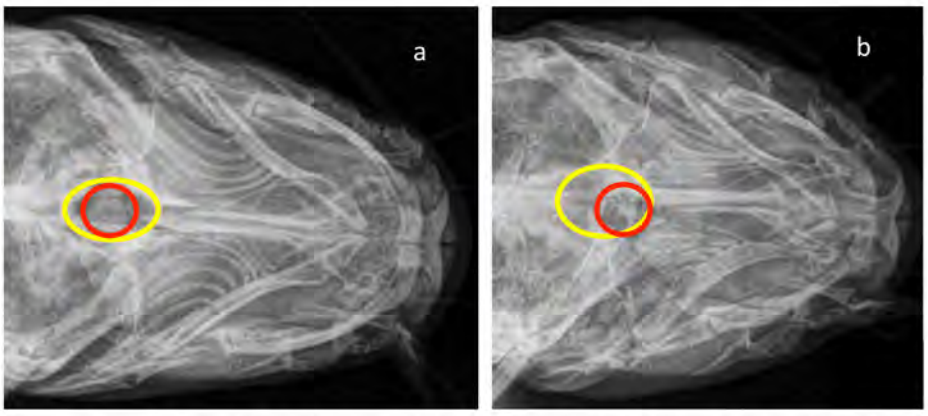

Fig 1. X-ray of an African catfish hit by the captive bolt device in the middle of the brain (a) and X-ray of an African catfish with a shorter skull hit in the rostral part of the brain. The localization of the brain is marked in yellow and the localization of the punch hole is marked in red.

(Continued on next page)

Results and discussion

All fish were stunned successfully by the captive bolt device. After shooting the fish were showing tremors of muscles in the back and of the fins for up to 30 seconds. After this time movement was no longer observed. On the X-rays it could be seen that the brain was destroyed in all fish. Nevertheless, in fish with a shorter skull, the captive bolt device was hitting the brain slightly too rostrally (fig. 1).

Conclusion

Stunning of African catfish with a captive bolt device is an effective and reliable method to achieve unconsciousness in the fish before slaughtering. The exact position of the brain has to be known and the procedure has to be adapted in fish showing alterations of the skull morphology, like shorter skulls. Nevertheless, also in these fish the brains were destroyed almost completely and a successful stunning could be achieved.

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FEEDING REGIMES FOR AN OPTIMAL UPTAKE TO OBTAIN HERD IMMUNITY IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Numerous diseases continue to cause economic and ecological problems in aquaculture. Injection vaccination of fish entails high effort and fish stress and many nations resort to other, ecologically questionable treatment methods. An ideal fish vaccination method is oral vaccination and research on the topic has been performed since the 80s with limited success. Although good immune responses can be achieved using the oral route (Romalde *et al.* 2004, Ballesteros *et al.* 2014) crucial long-term protection could not be induced. One of the bottlenecks of oral vaccination is the ingestion and digestion of the vaccine-particle (Ballesteros *et al.* 2015). The aim of this study is to investigate the early stages of an oral vaccination process and elaborate the optimal feeding regime for salmonids. At present, a large amount of research is dedicated to feed intake and feed composition of salmonids. However, little attention was drawn to the variance of feed uptake amongst and between individual fish, which is key to consistent vaccination. The information presented in this study is intended to form the foundation for successful induction of herd immunity for salmonids in aquaculture using effective oral vaccines

Materials and Methods

To evaluate the feeding regime, three trials were conducted, focusing on starvation period, portions size and daily feeding events. Fish weight ranged from 23g, 27g and 31g for the trials, respectively. Rearing temperature was 15°C and aeration was 0.1l • h^{-1} for each replicate. To determine the individual feed intake and the intake variance, quadruplicates were used with seven marked fish in each replicate. All feeding events were video-recorded and evaluated post-experiment. To evaluate the video files of each trial, the consumption of the food pellet was assigned for every fish independent .

Results and Discussion

Results of the starvation experiment showed no significant differences in pellet uptake, however a correlation of intake variance and starvation period was present. At this point of time, the video evaluation is still in progress. Initial results indicate significant differences in individual feed intake variance, while minimal effect is observed due to the starvation period.

Conclusion and Impact

Even though research on feeding regimes for salmonids has been performed, progress in oral vaccination requires a different approach than those experiments already undertaken and must include optimisation of oral uptake. The impact of an applicable oral vaccine will have a great impact on fish heath, welfare and labour matters in the global aquaculture industry. Consequently, the increase in fish robustness would decrease the pollution to the ocean by antibiotics in open-water systems.

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Argyrosomus regius IN THE MEDITERRANEAN AQUACULTURE: IS IT A SILENT CARRIER AND CONTAMINANT SPECIES FOR THE TWO GRAM POSITIVE BACTERIAL TYPES NAMELY MYCOBACTERIUM & NOCARDIA? WHAT ARE THE IMPLICATIONS TO MARINE FARMING?

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Production of *Argyrosomus regius*, introduced as a new species in to Mediterranean mariculture has spread widely in the last 15 years. It was introduced as a supplementary new species in to the traditional seabass and seabream producing hatcheries and cage farms.

Its culture as a futuristic fast-growing fish to take over an over unexpected niche market on a wider scale has faced with technical and biological problems leading to a slow abandonment of this species culture by most small-scale farmers and a number of larger farmers due to decimation of enthusiasm as a whole.

Apart from the technical problems of culturing this species in parallel with existing sea bass and seabream farms, at required low stocking densities (requiring larger cage space for a given biomass), growth and FCR issues have not been satisfactory. Due to limited market demand, need to harvest small volumes in irregular frequencies, makes this species mass culture more uneconomical in general based on all the above said factors.

While Biological constraints due to serious parasitic, bacterial and nutritional diseases have caused greater concern to the future viability of this species sustainability, serious Biosecurity concerns have surfaced exposing other species such as Sea bass and cultured sparids to gram positive bacterial infections.

The work will present the Disease problems faced in *Argyrosomus regius* aquaculture since its inception, and the serious biosecurity risks to the traditional Mediterranean Aquaculture species.

EFFECTS OF RAPESEED PROTEIN PRODUCTS SUPPLEMENTED WITH GLUCOSINOLATES AND PHYTIC ACID ON THE GROWTH PERFORMANCE OF RAINBOW TROUTS (*Oncorhynchus mykiss*)

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Introduction

With growing demands and a limited availability of fishmeal, the search for suitable alternatives has never been more urgent (Tacon et al. 2010; Hermann et al. 2016). Proteins from rapeseed and canola have been extensively studied in the past; however, anti-nutritive substances like glucosinolates and phytic acid present in rapeseed have limited its use as an ingredient in fish feeds (Francis et al. 2001; Thiessen et al. 2004). The present study focusses on determining thresholds at which aforementioned anti-nutritive substances are no longer impairing growth performance of rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Juvenile rainbow trout (mean weight 12g) were fed twice a day to apparent satiation for 38 days with nine basal diets containing rapeseed protein concentrate, supplemented with different levels of glucosinolates (GLS 0.5; GLS 1; GLS 1.8 in µmol/g) and phytic acid (PA 1.7; PA 2; PA 2.6; PA 2.9; PA 3 in %) and one control diet without rapeseed protein concentrate. Fish were held in a recirculation system with freshwater at 13°C and an average pH of 7.4.

Results

Results show a significant decrease in growth performance with increasing inclusion levels of phytic acid

The inclusion of 3% phytic acid shows a significantly lower daily feed intake (DFI, figure 1) and subsequently significantly lowered specific growth rate (SGR, figure 1) and final body weight compared with the rapeseed protein control group (CD RP).

There was no significant difference between the rapeseed protein control group and any of the glucosinolate groups (GLS0.5, GLS1, GLS1.8) in terms of growth performance.

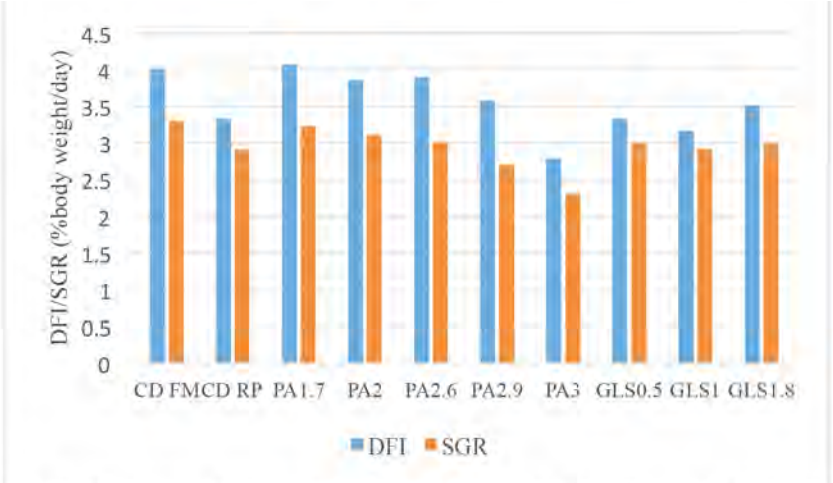

Figure 1: Daily feed intake and specific growth rate of rainbow trout for each treatment after 38 days of feeding. Specific growth rate = (Ln(final body weight)-Ln(initial body weight))/number of feeding days*100, daily feed intake = FCR*SGR.

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Discussion

Since no significant differences could be observed in feed conversion ratio (FCR), differences in growth performance most likely result from reduced feed intake. A reduced daily feed intake has been observed in different studies when rapeseed protein was used as a fish feed ingredient (Nagel et al. 2012; Bu et al. 2017). Glucosinolates, tannins and sinapine are considered to impair the organoleptic properties of certain ingredients in different species (Francis 2001 et al. 2001; Tan et al. 2011). However, effects on growth performance were most commonly observed by inclusion of different rapeseed proteins in fish feed, hampering to find which anti-nutritive substances are responsible for a reduced feed intake. Denstadli et al. (2006) also observed a reduction in daily feed intake when feeding Atlantic salmon (*Salmo salar*) graded levels of phytic acid. Atlantic salmon showed a significantly reduced daily feed intake at 2.07% inclusion after 80 days of feeding (Denstadli et al. 2006).

Since our results show no significant impact of glucosinolates on growth performance, phytic acid might be a main anti-nutritive substance influencing daily feed intake and therefore growth performance at certain inclusion levels of both anti-nutritive substances.

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INFLUENCE OF DIETARY ASTAXANTHIN ON THE OXIDATIVE STRESS RESPONSE CAUSED BY EPISODIC HYPEROXIA IN RAINBOW TROUT

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Introduction

Among carotenoids, astaxanthin (AX) is the most used in aquaculture nutrition, principally as a pigmenting feed additive. Recently, more attention has been paid to the potential health benefits of AX in part related to its free radical scavenging, singlet oxygen quenching and antioxidant properties (Dose et al., 2016). Aquaculture species are continuously exposed to several types of challenging conditions, all generators of oxidative stress. Based on the hypothesis that dietary AX provides physiological benefits to rainbow trout antioxidant response, the present study aimed to compare the liver oxidative status of this species fed an astaxanthin depleted diet (CTRL) and fed an astaxanthin diet (CARO), under normoxia conditions and in response to episodic hyperoxia (termed 8HYP:16NOR). In addition, we assessed if the antioxidant response of rainbow trout was associated with carotenoid-based pigmentation of the skin and muscle.

Material and methods

Two iso-nitrogenous, iso-lipidic and iso-caloric feeds were tested with triplicate groups of rainbow trout juveniles of 30 fish per 800-L tank (initial weight ~ 308.5g). The differences among experimental diets was the AX content, CTRL diet had no AX and the CARO diet was supplemented with 100 mg AX (DSM-Carophyll Pink) per kilogram of feed. This feeding trial was conducted for a period of 92 days; the first 85 days, fish were fed on the respective CTRL or CARO diet and reared under normoxic conditions. The following seven days, fish continued to be fed with the same diet and were exposed to hyperoxia. For hyperoxic conditions, oxygen supply was increased from 8 to 13 mg/l and between 9:00 to 17:30, afterwards oxygen levels returned to normoxic conditions. A factorial design 2x2 was performed, comparing normoxia treated fish (termed N) and hyperoxia (termed H), fed two distinct diets (CTRL and CARO) to produce four experimental treatments N-CTRL, N-CARO, H-CTRL, H-CARO.

Table 1

Skin and muscle redness, muscle and liver TBARs (umol MDA/g tissue), liver gene expression and enzyme activity GR (mU mg pt^{-1}), SOD (U mg pt^{-1}) and CAT (U mg pt^{-1})

Environment	Normoxia		Hyperoxia		Two way ANOVA		
Diet	N-CTRL	N-Caro	H-CTRL	H-Caro	E	D	EXD
a*-values							
Skin	6.8±0.3	10.3±0.5	6.7±0.4	10.3±0.4	ns	***	ns
Muscle	1.5±0.2[a]	12.4±0.4[b]	1.2±0.1[a]	15.2±0.3[c]	***	***	***
TBARs							
Muscle	2.2±0.1	1.8±0.1	2.5±0.2	2.1±0.1	*	*	ns
Liver	1.8±0.2	1.5±0.1	1.8±0.1	1.5±0.1	ns	*	Ns
Gene expression							
GR	1.0±0.2	1.4±0.5	1.0±0.2	1.3±0.4	ns	**	ns
Gclc	1.0±0.3	1.3±0.3	1.0±0.3	1.3±0.3	ns	*	ns
TR	1.1±0.4	1.5±0.6	1.3±0.4	1.7±0.6	ns	*	ns
Enzyme activity							
GR	10.4±0.6	13.4±0.6	8.8±0.7	10.3±1.2	**	**	ns
SOD	60.7±4.0	58.6±4.5	42.5±4.3	49.4±5.9	**	ns	ns
CAT	1279±135	1336±144	1027±94	1120±87	*	ns	ns

Values are presented as means ± SEM. Different superscript letters within a row denote significant differences among treatments determined by two-way ANOVA ($P< 0.05$). * $P< 0{\cdot}05$; ** $P< 0{\cdot}01$; *** $P<0{\cdot}001$.

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Results

Skin redness was only influenced by the diet (Table 1). In the muscle, environment, diet, and their interaction enhanced ($P<0.05$) muscle redness, having H-Caro fish significantly higher values. TBARs values in muscle and liver were affected by the diet; being lower in CARO fish. In contrast, environment also influenced TBARs in muscle, being higher with hyperoxia. A negative correlation was observed between skin a*-values and TBARs in muscle ($P<0.01$; $r=-0.32$) and liver ($P<0.05$; $r=-0.32$); furthermore, between muscle a*-values and TBARS in muscle ($P<0.05$; $r=-0.35$) and liver ($P<0.05$; $r=-0.36$). From the genes studied, CARO diet exerted an up-regulating effect on glutathione reductase (GR), glutamate-cysteine ligase catalytic subunit (Gclc) and thioredoxin reductase (TR). Correlations were found between liver GR gene expression and skin ($P<0.05$; $r=0.35$) and muscle ($P<0.01$; $r=0.48$) a*-values, and between TR and skin ($P<0.05$; $r=0.34$) and muscle ($P<0.05$; $r=0.42$) a*-values. In the case of Gclc, only a significant correlation was observed with muscle a*-values ($P<0.05$; $r=0.36$). Environment lowered liver antioxidant activity of SOD, CAT and GR. GR was also influenced by diet, rainbow trout fed on the CARO diet presented significantly higher values than CTRL treatment. A correlation was only found between liver GR activity and skin a*-values ($P<0.05$; $r=0.4$).

Discussion and conclusions

Reduced and oxidized glutathione (GSH/GSSG) and thioredoxin (Trx/TrxSS) redox couples play important roles in antioxidant defense (Circu et al., 2011). In this study dietary AX up regulated important hepatic GR and TR both key enzymes in restoring reduced forms of glutathione and thioredoxin, respectively; and Gclc an active participant in de novo GSH synthesis. However, the activities of other enzymes were not affected by dietary AX, being lower in trout undergoing hyperoxia. Regarding muscle and skin color, there seems to be no trade-off in the allocation of AX between avoidance of oxidative stress and color expression. In accordance to our results, birds exposed to mild levels of oxidative challenge also developed redder ornaments (García-de Blas et al., 2016). In conclusion, results in this study suggest that dietary AX can enhance rainbow trout liver antioxidant response.

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TRANSMISSION INTERRUPTION OF THE CILIATE *Ichthyophthirius multifiliis* AS AN ALTERNATIVE, NON-THERAPEUTIC CONTROL METHOD

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Introduction

Infections with the ciliate parasite *Ichthyophthirius multifiliis* regularly lead to serious losses in fish farms worldwide. At present, there are no effective means available against this pathogen in the EU. Thus, there is a therapeutic emergency for this disease up to date, which is unacceptable in terms of animal welfare and considering the economic risks. In the course of this project, the interference in host transmission of the fish-infecting theront stages using solubilized active component mixtures for water treatment was tested as an alternative control strategy against *I. multifiliis* in trout farms without the use of pharmaceutical therapeutics.

Materials and methods

Basis for the trials was the identification of natural substrates that activate the unique host search movement-pattern in vital theronts. This fast rotating swimming activation and host recognition behaviour was intended to disturb host transmission either by a decoy effect, failure of recognition or by the greatly increased energy consumption due to motility and subsequently earlier onset of vitality loss.

An initial small scale experiment with brown trout (*Salmo trutta*, 4-5 cm) fingerlings utilized direct dispersion of substances activating theront host finding behaviour to disrupt transmission success. For this purpose, 10 fish each were exposed as separate groups in control/preincubated or theront solutions (3.5 l) at 16°C and were transferred to 15 l aquaria after 5 hours. Active substance addition was conducted on one hand with simultaneous addition of active ingredient solution (A) and fish to parasite containing infection compartments, on the other hand with exposure of the fish only after preincubation with active ingredient for seven hours before fish addition. The admixture of active ingredient to the batches was done stepwise hourly. The trial was ended after 7 days. For the large-scale experiments, six rectangle tanks (280 x 60 x 60cm) were used and every group was arranged in triplicates. The tanks (production volume: 0.5m^3) were arranged as flow through system at 10°C. All rearing units were continuously aerated during the experiments and water quality corresponded with physiological requirements for trout fingerlings. For exposure, 11 500 theronts were added per tank with flow-through closed and 60g of active ingredient were stirred-in as solid in the treated group. After 24 hours, six rainbow trout fingerlings were stocked per tank and incubated overnight before flow-through was turned on. The trial was ended after 10 days. In both trials, all experimental fish were euthanized and fixed in 70% ethanol for later parasite stage enumeration on the body surface by stereomicrocopy.

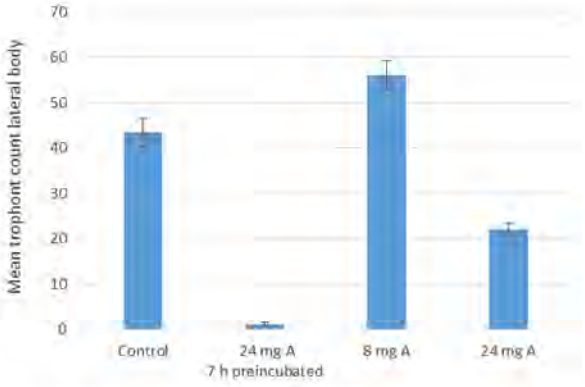

Fig. 1. Mean trophont numbers (± SEM) on rainbow trout fingerling body surface after 7 d incubation upon prior exposure to equal amounts *I. multifiliis* theronts. Fish were exposed to theronts under repeated cumulative addition of Activation Solution Mixture (A, mg final concentration per infection compartment) or after preincubation of theronts to A without fish (preincubated).

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Results

Counting the infestation rate at one week in the small-scale setup showed a highly significant reduction in epidermal trophic stages down to a fraction of those on the untreated group after 7 hours pre-incubation of theronts with active compound administration (Fig.1). Control theronts incubated the same period under equal conditions remained vital. The simultaneous addition of experimental fish and active substance with repeated administration to a final concentration of 24 mg per infection compartment (~7 mg/L) already resulted in a significant reduction of the infection rate by more than 50%. However, there seemed to be a critical concentration threshold, since lower final concentrations of 8 mg A did rather promote host invasion rates when fish and parasites were added simultaneousl .

In the large scale experiment, the result of the small-scale preincubation trial could be further confirmed, since the number of *I. multifiliis* trophonts on fish exposed in the group with prior dispersal of active substances could be significantly reduced compared to the control group. Individuals from treated tanks had over four times less trophonts on their body surface ($P < 0.0001$; Tukey t-test, $N = 18$ per group).

Discussion and Conclusion

Mortality by Ichthiophthiriasis is usually caused by overinfection via quick reinfection mostly at elevated temperatures. However, theronts are short-lived transmission stages that bear limited resources to achieve successful transmission. They rely on a distinct behavioural pattern for host finding and recognition upon chemical and physical stimulants (Haas et al. 1999, Hofmann 1995). This means may serve as a possibility that could be utilized for alternative control strategies, without the use of pharmaceuticals. Our results show that only by artificial activation of the natural behaviour of theronts in vicinity of a putative host can interrupt transmission to a considerable degree. This offers a chance for fish to cope with reduced numbers of parasites while acquiring a stable immunity status during this critical phase. These findings can be exploited in future management and control efforts.

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IMTA AND SMARTER MONITORING FOR GREENER AQUACULTURE.

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Introduction

The major challenges to EU aquaculture growth can be summarised to adaptation to market changes and competition, as well as need for technical improvements (maintaining health / welfare of livestock, integration of activity with the environment, optimizing resource use and spatial planning) (Anon. 2014). The production of aquaculture food and equipment must be supported by the most advanced research and technology, and the EU must maintain a strong research and technological edge to stay at the forefront, and improve the competitiveness, of the aquaculture sector by using innovative technologies and management techniques (Anon, 2009).

IMTA offers an attractive approach to aquaculture with the benefits of; environmental sustainability through bio-mitigation; economic stability through product diversification and risk reduction; spatial optimisation by increasing site productivity and; social acceptability through better management practices. The management of large-scale IMTA areas remains difficult, even in countries where it is currently practiced commercially (Fang *et al*, 2016), due in principal to limited knowledge of how the separate components in the IMTA ecosystem interact and function as a whole, as well as the impact on the environment and the broader ecosystem. Using new and emerging technologies and innovations in the monitoring and management systems can provide data and guidance to adaptively manage IMTA systems to ensure on-going sustainability.

Methods

The H2020 Impaqt project, Intelligent Management system for integrated multi-trophic Aquaculture, aims to maximise the growth rate and minimise the production costs, through the optimisation of production systems, while ensuring seafood product quality, optimal resource use, and minimisation of environmental impact. It will develop and validate in-situ a multi-purpose (inland, coastal and offshore productions), multi-sensing (heterogeneous sensors and new/emerging technologies) and multi-functional (advanced monitoring, modelling, data analytics and decision making) management platform for sustainable IMTA production.

The project is developing three main interacting subsystems:

1. the autonomous data acquisition and communication system - data sources from chemical, physical and attached sensors, remote sensing algorithms and satellite observation, crowd-sourcing observation and in vitro species characterisation.
2. the advanced IMTA model - yielding spatially explicit information on the interaction of farm components with the environment on the ecosystem scale, for management and regulatory planning.
3. the integrated management system - operating at the farm scale, comprising data and predictive analytics, decision making and actuation to enable improved operational decisions for animal welfare, production optimisation, environmental protection, food quality and sustainable productivity.

Results and Discussion

Impaqt will help to identify and quantify the benefits to be gained from IMTA and combining trophic levels. Impacts and interactions will be specifically assessed at an ecosystem scale, rather than just at the scale of individual farms. The integrated management system will also enable real time assessment of current conditions and respond to production and environmental challenges timely at the farm scale. It will enable a more efficient IMTA practice, informing the selection of optimal sites and optimal spatial configuration for the various aquaculture components

Impaqt will rely on an Open Systems approach and an "everything-as-a-service" thinking, while Semantic Aquaculture Data Interoperability and Harmonization will enable data federation and knowledge exchange between different systems.

This high level ambition is to drive a paradigm shift in the European Industry and its acceptance of IMTA as a viable approach, paving the way to a more environmentally friendly and more efficient/higher yielding European Industr .

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INVESTIGATION OF EARLY INTRODUCTION OF DRY FEED ON GILTHEAD SEABREAM *Sparus aurata* LARVAL DEVELOPMENT AND GROWTH PERFORMANCE AFTER METAMORPHOSIS

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Introduction

Marine larval feed technology is a fast changing field with great progress in manufacture technology. The challenge is to produce small enough feed particles that cover the nutritional needs of newly hatched larvae and have high acceptability and palatability, water stability and low nutrient leaching (Hardy and Barrows, 2002). Nowadays, many of the fish larval feed industries do provide such small-sized dry feeds which, in the hatchery practice of gilthead seabream *Sparus aurata*, are progressively introduced to fish larvae not earlier than 15 days post hatch (dph) with the concomitant use of live feeds (rotifers, *Artemia*) until weaning. However, compared to live feed, dry feeds appropriately manufactured (i.e. physical and nutritional properties) are expected to better provide for all nutritional needs of altricial fish species, such as gilthead seabream. The aim of the present study was to investigate the introduction of dry feed as early as on 4 dph on growth and digestive physiology of gilthead seabream larvae, as well as to monitor fish growth performance after metamorphosis

Materials and methods

The experimental trial was conducted in two stages: Stage 1, Hatchery rearing (Hr), was performed in a commercial marine fish hatchery. Four tanks of 10 m^3 were stocked with eggs within three days. In two of the tanks a commercial dry feed (Larviva Prostart, Biomar) was introduced on 4 dph (DF4), while in the other two tanks the dry feed was introduced on 15 dph (DF15). In all experimental tanks, the larvae were fed with rotifers from 4 dph up to 24 dph and with *Artemia* from 13 dph up to 58 dph. From 59 dph larvae were fed dry feed only. Feeding with live and dry feed followed the hatchery protocol and larvae samples were observed under microscope to confirm food consumption. During larval rearing, water quality was monitored daily and larvae were sampled every 5 days from 5 to 60 dph, to estimate larvae length, phenotypic deformities and digestive function (i.e. lipase, amylase, trypsin, chymotrypsin, pepsin specific activities). Stage 2, Laboratory rearing (Lr), was performed in a recirculating seawater system. On 61-62 dph fish larvae of each duplicated tank were graded on hatchery to two size classes (Big, Small). The Big and Small fish of each duplicated hatchery tank were pooled and transferred to laboratory installations (on 64-66 dph). Nine hundred and seventy-six (976) fish from each size class and Hr treatment (DF4-Big, DF4-Small, DF15-Big, DF15-Small) were group weighed and randomly distributed in duplicated tanks (on 66-68 dph). Fish growth (i.e. body mass, survival, specific growth rate-SGR, thermal growth coefficient-TGC, mass variation) was monitored for two (2) months. All fish were fed the same commercial diet *ad libitum*. Water quality was monitored daily and fish were group weighed (app. 15-20 fish per group) every 15 days, while at the end of rearing fish were individually subjected to weighing and length measurement. Phenotypic deformities were also individually recorded. During the second month of rearing, food consumption was recorded to estimate feed efficiency (food conversion ratio-FCR).

Results

Stage 1, Hatchery rearing (Hr): Larval length on 60 dph was similar among experimental treatments. DF4 larvae had significantly lower variability in length and lower phenotypic deformities than DF15. Survival, as estimated upon grading on 61-62 dph, was similar (30-33%), but DF4 treatment provided more Big fish (56%) than DF15 treatment (42%). Lipase, amylase, trypsin and chymotrypsin specific activities showed similar patterns during 5-60 dph in both treatments. However, pepsin was first detected on 55 dph for DF4 larvae and on 60 dph for DF15 larvae. Stage 2, Laboratory rearing (Lr): First weighing (66-68 dph) showed that DF4-Big larvae were significantly larger (0.087 g) than DF15-Big larvae (0.074 g). Differences in body mass for Small fish were not significan (DF4-Small: 0.051 g, DF15-Small: 0.049 g). At the end of the two months rearing period, DF4-Big fish retained their growth advantage being significantly larger (5.36 g) than DF15-Big fish (4.84 g), while DF4-Small and DF15-Small fish body mass was similar. SGR, TGC and FCR showed no differences for DF4 and DF15 fish, either Big or Small. Total survival and total deformities were significantly higher and lower respectively in DF4 larvae than in DF15 larvae, no matter size class. Finally, body mass distribution was significantly different for DF4-Big and DF15-Big fish, DF4 fish resulting in higher percentage of the most numerous l ge size class.

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Discussion and conclusion

Present results showed that the early introduction of dry feed on 4 dph, instead on the most commonly used 15 dph, resulted in lower variation among larvae and reduced incidence of deformed fish. Seabream larvae ingested the feed and their digestive function was similar to patterns observed for gilthead seabream and other Sparidae species (Moyano et al., 1996; Suzer et al., 2008; Yúfera et al., 2011), thus confirming normal exogenous feeding. Gastric acid digestion (i.e. pepsin) was evidenced slightly earlier in DF4 and slightly earlier than previously reported (i.e. >60 dph: Elbal et al., 2004; Yúfera et al., 2011). Upon grading, DF4 treatment resulted in higher body mass of Big fish and higher percentages of Big fish than DF15, advantage that was maintained during further rearing up to 123-125 dph. Taking into consideration the better survival and the lower incidence of deformities that both Big and Small fish showed for DF4 treatment, obtained results suggest that the early introduction of dry feeds has benefits and indicate that a change of dry feed protocol during development may have significant e fects on subsequent post-larval production stages (e.g. Koedijk et al., 2010).

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ESTIMATING GENETIC INTROGRESSION OF FARMED SALMON – *P(wild)*; METHODOLOGY, ACHIEVEMENTS AND PROSPECTS

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Genetic introgression of escaped farmed salmon is a large threat to the viability of wild salmon populations. Development of molecular genetic tools to trace farmed to wild genetic introgression has been a prerequisite for understanding, not only the magnitude, but also how this genetic introgression affects important life history traits in wild salmon populations, how natural selection acts against farmed genetic introgression, which conditions determine the success of escaped farmed salmon, and how broodfish of farmed ancestry in stock enhancement programs might impact wild populations. Without the development of these genetic tools, none of these central topics could have been addressed.

Although a large effort has been put into uncovering the magnitude and consequences of farmed to wild genetic introgression, this work needs to continue; a second generation set of markers for estimating genetic introgression is under development. This new set of genetic markers will enable us to monitor farmed to wild genetic introgression with higher precision, its consequences and to understand the relative importance of different factors in determining the success of escaped farmed salmon in different rivers and populations.

Methodology
In 2011, the first set of genetic markers specifically identified for generically differentiating between Norwegian farmed and Norwegian wild salmon populations was published (Karlsson et al. 2011), and in 2014 a statistical method was developed for quantifying farmed ancestry in individual salmon (Karlsson et al. 2014).

Achievements
The method for tracing farmed ancestry in wild salmon has been used extensively in many different projects: 1.The level of genetic introgression of escaped farmed salmon in Norwegian wild salmon populations has been quantified (Karlsson et al. 2016), and include analyses of more than 40 000 individuals from 225 populations (Diserud et al. 2019). 2. By relating individual data of genetic introgression and data from scale reading we demonstrated that genetic introgression has a large effect on age at maturity and growth, and that the effect of farmed genetic introgression depended on the phylogenetic origin (Bolstad et al. 2017) 3. Tracing farmed ancestry in naturally born salmon has since 2014 been included as a mandatory test of all brood-salmon used for stocking to exclude salmon of non-wild origin 4. We have demonstrated that hatcheries have inadvertently used brood-salmon of farmed ancestry for stock enhancement and that under hatchery conditions these have outcompeted salmon of pure wild origin (Hagen et al. 2019). 5. By estimating the level of introgression in a cohort, at different ages, we have demonstrated a lower survival of individuals of farmed ancestry (Aronsen et al. 2017). 6. By relating the level of genetic introgression and proportion of escaped farmed salmon in a river with different river specific and population specific parameters we have improved our understanding of why some populations show a higher level of genetic introgression that others.

Prospects
A second generation set of markers for tracing farmed to wild genetic introgression is under development. This set of markers will improve the precision when estimating the level of farmed to wild genetic introgression and will enable us to better understand the consequences on the viability of the wild salmon populations, and the role of natural selection in counteracting negative effects of the genetic introgression.

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ROCKFISH (*Sebastes schlegelii*) MYD88 MOLECULAR IDENTIFICATION AND FUNCTIONAL ANALYSIS

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Introduction

Studies on innate immune signal transducers hold paramount importance in fish vaccine development (Priyathilaka et al., 2018), wherein this study, economically important rockfish (Md Mizanur and Bai, 2014), signal transducing adaptor molecule Myd88 (*SsMyD88*) was characterized *in silico*, *in vivo* and *in vitro*.

Materials and methods

Cording sequence of *MyD88* was identified from a previously constructed rockfish transcriptome database. To evaluate the expression under no immune stimulant, tissue samples were obtained from healthy rockfish. To evaluate immune stimulants, rockfish were injected with immune stimulants including polyinosinic-polycytidylic acid (poly I:C), lipopolysaccharide (LPS) and *Streptococcus iniae* (*S.iniae*) then the post injected spleen tissue samples were obtained. RNA extraction followed by cDNA synthesis were carried out and the relative gene expression were determined by the qPCR. *SsMyD88* bearing vector was transiently transfected into the FHM cells along with vector bearing NF-κB luciferase construct for the luciferase reporter assay.

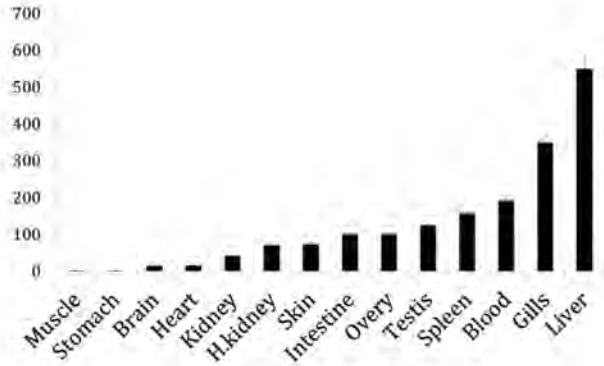

Fig. 1. Unchallenged expression of the SsMyD88 in different tissues/organs of the fish. Rockfish elongation factor-1-α was used as the internal control.

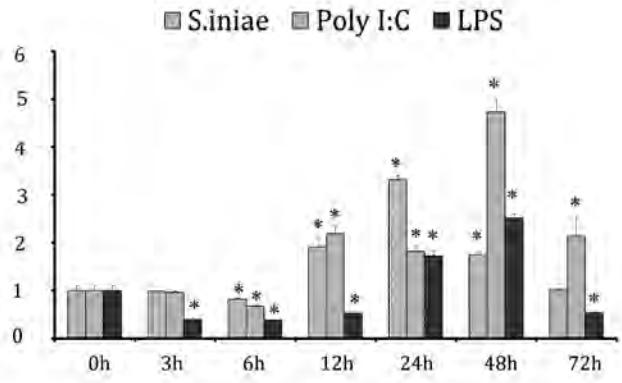

Fig. 2. Challenge expression of the SsMyD88 in spleen. Rockfish was challenged with three immune stimulants including *S.iniae*, Poly I:C and LPS. Rockfish elongation factor-1-α was used as the internal control.

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Results

SsMyD88 is a protein with 288 amino-acids, its pI and molecular weights respectively, 5.1 and 33.1 KDa. SsMyD88 is observed to contain two main domains, N-terminal death domain (DD) and C-terminal Toll/interleukin-1 receptor (TIR) domain. Expression under no immune stimulant revealed highest expression of *SsMyD88* in liver, whereas other important immune organs similarly, spleen and gills disclosed relatively high expression (Fig. 1.). Immune challenge experiment revealed upregulation of transcription resulted by poly I:C, LPS as well as *S.iniae* in spleen (Fig. 2.). According to the luciferase assay rockfish MyD88 has shown a significant and enhanced activation of N κB compared to the control.

Discussion and conclusion

SsMyD88 was found to express in enhanced levels in important immune organs compared to non-immune organs, revelling its importance in immune pathways. According to the immune challenge, *SsMyD88* expression was induced by all the distinct immune stimulants used, therefore SsMyD88 may be involved in diverse range of immune pathways. Luciferase assay results further confirmed the capability of SsMyD88 to activate the NF-κB. All these findings reassure diverse and important immune role of the SsMyD88 during the innate immune defence.

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ENVIRONMENTAL IMPACT ASSESSMENT: THE IMPACT OF COPPER ANTIFOULANTS AND SEA LICE THERAPEUTANTS ON THE BENTHIC SPECIES BIODIVERSITY SURROUNDING ASC-CERTIFIED NORWEGIAN SALMON FARMS

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Introduction

Norway is the biggest aquaculture producer in Europe, contributing 66% of the total production volume, of which Atlantic salmon (*Salmo salar)* is the predominant species (Rico et al., 2018). In recent years this aquaculture industry has intensified and expanded considerably, entailing an increased likelihood of major disease problems (Bondad-Reantaso et al., 2005). In order to promote optimal fish health conditions, a variety of therapeutants are used on Norwegian salmon farms. This research project will focus on the substances used to treat sea lice (*Lepeophtheirus salmoni*), including hydrogen peroxide, emamectin benzoate, deltamethrin, azamethiphos and cypermethrin. Another common problem Norwegian salmon farmers are facing is biofouling, which can also have significant production impacts, as the fouling restricts water exchange in cages, causes deformation of cages and increases disease risk (Fitridge et al., 2012). Copper-based antifoulants are mainly used to mitigate the effect of biofouling (Guardiola et al., 2012). However, concerns have been raised about the possible environmental impacts of the use of therapeutants and copper (Guardiola et al., 2012; Rico & Van den Brink, 2014). These chemicals may harm the biodiversity and functioning of aquatic ecosystems surrounding aquaculture farms, and may compromise the environmental sustainability of the aquaculture sector (Guardiola et al., 2012; Rico & Van den Brink, 2014). This research project was undertaken as part of the EU H2020 TAPAS project (Tools for Assessment and Planning of Aquaculture Sustainability) in order to assess the environmental impact of therapeutants and copper by investigating the relationship between copper and therapeutants and the benthic species biodiversity surrounding Norwegian salmon farms.

Materials & methods

As part of the certification process of the Aquaculture Stewardship Council (ASC), the ASC requests salmon producers to submit a comprehensive set of farm data to them. Included in this data are the results of benthic sampling for biotic diversity and copper concentrations. This data will be used to assess the impact of copper and therapeutants on the benthic biodiversity surrounding Norwegian salmon farms. The used benthic quality data includes biotic indices, copper concentrations and counts of macrofaunal taxa. Data concerning therapeutant use will also be used in this research project. A bivariate statistical analysis will be conducted to analyse the relationships between both the benthic copper concentrations and therapeutant dosage and the Shannon-Wiener (SW) index. Another aspect of this research project will look at whether the MAMPEC model is suitable, given the salmon data collected to date by the ASC, to assess environmental impacts of copper and therapeutants. The MAMPEC model is a chemical fate model, designed to predict environmental concentration of antifoulants, and may also be adapted to perform risk assessments of therapeutants used in aquaculture (Rico et al., 2018).

Results

Data analysis is still ongoing this stage of the research project, therefore the complete results will be presented in the updated abstract.

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Conclusion and discussion

As mentioned above, data analysis is still ongoing therefore no conclusions can be drawn yet. These will instead be presented in the updated abstract. Statistical testing of the associations or correlations between different concentrations of therapeutants and copper and the benthic species biodiversity will help to further scientific understanding of the environmental impacts of therapeutant and copper use on marine cage salmon farms. Use of environmental risk assessments in aquaculture is currently somewhat limited in many European countries (Rico et al., 2018), meaning it is important to improve chemical exposure and effect assessments of therapeutants and copper in order to help assure the sustainability of the aquaculture sector.

Based on this research, it may be possible for the ASC to alter its data collection system in a way that this data can be fitted into environmental impact models such as MAMPEC. This research project and further research therefore not only makes a contribution to the work of TAPAS, but also to the work of the ASC in appraising their collected salmon farm data.

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AquaIMPACT - GENOMIC AND NUTRITIONAL INNOVATIONS FOR GENETICALLY SUPERIOR FARMED FISH

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Introduction

AquaIMPACT is a Innovation Action project funded by EU's Horizon 2020 programme under the call 'Sustainable European aquaculture 4.0: Nutrition and Breeding'.

AquaIMPACT is a major effort to integrate the fields of fish breeding and nutrition to increase the competiveness of EU's aquaculture of Atlantic salmon, rainbow trout, gilthead seabream and European seabass, to ensure food and nutrition security and to satisfy consumer demands for high-quality seafood with limited environmental impact. AquaIMPACT is also the initiator of digitalisation to make aquaculture operations more cost-effective and with new services and products.

The AquaIMPACT concept is (Fig. 1):
• To demonstrate implementation of **genomic selection technology** in commercial breeding programmes, focusing especially in improving the traits related to feed utilisation, nutritional quality and disease resistance.
• To quantify the way current breeding programmes have changed fish traits, and to quantify the consequent need for associated changes in present and future fish nutrition, and to demonstrate the already-realised benefits for **the industry profit and environment**.
• To tailor-make **feed formulae and feeding practices for the genetically selected fish improved by breeding programmes**, based on their nutritional needs, focusing especially on improving the traits related to feed utilisation, nutritional quality, disease resistance and their interactions.
• Using the current breeding programmes as a starting point, to quantify **the break-even points and cost-efficiency ratios for investing, and to find optimized designs** to implement the novel genomic methods.
• **Develop methods for digitalisation, spectroscopy, smart-software, machine learning, and IoT**, especially for recording novel added-value traits, reducing costs of recording fish traits, reducing costs of genotyping, and to develop feed formulae to produce added-value fish products (with higher omega-3, balanced body composition, minimum waste) without the need to grow the fish in practice
• To interactively **communicate with stakeholders and end users** to promote the use of the developed methods for solving industry, societal and environmental challenges, and **increase awareness of consumers** on sustainable aquaculture practices.

Expected impact

In AquaIMPACT, we follow the Innovation Action principles to validate and demonstrate the use of existing knowledge, pre-products and technologies within companies, by overcoming the bottlenecks of their implementation, to meet the demands of companies, consumers, and society. The expected impacts are:

• Productive and resilient breeding and feed industry, and increased self-sufficiency in blue bioeconomy within E
• Increased potential for emerging raw materials (algae, single-celled, insect meals), novel additives in feeds, and novel technologies
• Environmental impact, fish welfare and resource efficien .
• Increased awareness and acceptability on aquaculture, fish breeding, genomics and nutrition

The consortium

The consortium consists of 11 companies and 13 research institutes, with solid track-record for developing technological advancement for the benefit of industries and consumers. The consortium members come from 9 countries: Finland, Norway, France, Spain, Portugal, Italy, Netherlands, Belgium and United Kingdom. The Stakeholder Group consists of FEAP - Federation of European Aquaculture Producers, FEFAC - European Feed Manufacturers' Federation, and EAS - European Aquaculture Society. The consortium is described at our website www.luke.fi/aquaimpact

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FISH CELL CULTURES AS A ROBUST TEST SYSTEM IN AQUACULTURE RESEARCH

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Introduction

Fish culture and production is the fastest growing area of food production with a steady expansion of 8.4% since the 1970s (FAO 2016), aiming to improve the food supply for the increasing world population. In order to establish a sustainable aquaculture production with high product quality and adequate animal welfare, the understanding of fish growth and its development needs to be deepened. For this purpose, the external influences that fish larvae are exposed to during development, like changing abiotic parameters due to climate change, environmental pollution or illnesses, need to be investigated. Already in the early ‘60s the first fish cell line was established by Wolf & Quimbly (1962) to examine these factors. Since then, the number of fin fish cell lines has strongly increased, reflecting the scientific interest in these species.

Even though capabilities of *in vitro* models have greatly improved, the numbers of fish species used in research is very low. The Federal Ministry of Food and Agriculture declared that in between 2015 and 2016 the number of used fishes in research increased by 72% (https://speakingofresearch.com/facts/animal-research-statistics/german-animal-research-statistics/). During the last years, we showed that these cell lines can be useful tools for studying the effects of different biotic and abiotic factors (Grunow et al., 2011a, Grunow et al., 2012, Grunow et al., 2015), like temperature changes and viral-host-pathogen interactions and pathogenesis (Noguera et al., 2017).The aim of our work is to investigate cell lines established from different economically important fish, like Atlantic sturgeon (Acipenser oxyrinchus), rainbow trout (Oncorhynchus mykiss), maraena whitefish (Coregonus mareana) and Atlantic salmon (Salmo salar), regarding their potential to serve as relevant *in vitro* models in aquaculture related research.

Methods

Cell cultures obtained from the eye-point egg stage or yolk-sac larvae of the fish species of interest were generated. Additionally, cell cultures were obtained from the German Cell Bank for Wildlife “CRYO-BREHM”. To elucidate the effect of temperature changes on cell growth, the proliferation of cells generated from Atl. sturgeon, trout and mareana was examined at 4 different temperatures (16°C, 20°C, 25°C, 28°C). These analyses were performed via impedance measurements using the xCELLigence RTCA SP instrument. Additionally, spontaneously contracting cardiac cell aggregates (SCC) were generated in order to examine the effects of temperature changes on cardiac cells. Further analyses were performed by using qPCR, immunocytochemistry as well as Mass Spectroscopy and Electron microscopy. As a last step, SCCs were infected with cardiotropic viruses (Infectious salmon anaemia virus and Salmonid alphavirus) and the viral gene expression was analyzed by qPCR.

Results

We established a well proliferating cell population which have been passaged and propagated over several years showing a uniform cell morphology with increasing numbers of passages. The cells from Atlantic sturgeon revealed the highest cell indices and therefore the optimal growth conditions at 25°C with a cell impedance of 19.68 reached after 35.22h of incubation. Using cells from sturgeon that were kept at 20°C, the highest cell impedance level was reached three-times in the following, having an index 5.3% lower than at 25°C (Grunow et al., 2011b). During passage 0, the generation of spontaneously contracting cell aggregates (SCC) could be observed after cell isolation of several fish larval species, like trout, salmon, sturgeon and maraena, with up to five SCCs per specimen (Grunow et al., 2011a, Grunow et al., 2012). The characterization of the cells exposed fully developed myocardial as well as endothelial and fibroblastic cells which were electrical and mechanical connected to each other (Grunow et al., 2011a, Grunow et al., 2015, Noguera et al., 2017). SCCs can be maintained with minimal support retaining their contraction capacity and displaying beating frequencies similar to the fish heart for up to 6 months in culture. The influence of the temperature showed that the spontaneously beating frequency of SCC generated from rainbow trout larvae will increase from 59.5±15.6 bpm at 16°C to 90±24.38 bpm at 25°C showing an increase of 66%. Furthermore, viral infection analysis via qPCR revealed that SCCs can be infected with different SAV (F93-125, MS 4640 and F97-220) and ISAV isolates (ISA 390-98) (Noguera et al., 2017).

(Continued on next page)

Discussion

Fin fishes possess an enormous diversity as well as different fish species will react in a disparate manner to external influences. Therefore, fish cells are an ideal tool to study various influencing factors in a closed and verifiable environment. We were able to show that *in vitro* models represent a bioethical and cost effective tool to study the impact of changing climate factors or the interaction between the host and viral pathogen. Our observations confirmed that cells will react differently based on their source of isolation. For forthcoming, our goal is to establish stable cell lines from a taxonomic broad range of fish species and to answer the question, whether adapted *in vitro* tools can narrow the gap between *in vitro* and *in vivo* models.

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SALMOSIM: BUILDING ARTIFICIAL ATLANTIC SALMON (*SALMO SALAR*) GUT SYSTEM

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Introduction

The aquaculture in Scotland is dominated by Atlantic salmon, which has increasing significance both in terms of economics and food sustainability. The constant decline in wild Atlantic salmon stocks has resulted in an increasing demand for farmed fish, however, the expansion of the Salmonid aquaculture industry is unsustainable due to two reasons: the reliance on over-exploited wild fish stocks as the main protein and lipid food source, and, the poor growth efficiency on alternative plant-based feeds (Ytrestøyl, Aas and Åsgård, 2015; Hemre *et al.*, 2016)two regression experiments, with parr and post-smolt, were conducted. A control diet was included to evaluate if ingredients supplied sufficient nutrients without any added nutrient package (NP. In order to overcome both of these problems the growth efficiency of fish fed on plant-based diets must be improved, and this requires a novel experimental approach.

We are developing a synthetic, continuous salmon gut microbial fermentation system, called SalmoSim, simulating salmon gut compartments representing generalized marine lifecycle stages. The SalmoSim project aims to provide an in vitro platform to study the link between the gut microbiota and digestion in Atlantic salmon (Salmo salar).

Materials and methods

SalmoSim is comprised of three series-linked Applikon MiniBio 500 reactors running anaerobically, simulating three major compartments in salmon gut: stomach, pyloric caecum and mid intestine. They are characterised by a constant flow of media, constant reactor volume, constant temperature and pH control of the individual reactor vessels. The bioreactor vessels are inoculated using a small homogenate of the gut contents collected from the corresponding gut compartments of adult farmed Atlantic salmon.

In order to validate the SalmoSim system, samples from a feed trial performed by Marine Harvest in Averøy were used. During this trial adult salmon were fed a fish meal positive (FM+) diet until they reached 1.5kg, and then they were switched to a fish meal zero (FM0) diet until they doubled in size. This experimental procedure was replicated within the SalmoSim system. Reactors inoculated with the gut contents from fish fed on the FM+ diet were left to stabilise over a 20 day period, whilst continually supplied with FM+ media. They were then switched to a FM0 diet over a following 20 day period, in order to see if the bacterial community changes seen in real salmon, can be replicated inside the SalmoSim system.

During the length of the validation experiment samplings were performed every second day, during which bacterial activity (protein, ammonia and volatile fatty acids concentrations) and bacterial population changes (qPCR and next generation sequencing) were measured.

Results

Preliminary results indicate that in both real salmon and SalmoSim the switch in feed from FM+ to FM0 diet results in similar response in several different bacterial groups, such as Actinobacteria, Betaproteobacteria, Firmicutes, Gammaproteobacteria, Lactobacillus and Mycoplasma. Currently next generation analysis on real salmon and SalmoSim samples is being performed, in order to see more detailed results. Results from this experiment and others in progress will be presented.

Discussion and conclusion

The observed similar bacterial community changes during the switch of the fish meal from FM+ to FM0 in both real salmon gut and SalmoSim system, indicate that artificial salmon gut system is functioning as expected. Once the system is validated, it has the potential to provide a platform with a broad range of applications including the study of microbial population dynamics, the evaluation of the performance of different feed formulations, the comparison of the fermentative capacity of the gut microbiome of lean and obese fish (possibly leading to oral transplant studies), the evaluation of the effects of antibiotic/disinfectant/antiparasitic treatments on microbial gut population composition, the evaluation of pro- and syn-biotic approaches as well as the study of antimicrobial resistance transfer. The system's physicochemical parameters can also be adjusted to simulate the gastrointestinal tract of other teleost fish of commercial or biological interest

Discussion and conclusion

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AUTONOMOUS UNDERWATER ROBOTS FOR SAFER AND MORE EFFICIENT OPERATIONS IN FISH FARMS

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Introduction

Fisheries and fish farming are important global contributors to the production of seafood for human consumption. These industries are also known for substantially higher HSE risks and work-related injury frequencies which implies that increasing the automation level of high-risk operations within fisheries and aquaculture would lead not only to economical but also social and ethical benefits [1]. Using intelligent machines and robots to replace humans in challenging and dangerous environments may improve the HSE situation in these industries, which will benefit society. Increased automation will improve the level of control humans have over operations in aquaculture by facilitating increased future use of autonomous underwater robots in the industry [2]. This paper aims to address challenges on how autonomous operations in complex and dynamically changing environments can be realized by enabling underwater robots to adapt their actions by considering interaction with fish, deformable flexible structures and environmental disturbances. This harmonises with the currently emerging Precision Fish Farming (PFF) concept, which outlines how innovative technologies and automation principles may be used to industrialise, digitise and improve operations in fish farming [3]

Material and Methods

The CageReporter project (RCN 269087) headed by SINTEF Ocean AS aims to develop a tetherless permanent resident underwater robotic system [4]. The integrated system consisting of an advanced underwater positioning system, a vision system able to obtain high quality data and novel control functions is developed in order to perform data capture in interaction with the fish, infrastructure and production environment. To this end, adaptive and bio-interactive control concepts have been developed for underwater robots performing autonomous operations. The concern of the bio-interactive based inspection and intervention operations/tasks is addressed in the context of intelligent bio-interactive path planning, which is the task of planning a path that allows the vehicle to inspect the area of interest while avoiding collision with the infrastructure inside the fish cage and avoid 'scaring the fish during each operation

Results

The developed control framework consists of three components: a) estimation of cage structure and fish behavior, b) path planning and c) path following control approach. Estimation techniques combining measurements from underwater positioning system have been used to estimate the deformation of the structure during planned operations in fish farms. In particular, the obtained positions of different locators installed at different places of the fish cage have been combined with a prior knowledge of the fish cage shape in order to estimate the current profile and thus the deformation of fish cages at each time step (Figure 2). For the estimation of fish behavior, underwater vision sensors combined with advanced image processing techniques have been used in order to calculate the fish swimming speed and the distance of the vehicle from the fish (Figure 3). Afterwards, the knowledge of the structural deformation and the fish related behavior have been used in order to propose bio-interactive path planning concept in this paper. To accomplish the planned operation, the off-line path has been generated using a prior knowledge of the fish cage structure. Then, the adaptation of the generated path is performed at each time step considering the inputs for the constraint functions obtained by using the online estimation of structural deformation and fish behavior. The estimated path is fed to the control systecm as reference point to be followed using the proposed path following control approach during the planned operations (Figure 2).

Discussions and Conclusion

Underwater robots are today used in a variety of different applications in different industrial segments (e.g. oil and gas, shipping and conservation/oceanography). In most present applications, the vehicle is beneath the wave zone, where environmental impacts are less challenging, and relates to fixed features (e.g. seabed, permanent bottom installations and rigid ship bodies). However, the external features a robotic system faces in an aquaculture situation differ from those encountered in conventional operations. Motivated by the PFF concept and following the needs of future unmanned aquaculture operations, this paper targets a novel research area by investigating the challenges of using underwater robots in "application-realistic" environments such as fish farms, where structures are flexible, and robotic systems must interact with animals during operations.

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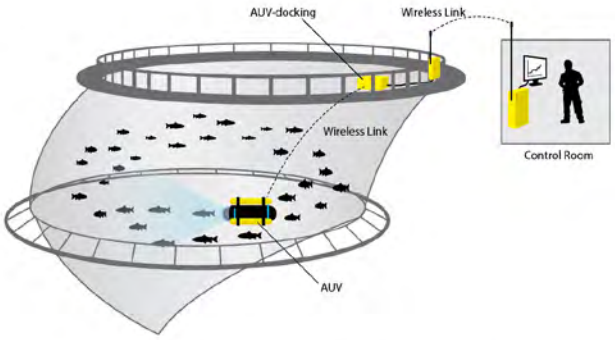

1 Permanent Resident Underwater Robotic System

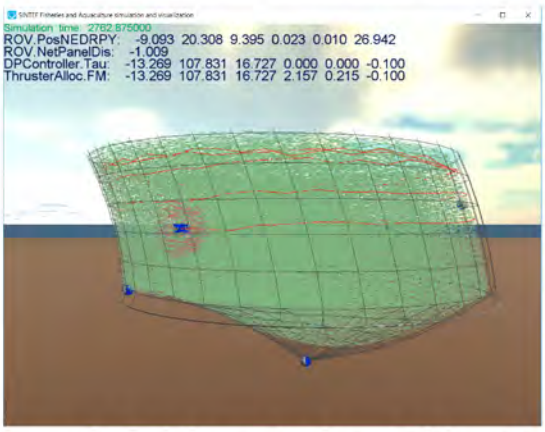

Figure 2 Real-time estimation of deformable structures and bio-interactive path following

Figure 3 Identification of fish behavior using high-quality vision data and artificial intelligent techniques

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INVESTIGATION OF THE BIODEGRADATION EFFICIENCY OF T-2 MYCOTOXIN-DEGRADING BACTERIA BY MICROINJECTION ON ZEBRAFISH (*Danio rerio*) EMBRYOS

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Introduction

Extreme weather conditions resulting from climate change greatly influence the appearance of microscopic molds and the amount of mycotoxins produced on agricultural crops (e.g. fruits). Mycotoxins are not only the reason of economic losses but also a source of public health threats. One of the most promising methods of mycotoxin decontamination is degradation and detoxification by microorganisms or by their enzymes. The method allows the removal of mycotoxins without using harmful chemicals and without significant loss in nutritional value. According to EFSA's recommendation, in addition to the degradation potential of the microbes used for this purpose, the metabolites from the parent compound should also be investigated by ecotoxicological tests. Not all toxins and microbes have a fast, reliable and cost-effective bioassay/ecoassay, this is also relevant for the T-2 toxin we tested, which is one of the most important mycotoxins from an agricultural point of view. Due to its immunological, dermato- and neurotoxic effects, it represents a potential hazard to both human and animal health.

Materials and methods

Our study covers if the seven selected T-2 degrading microbial strains have biodetoxification potential. The aim of our experiments was the bacterial degradation and biodetoxification of T-2, so the elimination of its harmful effect. The residual biological activity of the degradation samples was tested on zebrafish embryos by microinjection (120 hour test).

Results

Based on the results of our experiments, it can be stated that only the normal metabolite and the degradation by-product of *R. erythropolis* NI1 were not toxic to the treated fish. The normal metabolite of *R. rhodochrous* NI2 was found to be harmful, whereas the degradation by-product was not.

Conclusion

In conclusion, metabolic products produced by degrading bacteria under normal living conditions are also important to study, and considering the EFSA recommendation is recommended in all similar cases. In addition, the strain NI1 may be used in the future to decontaminate contaminated feed indoors.

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ECOSYSTEM SERVICES PROVIDED BY FRESHWATER FISHPONDS IN RELATION TO CLIMATE CHANGE

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Introduction

Fishponds in Central and Eastern Europe are centuries-old human constructions, however, beyond using them for fish production freshwater ponds are sites of valuable functions which are essential to society. Ecosystem services, defined as 'benefits people obtain from ecosystems' (MA, 2005), are often unrecognized in the case of fishponds. As nature-like shallow waters, fishponds are highly exposed to recent and forecasted changes in climate and therefore the ecosystem services may suffer considerable drawbacks. The objectives of our study were to identify ecosystem services (ESs) provided by the second largest pond system in Hungary and survey its importance for local residents. Moreover, we estimated the impact of climate change on identified ESs.

Materials and methods

The study area was the Biharugra Fishponds, located in the southeast of Hungary with a territory of 1920 hectares. The ponds are semi-intensively managed with an average stocking density of 350kg ha^{-1} in polyculture of warm water species, like common carp, silver carp, grass carp, European catfish, pike and pikeperch. During the three-year growing period, supplementary grain feeding is applied accompanied by manuring to enhance natural food resources. Because the favourable living and feeding conditions, fishponds are suitable for a great number of waterbirds and have a significant nature conservation value. To identify the fishponds ecosystem services we interviewed local key-informants of various stakeholder groups. Habitat mapping according to the Hungarian landscape types was implemented (Bölöni et al, 2011) as a base for ESs' visualisation. Biophysical and socio-cultural assessments were conducted on the revealed ESs. For biophysical evaluation, we have assigned indicators to every quantifiable service to determine their supply in a 3-5 years interval. During the socio-cultural assessment, we have performed 70 questionnaires among the people of the neighbouring settlement, to reveal local demand for services and their perceptions towards ESs. To assess ES capacity, we used a matrix model based mapping (Jacobs et al., 2015). To identify climate change effects on ESs the survey proposed by Foley et al (2018) was conducted by an expert panel.

Table I Ranking of identified ESs at Biharugra Fishponds and the effects of climate change (CC) (+ positive, - negative, +/- both)

Ecosystem service	Rank	CC effect
Fish production	1	+/-
Recreation	2	na
Aesthetics	3	-
Presence of protected/rare species	4	-
Reed production	5	+
Habitats for protected/rare species	6	-
Education	7	+
Air quality regulation	8	na
CO_2 absorption	9	+
Inspiration	10	+
Water storage	11	+
Groundwater recharge	12	+
Water quality regulation	13	+/-
Opportunities for research	14	+/-

(Continued on next page)

Results

Fourteen site-specific ESs were revealed through interviews with diverse stakeholders which were then ranked by the people of Biharugra the nearest village in the area (Table I). Our results showed that fish production, recreation and habitat provision were the most highlighted services (Palasti et al., 2018). Additionally, the interviews exposed the importance of other ESs like air quality regulation, education or water quality control. The potential use of the 14 services was mapped based on a matrix model and the actual use of ESs was also mapped and valued in biophysical terms in the described sub-habitats of the pond system which allowed us to build in stakeholders' ecological knowledge, as well.

According to the expert panel, almost all ESs will be impacted by climate change. Hungarian aquaculture will most likely benefit from higher temperatures in general through accelerated growth rates and elongated production seasons. However, increased occurrence of extreme weather events, new pathogens will stress fish and food organisms and also may amortize physical infrastructure. Altered precipitation patterns, especially drought will cause water scarcity and conflicts between fish farmers and operators of the irrigation sector. Assumed positive effects in some cases (education, water storage, inspiration) predict a need for increased efforts and attention towards semi-natural aquatic and wetland ecosystems.

Conclusions

The numerous ESs identified in our study site prove that freshwater fishponds are important ecosystems in rural areas in Hungary and in Central Europe as well. Climate change, even if positive effects were found, requires special planning and management efforts and additional costs to keep sustainable this segment. Working with diverse stakeholder groups also revealed future possibilities for further utilization such as water storage, ecotourism and environmental education.

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MOLECULAR GENETIC DIVERSITY OF HUNGARIAN SILVER PRUSSIAN CARP (*Carassius auratus gibelio)* POPULATIONS BASED ON MITOCHONDRIAL D-LOOP SEQUENCES

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Introduction

Silver Prussian Carp (*Carassius auratus gibelio,* Bloch, 1782) is a non-native, invasive fish species in the Hungarian water bodies. Because of their gynogenetic reproduction they spread very fast and mean a serious food competitor to native fishes and economically important species, like the carps. In spite of these our knowledge very limited about their population genetic background. The aim of the present study to analyse the genetic diversity of the silver Prussian carp populations on the water basin of lake Balaton and some additional Hungarian populations, based on mitochondrial D-loop sequences.

Materials and methods

134 samples were collected from six places in Hungary, the inner region of the Lake Balaton (Siófok;n=29), two regions of the Kis-Balaton (Ingó (n=18), Kányavár (n=18), Hőgyész (n=31), Török (n=19) and Őszödi-berek (n=19). From the samples, DNA was isolated by E.Z.N.A Tissue DNA Kit following the producer protocol. PCR was made with a general D-loop primers from Cyprinidae, Carp-pro2-F (5'-TCACCCC TGGCTCCCAAAGC-3') and Carp-Phe2-R (5'-CTAGGACTCATCTTAGCATCTTCA GTG-3') (Wang et al. 2010).

The purified amplification product was sequenced by using Big Dye Terminator v. 3.1 Cycle Sequencing kit (Applied Biosystems) and ABI PRISM® 3100 Genetic Analyzer. Sequence alignments were made by ClustalW algorithm with MegaX software (Kumar, 2018). Based on the polymorphic sites haplotype groups were calculated by DnaSP6 version 6. software and polymorphic tree was made by MegaX software. Different haplotypes were compared and checked in the NCBI database.

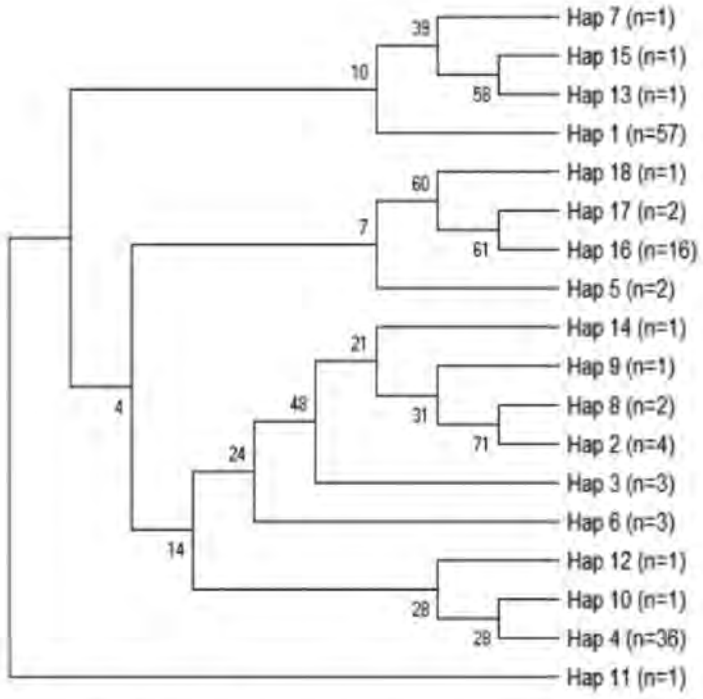

Fig 1. Silver Prussian Carp haplotypes in Hungary

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Results

A total of 18 haplotypes were identified in the 134 sequences with 50 variable sites. Haplotype diversity was 0,735. The most common group were contained (Haplotype 1) 57, the second (Haplotype 4) 36 and the third 16 individuals (Figure 1.). The remaining 15 haplotypes were present only 1, 2 or 3 samples. Populations from the different area were distributed mixed in the haplotypes expect the samples from Öszödi-berek. The Haplotype 17 and Haplotype 19 were found only in this population. Based on the NCBI Blast, there were 4 groups which despite their phenotypes show higher similarity with other *carassius* subspecies, than the *Carassius auratus gibelio*.

Discussion and conclusion

In conclusion, 18 haplotypes were successfully identified in six *Carassius auratus gibelio* populations in Hungary. More than one haplotypes were identified in all populations showing the maternal lines in populations. Probably live connections are present between the Balaton and its influencers based on the distribution of haplotypes. Only the Öszödi-berek forms an independent group. Interestingly 5 individuals carried goldfish originated haplotypes (*C. a auratus; C.a. buergeri*) showing signs of hybridization with other carassius species. However, these ornamental species are not natives in Hungary they can survive in nature and escape or release from ornamental ponds are proved several times. There was not identified the native *Carassius carassius* specific haplotypes. Larger sample size and nuclear genomic analyses are required for more detailed analyses of hybridization with the native relatives.

Acknowledgements:

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SLAUGHTER QUALITY OF ATLANTIC SALMON (*Salmo salar*) USING COMPUTERIZED TOMOGRAPHY

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Introduction

Currently, genetic evaluation of slaughter quality of Atlantic salmon (*Salmo salar*) is based on costly, time-consuming and laborious yearly test-slaughter of thousands of siblings of the breeding candidates. The potential of genetic progress in slaughter traits is limited, as the traits are recorded only from relatives, and because the manual dissection phenotypes are likely to be coupled with a measurement error.

Earlier studies have reported that computerized tomography (CT) scans accurately predict fat and protein percentage in rainbow trout (Gjerde, 1987) and Atlantic salmon (Rye, 1991), but so far this methodology is not implemented in salmonid breeding programs. Our objective is to create a link between the manual slaughter traits and CT scanning of live fish to assess the potential benefit of utilizing C technology for breeding evaluation of slaughter quality in Atlantic salmon.

The first part of the project focused on coupling traditional test slaughter measurements of body weight and fat with the CT-scan phenotypes to get deeper insight of the genetic variation in body composition, and to assess whether the ranking of the families relative to slaughter traits is similar using these two measuring approaches.

Material and methods

Data comprised records from 2692 (1685) individually tagged progeny of 144 (112) sires and 276 (178) dams from manual dissection (CT). The average weight of the fish at slaughter was 4.2kg. For each individual harvest weight (MHWT), gutted weight (MGWT) and fillet weight (MFWT) were recorded, and fat percentage (MFAT) in the fillet was measured using near infrared spectroscopy (NIR).

All CT scans was performed using a Toshiba Aquilion 16 CT scanner (Toshiba Medical Systems, Japan). The CT protocol selected for the salmon was with an energy of 100kVp, X-Ray tube current of 150mA, 750ms exposure time, standard reconstruction kernel (FC01), 2mm slice thickness and an in-plane-pixel spacing of 0.781/0.781mm. The data processing was conducted using a custom-made program removing the organs and cutting the head and tail off by applying a virtual three-dimensional mask to the CT scan. Individual scans were further segmented into volume of different tissue types (fat, meat and bone) for whole, headed-gutted fish and for the fillet, and multiplied by densities estimated from the round weight to convert into weight of the virtual cuts. Virtual phenotypes of fillet weight (FWT_CT), fillet fat percentage (FFAT_CT) and intestinal fat (INTFAT_CT) are presented in this abstract.

Genetic parameters were estimated using ASReml (Gilmore et al. 2015) with a bivariate animal model:

$$\begin{vmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{vmatrix} = \begin{bmatrix} \mathbf{X}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_2 \end{bmatrix} \begin{vmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{vmatrix} + \begin{bmatrix} \mathbf{Z}_{1_A} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_{2_A} \end{bmatrix} \begin{vmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \end{vmatrix} + \begin{vmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{vmatrix} \begin{vmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{vmatrix} = \begin{bmatrix} \mathbf{X}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_{1_A} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_{2_A} \end{bmatrix} \begin{vmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \end{vmatrix} + \begin{vmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{vmatrix},$$

where, $\mathbf{yy}$ is the vector of the phenotypic observations; $\mathbf{bb}$ is the vector of fixed effect of sex (male and female); $\mathbf{aa}$ is the vector of random additive genetic effects; $\mathbf{ee}$ is the vector of random residual effects. The $\mathbf{XX}$ and $\mathbf{Z}_A\mathbf{Z}_A$ are the design matrices assigning observations to the levels of fixed effect and additive genetic effects, respectively. Variance components were also estimated with a univariate animal model, and an additional random family effect was fitted for manual slaughter traits.

Results

Estimates of heritability for MHWT, MGWT, MFWT and MFAT from a univariate model were moderate, ranging from 0.28 to 0.32. Proportion of family variance from phenotypic variance varied between 0.04-0.07 and connected with a high standard error. Genetic correlations between the body weight traits were close to unity (0.96-0.99).

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Estimate of heritability for FWT_CT was similar (0.30) and for FFAT_CT higher (0.63) than for traits measured in the manual dissection. Heritability estimate for INTFAT_CT was 0.35.

MHWT and FWT_CT were genetically the same trait. Genetic correlations between MFAT and MHWT/MFWT were moderate (0.42-0.53), whereas high correlations were estimated between FFAT_CT and MHWT/MFWT (0.82-0.83). FFAT_CT and MFAT were phenotypically and genetically highly correlated with estimates of 0.64 and 0.91, respectively. Weight traits MHWT, MFWT and FWT_CT were all unfavourably phenotypically (0.51-0.63) and in lesser extent genetically (0.28-0.53) correlated with INTFAT_CT.

Discussion

The results presented here are part of a large project assessing the potential of using CT scans to evaluate the breeding candidates relative to the slaughter quality in Atlantic salmon. If successful, CT technology would enable finetuning the optimisation of body composition and slaughter quality of salmon through selection and offer potential for incorporation of non-invasive new phenotypes as selection criteria without resource- demanding test-slaughter.

As a first step, we performed a test-slaughter combining CT-scanned and manually dissected phenotypes. The genetic parameters show clearly the potential of using virtual cuts from CT scanning as selection criteria for improved slaughter quality. Unity genetic correlation between MHWT and FWT_CT verifies the difficulty of genetic improvement of fillet yield when selecting for body weight. MFAT has been shown to be highly correlated with chemical references (Segtnan et al. 2009). High genetic correlation between MFAT and FFAT_CT implies reliable ranking of individuals on MFAT based on non-invasive FFAT_CT phenotypes. Selection for rapid growth causes genetic increase in MFAT in lesser extent than in FAT_CT. Difference may arise from the definition of CT phenotypes (voxel threshold value and conversion through density) or that CT phenotype captures components other than lipids, which are highly genetically correlated with rapid growth.

In the next phase of the project we will CT scan live fish to validate the methodology and estimate the genetic correlations between live fish virtual cuts with manual dissection. High genetic correlations would speak for replacement of manual slaughter and sib information by live CT scanning of breeding candidates to recorded individual phenotypes for slaughter quality.

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POWER CALCULATIONS FOR OPTIMISATION OF THE EXPERIMENTAL DESIGN TO DETECT G X E: SALMON EXPERIMENTS IN FUTUREEUAQUA

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Introduction

The objective of the WP1 in FutureEUAqua is to assess how the current breeding programmes for salmon, seabass, seabream and rainbow trout can respond to future demands for novel feed compositions, and to make further improvements to disease resistance, climate resilience and animal welfare. For Atlantic salmon (*Salmo salar*) we estimate the genotype by diet/climate interactions (GxE) in semi-commercial salmon production system and validate best selection methods in salmon breeding programs by comparing traditional BLUP selection with GS/MAS for production and robustness traits. Estimated correlations will be taken as indicators of the magnitude of re-ranking of genotypes across diets/environments.

Power calculations are elementary part of the experimental design but unfortunately often de-prioritized, compromising the critical interpretation of the results. *A priori* power calculations of genetic studies are characterized with multiple uncertainties, such as true relationship structure, number of families and individuals at the time of registration and unknown heritability of the traits of interest. This said, we argue that by performing a range of power calculations it is possible to frame the true power of the experiment and improve the probability of executing scientifically solid experiments with given restrictions of resources. We demonstrate the optimisation of the experimental design in order to have adequate power to detect significant GxE (diet/climate aggregate) given FutureEUAqua WP1 resources.

Material and methods

WP1 experiments will be run on two geographical locations in order to study both genotype by feed and genotype by climate interactions. Given the WP1 resources each treatment will be run in two parallels. We assessed the power of detecting heritability (0.05-0.35) in experiments using different number of families utilizing the maximum cage capacity of 750 fish. Same range of true trait heritability and true genetic correlation ranging from 0.1 to 0.9 was used to estimate power detecting genetic correlation (r_g) different from 1 (one). Standard error of heritability was calculated assuming full-sib family structure, thus multiplying the standard error of the intraclass correlation by two: $\sigma_{h^2} = 2\sigma_t = 2\sqrt{\frac{2[1+(n-1)t]^2(1-t)^2}{n(n-1)(N-1)}}$ $\sigma_{h^2} = 2\sigma_t = 2\sqrt{\frac{2[1+(n-1)t]^2(1-t)^2}{n(n-1)(N-1)}}$ (Falconer 1989, p182). Standard error of the genetic correlation is dependent on the heritability of the traits (assumed identical), the standard error of the heritability and the genetic correlation: $\sigma_{(r_A)} = \frac{1-r_A^2}{\sqrt{2}}\sqrt{\frac{\sigma_{(h_x^2)}\sigma_{(h_y^2)}}{h_x^2 h_y^2}}\sigma_{(r_A)} = \frac{1-r_A^2}{\sqrt{2}}\sqrt{\frac{\sigma_{(h_x^2)}\sigma_{(h_y^2)}}{h_x^2 h_y^2}}$ (Falconer 1989, p317). Power was calculated in the context of one-tailed test and 0.95 cumulative probability.

Results

Assuming equal full-sib family size, power of 0.8 to detect true heritability of 0.15 or above was achieved with 20 or more families. To obtain similar power to detect significant GxE ($r_g \leq 0.8$) a minimum of 35 families was required when both traits have true heritability of 0.2 or larger, (figure 1). For genetic parameter estimation it is common to choose the design aiming at 10 fish/famil at registration. With this design and 0.2 heritability, we would be able to detect significant GxE for $r_g \leq 0.9$ or below with power of 0.80, whereas for heritability 0.15 true genetic correlation of 0.6 is needed to detect significant re-ranking of genotypes with identical power.

If the available capacity is only 500 fish, the power of detection of 0.15 heritability would drop down to 0.75. Alike, the maximum probability of detecting significant GxE ($r_g \leq 0.8$) for trait heritability 0.2 reduces to 0.7. For 10 fish/family design, $r_g \leq 0.55$ or $r_g \leq 0.30$ would allow to conclude re-ranking of genotypes with 80% power for traits with trait heritability of 0.2 and 0.15, respectively.

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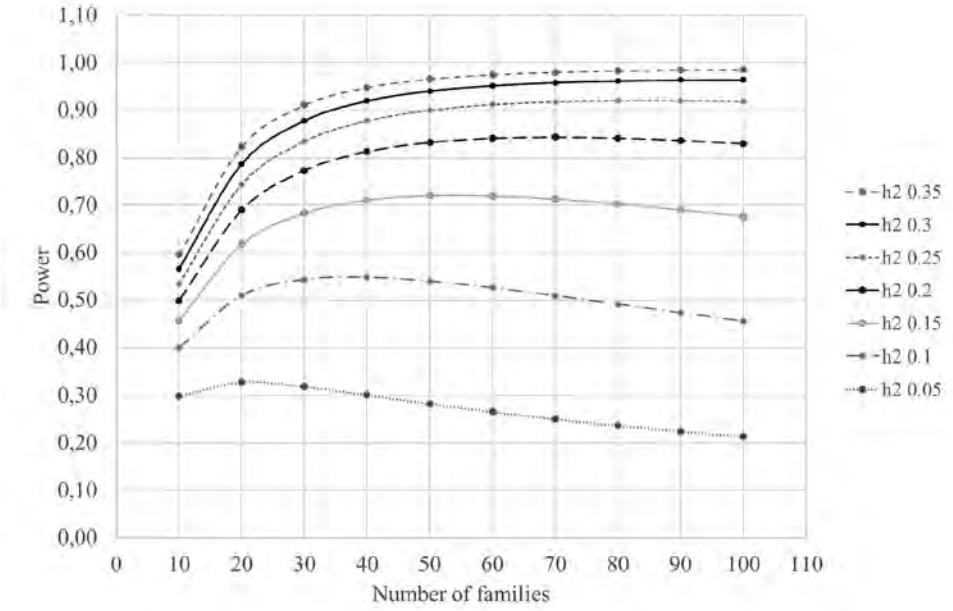

Fig. 1. Power to detect significant GxE, defined as r_g=0.8 (and below), with different number of families and true trait heritability given total capacity of 750 fish.

Discussion

Power calculations, demonstrated here, will allow appropriate reflections of the magnitude of r_g relative to the existence of GxE. The estimates presented here are based on full-sib family structure. In practical breeding programs the genetic material includes half-sib groups and/or related parents. Additionally, relationships are increasingly based on genomic information. Half-sib family structure will reduce the power, counteracted by the genomic relationship information. The true experimental design includes parallel cages to be able to detect treatment effects, whereas we have presented conservative calculations based on one cage only. Despite uncertainties, by performing a range of power calculations it is possible to frame the true power of the experiment with given restrictions of resources. Magnitude of power considered acceptable should be reflected and aligned with the overall objectives and resources of the project

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SYNERGETIC EFFECT OF DIETARY ESSENTIAL AMINO ACIDS (LYSINE & METHIONINE) ON THE GROWTH, BODY COMPOSITION AND ENZYMES ACTIVITIES OF GENETICALLY MALE TILAPIA (GMT)

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This study was conducted on genetically male tilapia (GMT) fry reared in glass aquariums for three months to examine the synergetic effect of essential amino acids (EAA) supplementation on growth, body composition and enzyme activities. Fish having average body weight of 16.56±0.42g were fed twice a day on artificial feed (20% crude protein) procured from Oryza Organics (commercial feed) supplemented with EAA; methionine (M) and lysine (L) designated as T1 (0.3%M & 2%L), T2 (0.6%M & 4%L), T3 (0.9%M & 6%L) and control without EAA. Significantly higher growth performance was observed in T1 followed by T2, T3 and control. The results showed that whole-body dry matter and crude protein were significantly higher ($P \leq 0.05$) in T3 (0.9% & 6%) feeding fish, while the crude fat was lower ($P \leq 0.05$) in the same group of fish. Additionally, protease, amylase and lipase activities were also observed maximum ($P \leq 0.05$) in response to T3 than other treatments and control. However, the essential and non-essential amino acids, especially methionine and lysine were found significantly higher ($P \leq 0.05$) in T1 compared to other treatments. Conclusively, the addition of EAA methionine and lysine in feed not only significantly enhanced the growth performance of GMT fry, but also improved body proximate composition and essential amino acid profile.

PROGRESS IN HĀPUKU (*Polyprion oxygeneios*) PRODUCTION FROM F1 BROODSTOCK

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The wreckfish hāpuku (*Polyprion oxygeneios*) is a high-value commercial species found in the waters of the southern hemisphere. A research breeding program was started to develop the species as for aquaculture in New Zealand, that showed the amenableness of wild caught broodstock to reproduce in captivity, with the key production bottleneck being the highly variable and often very low fingerling survival. However, sufficient numbers of F1 were obtained which were subsequently raised as broodstock. Here, we provide a broad description of the development of reproductive performance of F1 broodstock relative to wild-caught broodstock.

Wild and F1 broodstock were kept in 20-70 m^3 photothermal controlled tanks. Wild and F1 broodstock were given wet feed and pelleted feed, respectively. Spawning occurred between August and December, with sufficiently viable eggs carried through incubation and larvae rearing. The 2008 F1 cohort was part of a study to describe the physiological control of reproduction and were periodically handled for gonad biopsies, measurements, blood sampling and hormonal induction (GnRHa and hCG). The 2010 cohort acted as a minimal disturbance group for comparison. Production and quality parameters of eggs and larvae for each batch (e.g., fertilization, hatch and survival rates) were recorded.

F1 broodstock can be induced to spawn in captivity from 5-6 years using GnRHa implants, with yearly improvements in egg fecundity and quality. After 7 years, particularly when disturbance was kept minimal in the preceding years, F1 broodstock consistently outperform wild broodstock in terms of fingerling survival (5-7% yearly average) by a factor of 5. At 8 - 9 years, spontaneous spawning occurred without exogenous hormone. The high fingerling production subsequently revealed another bottleneck at the early juvenile stage associated with *Vibrio* proliferation, which was overcome by improvements in microbial control practices. Thus, we achieved fingerling and juvenile survival of 6.6% and 4.1% in the last production season from F1 broodstock.

Conclusion

The ongoing research program has now demonstrated that captive-bred (F1) broodstock are a viable and sustainable source for high quality gametes in hāpuku. However, commercial-level natural production does not take place until at least 7 years.

COMPARISON OF SURVIVAL AND GROWTH RATES OF ABALONE, *Haliotis discus hannai*, IN THE DISCHARGE AND INTAKE CHANNELS OF IN POWER PLANT

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Introduction

The aquaculture potential offered by the thermal discharges of power stations is widely recognized. Their suitability for fish farming largely depends on the cooling water sources and on the design and operation of the power station. Water of varying quality and salinity is used to generate steam and drive the turbines, which then drive the generators, and the waste steam is fed to a condenser and discharged as thermal effluent.

The heated water that is discharged from steam-electric generating stations has been considered a nuisance and a direct threat to aquatic life. The survival rates of caged fish in discharge canals have been extremely poor, and, thus, it is necessary to develop an aquaculture system for culturing new species such as abalone that can tolerate high-temperature environments relatively well compared to fish. This development would transform "waste water" into an aquaculture resource.

Materials and methods

Survival and growth rates of abalone were investigated in the cages installed in the discharge and intake channels for a total of 98 days from December 17, 2014 to March 25, 2015. The total number of abalone stocked in the 240 shelters installed in the container system was approximately 115,000, with about 479 abalone per shelter. The average shell length of each abalone was 37.87 ± 3.29 mm, and the mean wet weight was 6.29 ± 1.74 g. In order to compare the survival and growth rates of the abalone in the hot-water drainage channel and the intake channel, one cage for the control was installed in the intake channel. In the control cage, 500 abalones were enclosed within the abalone shelter, with an average shell length of 38.31 ± 3.35 mm and an average wet weight of 6.62 ± 1.86 g. Feeding was done about once every 10 days. To prevent deterioration of water quality in the shelter of the cage facilities, the abalone was fed and the remaining feed was removed at the next feed. Bubbles and biofouling that occur in the channels would interfere with the flow in the container; therefore, the containers were raised above the water surface and cleaned at three- or four-day intervals using a high-pressure washer.

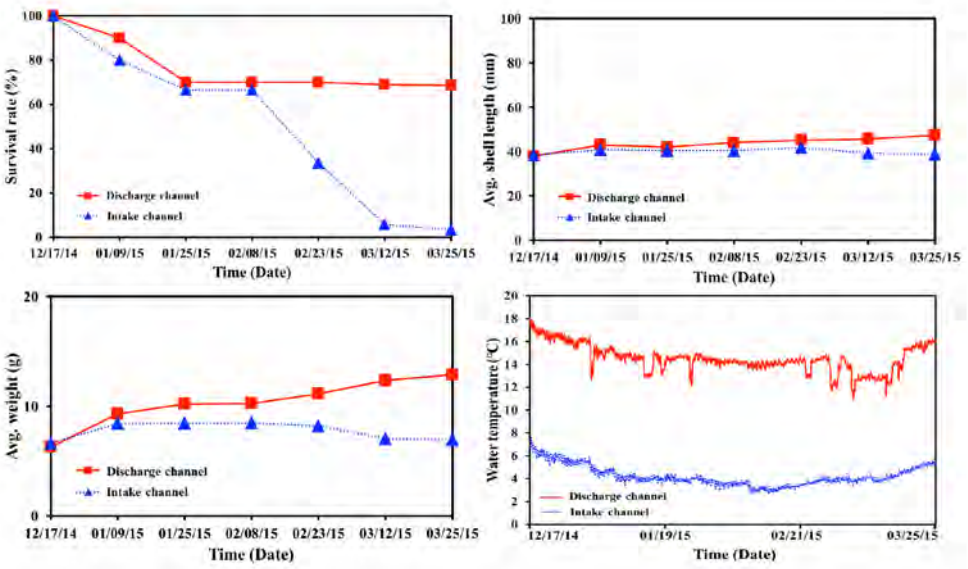

Fig. 1. Survival and growth rates of abalone plotted with changes in water temperature in the discharge and intake channels.

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Results and discussion

About 10 percent of the total stocks (approximately 11,500) were dead three days after stocking the abalone in the discharge channel, and most of the individuals with poor initial health died. After the initial 10 percent die-off, the mortality rate increased, and the final survival rate of abalone was estimated to be 68.71 percent in the discha ge-channel cage.

The survival rate of abalone in the control cage located in the intake channel began to decrease rapidly from February 23, 68 days after the abalone stocking, and the final survival rate of control abalone was 3.4 percent.

In terms of growth rate for the abalone in the discharge channel, the average shell length was 47.34 mm, representing a growth of 9.47 mm (125 percent), compared with 37.87 mm for the control cage. The average wet weight increased by 6.66 g (193.54 percent), from 6.29 to 12.89 g.

In contrast, the average shell length of abalone in the control cage grew from 38.31 to 38.9 mm, an increase of 0.59 mm (101.54 percent). The average wet weight of abalone in the intake channel increased by 0.28 g (104.84 percent), from 6.62 g to 6.94 g.

Conclusion

The successful performance of the abalone cage system in the discharge channel during the tests shows promise for feasibility in aquaculture to maximize growth rates of abalone in the winter season.

EFFECT OF DIETARY MONOBASIC POTASSIUM PHOSPHATE (MKP) ON GROWTH OF FAR EASTERN CATFISH (*Silurus asotus*) AND LEAFY VEGETABLES IN HBFT (HYBRID BFT) AQUAPONIC SYSTEM

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Introduction

To date, recirculating aquaculture system (RAS) and biofloc technology (BFT) have been the representative methods of aquatic animal production through the reuse of water in the rearing tank (Avnimelech et al., 2015). Aquaponics is an integrated production system which combines the aquaculture of fisheries with hydroponics of agriculture. In the system, around 30% of feeds supplied to animals such as fish and prawn are assimilated in their body. The remaining 70% are excreted outside the body (Emerenciano et al., 2017) and decomposed and formed into various minerals and nitrates. Successively, such nutrients are utilized by plants and water quality is optimally maintained. In general, plants require 13 kinds of nutrients (N, P, K, S, Ca, Mg, Fe, Mn, Cu, Zn, B, Mo and Al) for their growth in aquaponics but aquafeed contains a limited levels of Ca, K, Fe and P for growth of plants (Bittsanszky et al., 2016). The UVI (University of Virgin islands) aquaponic system has been proposed to artificially add the above-mentioned deficient nutrients for plant growth (Rakocy et al., 2006). The purpose of this study was to suggest a direction to simplify the present aquaponic system by supplying nutrients to the plants through diet only. Therefore, we tried to improve productivity of both of fish and plant by replacing the phosphate source being used in conventional diet with monobasic potassium phosphate (MKP) as P and K sources.

Materials and methods

Graded MKP of 1, 2, 3 and 4% was added to each of the experimental diets containing 45% protein and 7% lipid designated to MKP1, MKP2, MKP3, and MKP4. The first experiment (Exp. 1) was conducted to investigate the differences among three commercial diets (CA, CB and CC) and one experimental diet (MKP2) for growth of Far Eastern catfish (*Silulus asotus*) and two leafy vegetables (Lollo-bionda and Lollo-rossa). Following a 24 h fasting, 400 fish with a mean body weight of 225 g were randomly allotted to 4 groups in two replicates, whereby 8 tanks (1.5 × 1.5 × 0.6 m, water level of 1.35 ton) and 8 plant beds (1.5 × 1.5 × 0.1 m, water level of 0.22 ton) were employed. Diets at the level of 0.8% body weight/d were supplied twice a day at 08:00 h and 17:00 h, respectively, for 4 weeks. Subsequently, the second experiment (Exp. 2) was designed to determine the optimum levels of MKP as growth promoter for fish and four leafy vegetables (Lollo-rossa, Avatar, Caipira and Heuk-Romaine) during 10 weeks. Using the same system as the Exp. 1, 480 fish with a mean body weight of 186 g were randomly allotted to 4 groups in two replicates. Diets at the level of 0.5% body weight/d were supplied twice a day at 08:00 h and 17:00 h, respectively, for 10 weeks.

Results

After 4-week feeding trial of the Exp 1, there were no significant differences in weight gain (WG), feed efficiency (FE), specific growth rate (SGR) and protein efficiency ratio (PER) among fish groups fed MKP2, CA and CC ($P>0.05$) except CB. Growth (total leaf weight) of two leafy vegetables (Lollo-bionda and Lollo-rossa) was the highest in fish group fed MKP2 among treatments (Table 1). After 10-week feeding trial in the Exp 2, WG, FE, SGR and PER were higher in fish groups fed MKP2 and MKP3 than in other groups ($P<0.05$). Similarly, growth of four leafy vegetables was higher in fish groups fed MKP2 and MKP3 than in other groups.

Discussion and conclusion

The productivity (total leaf weight, g) of two leafy vegetables was higher in the MKP2 diet group compared to the three commercial diet groups (CA,CB and CC) in the Exp 1 and it was suggested that the addition of monobasic potassium phosphate (MKP) to the diet could improve plant productivity. In the Exp 2, all leafy vegetables grew well to such an extent that whole harvest was possible 23 days after the initial seeding. It was suggested that the supplementation of the MKP in diet would increase the levels of P and K in the system. Second order polynomial regression model analysis indicated that the dietary optimal MKP level could be 2.77% to 2.89% on the basis of WG of far eastern catfish and four leafy vegetables (Fig. 1 and Fig. 2).

(Continued on next page)

Table 1. Growth of two leafy vegetables in aquaponic system for 4 weeks (Exp. 1).

Item	Diets							
	MKP2		CA		CB		CC	
Species (No. 48)	Lollo-bionda	Lollo-rossa	Lollo-bionda	Lollo-rossa	Lollo-bionda	Lollo-rossa	Lollo-bionda	Lollo-rossa
T. wt (g)	1,868	1,496	1,220	868	1,783	1,179	1,597	1,051

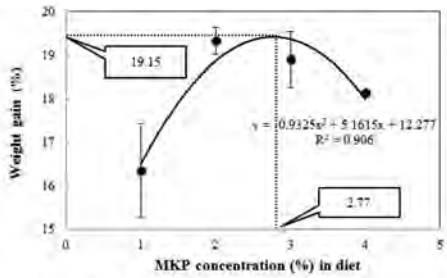

Fig. 1. Polynomial regression analysis on weight gain (WG, %) of Far Eastern catfish to dietary monobasic potassium phosphate (MKP) levels.

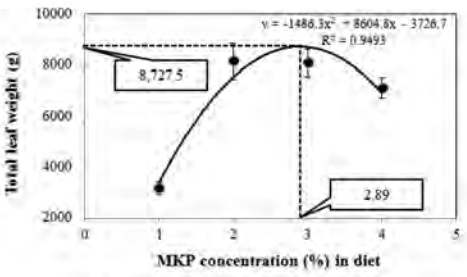

Fig. 2. Polynomial regression analysis on total leaf weight (g) of four leafy vegetables to dietary monobasic potassium phosphate (MKP) levels.

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EFFECT OF LOW PH AQUAPONICS USING MIXED MICROORGANISMS ON WATER QUALITY AND BLOOD CHARACTERISTICS OF FAR EASTERN CATFISH (*Silurus asotus*)

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Introduction

Aquaponic system is designed to keep water quality optimal for growth of both of fish and plants and water quality control has been carried out by a mechanical filtration filter which removes solids and a biological filtration which induces a nitrification process. Biofloc tech (BFT) is a recirculating aquaculture method that was introduced in South Korea in the 2000's. One of the pollutants generated by aquaculture is ammonia (NH_3-N) and the ammonia is removed by using organic carbon (molasses, glucose, etc.) to induce proliferation of microorganisms through C:N ratio (15:1 or more) control. To date, recirculating aquaculture system (RAS) does not completely reuse rearing tank water and it is common that less than 10% of the total circulation is replenished every day. RAS is slow to proceed with industrial expansion due to high initial capital investment. For this reason, fresh water aquaculture farms with a simple pollutant settling facility in South Korea prefer the use of the flowing water taken from river and underground. Farms that operate the RAS system are restricted to rearing high-priced fish species such as eel. The UVI (University of Virgin islands) aquaponic system, which has been universally adopted, has been operated in a way that combined hydroponic cultivation with the RAS production method of aquaculture (Bailey and Ferrarezi, 2017) but it has included a problem of facility cost and complexity of system operation. The purpose of this study was to investigate a low pH aquaponic system that utilizes useful microorganisms mixed with heterotrophs and autotrophs to increase the production efficiency and to simplify the current universalized system

Materials and methods

Far Eastern catfish (*Silulus asotus*) of 480 with a mean body weight of 186 g were randomly allotted to 4 groups in two replicates, whereby 8 tanks (1.5 × 1.5 × 0.6 m, water level of 1.35 ton) and 8 plant beds (1.5 × 1.5 × 0.1 m, water level of 0.22 ton) were employed. Diets (graded monobasic potassium phosphate of 1, 2, 3 and 4% was added to the experimental diet containing 45% protein and 7% lipid designated to MKP1, MKP2, MKP3, and MKP4) at the level of 0.5% body weight/d were fed twice a day at 08:00 h and 17:00 h, respectively, for 10 weeks. Product of BFT-ST (EgeeTech, Ltd., USA) was used for low-pH aquaponics water quality management. BFT method (Emerenciano et al., 2017) was applied for 14 days after fish stocking and glucose as carbon source was added into water. Then, the supply of organic carbon was stopped and a certain amount of carbon dioxide (inorganic carbon source) was continuously supplied into the sump tank until the pH dropped below 6.0 using an automatic regulator. From pH 6.0 or lower, useful microorganisms were added when NO_2-N increased and stopped when water quality maintained stable. Water quality (DO, pH, water temperature, electrical conductivity, turbidity, TAN, NO_2-N, NO_3-N, and PO_4-P) measurement was performed 6 times a week using a portable water quality meter (YSI PRODSS, YSI Inc., USA) and reagent measurement (DR5000, HACH Ltd., USA). Blood samples were taken from the caudal vessels of six fish from each group by using a heparinized syringe after fish were starved for 24 h and anesthetized with MS-222.

Results

In present study, the pH value began to decrease following 3 weeks and maintained less than 6.0 from 6 weeks later in all experimental groups. The values of TAN (4.58-20.40 mg/L), NO_3-N (24.12-52.40 m /L), and PO_4-P (20.38-48.48 mg/L) increased and NO_2-N value remained below 0.1 mg / L (Table 1). Blood analysis of fish was shown in Table 2.

Discussion and conclusion

The most important characteristic of the low pH aquaponic system with hybrid BFT is the change of TAN, NO_2-N and NO_3-N according to the nitrific tion process. The results of this experiment which were different from the previous study (Thorarinsdottir, 2015) showed that TAN and nitrate were elevated at the same time and this is presumed to be the result of the nitrificat on process by the heterotrophic bacterium such as genus *Bacillus* at low pH (5.0~6.0) condition. The largest hematologic features are PCV and Hb values which are main indicators of oxygen transport capacity and nutritional anemia, respectively, in fish. Compared to previous researchers, the high value of these two items seems to be the result of adaptation to the environment due to the low pH state of the closed environment.

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Table 1. Change of water quality (pH, TAN, NO_2-N, NO_3-N and PO_4-P) of MKP2 group in aquaponic system with hybrid BFT (biofloc tech) for 10 weeks.

Diet	Week	pH	TAN (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	PO_4-P (mg/L)
MKP2	1	7.80±0.09	0.12±0.07	0.245±0.132	3.33±0.48	0.56±0.19
	2	7.56±0.21	0.04±0.01	0.397±0.146	3.20±0.80	0.46±0.07
	3	7.84±0.10	0.07±0.02	0.092±0.045	4.03±0.97	0.72±0.12
	4	7.65±0.06	0.13±0.04	0.099±0.016	6.22±0.70	1.69±0.37
	5	6.69±0.79	1.00±1.01	0.101±0.039	14.74±4.23	7.43±3.59
	6	5.83±0.21	5.34±1.49	0.046±0.017	24.12±2.10	26.00±5.69
	7	5.40±0.16	9.40±0.91	0.026±0.004	34.66±3.45	38.30±2.50
	8	5.26±0.13	12.82±0.97	0.032±0.008	41.20±1.48	38.92±3.48
	9	5.30±0.19	14.66±0.50	0.045±0.009	39.40±4.77	41.56±2.06
	10(3day)	5.23±0.04	20.40±2.81	0.055±0.015	52.40±7.40	47.98±6.24

Table 2. Hematological analysis of Far Eastern catfish fed graded levels of monobasic potassium phosphate (MKP) in aquaponic system with hybrid BFT (biofloc tech) for 10 weeks.

Blood parameters	Diets			
	MKP1	MKP2	MKP3	MKP4
Average Fish weight (n=6)	203.1±26.6^{ns}	226.7±25.0	217.2±33.2	212.8±1.5
PCV (%)	45.83±7.31^{ns}	45.33±4.23	45.67±4.89	45.33±2.94
Hb (g/dL)	14.13±1.85^{ns}	14.03±1.41	14.47±1.99	14.50±1.02
Pi (mg/dL)	14.18±0.83^{a}	15.72±0.46^{b}	15.47±0.52^{b}	14.12±0.65^{a}
K (mEq/L)	0.57±0.08^{a}	0.80±0.11^{b}	0.92±0.19^{b}	0.72±0.08^{b}

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ROLE OF SMART AQUACULTURE RESEARCH CENTER IN REPUBLIC OF KOREA

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Smart Aquaculture Research Center (SARC) as a part of professional human resource training project of Ministry of Oceans and Fisheries is aiming to revitalize high-tech aquaculture industry and cultivate convergent professional manpower through the development of core smart aquaculture technologies and the cultivation of professional human resource.

SARC plans to develop the following three core technologies for smart health-care based on aquaculture biology. First, integrated monitoring and optimal feeding system based on ICT (Information and Communications Technologies)/ IoT (Internet of Things) function for control of breeding environment, growth, working environment, and aquaculture structure in aquaculture ground will be developed to increase productivity and reduce labor force. Second, water treatment technologies to increase re-use rate of breeding water in land based aquaculture will be developed such as nutrient treatment technology of breeding water, process development for efficien removal of waste products, and optimization technology of disinfection process of breeding water. SARC also commercialize IoT-based water quality factor analysis technology to treat pollution sources of aquaculture farm and will develop big data-based water quality analysis and prediction technology. Third, smart aquaculture environment technology to maximize production and for efficient production and management of safe and healthy on cultured fish will be developed by applying ICT to aquaculture process and optimizing breeding methods. SARC build big data on the growth factors of cultured fish growth stages and will develop big data-based health care model on fish growth stages. Moreover, ICT-based high sensitivity rapid diagnostic kit (Point-of-Care Tesing; POCT) and standard manuals for smart health-care will be developed.

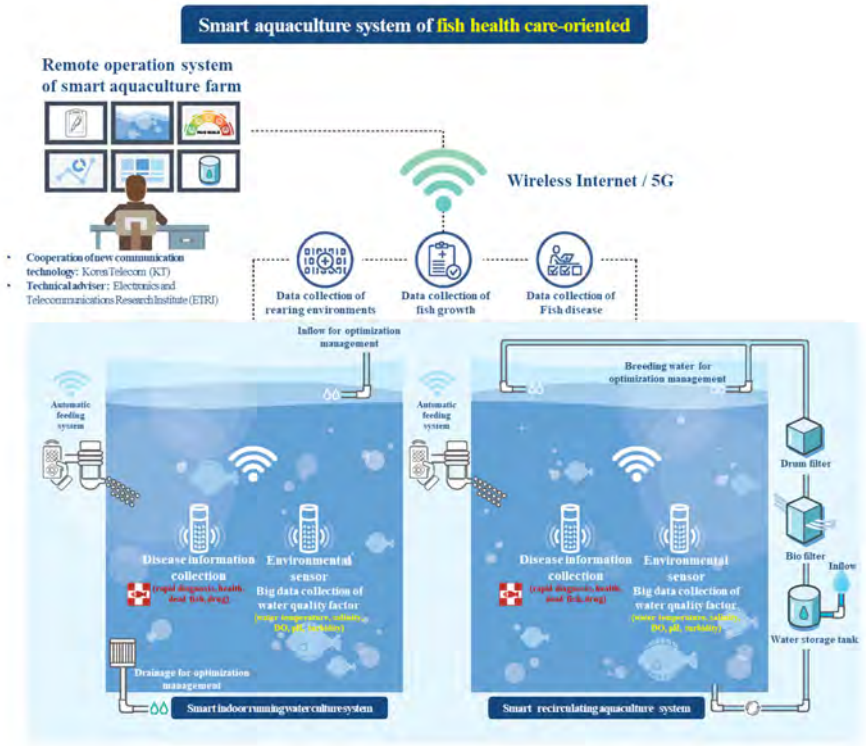

Fig. 1. Concept of smart aquaculture system.

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As above, the core goal of this project is to cultivate convergent professional human resource through training and technology transfer for the technologies developed at our center. The Smart Aquaculture Research Center plans to develop AI-C (Advanced Intelligent - Convergence) type professional human resource programs and operate three type programs such as advanced programs for attracting undergraduate students to graduate courses, intelligent programs for strengthening the research power of graduate students of master's and doctoral courses, and convergence programs for strengthening the practice through re-training of industrial manpower and for employment an start-up. In addition, a certification system for smart aquaculture training will be introduced in order to verify the ability of professional human resources who have completed the training of the AI-C type programs.

It is expected that there will be economic and technological ripple effect and job creation effect of Korea's fisheries industry through the development of smart fishery aquaculture technology and fostering of professional manpower that are aimed at Smart Aquaculture Research Center.

A STUDY ON POLY-CULTURE OF SEA Squirt *Halocynthia roretzi* AND SEA CUCUMBER *Apostichopus japonicus* UNDER A HANGING CULTURE SYSTEM

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Changes in the natural environment and high density aquaculture in limited fisheries ground resulted in lower productivity and mass mortality. Sustainable aquaculture farms based on eco-friendly style are needed. Therefore, it is necessary to develop aquaculture technology to improve the up-bottom use of water and the environment in the sea cucumber polyculture. We have developed polyculture techniques using oysters, sea squirts, and sea cucumbers.

The rate of inhabit test in Tongyeong was 45.6% in the oyster shell test, 30.1% in the black tube test, and 33.1% in the rubber tube. In the summer, the oyster shell test was 20.4%, the black tube test was 22.2%, the octopus port shelter test was 17.7% and the rubber tube test was 15.5%. The oyster shell test - 79.6%, black tube test -77.8%, octopus port shelter - 82.3% and rubber tube test plots - 84.5%. In August, the rate of inhabit began to increase gradually. In the November survey, 29.4% in oyster shell test, 35.4% in black tube test, 27.5% in octopus port shelter and 29.2% in rubber tube test. In December, the rate of oyster test was 34.4%, that of black tube test was 37.5%, that of octopus port shelter was 31.7%, and that of and rubber tube test was 32.5%.

The discharge density at the beginning of the test at Tongyeong - si oyster farm ground was 2.51 per m^2. In the March survey, which was one month period, the densities of the oyster shells were 1.06 m^2, 0.75 m^2 for the oyster shell, 1.13 m^2 for the octopus port shelter, and 0.83 m^2 for the rubber tube. The densities of the August survey in summer were the lowest at 0.51 m^2 for oyster test, 0.55 m^2 for black tube test, 0.44 $m^2$for octopus port shelter, and 0.39 m^2 for rubber tube. In October, when the water temperature dropped, the density gradually increased from 0.68 m^2 in the oyster shell test area, 0.83 m^2in the black tube test area, 0.58 m^2 in the octopus port sheltert area, and 0.59 m^2 in the rubber tub test area. In the December survey, the density was 0.86 m^2 in oyster shell test, 0.94 m^2 in black tube test, 0.79 m^2 in octopus port shelter, and 0.81 m^2 in rubber tube, showing similar density as March.

MOLECULAR CHARATERIZATION OF OMEGA AND KAPPA CLASS GLUTATHIONE S-TRANSFERASE FROM REDLIP MULLET (*Liza haematocheilus*) AND ENZYME KINETICS, OPTIMUM CONDITIONS

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Introduction

The redlip mullet (*Liza haematocheilus*) is a saltwater fish which holds paramount importance as food fish worldwide as well as in Korea. Present study, we characterized the GST kappa (LzGSTκ) and omega (LhGSTω) from redlip mullet. Functional studies were carried out with the recombinant protein to determine its enzymatic and antioxidant properties. In addition, the transcriptional levels of *LzGSTκ*, *LzGSTω* were determined under normal physiological conditions and immunologically challenged conditions.

Materials and methods

The transcriptome database of mullet cDNA sequences was developed using de novo assembly. Mullet fish (~100g, length 24 cm) purchased from Sangdeok fish farm in Hadong, Korea. The fish were acclimatized in the laboratory aquarium tanks at 20 °C for a week prior to the experiment. Total RNA was extracted by using RNAiso plus (TaKaRa, Japan) and cleaned with RNeasy spin column (Qiagen, Germany). Quantitative real-time PCR (qPCR), with specifically designed primers were performed to analyze the expression profile of *LzGSTκ, LzGSTω*. The coding sequence (CDS) of the cDNA fragment was amplified by using gene-specific cloning primers. The size of the amplicon were 687 bp (LzGSTκ) and 720 bp (LzGSTω) respectively. Digested PCR products were ligated into the pMAL-c5X vector. The ligated product was then transformed into *Escherichia coli* (*E.coli*) DH5α and the coding sequence was confirmed by sequencing. To express the recombinant LzGSTκ, LzGSTω protein (rLzGSTκ, rLzGSTω), the pMal-c5X/ LzGSTκ, LzGSTω construct was transformed into *E. coli* BL21 and incubated at 37 °C in LB broth medium containing 100 μg/mL ampicillin, until the OD600 reach 0.6. Isopropyl-β-galactoside (IPTG) was then added to the culture (final concentration 0.5 mM) and incubated for 24 h at 15 °C to induce the expression of the recombinant protein. After incubation, the cells were harvested by centrifugation. The rLzGSTκ, rLzGSTω protein was purified from the supernatant using maltose affinity chromatography. Enzyme activity was measured separately using CDNB as substrates. The absorbance of the reaction was measured, immediately and 5 min after addition of the substrate.

Result

To understand the potential endogenous functions of LzGSTκ, LzGSTω, its relative expression was examined in different tissues. Analysis of the expression profiles from the redlip mullet revealed that *LzGSTκ* was strongly expressed in the heart, while the lowest expression was observed in the head kidney (Fig.1.A). *LzGSTω* was strongly expressed in intestine, whereas the lowest expression was observed in the head kidney. (Fig.2.A) Activities of rLzGSTκ, rLzGSTω and MBP against different substrates, including CDNB(2,4-Dinitrochlorobenzene), DCNB(2,4-Dinitrochlorobenzene), 4-NPB(4-nitrophenethyl bromide), 4-NBC(4-nitrobenzyl chloride), and ECA(ethacrynic acid**)** were then measured. Only detectable activity was observed, when CDNB used as the substrate. No significant activity was detected for MBP against any of the substrates and therefore it was treated as a control. rLzGSTκ was shown to have GSH:CDNB conjugating activity at pH range 6 to 8 (Fig.1.B). Its highest activity was observed at pH 7. For RLzGSTω, GSH:CDNB conjugating activity observed at pH range 6 to 9 (Fig.2.B), wherein the highest activities were observed on pH 8.

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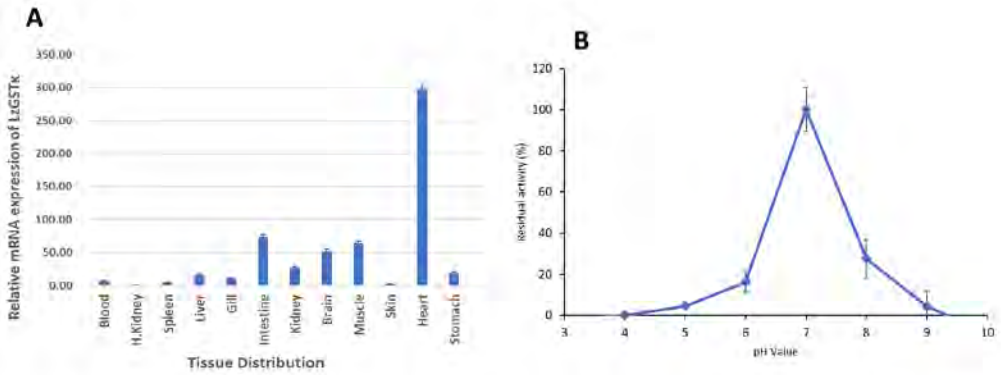

Fig. 1. (A)Relative mRNA expression level of GSTκ in different tissues. The tissues were collected from healthy mullet fish, and expression levels in each tissue were analysed using real-time qPCR. (B)Effect of pH on the GSH conjugating activity of LzGSTκ.

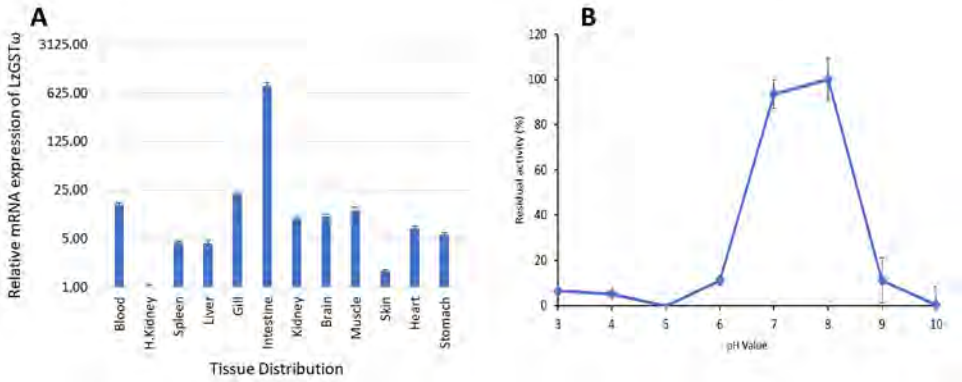

Fig. 2. **(A)**Relative mRNA expression level of GSTω in different tissues. The tissues were collected from healthy mullet fish, and expression levels in each tissue were analysed using real-time qPCR. (B)Effect of pH on the GSH conjugating activity of LzGSTω.

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LOCAL ACCEPTANCE OF MUSSEL CULTIVATION IN THE BALTIC SEA

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Introduction

It is a recurring problem in land use management, including also coastal management, that the location of all kinds of infrastructural installations and production plants can cause conflicts. Such siting conflicts arise even when new facilities provide general societal benefits such as reduction of greenhouse gas emissions or of nutrient loads in fjords and seas. Discrepancies between greater societal concerns and local resistance sometimes lead to accusations of so-called NIMBYism – i.e. 'Not in my back yard'-ism – implying that local interests are egoistic when they acknowledge the greater good of a facility but just want it located elsewhere. The term is, however, more useful as abuse than as analysis, because it completely blocks any consideration of the legitimacy of local concerns and also blocks the integration and negotiation of such concerns in land use planning (Devine-Wright 2009).

The accumulated experience regarding facility siting has revealed a pattern of recurring issues that are at the root of potential conflicts. Thus, residents, permanent as well as seasonal, can feel a strong attachment to the place in which they reside. This is not least true of seasonal residents, i.e. summerhouse owners and other recurring visitors in an area who may have a longer connection to the area than they have to their permanent residence, and who are particularly concerned about the landscape of their leisure time not being ruined by any changes (Stedman 2006; Trentelman 2009).

It can therefore be perceived as an intrusion when a new facility causes significant changes in the landscape and scenery, whether it is as foul smell, noise, increased traffic, large unappealing structures, increased exposure to health risks, reduced air or water quality or something else. Local residents and visitors may also feel that while they have to live with the nuisances and damages of a facility, its benefits are located elsewhere. Moreover, a new facility can potentially disrupt existing activities and business interests, for instance if fish banks are disturbed by offshore wind farms or if the access to a harbour is disturbed by an aqua culture facility (Michler-Cieluch & Kodeih 2008).

On the other hand, local residents may also embrace the location of a new facility, when they feel involved in the planning process, if they believe a facility is in accordance with local values and if they think the facility in other ways will benefit themselves and their community. Following the EU strategy to increase the Blue growth of the marine and maritime sector, e.g. new aquaculture facilities, it is therefore important to consider and adapt to local reactions to the siting of a new facility in their area. (Barrington et al. 2010)

Material and methods

As part of the BONUS OPTIMUS project, we have investigated the issue of local acceptance in three case areas, where mussel farms have been established: one in Hagensche Wiek as part of the Greifswald Bay in Germany and two in Denmark - in As Vig, which is part of Horsens Fjord and in Skive Fjord, which is part of Limjorden. Here we have conducted expert interviews, questionnaire surveys of summerhouse owners and seasonal residents as well as stakeholder workshops and media analysis of local newspapers in the period from autumn 2017 until spring 2019.

Results and discussion

It appears from the studies that mussel cultivation has a chance of being met with local acceptance, although locations of the facilities have to consider various concerns and interests. It should be mentioned that mussel cultivation has a longer history in Denmark than in Germany. This means that in Denmark there are already local experience with mussel farms, but also with other forms of aquaculture.

In both case areas there is a level of ignorance about mussel cultivation. Respondents are to some extent unaware that there is a mussel farm in their vicinity. Another recurring pattern is the importance attributed by respondents to the quality of water and beach. Especially for the Danes, water clarity was very important. Half of the Danish respondents also found that water quality had deteriorated over the years. Danes in particular blamed fish farms (and not mussel farms) in the bay for the reduction in water quality. 82 % indicated that fish farming reduces water quality, 67 % indicated that mussel

(Continued on next page)

farms to some or to a high extent spoil their experience of the landscape, 57 % find that aquaculture facilities spoil their view a little or a lot, and in their open replies they blamed fish farms for cloudy water, for a muddy, slimy seabed and for the disappearance of flatfis in the bay. Some of this frustration with fish farms may have rubbed off on their somewhat more sceptical attitude towards mussel cultivation. While 74 % of the German respondents agreed partly or fully with the statement "I don't mind that there is mussel farming in this area", only 30 % of Danes agreed and 41 % disagreed fully or partly. From our media study, we also learned that there is a concern in the local public that mussel farms, due to their water cleansing capacity, will be used as a lever to introduce even more fish farms in Horsens Fjord including As Vig.

In conclusion, we would suggest that a strong emphasis on water quality would be useful, if developers of mussel cultivation want the acceptance of local stakeholders as well as local seasonal and permanent residents and want to include them in processes of planning, establishing and managing mussel farms.

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MULTIVARIATE GENOMIC MODEL FOR DIPLOID AND TRIPLOID GROWTH PERFORMACE IN ATLANTIC SALMON (*Salmo salar*)

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Introduction

Triploid salmon is to date the only available product, for producing sterile Atlantic salmon. To our knowledge there exists no genetic program specifically selecting for triploid performance in Atlantic salmon, and selective breeding is thus based on the assumption that triploid and diploid genetic effects are closely correlated. This has yet to be shown in a proper genetic model.

Studies on salmonids have investigated performance traits among families or strains in response to triploidy, with some re-ranking of families being observed when going from diploid to triploid genotypes (Blanc et al. 2001; Johnson et al. 2004). But here has been a dissidence in conclusion on whether the selection should be on triploid phenotype (Friars et al., 2001), or not (Taylor et al. 2013; Harvey et.al 2017; Leeds et al. 2019).

One of the problems of genetic analysis of triploids is that the genetic contribution of sires and dams are different, as the dam contribute with two copies of each chromosome, while the sire contribute with one copy of each chromosome (as in diploids). Hence, triploid and diploid family members are indeed genetically different, and substantial re-ranking among families should be expected even if the traits as such are genetically equivalent. Furthermore, the two chromosomes inherited from the dam are not independent and will only differ through recombination (depending on recombination rate and location of recombinations). The variance of the Mendelian sampling deviation is thus unknown, unless modelled though a proper genomic model. Finally, genotyping of triploids using standard software packages for genotype calling has so far not been possible. A new software tool was therefore developed for this purpose.

The aim of this study is to evaluate the genetic correlation between performance on growth for diploid and triploid, by the use of proper genomic relationships involving both diploid and triploid full-sib groups, evaluated for growth performance within the same environment.

Material and methods

A random subset of eggs from approx. 75 families of the year class 2014 nucleus of AquaGen, where immediately after fertilization treated with hydrostatic pressure (5 min of 9500 psi) to induce triploidy. The triploid group of approx. 2000 animals were reared as a separate group until they were mixed with diploid full sibs after vaccination. As smolt the fish were put into a single cage at sea were the fraction of triploids where 3%. The group were on-growing in one cage until harvest.

Growth performance were measured by individual weight registrations after 14 months in sea, and tissue samples for genotyping where sampled and genotyped using a 70K SNP-chip. Special software was used to call genotypes for triploids, and methods were developed to assign triploid offspring to diploid parents.

Table I. Descriptive statistics, number of observations, mean etc of families with diploid and triploid offspring.

Group	N	Mean	Std Dev	Minimum	Maximum	CV
Triploid	743	3687	850.4	1080	5740	23.06
Diploid	7883	4588	949.2	200	10240	20.69

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A genomic animal model will be used to model diploid and triploid performance:

$$\begin{bmatrix}\mathbf{y_{2X}}\\ \mathbf{y_{3X}}\end{bmatrix} = \begin{bmatrix}\mathbf{1_{2X}} & \mathbf{0}\\ \mathbf{0} & \mathbf{1_{3X}}\end{bmatrix}\begin{bmatrix}\mu_{2X}\\ \mu_{3X}\end{bmatrix} + \mathbf{Z}\begin{bmatrix}\mathbf{g_{2X}}\\ \mathbf{g_{3X}}\end{bmatrix} + \begin{bmatrix}\mathbf{e_{2X}}\\ \mathbf{e_{3X}}\end{bmatrix}$$

where $\begin{bmatrix}\mu_{2X}\\ \mu_{3X}\end{bmatrix}$ are the overall means of diploid and triploid salmon, $\begin{bmatrix}\mathbf{g_{2X}}\\ \mathbf{g_{3X}}\end{bmatrix} \sim N(\mathbf{0}, \mathbf{G_0} \otimes \mathbf{G})$ is a vector of random diploid and triploid additive genetic effects with respect to growth, $\mathbf{Z}$ is the associate incidence matrix, $\begin{bmatrix}\mathbf{e_{2X}}\\ \mathbf{e_{3X}}\end{bmatrix} \sim N\left(\mathbf{0}, \begin{bmatrix}\mathbf{I_{2X}}\sigma^2_{2X_e} & \mathbf{0}\\ \mathbf{0} & \mathbf{I_{3X}}\sigma^2_{3X_e}\end{bmatrix}\right)$ is a vector of random residuals, $\mathbf{G} = \frac{1}{\mathbf{2p(1-p)'}}\mathbf{MM'}$ is the genomic relationship matrix, $\mathbf{G_0}$ is the additive genetic (co)variance matrix, $\mathbf{M}$ is a matrix of centered marker genotypes, including both diploid and triploid individuals. The centered genotypes of an individual *i* (*i*th row in $\mathbf{M}$) is defined as:

$$\mathbf{m_i} = \mathbf{q_i} - t_i\mathbf{p}$$

where $\mathbf{q_i}$ = row vector of genotypes for the individual, t_i = ploidy of the individual, $\mathbf{p}$ = row vector of allele frequencies for all loci.

Results

The triploid group of salmon were offspring of 65 sires and 63 dams, and weight was measured on 1063 triploid salmon with average of 3635g. The average weight of the diploid was 4257g. Comparison of weight and variation for offspring from the same families are given in table I.

Genetic correlation between performance on growth for diploid and triploid will be presented.

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TURBULENCE AND FLOW FIELD ALTERATION IN THE WAKE OF AQUACULTURE SEA CAGE

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Introduction

Current flow dynamics within and surrounding fish cages are highly complex (Klebert et al. 2014, 2015). Flow regimes within cages are dependent on both the incoming current (Klebert et al. 2012, Gansel et al. 2012, Rasmussen et al. 2014) and fish behavior (Johansson et al. 2014), however no clear flow pattern has been found due to flow-net-fis interactions driving complex 3D turbulent regimes. Physical factors that influence hydrodynamic flows at the farm-scale include water depth, current flow velocity, and water stratification (salinity and temperature). The present study was undertaken to characterize the flow pattern and the 3D turbulence regime in the wake of aquaculture sea cages

Materials and methods

By using different sensors, such as a 3D current flow profiler (ADCP), micro-turbulence profile , and High precision CTD (Conductivity, Temperature, and Depth) instruments, echosounders (fish distribution in cages), full-scale measurements of detailed 3D current flow patterns have been carried out on the wake of salmon-farming sea cages with and without fish. The cages were using a skirt in the upper 10 m of the water column in order to prevent Sea-lice infestation.

Results

The field observations mapping spatial and temporal variations in the hydrodynamic flows surrounding the cages showed a strong variation of the turbulence levels in the lee of the cage across a range of different background flow conditions. Turbulence levels over the upper 20 m of the water column (depth of cage) and in the lee adjacent to the cage, showed highly elevated rates of turbulence up to several orders of magnitude greater than those observed several hundred meters upstream of the cage. More precisely, higher turbulence levels were also measured over the upper 10 m of the water column just behind the cage, corresponding to the depth of the skirt, compared to upstream values and depths below the cage. In addition, measurements with a moving ADCP, shown a current flow reduction in the vicinity of the cage. These results show that the presence of stocked aquaculture sea cages significantly and consistently altered the intensity and distribution of turbulence levels, and hence the mixing regime, on the wake of cages: this will strongly affect the spatial and temporal distribution of particulate organic matters in the vicinity of the fish farm

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UTILIZATION OF EFFLUENT STREAMS FROM RECIRCULATING AQUACULTURE SYSTEMS BY VERMIFILTRATION

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Introduction

With increasing production of farmed animals, treatment of their effluents becomes crucial for the ecological sustainability of the industry. In many countries the nitrate load of soil and groundwater already is critical or exceeds the official guideline values. The random distribution of manure as fertilizer accounts for large parts of this load. Land based aquaculture producers contribute to that to some extent by spreading process sludge on agricultural grounds. However efforts are being made to treat effluents streams on site and recycle the process water effectively. However, filtration often requires sophisticated technology and may be very energy consuming. Aquaponic producers are using fish based excretions as nutrient source for plant culture and cycle those within their system. However, only the dissolved nutrients are being used at the moment and some nutrients are often lacking in the solution. The solids usually are disposed within the municipal sewage system or being spread on agricultural grounds. Nutrients in effluent sludge are carbon-bound and have to be treated in order to make the nutrients available for crop production. A very promising on-site and low-energy treatment option for aquacultural sludge could be vermifiltration, hence worm digestion of effluents from fish production. Former studies showed high potential of this technique regarding physical and biological filtration and mineralization of non-solvent plant nutrients (Sinha et al., 2007 & Gardner et al., 2009).

Material and Methods

System Design

Nine individual vermifiltration devices were set up. The devices were made out of plastic boxes (36 x36x12cm) and arranged in a drawer system style. Each system had 4 drawers and a liquid catch tank. Three drawers per device were filled with 12 liter beech wood chips each as worm substrate and the remaining drawer, inaccessible to the worms, was filled with "Kaldnes K1 Micro" Moving Bed Filtermedium.

Out of the nine systems three groups were formed and stocked with different worm densities. The intensive group was stocked with 25 g/l substrate volume. The extensive group was stocked with 15 g/l substrate volume and the third group (control) was not stocked with worm biomass. Each filtration device was fed African Catfish RAS sludge. The sludge/water was recirculated twice a day via a pump sitting in the catch tank and spread again on the top drawer, from where it took three hours to trickle down to the catch tank again.

Sludge was collected at the semi-intensive aquaculture unit in the FishGlassHouse at the Faculty of Agriculture and Enviromental Sciences at the University of Rostock stocked with 630 African catfish with ≙ 500 g fis $^{-1}$. The experiment was carried out for four weeks and at the end fish biomass within the system was ≙ 441 kg. Dry matter content within the fed sludge varied between 2, 68 and 3, 52 %. The sludge was collected from the sedimenter units. Fish were fed with Coppens Meerval Special PRO EF feed.

Analysis and Sampling

Each filtration device was fed with 3l sludge per week. After one week the liquid fraction was collected at the catch tank and kept for sampling. Solids remained trapped within the filter substrate

The chemo-physical water parameters (dissolved oxygen level (DO), pH-value, temperature, redox potential, conductivity and salinity) were measured with a multimeter (HACH LANGE HQ40d) in input sludge and output effluent after one week of filtration.

Conducted and planned analysis include Chemical Oxygen Demand (COD), Total Organic Carbon (TOC), Total Nitrogen bound (TNb), Macronutrients for input sludge and output effluent. Furthermore the compost value (rotting degree, bound and solvent nutrients) and dry matter plus organic dry matter will be analysed within the substrate after the experiment.

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Results

Most of the analysis is yet to be done. First results are promising. Both intensive and semi-intensive vermisystems were able to reduce the organic load. TOC values were reduced up to 30 % and COD values up to 53 % more than in control filters (no worms). Furthermore the vermifiltration systems have strong pH regulating effects on input sludge/water. The pH in the vermisystems evened out at neutral levels around 7, whereas the control group filters were not able to significantly change input pH. Substrate condition was significantly impacted by worm burrowing activities, too. Substrate in vermifilters was loose and kept aerated, whereas substrate in control group was dense, compact and clogged.

Discussion and conclusion

To analyze the potential of vermifiltration for aquaculture and aquaponics a pilot-scale setup was build. This study shows that "worm filtration" effectively reduces Chemical Oxygen Demand (COD) Total Organic Carbon (TOC) and Total Nitrogen bound (TNb) loads in aquaculture effluents. Regarding this, the application of worms and microorganisms can be seen as a low energy additional filtration device for aquaculture operations.

Whether worms and microorganism together are able to transform aquacultural sludge and filter substrate into high quality biological fertilizer or worm digestion supports the mineralization of organic bound nutrients, has yet to be proved (analysis of compost value will start in Mid-May) .

For future studies one should not neglect the fact, that several authors mention worm filtration could be used to remove contaminants from organic effluents due to bioaccumulation (Taylor, 2003). Also the worm biomass, which contains high-quality lysine-rich protein (Xing, 2006), may act as a suitable source for substituting fishmeal in future fish diet

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THE NATIONAL ALGAEPILOT MONGSTAD: PRODUCTION OF MICROALGAE FOR AQUACULTURE

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Goal of the National Algaepilot Mongstad is to produce different microalgae species and optimize the production process, using CO_2 captured by the co-sited Technology Centre Mongstad. The pilot facility is used for upscaling the cultivation work of the microalgae research group in Bergen, a close collaboration between NORCE and the Department of Biological Sciences of the University of Bergen. The microalgae group focusses on developing a commercial and sustainable production chain for industrial application of microalgae, such as aquaculture feed, food products and chemicals.

Most of our research projects are interdisciplinary, aiming at integration of production and processing chain into final products, including use of waste streams as nutrient sources. This encompasses captured CO_2 from flue gas, but also recovery of nutrients from waste streams from aquaculture (fish manure), insect production (insect frass) and municipality waste, where we achieved promising results. We work on bioprospecting, strain selection and optimization, cultivation and process optimization. For bioprospecting, we make use of interesting inhabitants of local fjords and cold coastal Arctic waters, looking for cold-adapted species high in omega-3 fatty acids and other interesting components. Within two projects, CO2Food and Algae2Future, we have been optimizing the production process for a local *Phaeodactylum tricornutum* strain, and compared it to the production of two commercial strains; *Nannochloropsis gaditana* and *Tetraselmis chuii*. Moreover, biomass of these strains was produced for feed trials with salmon. Together with research and industrial partners in food and feed processing and the aquaculture sector, we produced and processed microalgal biomass into aquafeed and performed feed trials to determine suitability of omega-3 rich microalgae to replace fishoil and fishmeal. The first results appear to be very promising, though there are significant differences between algae species and e.g. the digestibility in salmon. Next to salmon feed, also applications as greenwater and rotifer feed have been tested, in the production of Ballan wrasse.

Through these application tests, the value of the microalgae biomass was determined. This information was combined with a detailed techno-economic analysis of the production chain, where production costs of the microalgae feed ingredient were calculated for production sites of 1, 10 and 100 ha in Spain and Norway. The experimental data gathered at the National Algaepilot Mongstad were used for the cost projections, combined with data from literature, directly from suppliers, or standard engineering estimates. Current production prices are still higher than both fish oil and omega-3 rich oil from heterotrophic microalgae, but we foresee that through further strain and process optimization the cost price can become significantly lower than both fish oil and heterotrophic microalgae oil. Using the techno-economic analysis, we could pinpoint the major cost factors that we will address in follow-up projects to achieve the desired cost price reduction.

USE OF BACTERIOPHAGES TO CONTROL *Yersinia ruckeri* IN SALMON FARMING

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Bacterial pathogens cause serious challenges in all aquaculture farming industries. *Yersinia ruckeri* is one important pathogen in salmonid farming, where it causes ERM/yersiniosis. The disease is usually controlled by keeping a healthy balance between infection pressure (e.g. hygiene, biosecurity and water quality) and the animal's tolerance for the pathogen (e.g. vaccination and good animal welfare). However, some farm operations, such as sorting, vaccination and transport, challenge this critical balance and can trigger disease outbreaks. During such operations, *Yersinia ruckeri*-specific bacteriophages can be used to control of the infection pressure from the pathogen, thereby maintaining the critical balance and avoiding disease outbreak.

Bacteriophages, viruses which infect and kill bacteria, are nature's own biocontrol tool, making sure no one bacterium becomes too dominant in an environment. Since bacteriophages have been specialized through an evolutionary arms race against their host bacteria, each bacteriophage shows very limited host range. Most phages can only infect and kill one specific bacterial species, some are limited to single bacterial strains. This trait makes bacteriophages particularly suited for use in environments where protection of the commensal microflora is equally important as removing the unwanted

Here, we present data from field trials carried out at salmon farms in Norway, showing that a novel bacteriophage-based product, CUSTUS™$_{YRS,}$ can effectively control infection pressure from *Yersinia ruckeri* in water, and thus avoid bacterial infection and disease outbreaks after stressful farm operations.

AQUAPONICS (S.L.) PRODUCTION OF SPEARMINT (*Mentha spicata*) AND BASIL (*Ocimum basilicum*) WITH BIOHUMIN® AND AFRICAN CATFISH (*Clarias gariepinus*)

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Introduction

The quality of plants in aquaponics depends on the nutrient composition of the process water, which generally differs from standard hydroponic solutions or soils for plant production. Specific macro- and micro-elements are usually higher in hydroponics such as N, K^+, PO_4^{3-}, Mn^{2+}, Fe^{2+} and Mo (Bittsanszky et al., 2016). Nevertheless a better incorporation of calcium, potassium, magnesium and sodium was recorded from leafs of romaine lettuce (*Lactuca sativa* L. cv Integral) under aquaponics with partly comparable chlorophyll values (SPAD) to lettuce grown under hydroponics (Pantanella, 2012).

In aquaponics, the output and quality of plants increases by partial addition of fertilizers. Ru et al. (2017) described 1.8 times more yield (or 83.6%) of pak choi (*Brassica campestris* L. subsp. *chinensis*) in coupled aquaponics with tilapia (*Oreochromis niloticus*) and the supplementation of partial optimized micro- and macro-nutrient solution with K, Ca, Mg, B, Mn, Zn, Cu, Mo and Fe, respectively. On the other hand, artifi ial fertilizer addition contradicts the "organic" production in aquaponics.

A cost-effective sustainable alternative to liquid fertilizer is Biohumin® (Bioverde AG, Switzerland), a 100% natural product from livestock farming, forestry and soil science. Main components are carbon (30%), calcium (5%), silicium (30%), organic matter (30%) and different natural macro- and micro-nutrients as well as vital cell substances. In this study we evaluated the effectiveness of 15% Biohumin® as a peat substitute on the growth of spearmint (*Mentha spicata*) and basil (*Ocimum basilicum*) under aquaponics (*s.l.*) farming conditions ("gardening plants") following Palm et al. (2018) and the EU Aquaponics Hub COST Action FA1305.

Material and Methods

The experiment was conducted in the FishGlassHouse, an experimental aquaponic facility in Northern Germany (Mecklenburg-West Pomerania, University of Rostock) in spring 2018. Fish were fed with Alltech Coppens Special Pro 4.5mm (42.0% protein, 13% fat, 1.5% crude fibe , 7.5% ash, 1.02% total P) and *C. gariepinus* was held extensive (EAU: 35 fish $tank^{-1}$) and intensive (IAU: 140 fish $tank^{-1}$) in two different aquaculture units. Garden pots with peat soil and seedlings of mint (*M. spicata*) and basil (*O. basilicum*) were repotted in a mix of ≈ 15% Biohumin® natural humus substrate (mean 37.55g = 15.05%, N = 33) and ≈ 85% zero-nutrient-soil substrate (211.89g = 84.94%, N = 11; "Typ 0", Einheitserdewerke Patzer GmbH & Co. KG, Germany).

The plants were cultivated on ebb-and-flood tables with 15 plants per group in triplicates (45 plants per group, 135 plants in total). The hydroponic control group (Control) was adjusted with commercial fertilizer Universol® Orange 16–5–25+3MgO+TE (Everris International BV, The Netherlands) to an electric conductivity of 2 000 µS cm^{-1} (± 50). Nutrient solutions in hydroponics were adapted to pH of 6.0 (± 0.2) by automated Bluelab controllers (New Zealand, USA).

parameters	Control	EAU	IAU	M (I)	M (II)	M (III)
SPAD mint	53.8 ± 7.3^{a}	38.9 ± 2.7^{c}	43.4 ± 3.0^{b}	41.1 ± 3.1^{bc}	43.7 ± 3.1^{b}	29.5 ± 3.2^{d}
height (cm)	63.0 ± 10.5^{a}	51.5 ± 4.3^{b}	53.5 ± 4.7^{b}	33.5	35.5	22.8
SPAD basil	37.3 ± 3.0^{a}	33.6 ± 3.1^{b}	33.9 ± 3.1^{b}	46.7 ± 14.1^{a}	50.0 ± 11.4^{a}	33.3 ± 5.2^{b}
height (cm)	61.2 ± 5.5^{a}	40.5 ± 3.8^{c}	48.2 ± 5.4^{b}	28.5	25.9	-

(Continued on next page)

Plant quality was compared to plant samples from local markets (Grönfingers GmbH, Germany; Fossa Eugenia, The Netherlands; Kaufland Dienstleistung GmbH & Co. KG, Germany) by using the SPAD-index (SPAD-502Plus Chlorophyll-Meter, Konica Minolta).

Results and Discussion

Plant quality of *M. spicata* cultured in aquaponics (*s.l.*) with 15% Biohumin® natural humus substrate reached SPAD values of 38.9 (EAU) and 43.4 (IAU) compared with market samples (41.1-43.7, Table I). SPAD levels with water from the intensive *C. gariepinus* production were insignificant while the water from extensive production resulted in significant less SPAD values compared with one market plant sample (except M III). *O. basilicum* SPAD values were comparable to cutted leaves but not to marketed plants. Plant growth and quality parameters of the control groups were better due to the high nutrient content of the fertilized water that was obviously above the required EC level for the marketed mint plants (Table I).

The positive qualitative (SPAD) effect of Biohumin® in combination with nutrient water from *C. gariepinus* production was more evident in mint (different SPAD of EAU & IAU; equal plant height). In basil, the height of the IAU was significant better compared with the EAU, however, much less than the control group. The relative agronomic efficiency of the plant heights compared with the fertilizer control groups (according to Brod et al., 2017) was higher in mint (EAU: 81.7%; IAU: 84.9%) compared with basil (EAU: 66.2%; IAU: 78.8%). Consequently, Biohumin® is a useful supplement for mint in extensive and intensive aquaponics (*s.l.*) production while the chosen amount of 15% peat replacement appeared to be too low to reach adequate basil growth and quality. Biohumin® is a new option to increase plant quality and growth in aquaponics (*s.l.*) and should be tested in more detail in future experiments to develop marketable aquaponics (*s.l.*) produce.

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EFFICIENCY OF RAINBOW TROUT PRODUCTION IN NORTH-WEST GERMANY

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Introduction
Aquaculture is among the most rapidly growing food production sectors in the world. However, in Germany production growth is hampered by conflicting policy frameworks. In particular, strict environmental, water and building restrictions are sometimes at odds with the national sector development goals. Furthermore, missing international level playing fields with regard to standards and subsidies contribute to this trend. An extensive overview on the current situation and perspectives for Germany can be found in the German study by AFC&COFAD (2017). Nevertheless, marked differences exist between individual fish farms, with other farms growing at impressive rates. This heterogeneity begs the question what drivers are underlying this behaviour, and in particular, whether individual management capacities or regional regulatory pressures are more important. The aim of this study is to provide an overview on the production and marketing structures including efficiency analysis in this market and to support producers and authorities in future investment, management and regulatory decisions.

Method
Against this background, a questionnaire-based production survey with a sample of 39 representative rainbow trout farms in north-west Germany was conducted in 2019. The in-person interviews were usually connected with farm visits, which showed a representative view into the operation conditions of the farms. Here, further problems beyond the scope of the questionnaire could be discussed. This unique dataset allows an in depth view into specific aquaculture technologies and the effects of various external factors. The data depicts the production process in detail from hatching to sale. Additionally, key external impact factors like the time spent on mandatory regulatory requirements and environmental factors were included. Another important part of the survey was to investigate the effects of the extreme weather conditions of 2018 on the farms` performances. Here, one goal was to see if the best performing farms also showed a higher resilience against the extreme weather conditions. Following the efficiency literature in the aquaculture sector (see for example Asche.et.al (2009)) the data was then used to estimate the farms` efficiency using the Data Envelopment Analysis (DEA) approach. This method has the advantage to perform well despite the great diversity in production in the sector. Here, the best performing farms are used to estimate a frontier against which all other farms are benchmarked.

Results
First results demonstrate the sector's great diversity. Interviewed farms range from part-time enterprises with a few tons output to big farms with hundreds of tons of output and more than 10 employees. Production systems and distribution also greatly differs. While some divert large shares of their working hours to processing or selling their goods on farmer's markets, others concentrate on rearing the fish until slaughter. This often relates to the farm size, water rights and the intensity of production. Furthermore, the data also shows the trend to outsource the reproduction, with 75% of farms exclusively buying eggs or juvenile fish from other farms to fatten up

Regarding the extreme weather conditions of 2018 only a minor part experienced the effects as an existential thread for the viability of their businesses. Furthermore, most farms stated reduced yields and mortalities below 10% with only a few mentioning up to 60%.

Discussion and Outlook
The research indicates the great diversity in north-west German rainbow trout production affecting the future development and the expectations and goals of the different farmer. What unifies many of them is the uncertainty about the future of their operations regarding regulatory changes and the water availability. These are stated to be the main constraints decreasing their willingness to invest, thereby limiting the possible sector development. In depth efficiency analysis will shed light on the underlying interactions between the different drivers.

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The future will tell if the sector will see a similar structural change as it is going on in the agricultural sector.

Due to climate change, the competition for water resources between aquaculture and agriculture production constitutes an increasing field of conflict and unpredictability. The elimination of major uncertainties is paramount in order to foster key investments needed to reach the national strategic goals. To assist authorities and farmers in estimating the effects of their decisions this research will concentrate on the underlying interactions regarding the production and further external factors.

In the future the environmental interactions will be taken into account next to the results of the research project which focus on the replacement of fishmeal and oil by algae and insects

In regard to policy implementations it can be said that the sector would profit from a unified administration which clarifies and simplifies the decision making and predictability for all actors. For example, the state of Schleswig-Holstein took a step in this direction by establishing the competence network of aquaculture (KNAQ).

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UTILISATION OF FIBRE-REDUCED COLD PRESSED RAPESEED CAKE FOR THE PRODUCTION OF HIGH-PERFORMANCE FISH DIET FOR RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Over the last decades fishmeal substitutes has been an essential element in the production of new formulated fish diets and oilseed cake-based substitutes are a promising new ingredient with a lot of attention in the past decade. Cold pressed oilseed cake is not only rich of lipids, proteins, vitamins and anti-oxidants but also contains antinutritional factors (ANF) like fibre, phenols, phytohormones and further secondary plant materials (Francis *et al.* 2001). ANF´s can have a negative effect on fish growth and health, meat quality, as well as food acceptance. Therefore it is important to keep the proportion of ANF´s as low as possible. This study was conducted to observe the impact of fibre-reduced rapeseed cake as substitute for fishmeal. Two main questions should be answered. How much fishmeal can be substituted with rapeseed cake in formulated diets for rainbow trouts (*Oncorhynchus mykiss*) until the negative effects of ANF´s take over the positive aspects? And secondly, how do the different concentrations of rapeseed cake in diets impact the growth performance and health of rainbow trouts?

Materials and methods

The experiment took place in the trout farm of the Institute of Fisheries (IFI) of the Bavarian State Research Center for Agriculture (LfL) in Starnberg, Germany. Two production lines (F and T) based on different formulated basal diets were produced. Each line consisted of a reference diet and two experimental diets. Line F was produced at the Fraunhofer Institute for Process Engineering and Packaging using cold pressed cake of organically grown rapeseed. While line T was produced at the "Teutoburger Ölpressmühle" and an organically grown premium rapeseed cake was utilized. Therefore, a total of six groups were monitored during the 9 week experiment. Each group was replicated four times. Twenty-four circular tanks, with a production volume of 450 l each, were stocked with 40 rainbow trouts with an initial weight of 161 ± 0.5 g. Feeding was done by hand once a day at 9 a.m. up to a maximum of 1.2% LW/day. Afterwards the oxygen content and temperature were measured and the daily consumed diet per group was calculated. Every three weeks the growth of ten trouts per tank was examined. At the end of the trial all fish were weighed and a representative number of fish per group were processed for further analysis of length, FCR, organ and general health condition, slaughter yield, body composition and fatty-acid profile.

Table 1: Performance parameters of each diet after 9 weeks.
Statistical analysis was performed using JASP v.0.8.2, Post hoc-Test = Tukey, α = 0.05.

Diet	F REF	F 9 %	F 18 %	T REF	T 17 %	T 34 %
Initial Weight [g]	160.9 ± 8.24	161.2 ± 7.85	161.1 ± 7.94	160.9 ± 7.38	160.7 ± 7.72	160.7 ± 7.95
Final Weight [g]	338.2^{a} ± 34.05	$332.4^{a,b}$ ± 35.86	338.6^{a} ± 32.17	341.3^{a} ± 33.73	$329.4^{a,b}$ ± 34.91	319.9^{b} ± 34.28
Growth [g]	$177.6^{a,b}$ ± 8.16	$166.8^{b,c}$ ± 5.52	$177.5^{a,b}$ ± 7.27	180.4^{a} ± 3.86	$168.8^{a,b,c}$ ± 4.37	$159.3^{b,c}$ ± 5.12
Mean Intake per Group [g/d]	106.5 ± 22.61	107.1 ± 22.95	112.8 ± 22.13	107.1 ± 22.65	104.9 ± 21.59	105.5 ± 23.44
FCR	0.60^{a} ± 0.013	0.64^{b} ± 0.020	0.64^{b} ± 0.012	0.60^{a} ± 0.015	$0.62^{a,b}$ ± 0.010	0.66^{c} ± 0.007
Specific Growth Rate [%/d]	$1.2^{a,b}$ ± 0.04	$1.1^{b,c}$ ± 0.03	$1.2^{a,b}$ ± 0.03	1.2^{a} ± 0.02	$1,1^{a,b,c}$ ± 0.02	$1.1^{b,c}$ ± 0.03

[a,b,c] lowercase letters indicate statistically significant differences between groups.

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Results

The conditions of the experiment fulfilled the common physiological requirements o
rainbow trout. The data of the performance parameters which were collected at the end of the experiment are listed below in Tab. 1:

The results of the health, nutrient and fatty acid of the whole body and muscle analysis will be completed in the first half of 2019.

Both reference diets achieved the best results for all parameters. Treatment diet F 18 % showed the same specific growth rate as both reference diets, whereas diet T 17 % achieved the best FCR of all treatment groups which was only 0.2 lower as the FCR of both reference groups. Diet T 34 % showed the most significant differences in final weight, growth, FCR and Specific Growth Rate to all groups.

Discussion and conclusion

All fish achieved a satisfying performance, irrespective of their diet. The statistically significant differences in the growth, FCR and Specific Growth Rate between all treatment diets were still in the margin of high-performing industrial diets. The impact of the ANF´s on the performance and growth of trouts seems to start from a percentage of ~ 34 % rapeseed cake on the dry weight. This can be attributed to the rising proportion of fibre, which provided no nutritional value for the trouts.

Over the last five years the price of rapeseed cake was relatively stable and costs ca. 1.000 USD/t less than fishmeal which is sold at 1,486.35 USD/t (IndexMundi March 2019). Therefore, rapeseed cake appears to be a promising partial substitute for fishmeal in high-performance fish diets with an overall lower ecological and economical impac

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PHARMACOKINETIC COMPARISON OF DIFFERENT DOSING STRATEGIES OF IN-FEED ADMINISTERED PRAZIQUANTEL IN GREATER AMBERJACK, *Seriola dumerili*

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Introduction

Praziquantel (PZQ) is a broad-spectrum anthelmintic used in both human and veterinary medicine. In captive fish, PZQ has proven an effective chemotherapeutic against monogeneans infecting the gills, skin and branchial cavities (Hirazawa et al., 2004; Sharp et al., 2004). Orally administered PZQ preparations at doses of 50 to 400mg/kg BW daily for up to 20d have been tested in a variety of cultured fish species (Kim et al., 2001; 2003; Tubbs and Tingle, 2006; Shirakashi et al., 2012). In order to design an effective oral dosing regimen, plasma and tissue (especially at the site of activity) PZQ concentrations can be a useful tool. However, to our knowledge, there is no literature examining the pharmacokinetic properties of PZQ in greater amberjack, *Seriola dumerili* which is a promising emerging farmed finfis species for the Mediterranean area suffering from monogenean infections such as those caused by *Zeuxapta seriolae*. Thus, the aim of this study was to investigate the distribution profile of dietary administered PZQ in greater amberjack following a multiple oral dosing as a first step to optimise PZQ dosing regimens for this species

Materials and methods

Three hundred and twelve healthy greater amberjack of an average body weight of 84±12g were distributed in four land-based seawater cages (78fish/cage) and allowed to acclimate for ten days prior to the beginning of the trial. Both studies were performed at 24.5±0.5°C. During acclimatization fish were received a drug-free commercial diet in amounts of 2% BW per day and starved for 24h prior to administering the medicated feeds. Two distinct medicated diets were made by mixing the active substance (0.75g PZQ/kg feed for the first feed and 1.5g PZQ/1kg feed for the second one) homogeneously with all the dietary ingredients prior to the cold pelleting process with a Hobart food pellet mill. The final dosing regimens of administered PZQ were 75mg/kg BW (Trial A) and 150mg/kg BW (Trial B). Fish were fed the two experimental diets daily for 5 consecutive days. On the first day of treatment, plasma samples were collected from 2h to 24h, while on the other intervention days, plasma samples were collected after 24h of drug administration. Approximately, 2ml of blood was drawn from the caudal vein of from 5 individuals/sampling point. Plasma was separated from blood samples by centrifugation at 14000rpm for 10min at 4°C. An HPLC-UV method has been developed for PZQ measurements in plasma samples of individual fish at each time point. Separation was performed on a Luna-C18 column using an isocratic mixture of 35:65v/v acetonitrile: water as the mobile phase with a constant flow rate of 1ml/min. Eluant was monitored at 210nm.

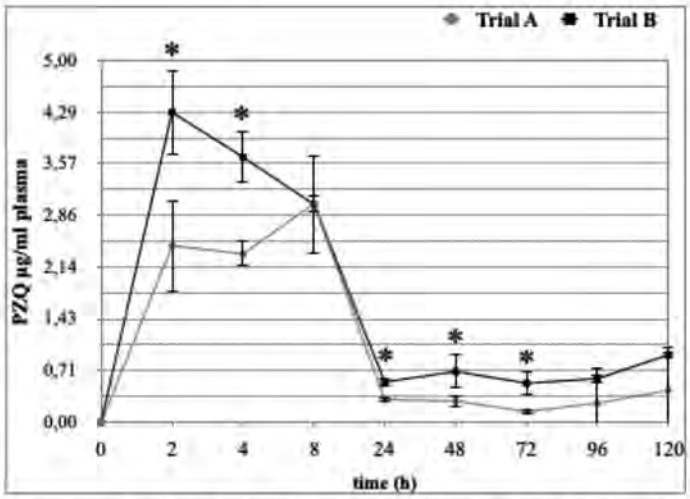

Fig 1 Plasma concentration (µg/ml) of two PZQ doses (Trial A=75mg/kg BW, Trial B=150mg/kg BW) in greater amberjack following multiple dose administration at 24h intervals. Values shown are mean±stdev (N=5). * indicates a statistically significant difference. Significance level was set at $P < 0.05$.

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Results

The recovery rates of PZQ for plasma samples were 88.5%-101.6%. PZQ was readily detected in all plasma samples. Plasma concentrations of PZQ in greater amberjack revealed values around 2.6μg/ml and 2.3μg/ml at 2 and 4h post feeding, respectively for Trial A and 4.3μg/ml and 3.7μg/ml at 2 and 4h post feeding, respectively for Trial B. Maximum plasma concentrations (C_{max}) of PZQ for fish fed 75mg/kg BW were achieved at 8h post feeding (3.0μg/ml), while C_{max} of PZQ for fish fed 150mg/kg BW were achieved 2h post feeding (4.3μg/ml). This indicates that the clearance of PZQ for Trial A did not follow a simple decay model. Additionally, a two-fold increase in the administered dose only led to an approximately 76% increase in the mean plasma concentration of PZQ 2h post-treatment. Due to the large variance, no significant differences were found between the mean PZQ concentrations of the two doses at 8h post feeding. Withdrawal of PZQ occurred rapidly in greater amberjack as its concentrations diminished 24h post-treatment in circulation (0.15-0.44μg/ml and 0.54-0.93μg/ml for Trial A and Trial B, respectively). Furthermore, no significant differences were found between the mean PZQ concentrations of either the 75mg/kg or 150mg/kg dose during days of treatment. However, as it shown in Fig. 1, mean plasma concentrations of PZQ were higher on the 5th day of treatment compared with the first, indicating that some low accumulation of the drug occurred during this period for both dosing regimens.

Discussion & conclusion

The results of the present study showed that PZQ was readily absorbed in plasma of greater amberjack while its accumulation was limited for both dosing regimens examined. Rapid absorption of orally administered PZQ preparations has been also demonstrated in other *Seriola spp.* such as kingfish, *S. lalandi* (Tubbs and Tingle, 2006). Low PZQ accumulation was evident in the present work which has been seen also in other farmed fish such as rockfish, *Sebastes schlegeli* (Kim et al., 2001; 2003). This kinetic profile is most probably related to the rapid clearance of the drug, rather than poor absorption. In conclusion, the results of present study revealed that under a 24h dosing interval for 5 consecutive days, the anthelmintic concentrations exhibit significant fluctuations in greater amberjack plasma. Based on the information obtained from the PZQ analysis there is an apparent benefit from the double dosing schedule (split in 2 feedings per day) as seen from the higher achieved plasma PZQ levels and thus this would be the suggested dosing against *Z. seriolae*. Its efficacy however remains to be confirmed in field trial

Acknowledgement

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A LABORATORY *IN-SITU* BIOASSAY FOR EVALUATING THE EFFICACY OF ANTIFOULIG PAINTS USING *Ectocarpus siliculosus*

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Introduction

Effective antifouling technologies are critical to maintain the fuel consumption efficiency of ships, minimizing the release of greenhouse gasses and other hazardous air pollutants and also for minimizing possible translocation of aquatic species through maritime trade (Schultz et al., 2007). In order to develop more effective antifouling paints for preventing biofouling of ships, it is very important to test and quantify the efficacy of new antifouling systems including antifouling agents. It is said that antifouling paints have been evaluated in the past mostly through field experiments on rafts and by patch-tests on ship hulls. In this manner, conditions of the marine environment influence test results (Berntsson et al., 2003). Consequently, biofouling data obtained from the field experiments conducted at different sites and in different seasons exhibit large variations, making it insufficient to conduct an objective and quantitatively evaluation of various antifouling paints. In this regard, it is necessary to establish a bioassay to assess antifouling paints under controlled laboratory conditions. The purpose of this study is to confirm whether the efficacy of the antifouling paints is directly reflected in an appropriate manner in a newly proposed evaluation method. In this study, the authors prepared test plates coated with different antifouling paints and tested their antifouling efficac es with a flow-through water system using macro algae. Furthermore, the validation by comparing results of the laboratory bioassay with antifouling efficacy results from field experiments on raft was also considered (Kojima et al., 2016).

Material and methods

5 types of antifouling paints with hydration type coatings and containing 0, 5, 10, 20 and 40wt.% of Cu_2O were prepared as test paints. Polyvinyl chloride plates used in a laboratory bioassay were 50mm x 50mm x 2mm in size. The test plates were coated on one side with the test paint. The panels were aged using a dynamic rotating device under controlled condition (water temperature: 20ºC, rotation speed: 10konts, period: 45days) prior to the laboratory bioassay (Figure1).

The unialgal culture strain of a filamentous brown alga, *Ectocarpus siliculosus*, which is one of the most common fouling macroalgae in biofouling of ships, was housed in Kobe University Macroalgal Culture Collection (KU-MACC) as KU-1372 for test organism. *E. siliculosus* was cultured on a nitro cellulose membrane filter (*f*=47mm, pore size 8.0mm, Millipore) in medium (PESI medium without tris-sodium glycerophosphate) at a temperature of 15ºC for about 7days in a still water condition before their use in the bioassay. The bioassay system consisted of the medium storage tank (volume capacity of 20L), peristatic pump, polypropylene bioassay tank (ca. 2L), and reservoir tank for waste medium. The laboratory bioassay with a flow-through water system was conducted under controlled conditions (flow rate: ca. 500ml/h, water temperature: 15ºC, light cycle: 12h. light/ 12h. dark with 1000 lux, period: 7days). The efficacy of antifouling paints was evaluated by the fluorescence strength of chlorophyll-*a* extracted from the adhered *E. siliculosus* on the surface of filter membranes on each test panel after incubation.

Results

The fluorescence strength generally decreased with an increasing of Cu_2O content. Statistical analysis showed that an inhibition of fluorescence emission was clearly observed at 20wt.% of Cu_2O concentration ($p<0.05$), and the significance difference between the control was extremely different at 40wt.% of Cu_2O concentration ($p<0.0001$) (Figure 2, A). Furthermore, the validation of the bioassay was evaluated compared to field experiments and showed the good agreement between them (Figure 2, B).

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Fig. 1. Photographs of the dynamic aging apparatus. (A): the cylinder with test plates fixed on it, and (B): the cylinder with a light shielding sheet while aging.

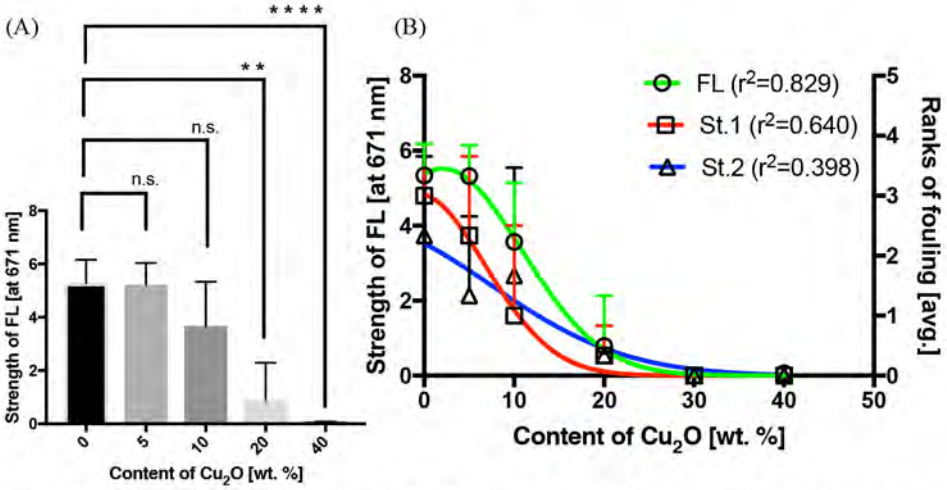

Fig. 2. The validation of the bioassay. (A): The variation of the fluorescence strength from *E. siliculosus* against content of Cu_2O, and (B): relationship between the fluorescence strength vs ranks of fouling (St.1: Miyajima, Hiroshima, Japan, St.2: Tamano, Okayama, Japan).

Discussion and conclusion

A reproduceable and effective laboratory bioassay was established by evaluating the antifouling efficacy of test paints that were prepared with varying contents of Cu_2O. To simulate the actual condition of ship hulls, dynamic aging of test plates was conducted. A positive correlation between the Cu_2O content and the repellant effect of the paint on macro algae was observed at the concentration of more than 20wt.%. Comparison of the results between laboratory bioassay using algae and of filed experiments revealed a highly consistent relationship between the two. This study also proved to be a significantly consistent method for assessing the effectiveness of present or future antifouling paints.

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RELEASE OF PHENOLICS AND OTHER ANTIOXIDANTS FROM MICROALGAE *Phaeodactylum tricornutum* AND *Tetraselmis chuii* FOLLOWING BEAD MILLING

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Introduction

Rigid cell walls may limit the availability of valuable nutrients in microalgae biomass for food and feed applications. Cell wall disruption may improve nutrient availability but also increase the risk of oxidative degradation of perishable compounds. Optimisation of cell wall disruption for *Phaeodactylum tricornutum* (*P. tricornutum*) and *Tetraselmis chuii* (*T. chuii*) by bead milling was previously studied by Kokkali et al. (2018a) and Kousoulaki et al. (2018). In the present study, we identified biomass phenolic compounds and their release following bead milling, along with lipids and proteins. Effects of processing on other quality characteristics of the differentially disrupted biomasses, such as viscosity, microbial load in *P. tricornutum,* total phenolics, antioxidant capacity and total carotenoids in both *P. tricornutum* and *T. chuii* processed by bead milling were also estimated, and selected data are presented below.

Material and methods

The microalgae biomass (*P. tricornutum* B58 and *T. chuii* UTEX LB232) were produced at the National Algae pilot Mongstad (NAM) (Mongstad, Norway) in a fed-batch process in four 800L photobioreactor systems (GemTube MK2-750, LGem b.v), harvested twice a week, concentrated by centrifugation to a paste (Evodos 50, Evodos b.v), vacuum packed and directly frozen at -20 ºC before further processing. The microalgae paste was thawed, diluted to different dry matter contents (12.5-22%) and processed by single passes through a Dyno-Mill Multi Lab bead mill (WAB, Muttenz, Switzerland) using a 0.6l chamber, glass or zirconium beads (0.25-0.4 and 0.3mm, respectively) at 80% chamber filling, following factorial designs, varying in a systematic way, besides biomass dry matter, also agitator tip speed (8-12 m/sec) and biomass flow rate (ca. 7-23kg/h). Samples from di ferent processing settings were frozen until further analyses.

Results and discussion

In *T. chuii* the polyphenols capsaicin, dihydro-p-coumaric acid, ethyl vanillin, benzoic acid, methoxyphenylacetic acid, 4-vinylphenol and 4-hydroxybenzaldehyde, sinapic acid were identified by AB SCIEX TripleTOF™ 5600 LC/MS/MS (Figure 1). In the case of *P. tricornutum*, lower number of polyphenols were present compared to *T. chuii*, and the main one was cinnamic acid, a known potent antioxidant, which was released in a linear way as compared to cell wall disruption % (P=0.001) (Figure 2). Aerobic bacteria in *P. tricornutum* culture were reduced by up to 86.9% from 980 000 to 128 000CFU/g wet biomass by bead milling using 0.3mm zirconium beads, with significant effects of flow rate and interaction between biomass dry matter and flow rate (P=0.000)

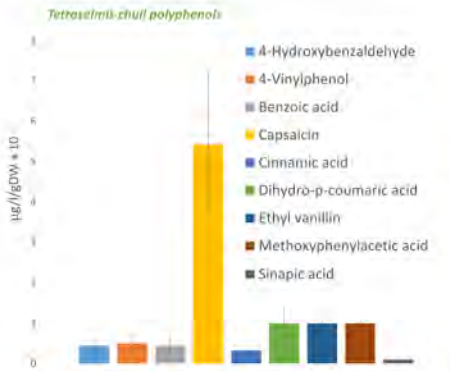

Figure 1 *T. chuii* polyphenols. Values are mean and standard deviation of analysed compound amounts in differentially disrupted samples by bead milling.

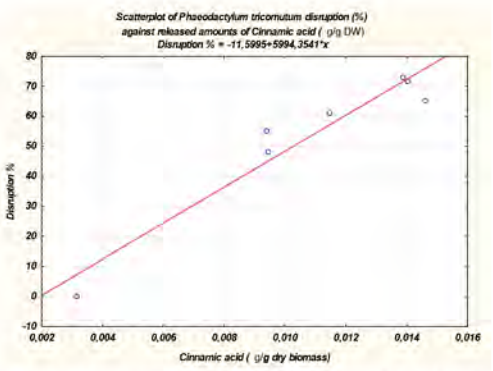

Figure 2 Identified levels of cinnamic acid in differentially disrupted *P. tricornutum* biomass, by bead milling.

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Conclusion

The *P. tricornutum* and *T. chuii* biomasses were further used for salmon feed and bread production, respectively. Thus, purity and availability of antioxidants and other nutrients and bioactive compounds are of outmost importance for commercial use of the biomasses, and all these parameters where improved by efficient cell wall disruption using bead milling

Acknowledgements

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EXPLORING EUROPEAN SEABASS GUT MICROBIOME AND ITS RESILIENCE TO IN-FEED ANTIBIOTICS

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Introduction

The constant increase in animal production in order to supply the growing population demands, has led to an extensive use of antibiotic in the livestock industries, as well as aquaculture. Antibiotics are used as growth promotors or to prevent and treat diseases, with adverse implications on the environment, animal health, as well as the commensal gut microbes (Okocha et al., 2018). Gut microbes are known to be important for many physiological functions of the host, thus evaluating such an impact is highly crucial. Examining the antibiotic impact on gut segments with different physiological roles, may provide significant insight into the effect of antibiotics on bacterial taxa in these microhabitats. Hence, we aimed to evaluate and understand the effect of commonly used feed-administrated antibiotics on the composition and metabolic potential of the microbial communities distributed along the gut of the European seabass (*Dicentrarchus labrax*), an important aquaculture species to be used as an experimental model. In the present study, we evaluated the impact of regular and high levels of in-feed antibiotics on the gut microbiome of European seabass juveniles, using quantitative PCR to measure the bacterial copy numbers and amplicon sequencing of the 16S rRNA gene to describe microbial community composition along the gut.

Materials and Methods

European seabass juveniles were housed in 250-L experimental indoor tanks equipped with recirculating systems. Five different treatment groups of fish were formed in triplicates: a group fed with the commercial diet (control group) and four groups fed with the commercial diets coated with two different mixtures of antibiotics of different range at concentrations 5 mg gr-1 of feed and 30 mg gr-1 of feed (excess): **Mixture 1**-Ampicillin, Kanamycin, Erythromycin, Ciproflaxine, Vancomycin; **Mixture 2**- Penicillin, Streptomycin, Lincomycin, Ciproflaxine, Vancomycin. The experiment lasted 7 days and at the end, pyloric caeca, midgut and hindgut were dissected from three randomly selected fish per tank

Bacterial DNA isolation, PCR amplification and 16S rRNA gene sequencing were performed using the protocol described by Kokou et al. 2018. Quantitative real-time PCR analysis was performed to investigate the relative abundance of bacteria inside each intestinal sample through amplification of their copy of the 16S rRNA gene. The microbiome composition along the gut was assessed by PCR amplification and sequencing of the V4 region of 16S rRNA using a MiSeq 2000 Next Generation system (Illumina). Data quality control and analyses were performed using the QIIME pipeline (Caporaso et al. 2011) with the default settings. In order to predict the functional content of the gut microbiome originating from the different groups, we used the PICRUSt tool (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; PICRUSt (Langille et al. 2013). Statistical analysis was performed in R.

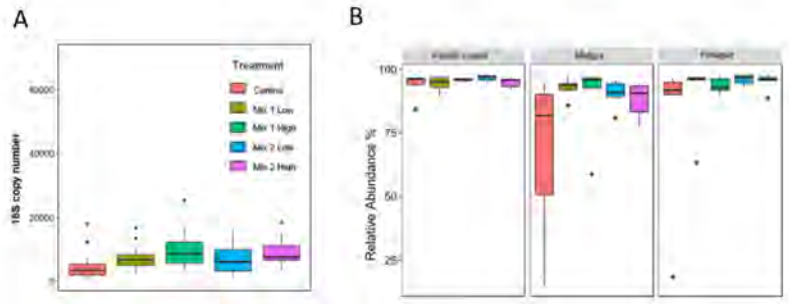

Figure 1. A. Quantitative PCR reveals an increase in the bacterial copy numbers with antibiotic resistance. **B.** European seabass possesses a highly abundant core microbiome which is represented by few bacterial species, that is resilient to antibiotic treatment.

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Results and Discussion

The results revealed that the antibiotics had a differential effect on the microbial composition across the fish gut, highlighting the variability of microbial niches. Antibiotic intake overall increased the bacterial counts compared to the control, mostly in Mixture 1 and in the high dosage of Mixture 2, suggesting a higher availability of microbial niches after antibiotic treatments, with opportunities for species expansion or invasion (Figure 1A). No differences were observed in the microbial diversity as revealed by the sequencing analysis, but an effect on the midgut and hindgut microbiome composition was found (Figure 1B). Analysis into the functional potential of the microbiome showed an increase in the antibiotic resistant genes in the treated groups, suggesting the presence of antibiotic resistant bacteria within the seabass gut. Lastly, our study reveals a highly abundant core microbiome in European seabass (<80% of the total microbiome abundance) that seem to be resilient to the examined antibiotic mixtures, thus indicating a high stability of the fish gut microbiome composition to perturbations.

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INVESTIGATION OF THE PROTECTIVE ROLE OF THE SEMINAL PLASMA IN COMMON CARP (*Cyprinus carpio*) SPERM

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Introduction

In vitro test systems are more and more widespread in ecotoxicology because of their ethical and practical advantages as well as their time- and cost-efficienc . Fish sperm is a feasible model for *in vitro* toxicology tests due to its easily measurable parameters (motility, antioxidant response etc.). Numerous studies have been published in this topic in the last years, however, they are different in many aspects. The most important difference in these is the presence or absence of seminal plasma. In several publications, seminal plasma was removed and only spermatozoa were exposed to the toxic substance in order to eliminate the protective role of the seminal plasma (Hulak et al. 2013, Shaliutina et al. 2017). However, in other experiments, the sperm including seminal plasma (not only the spermatozoa) was treated with xenobiotics (Chyb et al. 2001, Kollár et al. 2018ab) which raises difficulties to compare the results. Our goal was to investigate the protective role of seminal plasma in common carp (*Cyprinus carpio*) sperm.

Materials and methods

Common carp sperm was stripped individually (N=3) 24-48 hours after hormonal treatment. Three sub-groups were created from the sperm of one individual: one group was exposed to Hg in 1:1 (v/v) ratio (Treatment1). The two other groups were centrifuged (500 g, 10 min) and the seminal plasma was removed: in one group, seminal plasma was replaced by cyprinid immobilising solution before spermatozoa were exposed to Hg in 1:1 (v/v) ratio (Treatment2), while in the other group, seminal plasma was not replaced, only the spermatozoa were exposed to Hg in 1:1 (v/v) ratio (Treatment3). In each case, 4 concentrations of Hg (0.5, 1, 2.5, 5 mg/L) and one control group were tested. During the exposure period, progressive motility of sperm (PMOT, %) was measured in 30^{th}, 120^{th} and 240^{th} minutes by Computer-assisted Sperm Analysis system.

Results

No significant differences were found between Treatment1 and Treatment3 at any of the examined exposure times. Treatment2 differed significantl from Treatment1 at 1 and 2.5 mg/L concentrations at each exposure duration as well as at 1 mg/L in the 30^{th} minutes.

Discussion and conclusion

No differences were found between the groups where seminal plasma was removed and where it was not removed prior to Hg exposure. The only difference appeared when seminal plasma was replaced by cyprinid immobilising solution prior to exposure. It can be concluded that seminal plasma does not have a protective role against Hg exposure, however, its replacement with immobilising solution could significantly decrease the progressive motility of common carp sperm. Thus, the removal of seminal plasma is not justified during toxicology tests as it does not a fect the results.

Acknowledgement

The work was supported by the Fisheries Operative Programme III. axis „European Fisheries Fund for Renewable Fisheries" provided by the EU and Hungary, the EFOP-3.6.3-VEKOP-16-2017-00008 project co-financed by the European Union and the European Social Fund as well as the GINOP-2.3.2.-15-2016-00025 project co-financed by the European Regional Development Fund and the Government of Hungary.

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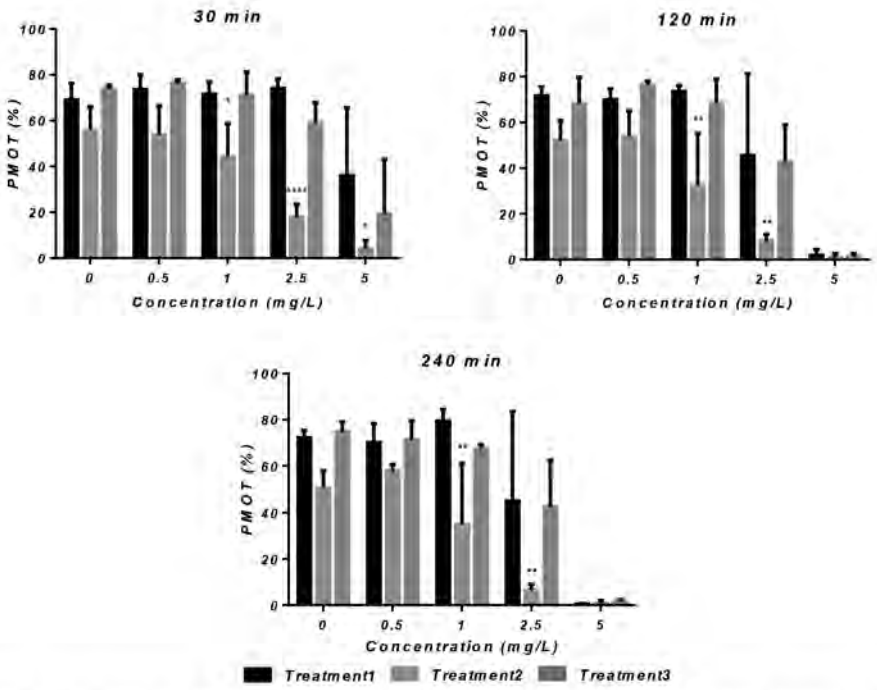

Fig.1. Progressive motility (PMOT, %) of common carp (*Cyprinus carpio*) sperm affected to different concentrations of Hg, next to 30, 120 and 240 minutes of exposure (N=3). Treatment1: sperm diluted in 1:1 (v/v) ratio with Hg, Treatment2: seminal plasma replaced by immobilising solution and sperm diluted in 1:1 (v/v) ratio with Hg, Treatment3: seminal plasma removed and spermatozoa diluted in 1:1 (v/v) ratio with Hg. Asterisks sign significant differences compared to Treatment1: * $p<0.05$, ** $p<0.01$, **** $p<0.0001$.

ESTABLISHING METHODS FOR THE DETERMINATION OF FISH QUALITY IN DOMESTIC FISH SPECIES OF MECKLENBURG-WESTERN POMERANIA AQUACULTURE

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Introduction

Aquaculture is the fastest growing area of food production with a steady expansion of more than 8% per year since the 70s (Burbridge et al. 2001; Knaus 2012). Besides this, fish flesh quality has gained importance among consumers and in the aquaculture industry due to its directly related impact on human health and nutrition. But so far, fish flesh quality of only few species (e.g. *Oncorhynchus mykiss, Salmo salar)* has been examined and this mostly in regard of the chemical composition. These results exhibited that lipid and protein composition in fish muscle varies greatly between species and is depending on age, sex, environment and season (Johnston 2001). Presently, there are no established methods and regulations to measure the physical properties of fish flesh. Therefore, our aim is to establish methods for a defined analysis of fish flesh quality and to adapt the protocols that are presently in use for beef, pork and chicken to fish, to analyze the physical parameters of fish flesh. For a first description two economically important fish species, the European perch (*Perca fluviatilis*, Percidae) and marena whitefish (*Coregonus maraena*, Salmonidae), were analyzed. Both species are present targets for aquaculture cultivation which will ensure the sustainability of our protocols for the future.

Methods

Fishes were cultured in a recirculation system in the Institute of Fisheries in Born, Germany. Adults of both sexes from *P. fluviatilis* (PFL, n = 15) and *C. maraena* (CMA, n = 15) were slaughtered by standard procedure in accordance to the German Animal Welfare Act (§ 4(3) TierSchG). Morphometric measurements of animals and filets as well as the physical measurements were taken immediately after death. Quality parameters of muscle like pH, isoelectrical conductivity and impulse-impedance were measured at 5 min and 1 h post-mortem using pH-Star (Matthäus), LF-Star (Matthäus), and Meat Check 150 (Sigma Electronic), respectively. The filet firmness was measured directly after death by Texture Analyser TA.XTplus (Winopal) with Warner Bratzler blade on the posterior filet, lateral on the horizontal septum, in triplicate. Color of muscle was measured three times with CR-300 Chroma Meter (Minolta) for the epaxial and hypaxial muscles separately. The water holding capacity (WHC) was determined by filte -press-method via Hypress (Grau and Hamm, 1953), in triplicate. Morphometric and quality parameters were statistical analyzed using SAS (v.9.2).

Results

At slaughter, PFL had a total length of 37.58 ± 0.37 cm and a weight of 777.07 ± 0.30 g. CMA exhibited a total length of 32.58 ± 0.27 cm and a total weight of 327.07 ± 7.59 g. For the consumers, especially the amount of free water in the fish filet, defined as WHC and texture, represented by the maximal shear force, is of interest. Under the present fish cultivation condition, the results showed a firmer texture (53.89 ± 7.76 N) with lower WHC (16.45 ± 7.76%) in the PFL filets. Compared to this, the CMA filets exhibit a softer texture with 24.74 ± 0.65 N and a higher WHC (20.90 ± 1.29%). Furthermore, also the impulse impedance showed higher values in PFL filets (able I).

Table I: Physical parameters of fish quality in European perch (*Perca fluviatilis*, PFL) and marena whitefish (*Coregonus maraena*, CMA) in aquaculture of Mecklenburg-Vorpommern.

PFL (n = 15)			CMA (n = 15)	
physical parameter	mean	SEM	mean	SEM
brightness	42.70	0.70	43.64	0.91
pH (5min)	6.68	0.04	6.87	0.04
electrical conductivity (5min)	4.79	0.26	4.05	0.17
impulse-impedance (5min)	70.93	1.54	60.33	1.30
water holding capacity [%]	16.45	0.60	20.90	1.26
shear force max. [N]	53.89	1.76[a]	24.74	0.65[b]

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Discussion

In the meat production, strict regulations for pork, beef, and poultry meat quality exist. Due to the high diversity of fish species, regulations for fish muscle quality have not been defined so far. With the present study, we showed that an adaptation of the used physical methods can be transferred to fishes as well. Therefore, the obtained results are a first step in order to develop consistent regulations and statements regarding the physical values of fish filet to define the quality of fish flesh

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GENETIC EVALUATION OF RESIDUAL FEED INTAKE IN THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei* USING PHENOTYPIC, PEDIGREE AND GENOMIC INFORMATION

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Introduction

Use of residual feed intake (RFI) as a measure of feed efficien y in animal production has raised much interest. Genetic evaluation of important traits in breeding programs of livestock and aquatic species traditionally use only phenotypic data and pedigree information. With the development of high-throughout SNP markers, genomic relatedness from high-throughput genotyping may offer more accuracy to genetic evaluation than pedigree information. The first genetic evaluation of RFI for *Litopenaeus vannamei* was implemented in the breeding population using phenotypic, pedigree and genomic information via a single-step genomic BLUP method (ssGBLUP).

Materials and Methods

Shrimp from 34 families were housed individually and residual feed intake was recorded during three weeks. A total of 479 individuals were genotyped using the 2b-RAD method. Two methods were used in the genetic evaluation. BLUP only used the classical pedigree-based relationship matrix A. ssGBLUP considered both genotypes at the SNP sets and pedigree, in which a combined pedigree-genomic relationship matrix H was constructed by augmenting the pedigree-based relationship matrix with the genomic relationship matrix G (Aguilar et al., 2010; Christensen and Lund, 2010).

Results

Shown in the heatmap (Figure 1), kinships estimated using both genomic and pedigree information were more accurate than those based on only pedigree, which also provides more reference information in genetic evaluation. Prediction accuracies of estimated breeding values (EBVs) for RFI based on A matrix and H matrix were carried out by the tenfold cross-validation (Figure 2), and prediction accuracy based on H matrix (0.41) was higher than that based on A matrix (0.340).

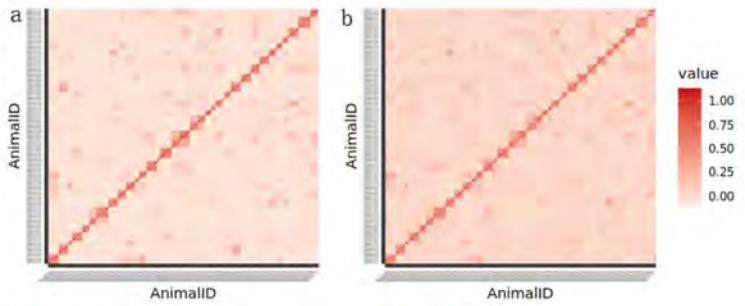

Fig. 1. Heatmap of kinships between individuals estimated using A matrix (a) and H matrix (b).

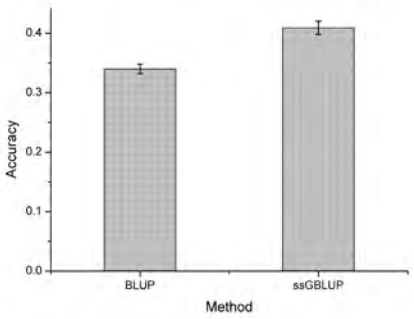

Fig. 2. Prediction accuracies of BLUP and ssGBLUP for EBVs of residual feed intake with standard error.

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Discussion and conclusion

In most cases, it is assumed that in a pedigree founder animals comprising the earliest generation do not share genes from older ancestors. Relationships from later generations are estimated as deviations from the founders' relatedness. In fact, however, founders are likely to share genes identical by descent, and if so, the results estimated from original assumption will be biased. Intuitively, pedigree errors may be expected to cause a bias of both heritability estimates and genetic covariances. Introduction of genomic information could reconstruct more real and right relatedness among some individuals (such as full-sib or half-sib individuals), which is expected to contribute to the improvement of evaluation accuracy. The cross-validation analysis implies that accuracy of EBVs of RFI increased by 20.59% using ssGBLUP compared to BLUP. As for its practical implementation, the use of ssGBLUP will depend on a cost-benefit analysis of individual genotyping against the expected gains of accuracy in breeding values.

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PROTEIN DEPOSITION IN NILE TILAPIA (*Oreochromis niloticus*): THE INTERPLAY BETWEEN DAILY PROTEIN AND ENERGY INTAKE

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Introduction

Protein is usually the most expensive macro-nutrient of a fish diet. Its retention into body tissue largely determines aquaculture resource-use efficie cy. Protein deposition is an energy-demanding process. Thus, for a given protein intake, raising energy intake leads to increased protein deposition (Haidar et al. 2018). Animal nutritionists aim at minimizing the use of dietary amino acids (i.e. protein) as energy substrates to improve resource and cost efficiency and minimize environmental impact. In terrestrial monogastric farm species such as pigs and poultry, protein deposition is often described by a linear-plateau model (Campbell et al., 1985). In these species, protein deposition increases with protein intake until energy intake becomes limiting. This model has important implications for animal feed formulation as it helps animal nutritionists balancing dietary protein, lipids and carbohydrates content to maximise protein deposition. The validity of such a linear-plateau model has not been tested in Nile tilapia. The present study intended to describe the interplay between protein and protein-free energy intake on protein deposition in Nile tilapia above 60 g of body weight.

Material and methods

The experiment consisted of a 42 days balance trial during which 16 diets were fed to all-male Nile tilapia with initial body weight = 63.4 g (SD = 1.3g). The diets were formulated and fed restrictively to achieve the following 2*8 factorial design: 2 levels of daily individual protein-free energy intake (18.3 and 25.6 kJ.d^{-1}) * 8 levels of individual daily protein intake (ranging from 0.5 to 1.35 g.d^{-1}). Each of the 16 diets was hand-fed twice daily to two randomly assigned 70-l tanks (30 fish per tank). All tanks were connected to a single water recirculation system. Initial and final body composition were measured from 20 fish and 10 fish per tank, respectively. Apparent digestibility was measured by the indirect method (using yttrium oxide as indigestible marker) based on faeces collected during the 2nd, 4th and 6th week of the trial. Generalized linear modelling was employed to analyse the data. Significance of model e fects was set at $P < 0.05$.

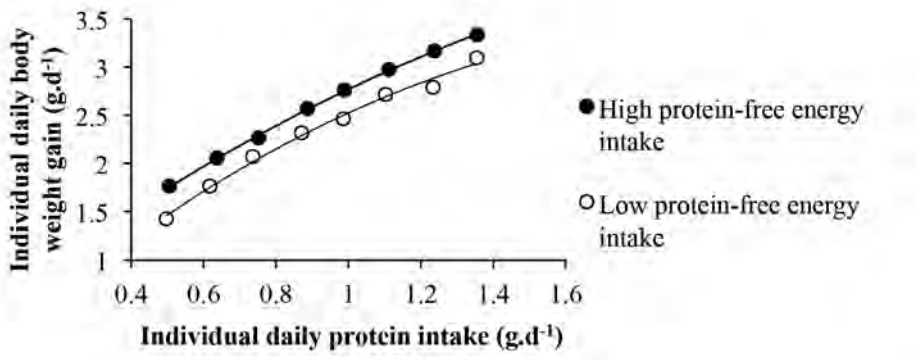

Figure 1. Daily body weight gain increases linearly with increasing protein intake.

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Results and discussion

Preliminary results indicate a linear increase of daily body weight gain (BWG, $g.d^{-1}$) with increasing protein intake (PI, $g.d^{-1}$), at both high (Eq. 1) and low (Eq. 2) protein-free energy intake:

$$BWG = 0.86\ (SE\ 0.05) + 1.87\ (SE\ 0.05) * PI \qquad r^2 = 0.99 \quad (1)$$

$$BWG = 0.62\ (SE\ 0.08) + 1.84\ (SE\ 0.08) * PI \qquad r^2 = 0.97 \quad (2)$$

At similar daily protein intake, fish receiving a high protein-free energy intake showed higher daily body weight gain than those receiving a low protein-free energy intake (figure 1). This clearly shows the protein-sparing effect induced by increasing protein-free energy intake which was already showed in Nile tilapia (Haidar et al., 2018; Saravanan et al., 2012).

The linearity of the relation between daily protein intake and daily body weight gain (figure 1) suggests that protein deposition does not follow a linear-plateau model as observed in terrestrial monogastric farm species. However, previous studies showed that Nile tilapia body composition – especially fat content – can be strongly affected by diet composition (Haidar et al., 2018). Body weight gain is therefore not the best read-out parameter for a cross-species comparison. Complete nutrient balances (including nutrient digestibility and body composition data) will provide a more precise description of the interplay between protein and energy intake on nutrient deposition rates and retention efficiencies during this experiment.

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NUTRIENTS INVOLVED IN DIGESTION AND TRANSPORT OF LIPID ACROSS THE INTESTINAL MUCOSA OF ATLANTIC SALMON (*Salmo salar* L).
PART 3: EFFECTS OF DIETARY CHOLESTEROL, PHOSPHOLIPIDS AND BILE SALTS ON INTESTINAL LIPID METABOLISM

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See abstract of Krogdahl et al.: Nutrients involved in digestion and transport of lipid across the intestinal mucosa of Atlantic salmon (*Salmo salar* L). Part 1: An overview.

EFFECTS OF HEAT-TREATED SOY & SUNFLOWER FLOUR FOR FISH MEAL SUBSTITUTION ON ENERGY RESERVES AND ANTIOXIDANT & DIGESTIVE ENZYMES IN THE GILTHEAD SEA BREAM (*Sparus aurata*)

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Introduction

Aquaculture has become one of the fastest growing food-producing sectors (FAO, 2018). However, the shortage and cost of fish meal supplies is limiting the further development of aquaculture. Thus, alternative protein sources have been studied in order to replace fishmeal in aqua-feeds. The potential alternatives of plant-derived nutrient sources are known to contain a wide variety of anti nutritional factors (ANFs) (Francis et al., 2001). Thermal processing is recognized to be a very successful method for inactivation of ANFs (Arndt et al., 1999; Ari et al., 2012).

Materials and Methods

Three isoenergetic and isonitrogenous experimental diets were formulated including a control (30% fish meal, 0% soy & sunflower), an untreated (12% fish meal, 25% soy+15% sunflower) and a treated (12% fish meal, heat-treated 25% soy+15% sunflower). Heat treatment included addition of 20-30% hot water (80-90^{o}C) to soy and sunflower flour and mixing, cooking at 60-65^{o}C for 15min with continues mixing and drying at 50^{o}C for two hours. A 14-week feeding trial with three groups of *S. aurata* juveniles (26.4 ± 0.5g) was conducted in 100 l tanks (28 fishes/tank). Livers and intestines were excised after properly sampling, placed in liquid N_2 and stored at -80^{o}C until analysis. Biomarkers responses of energy reserves (HSI) and antioxidant (GSH content and GPx, GR, GST activities) and intestine (ALP, Trypsin, Aminopeptidase activities) enzymes were determined.

Results and Discussion

Hepatosomatic index (HSI) values were affected by diet composition and treatment. HSI was higher in treated compared to untreated diet but significantl lower compared to control diet (Table 1.). GR & GST activities significantly induced in treated compared to untreated diet with a significant induction of GST indicated in treated diet compared to control. GSH content did not differ significantly in treated from untreated and control diet, whereas a significant reduction is showed in untreated compared to control diet (Fig.1). GPx activity was similar in treated compared to untreated diet but it was significantly reduced compared to control diet (Fig 1). In general, induction of antioxidant enzymes reflects activation of a compensatory mechanism to prevent a rising oxidative stress. Heat-treatment was positively affected some antioxidant enzymes.

Alkaline phosphatase (ALP) & aminopeptidase activity exhibited similar pattern. A significant reduction of ALP & aminopeptidase is shown in treated and untreated diets compared to control. Reduction in ALP activity is indicative of malnutrition (Estensoro et al., 2016). Trypsin activity was similar in all diets (Fig. 2). Trypsin activity can be

used for the prediction of digestive ability of the fish, which may in turn lead to differences in growth rate (Sunde et al., 2004). Heat-treatment had no effect on digestive enzymes.

(Continued on next page)

Table 1: Hepatosomatic index (HSI) for the three experimental diets.
(mean ± s.d with different superscripts are significantly different at p<0.05)

Diets	HSI
Control	1.65 ± 0.16^{c}
Untreated	1.15 ± 0.20^{ab}
Treated	1.28 ± 0.11^{ab}

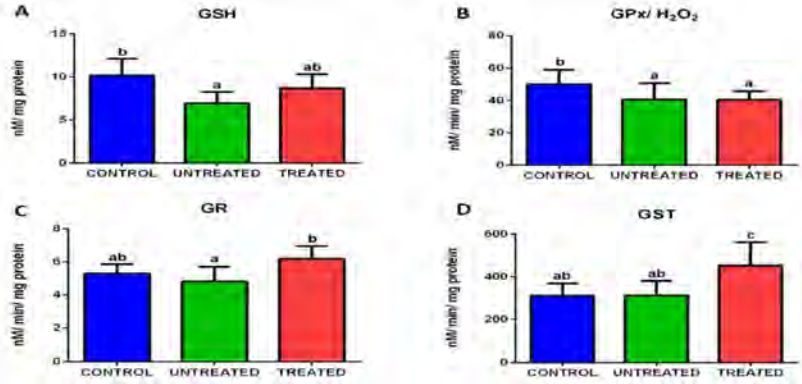

Fig. 1: Glutathione (GSH) (**a**), Glutathione peroxidase (GPx) (**b**), Gutathione reductase (GR) (**c**) and Glutathione-S-transferase (GST) (**d**) activities in *S. aurata* livers.
**different letters indicate significant differences at p < 0.05 level*

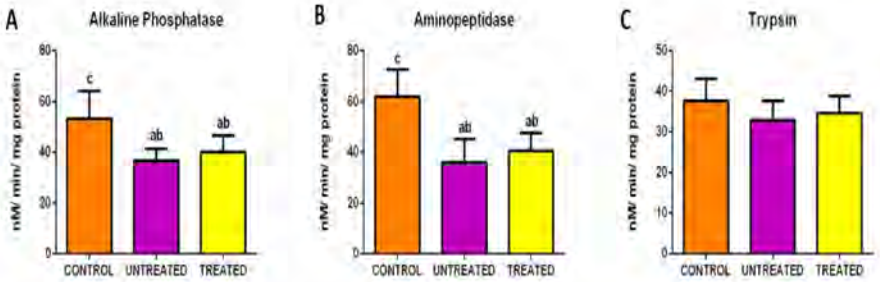

Fig. 2: ALP (**a**), Aminopeptidase (**b**) and Trypsin (**c**) activities in *S. aurata* intestine.
**different letters indicate significant differences at p < 0.05 level.*

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EFFECTS OF EXOGENOUS GONADOTROPIN TYPE (CARP VS. SALMON PITUITARY EXTRACT) ON OFFSPRING QUALITY IN EUROPEAN EEL

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Introduction

The catadromous European eel, *Anguilla anguilla*, has an unusual life cycle including a 5000-6000 km long migration to spawning areas in the Sargasso Sea. Whilst inhabiting continental habitats, complex hormonal mechanisms inhibit gonadotropic function (Vidal et al., 2004). This inhibition must be released in nature when reaching spawning habitats. Thus, captive reproduction apply exogenous administration of gonadotropins (Mylonas et al., 2010). Generally, female breeding protocols of anguillid species have been established using pituitary extracts of either carp (CPE) or salmon (SPE) varying in dosage. Such hormonal treatment schemes affect female reproductive developmental success and offspring quality (Mylonas et al., 2010). This study compares for the first time effects of applying either CPE or SPE to induce vitellogenesis in female European eel broodstock and evaluates efficiency as well as offspring quality under controlled experimental conditions. Expression of developmental genes are used to explore the largely unknown mechanisms affecting embryonic survival.

Material and methods

The experiment included migrating female silver eels caught at lower Bann, Toomebridge, Ireland (n = 28), migrating female silver eels from Lake Vandet, Denmark (n = 26) and male eels farmed from glass eels at a commercial fish farm. Vitellogenesis in the female broodstock was induced by weekly intramuscular injections of either CPE or SPE, each at 18.75mg kg^{-1} initial BW for 10-21 wks depending on response. Both treatment groups received 17α,20ß-dihydroxy-4-pregnen-3-one (DHP) as maturation inducing hormone, while males received human chorionic gonadotropin (hCG). Females were strip-spawned and eggs *in vitro* fertilized (Butts et al., 2014). Eggs from the floating layer were incubated in glass beakers (1 L) filled with filtered UV-treated seawater (FUV seawater; filter size: 10, 5, 1 µm) and supplemented with rifampicin and ampicillin (each 50 mg L^{-1}, Sigma-Aldrich, Missouri, USA) under controlled conditions at 36‰ salinity and 18°C. Embryonic survival was determined at 2, 3, 4, 5, 6, 7, 8, 16, 24, 32, and 48 hours post fertilization (HPF), where the number of dead and alive eggs were counted and expressed as a percentage. Furthermore, cleavage abnormalities were determined at 4 HPF by counting the number of eggs with regular and irregular cell cleavages. Hatch success was expressed as the number of hatched larvae divided by the total number of eggs. Samples for gene expression included ovarian tissue after spawning, unfertilized eggs, as well as embryos at 2, 4, 8, 24, 32, and 48 HPF. RNA was then extracted using the NucleoSpinR RNAKit (Macherey-Nagel, Germany) and expression of genes was analyzed using the qPCR BiomarkTM HD system (Fluidigm). Data were analyzed by repeated measures ANOVA models using SAS statistical analysis software.

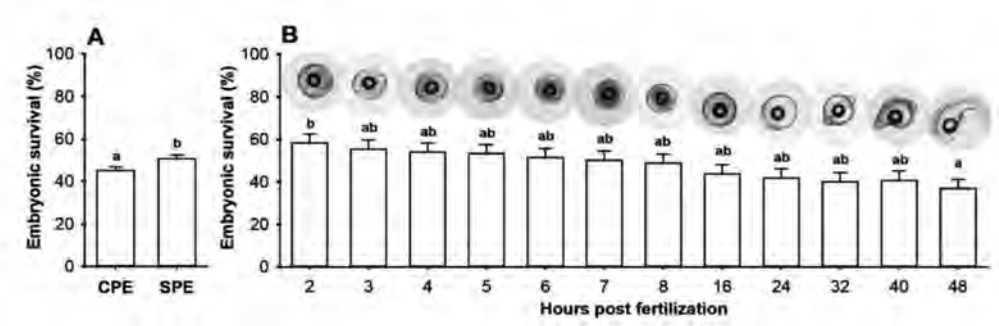

Fig. 1 Fertilization success and embryonic survival in European eel (*Anguilla anguilla*). Effects of hormonal treatment (A) and age (2 – 48 hours post fertilization, HPF; B) on fertilization success (2HPF) and embryonic survival. Bar plots represent means (± SEM) and lower-case letters significant statistical difference ($p < 0.05$).

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Results

Female responsiveness to CPE (96.4%) exceeded SPE (80.7%). However, the proportion of embryos obtained from SPE treated females and embryonic survival were significantly higher than for CPE females ($p = 0.029$, Fig. 1A). For treatments overall, the fertilization percent was 58.3%, while embryonic survival decreased gradually over time to 36.9% at 48 HPF ($p = 0.003$, Fig. 1B). Moreover, embryos obtained from CPE females showed significantly higher ratio of cleavage abnormalities at 4 HPF than those from SPE females ($p = 0.037$). In both treatment groups, a strong negative relationship was found between cleavage abnormalities and embryonic survival at 48 HPF (CPE: $Y = -1.31x + 87.83$; $R^2=0.64$, $p = 0.0003$; SPE: $Y = -0.66x + 52.80$; $R^2=0.76$, $p<0.0001$). The gene expression analyses underline differences between treatment and related maternal effects on embryonic developmental competence.

Discussion and conclusion

The study revealed enhanced offspring developmental competence from female European eel broodstock treated with SPE to induce ovarian development compared to those treated with CPE. The two hormonal product types are generally used as equal alternatives, although CPE is more frequently used for European eel, and SPE for the Japanese eel. However, this study reveals that while fertilization success is little affected, the PE origin has a profound impact on offspring quality. This may relate to genetic differences in hormone structure among species or the gonadotropin composition and content in the specific pituitaries. Data on the expression of developmental genes will be discusses in relation to possible causes for differences in developmental success and survival during early life history of captive bred eel offspring using assisted reproduction. Results of this study may be incorporated into future broodstock treatment improving offspring quality for future European eel breeding.

We thank the Lough Neagh Fishermen's Co-operative Society and Lake Vandet Eel Guild for donating silver eels. This study is supported financially by Innovation Fund Denmark through the projects: Eel Hatchery Technology for a Sustainable Aquaculture (EEL-HATCH; Grant no. 5184-00093B), and Improve Technology and Scale-up Production of Offspring for European eel aquaculture (ITS-EEL, Grant no.7076-00125B).

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'Wherefore art thou Aquaponics – The herd of Elephants in the Room': FUTURE AQUAPONICS IN EUROPE TOWARDS SURVIVAL, GROWTH AND SUCCESS

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Introduction

Aquaponics in the EU has developed significantly over the last 5 years, since the initiation of the 4 year long COST (European Cooperation in Science and Technology) FA1305, 'EU Aquaponics Hub' by Benz Kotzen in 2014 and who was chair of the project for the duration. The Action brought together 90 members from 30 countries and initiated 6 international conferences attended by hundreds of people and numerous papers (over 30 review papers, journal special issues, books and monographs), a white paper, and 59 videos. It has been the catalyst for numerous another EU projects, including 'Aqu@teach' Erasmus+ Strategic Partnership in Higher Education, led by the University of Greenwich in partnership with institutions in Switzerland, Spain and Slovenia. The project concluded with a White Paper and as a final output an open access publication titled 'Aquaponics Food Production Systems' edited by Simon Goddek, Alyssa Joyce, Benz Kotzen and Gavin Burnell. This publication highlights the core of aquaponics and cutting edge aspects in many areas but the future of aquaponics not only lies in technical and scientific innovation but as importantly in the areas of policy, social acceptance and representation. For aquaponics to succeed, we need to deal with the *'herd of elephants in the room'*.

Discussion - Aquaponics the Present and the Future

The project was always envisaged to kick-start real growth in aquaponics research and entrepreneurship, and it did so, but what it did not envisage is the next steps and this is what this paper is about. The important areas for the future of EU Aquaponics was set out in the White Paper, highlighting 8 key areas:

1. The promotion of continued research in aquaponics;
2. The commercialisation and funding of aquaponics
3. Urban aquaponics – promotion of aquaponics as social enterprise
4. Developing world aquaponics and refugees
5. Aquaponics policy and legislation
6. Aquaponics and education
7. Aquaponics health and safety including fish welfar
8. Initiating the EU Aquaponics Association

This paper concentrates on the 3 connected areas of commercialisation / funding with regard to aquaponics policy and legislation and the initiation of the EU Aquaponics Association and discusses the placement of aquaponics in the global and European niches of production. Whilst this issue has been visited previously, it is one of the largest of the elephants in the room in terms of furthering aquaponics acceptance and thus commercialisation. Post the EU Aquaponics Hub, an EU Association was to be set up, but this has floundered for a number of reasons. The discussion in this paper concludes on how best we as an aquaponics community, both research and commercially based can best set up a group. For the purposes of this paper, we will call in the 'New EU Aquaponics Hub', whose aims will be to represent and assist the EU aquaponics community to fulfil its potential, by lobbying and representing aquaponics in terms of polic , law making, identity etc.

GASTRIC EVACUATION, STOMACH DIGESTA MOISTURE AND CARCASS PROXIMATE COMPOSITION OF MEAGRE, *Argyrosomus regius*, FED DIETS WITH DIFFERENT DIETARY LIPID LEVEL

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Introduction

Digestion characteristics are species-specific and are based, although not strictly, on fish size, diet formulation and composition, feed processing, feeding frequency and water temperature (Dos Santos and Jobling, 1991; Venou et al. 2009). Gastric evacuation (GE), the process of digesta leaving the stomach, has been greatly studied in wild populations as a means to define prey-predator interactions, adaptive seasonal physiological behaviour and quantify stock populations. In contrast, in aquaculture species fed pelleted diets, it has only been vaguely examined. GE can provide an indication of feeding rates and frequency, contributing to the optimization of feeding regimes, as well as on feed deprivation time before harvesting. Diet formulation, ex. dietary protein/lipid ratio, can influence nutrient transport and absorption in the organism. In meagre, a limited number of studies have investigated the effect of diet formulation on nutrient apparent digestibility coefficients (Kounna et al. 2012; Velazco-Vargas et al., 2014), but no available references exist yet on the effect of diet on GE. Hence, the present study investigated the effect of dietary lipid level on: gastric evacuation time and rate (GET, GER); digesta moisture content and carcass proximate composition of juvenile meagre for the first time

Materials and Methods

A 4-week feeding trial was conducted in an open flow-through experimental system. Three isonitrogenous (46% crude protein, dry weight), practical extruded diets -A1, A2, A3- with increasing lipid level (15, 18, 24% dry weight), were offered to the experimental fish in triplicate groups. Average fish weight was 114.5g ± 5.0 (± s.e.m) and fish density was 4.1kg/m^3. The fish were acclimatized to the system and diets for 19 days prior to the experiment. Feeding was performed by hand to apparent satiation, once a day. On the sampling day, fish were fed once to satiety (ca. 1% body mass) and then 5 fish treatment were sacrificed at 0.5, 2, 4, 6, 8, 10, 12 and 23 hours post feeding. Stomach contents were collected and their dry weight was determined and expressed as % of wet fish body weight. GET and GER were evaluated using regression analysis. GET was estimated as the time needed for the fish to empty 50% of its stomach content. At the end of the trial 3 fish/tank were collected for proximate carcass (no viscera) composition analysis. Water temperature during sampling was 25.5 – 26.2ºC; salinity was 37ppm and photoperiod 12L:12D.

Results

The exponential model best described the pattern of evacuation of the stomach of meagre in all 3 treatments. GET and GER were similar for diets A2 and A3 (3.61, 3.65h; 3.20, 3.17g/min x 10^{-3}, respectively), but statistically faster ($p<0.05$, t-test) for meagre feeding on diet A1 (2.41h, 4.80g/min x 10^{-3}). As a result, the group fed diet A1, had completely evacuated their stomachs close to 12h postprandial, whereas the other two groups a bit later. By 23h postprandial the stomachs of the fishes from all groups were empty.

Table I. Juvenile meagre carcass proximate composition (% wet weight) (mean ± std). No statistically significant difference between diets ($p > 0.05$)

Diet	Protein	Total lipids	Ash	Moisture
A1	18.03 ± 2.33	6.14 ± 0.77	4.05 ± 0.32	71.78 ± 0.78
A2	16.98 ± 0.19	6.06 ± 0.63	4.09 ± 0.19	72.91 ± 0.73
A3	17.99 ± 0.82	6.59 ± 0.18	4.02 ± 0.34	71.40 ± 1.04

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There was an effect of time on digesta moisture content but not of diet (at $p = 0.05$).
% digesta moisture at 0.5h ranged from 68.0% (A2) to 69.8% (A3). An increase (2-8%) was noted 2h postprandial in all treatments and a further statistically significant increase (6-18%) at 6h –diet A1, at 4h –diet A2 ($p<0.05$, Welch test, Games-Howell) and at 12h -diet A3 ($p<0.05$, Kruskal-Wallis). Finally, after 23h it had reached maximum, 96 - 98.5%, in all treatments.

Carcass proximate composition of meagre, Table I, was similar between the treatments ($p>0.05$, one-way ANOVA).

Discussion

There was an effect of diet on GET and GER but not on carcass proximate composition. The exponential model best described GET of meagre fed diets with different lipid level, as in seabream and seabass by Nikolopoulou et al. (2011). The model implies a quick stomach evacuation the first hours postprandial, which then slows down as the residual content in the stomach decreases. The group fed the lower dietary lipid content exhibited the highest GER, whereas the medium and high lipid inclusion levels produced similar results, indicating that the effect on GE does not change from 18 to 24% lipid content, but lower (15%), probably increases GER. Nevertheless, it appears that GET and GER were adequate in all treatments for sufficient nutrient digestion and absorption to occur, yielding fish with similar carcass proximate composition characteristics. This is further supported by the lipid and protein digestibility coefficients that were not affected by dietary lipid level in a previous study (Kounna et al. 2012). Under the specific conditions, meagre required less than 23h to empty its stomach contents.

The increase in digesta moisture content in all treatments after feeding, prompts to possible fluid secretions in the stomach of the fish to facilitate digestion. An increase in digesta moisture content after feeding was also observed in seabream by Venou et al. (2009) and Nikolopoulou et al. (2011). In these studies, stomach hydration level was also affected by feed processing method and diet composition. In the present study, there was no effect of diet formulation, perhaps because diet moisture level and composition were similar amongst the 3 diets.

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MICROSATELLITE SET FOR PARENTAGE ANALYSES OF AFRICAN CATFISH, *Clarias gariepinus* (BURCHELL, 1822)

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Introduction

The African catfish or sharp tooth catfish (*Clarias gariepinus*) is farmed in numerous African, Asian, and European countries. Moreover, during the last decades its production has grown significantly worldwide. Currently, following the carp, this species is produced in the second largest volume in Hungary. Despite its economic importance, the stocks have been maintained without genetic control or guided breeding. Molecular genetic data on bred populations or strains are very limited. Microsatellite markers are particularly suitable for numerous applications including population genetics, parentage analysis or marker assisted selection. In order to investigate the genetic structure of the stocks, 49 new microsatellite markers were characterized and tested on a Hungarian farmed stock. Based on the results an effective set of markers were identified for parentage analyses.

Materials and methods

Thirty-two African catfish individuals were sampled from closed bred population of Szarvas, Hungary. The genomic DNA was isolated using E.Z.N.A. DNA Tissue Kit (Omega BioTek). Repeat enriched DNA libraries were produced from pooled samples of male specimens following the protocol of Glenn and Schable 2005. Rsa I, Hae III, Alu I, and HpyCH4 V endonucleases and the Box I linker (F:Phos-ATGTCTGAAGGTACCA CTGCTGTCCGAAA; R:CGGACAGCAGTGGTACCTTCAGACAT) were used for librarie preparation. The insert size was 300-1000bp. The sequences of the inserts were determined by 3130 Genetic Analyser (Applied Biosystems). Primer3Plus software was used for primer design. Tail and tail specific fluorescent primers were used to reduce the costs of detection, as it was described by Shimizu et al. (2002). A 17 basipair long tail (ATTACCGCGGCTGCTGG-) were added to the forward primers' 5' ends to provide an attachment site for fluorescent dye labeled (FAM or VIC or NED or PET) tail-specific oligos. MICROSATELLITE TOOLKIT VER. 3.1.1 was used to estimate mean number of alleles and polymorphic information content. The Hardy-Weinberg equilibrium (HWE) were estimated by GENALEX VER. 6.5. The parentage and minimum set of markers which provides a high resolution of parentage allocation and the probability of exclusion in paternity were tested by PARFEX v1.0., while the inbreeding coefficient (Fis) was calculated by FS AT 2.9.3.

Results

In total, 127 selected clones were sequenced from four CA-repeat enriched genomic libraries. 55 microsatellite sequences were selected and primers were synthesized for later PCR analyses. Forty-nine of the selected markers were used to genotype 32 individuals. None of the 49 markers was monomorphic. The number of alleles per locus ranged from 2 to 11, with a mean of 4.818 (SE: 1,973) alleles. Twenty-seven loci were highly polymorphic (PIC > 0.5), 18 were moderately polymorphic (0.2 < PIC < 0.5), and four showed low polymorphism (PIC < 0.2) in our bred population. The expected heterozygosity (HE) among loci ranged from 0.147 to 0, whereas the observed heterozygosity (HO) ranged from 0.031 to 1.000. Thirty-one loci showed significant deviation from HWE, probably due to selection practice, artificial propagation, fluctuations in population size or stocking. No parental relationships were identified by the exclusion or the maximum likelihood methods. However, the maximum likelihood analyses of relatedness showed a high probability of full-sib or half-sib relationships between individuals. All the individuals showed relationship with at least two specimens, while three of them had ten probable relatives. Nevertheless, the high number of related individuals can lead to spurious associations in the estimation of random distribution. The overall FIS values were 0.063, while 29 markers had positive and 20 negative Fis values. The calculation of the minimum set of markers for parentage allocation or for identification over 99% probability resulted in a marker set containing 14 (Cg10, Cg175, Cg132, Cg370, Cg3, Cg341, Cg214, Cg661, Cg287, Cg299, MS647, Cg352, Cg639 and Cg294) markers.

(Continued on next page)

Discussion and conclusion

In conclusion, 49 newly isolated *C. gariepinus* microsatellites were tested and characterised. The genetic effects of long-term intensive breeding were also investigated on the examined Hungarian African catfish population by the markers. The expected and observed heterozygosity differed significantly from each other. The inbreeding coefficient also showed the presence of heterozygote surplus and deficiency in a wide range (from -0.709 to 0.899) at different markers. All these can be the consequence of intra-population genetic structures (non random mating) or small founder group with rare and unsystematic crossbreeding with other strains. The presence of intra-population genetic structures was confirmed by the maximum likelihood estimation of relatedness and the parentage allocation. However, none of these two methods identified parental relationships among the individuals, the maximum likelihood analyses highlighted the presence of a high number of half-sib and full-sib individuals in the analysed group. During the parentage analyses the most efficient set of markers were also calculated. The marker set contains 14 markers usable for parentage allocation or for identification of individuals over 99% probability in exclusion analyses.

Acknowledgment

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EFFECT OF RATE OF FREEZING ON THE SPERM OF STURGEON

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Introduction
Studies of cell structures and their organelles indicate that the effect of deep freezing can affect any of their components, which is an important obstacle to the preservation of the integrity of cells and tissues during cryopreservation.

The aim of this study was to establish optimal freezing rates during cryopreservation of sturgeon sperm, ensuring the preservation of the structural components of reproductive cells.

Materials and methods
Reproductive cells of Russian sturgeon (Acipenser gueldenstaedtii Brandt&Ratzeburg, 1833) and sterlet (Acipenser ruthenus Linnaeus, 1758) obtained at sturgeon hatcheries of the Astrakhan region during the spawning campaign served as material for research.

The following freezing rates were studied: 3°C/min, 10°C/min and step mode (6**°C/min for 6 minutes, 10°**C/min, for 4 minutes, then the samples were immersed in liquid nitrogen).

Thawing of sperm was carried out in a water bath at a temperature of 38-40°C.

Results
The best freezing speed for the sperm of Russian sturgeon was the speed of 3°C/min, both in activity and in life time of sperm after defrosting. Other modes of freezing showed results worse, however, it is noted that all three investigated speeds were not reduced frozen-thawed sperm quality below fish production parameters. Thus, all these freezing rates can be used for cryopreservation of sperm of Russian sturgeon, depending on certain conditions of conservation.

When working with sterlet sperm, less damage after freezing-thawing occurred at a freezing rate of 10 ° C/min, while under the same conditions and relatively high sperm quality for Russian sturgeon, the optimal speed was 3 ° C/min. the Speed of 3 ° C/min for sterlet sperm was slightly worse. Step freezing mode was much worse in both cases.

Discussion and conclusion
For sturgeon from the tested freezing rates the most acceptable are 3 ° C/min for Russian sturgeon and 10 ° C/min for sterlet.

The results indicate that the choice of the sperm freezing rate of sturgeon is species-specific.

Work is performed with the use of the Bioresource collection of rare and endangered species SSC RAS No. 73602 with the financial support of RFBR, research project No. 19-016-00208 (ice formation) and grant MK-68.2019.11 (the freezing rate).

FUTURE PROFITABILITY OF TYPICAL FINFISH FARMS IN EUROPE

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Impacts of climate change on European marine and freshwater environments are already beginning to affect finfis production in Europe. Besides impacts on water quality and temperature that might influence growth performance, pathogen interactions, feed conversion ratio, and thereby the fish production itself, the aquaculture sector is also facing an uncertain future in terms of production costs and returns. Climate change, social and economic factors will determine future access to proteins and omega-3 fatty acids and thereby define the price of these crucial ingredients for fish feed. Another main expenditure within fish farming is attributed to energy, whose future price development is also dependent on various factors. Finally, the market price trends will have a major impact on future profitability within the aquaculture sector. This study analyses the future economic drivers of grow-out production of Atlantic salmon (*Salmo salar*), European seabass (*Dicentrarchus labrax*), Gilthead seabream (*Sparus aurata*) and Rainbow trout (*Oncorhynchus mykiss*) under four socio-political scenarios developed in the EU Horizon 2020 project CERES. Thereby, an established benchmarking approach was used to contrast today's economic performance of "typical farms" in selected European production regions with their future profitability under the different scenarios. The intra- and inter-species comparisons of different production systems for their future security is intended to create awareness among the aquaculture sector to prepare for the next decades.

THE EFFECT OF OZONE ON WATER QUALITY AND PIKEPERCH *Sander lucioperca* PERFORMANCE IN RECIRCULATING AQUACULTURE SYSTEM

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Introduction

Aquaculture and recirculating aquaculture system (RAS) will be very important production system for future seafood production to supply the ever-expanding human population. However, RAS can provide problem with a latent disease and exposure of fish to pathogens, bacterial and fungal diseases. Ozone can effectively inactivate a viral, bacterial, fungal and protozoan fish pathogens in RAS. But the effectiveness of ozone treatment depends on ozone concentration, length of ozone exposure (contact time), pathogen loads and levels of organic matter (Gonçalves et al. 2011). The aim of this study was to compare ozone and/or nonozone water treatment for pikeperch ongrowing culture under RAS.

Material and methods

The experiment was carried out using triplicate groups of 500 fish for two tested groups presenting two different conditions: ozone treatment of water (ozone +) and control group without ozone (ozone –). In total, the 3000 juvenile pikeperch (9.2 ± 2g, 102.5 ± 8.1 mm) were used for this study. Water temperature and pH were kept under similar values for both groups. Group ozone + was supplied with 2.5g ozone per hour (60g ozone) per day. The duration of the experiment was 231 days. Water quality parameters (ammonia, nitrites, nitrates, total N and P, chemical oxygen demand, biological oxygen demand, suspended solids), fish mortality and feed intake were recorded daily. At the beginning of the experiment and then every three weeks until the end of the experiment, total length (TL), standard length and fish weight (FW) was measured in representative samples of fish from each tank, specific growth rate (SGR) and Fulton coefficient (FC) were compared. At the end of the experiment, the blood biochemical analysis: cortisol, albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), total protein (TP), ammonia (NH_3), lactate dehydrogenase (LDH), lactate (LACT), Spleen Somatic Index (SSI), Hepato Somatic Index (HSI), Viscero Somatic Index (VSI) and fin erosion according Policar et al. (2016) were analyzed and compared between groups.

Results

At the end of the experiment, the average values of survival rate (ozone + and ozone –) were significantly different between groups ($P<0.05$). The group ozone + achieved higher survival rate 77 % in comparison with 67.2% in ozone –. No significant differences in FW, TL, SGR and FC were found (FW = 130.0 ± 26.2 g compared to 123.5 ± 21.3 g; TL = 258.0 ± 16.3 compared to 238.0 ± 15 mm; SGR = 1.15 ± 0.12 %.d^{-1} compared to 1.13 ± 0.1 %.d^{-1} and FC = 0.76 ± 0.05 compared to 0.91 ± 0.07 in ozone + and ozone – groups, respectively). The water quality parameters are shown in table 1.

Table 1. The average water chemical parameters in ozone – and ozone +. Values within column with different superscripts are significantly different ($P < 0.05$)

	NH_4^+- N(mg.l^{-1})	NO_3- N (mg.l^{-1})	NO_2- N (mg.l^{-1})	N-total (mg.l^{-1})
ozone –	0.37±0.1a	26.4±10.2a	0.09±0.03a	34.6±10,9a
ozone +	0.31±0.1a	23.6±9a	0.06±0.02a	29.4±8.9a
	P-total	COD_{Mn} (mg.l^{-1})	BOD_5 (mg.l^{-1})	suspended solids
ozone –	1.44±0.6a	10.7±1.6a	8.1±1.3a	8.17±6.2a
ozone +	1.22±0.6a	6.4±1.2b	5.32±1.8b	4.3±2.8b

Table 2. The blood biochemical profile in pikeperch.

	kortizol (ng/ml)	ALB (g/l)	ALP (µkat/l)	ALT (µkat/l)	AST (µkat/l)	TP (g/l)	NH_3 (µmol/l)	LDH (µkat/l)	LACT (mmol/l)
ozone–	124.5 ± 55.29	3.0 ± 1.10	1.4 ± 0.40	0.2 ± 0.07	3.0 ± 1.10	36.5 ± 4.32	931.0 ± 55.81	19.6 ± 2.07	3.5 ± 0,85
ozone+	151.8 ± 74.50	3.5 ± 1.38	1.3 ± 0.65	0.2 ± 0.14	3.9 ± 1.45	39.0 ± 2.61	872.5 ± 191.57	20.5 ± 1.55	4.0 ± 0.48

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The significant differences were detected in COD, BOD and suspended solids. Group ozone– was more organically loaded. The blood biochemical analysis is shown in Table 2. No significant differences in mentioned parameters were determined between tested groups.

In comparison SSI, HSI and VSI, the significant differences were observed in SSI and HSI. The bigger SSI 0.09 ± 0.03% was found in ozone + compared to 0.04 ± 0.05% in ozone–. The HSI 1.77 ± 0.2% was bigger in ozone– in comparison with ozone+ 1.34 ± 0.31%. Pikeperch from group ozone – were the mostly affected by fin erosion compared to group ozone +. The significa tly differences were observed in pectoral fin: 38-42% (degree 1) 8-10% (degree 2) compared to 55-65% (degree 1), 18 -20% (degree 2), in ozone + and ozone – respectively. Similar results were in first dorsal fin: 38% (degree 1) and 50% (degree 1) in ozone + and ozone –, respectively. The caudal fin was the most damaged fin in group ozone–. In this fin, the serious erosion 5% (degree 3) was detected. Degree 3 was not found in ozone + group. Degree 2 was 12 % and 27% and degree 1 was 76% and 67% in ozone + and ozone –, respectively. Other fins were not statistically different between groups.

Discussion

Ozone is a powerful oxidizing agent that can be put to numerous beneficial uses within RAS (Summerfelt and Hochheimer, 1997). In solids, the similar results achieved Davidson et al. (2011), who also demonstrated removing solids in water treated by ozone. Similar positive ozone treatment effect observed Powell et al. (2015) in turbot (*Psetta maxima*) and Davidson et al. (2011) in rainbow trout (*Oncorhynchus mykiss*). The present study showed better survival rate, quality of water and quality in fin erosion in ozone + compare to ozone– group. Furthermore, the biochemical parameters, growth rate, fitness condition were similar. In conclusion, we can recommend the using 2.5g per hour for on growing pikeperch fingerlings in RAS

Acknowledgements

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DESIGN CONSIDERATIONS AND RESEARCH CHALLENGES FOR CLOSED FISH CAGES IN CURRENTS AND WAVES

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Introduction

From a marine technology perspective, floating closed fish cages are novel structures with possible complex dynamic behaviour, where care should be exercised in the design process to reduce the risk of structural issues and failure. Floating closed fish cages have gained increasing attention and interest among fish farmers as a strategy to mitigate several challenges in sea-based farming of Atlantic salmon in open cages. Several new closed cage structures have been proposed by fish farmers, suppliers and others in development-permits applications to the Norwegian authorities. By enclosing the production volume the risk of sea-lice infections and need for medical treatment can be reduced, and deposits from the production collected. Further, the production efficiency can be improved by optimizing parameters such as water temperature, oxygen saturation, and acidity. The present work compiles results from previous and on-going research on floating closed fish cages to describe state-of-the-art in design of closed cages from a marine technology perspectiv

Design considerations of closed cages

In order to address the different structural and hydrodynamic properties of closed cage concepts, a categorization of closed cage structures based on their elastic properties was proposed in [1], where closed cages are defined as either rigid, elastic or fully flexible.

Stability: The free water surface inside the containment has a destabilizing effect on a floating closed cage, where the cage filling level is also a parameter. Furthermore, the hydrostatic restoring moment is typically small as the water-plane area of the structure is often limited to a narrow walk-way surrounding the cage. This can lead to stability related issues if it is not considered appropriately during design.

Water density: In fjord areas, where typically closed cages are installed, brackish surface water may cause density stratification in the upper part of the water column. Inlets of the water exchange system can be located at a distance below the surface, to reduce the risk of sea-lice contaminated water. If so, this can result in the contained water having a different water density compared to its immediate surroundings. The density difference combined with the internal filling level causes a hydrostatic pressure difference across the containment wall which can lead to discharge of contained water in damaged conditions. For flexible bag-type closed cages this can cause drainage and collapse if no countermeasures are taken (see e.g. in [2] and Fig. 1).

Sloshing: Closed cages are typically characterized with a large free surface that can be set into motion when the cage moves in response to waves. These induced motions of the internal water volume are known as sloshing and can have strong influence on the cage motions and structural loads. Particularly at resonant conditions when the cage is excited close to a natural period of sloshing (see e.g. in [1] and Fig. 2). From a design perspective, it is the first few highest natural periods that are of interest. They represent the most energetic conditions and may yield the largest structural loads. Hence, it is important to compare the expected exposure at the site in question to the estimated highest natural periods of sloshing for a given design. By modifying design dimensions and/or geometry the natural sloshing periods can be shifted away from the energic wave period, thereby reducing the risk of resonant conditions. Damping can also be used to limit the sloshing response. However, typical damping strategies for sloshing as used in ship tanks are considered less attractive for closed cages due to operational aspects. Whereas sloshing in rigid containers is well described (see e.g. in [3]), sloshing in elastic or flexible containers are still in the research front. Complex wave-structure interaction effects obtained from a 2D numerical model of a flexible type closed cage was presented in [4], where scaling issues of flexible bag models were also identified.

Parachute effect: Depending on the current conditions at the site, closed cages can be exposed to significant current-induced forces and moments. These can be static forces and moments, or dynamic loads e.g. due to flow separation from the structure. Current-induced loads on rigid type closed cages can be addressed by conventional design methods by the application of drag coefficients. However, for the flexible bag-type hydroelasticity matters, meaning that hydrodynamic loads and structural deformations are mutually dependent. For flexible bag-type closed cages in damaged condition with reduced filling ratio, bag deformation in steady current may cause a large increase of the current-induced drag compared to intact condition due to the so-called “parachute effect” [5].

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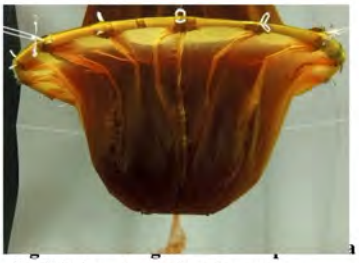

flexible bag-type closed cage.

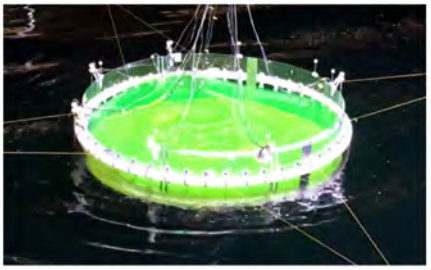

Fig. 2. Sloshing in an elastic closed cage.

Mooring analysis: Compared to traditional slender net cage structures, closed fish cages are large volume structures. Further, due to large displacement, closed cages with moorings represent a dynamic mass-spring system. This means that dynamic amplification of mooring forces due to excitation from slowly varying wave, wind and current forces should be considered as for conventional mooring analysis of large volume structures. First order wave induced motions of the cage, which are highly affected by sloshing, are also important. Due to small hydrostatic restoring moment, one should also be aware that unfavourable location of mooring line attachments may cause tilt moments that can result in static heel angles. Numerical modelling of wave response for moored closed cages in relevant sea-states is an on-going research activity.

Summary

Floating closed cages are novel structures with complex dynamic behaviour in a typical marine environment. In the present paper, important design considerations for floating closed fish cages resulting from recent experimental and numerical studies are presented. The importance of some of the design considerations depends on the category of the closed cage structure (rigid, elastic of flexible bag type). The main findings from these studies and relevant research activities are discussed.

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NUTRIENTS INVOLVED IN DIGESTION AND TRANSPORT OF LIPID ACROSS THE INTESTINAL MUCOSA OF ATLANTIC SALMON (*Salmo salar* L) PART 1: AN OVERVIEW

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For many years, symptoms of impaired lipid digestion, a lipid malabsorption syndrome (LMS), have been commonly observed in cultivated Atlantic salmon in Norway (Hanche-Olsen et al., 2013). Severe outbreaks are characterized by steatorrhea, visible in the environment as floating faeces accumulating on the water surface and on nearby seashores. In most cases, the symptoms are less pronounced and only visible upon opening of the abdominal cavity, macroscopically as foamy pyloric caeca, and histologically as hypervacuolation of the enterocytes. The occurrence of the symptoms appears to have increased as level of plant ingredients in the diets has increased, although the most severe symptoms have decreased in frequency. An ongoing survey will provide updated information on the situation in salmon farm sites along the Norwegian coast. The symptoms are accompanied by high content of lipid in the intestinal mucosa, indicating disturbance of lipid digestion, absorption and transport across the mucosa and/or delivery to the peripheral circulation. However, the mechanisms and structures involved in these processes in fish are not well described and clear differences, compared to mammals, exist (Bakke et al., 2010). Among the lipases responsible for lipid hydrolysis in the intestine, fish seem to lack pancreatic triacylglycerol lipase. This may affect the following micelle formation and lipid absorption including phospholipids, cholesterol, bile salts and other lipophilic compounds such as fat soluble vitamins. In the other end of the lipid absorption process, fish seem to differ from mammals by lacking a lymphatic system for conveying lipid to the peripheral circulation and tissues. The likely route of lipid transport from the intestine is, therefore, in the portal blood, as "portomicrons", to the liver, and onward in VLDL. However, this is not clearly documented. The same is the situation regarding the elements of lipid absorption into the enterocytes, lipoprotein assembly and export (Xiao et al., 2018). The core of lipoproteins formed in the enterocytes mainly contains triglycerides, esterified cholesterol as well as lipid soluble vitamins and other highly lipophilic compounds. The surface contains, besides a number of apolipoproteins, phosphatidylcholine and free cholesterol.

A series of feeding experiments has been conducted to find whether insufficient supply of compounds and metabolites involved in phospholipid and sterol metabolism might explain development of LMS. The figure below indicates relationships between the compounds in focus of these studies. The basal diets have been commercially relevant low fish meal, high plant and high lipid diets which have been supplemented with phospholipids, choline, cholesterol, bile salts and taurine. We have also investigated effects of supplementation with methionine and cysteine, involved in metabolism of methyl groups, which may be utilized in the synthesis of phosphatidylcholine from phosphatidylethanolamine (Schubert et al., 2003). The observed endpoints are growth, -nutrient digestibility, hypervacuolation as indicated by histology, expression of genes involved in nutrient digestion, cholesterol, bile salt and phospholipid metabolism, lipid transport, gut barrier functions as well as plasma metabolites.

Results from these studies will be presented under four titles:

1. Key components in intestinal lipid transport and metabolism. An overview. Å. Krogdahl
2. Report from an ongoing field survey of LMS in Atlantic salmon in Norway. E. Chikwati
3. Effects of dietary cholesterol and bile salts on intestinal lipid metabolism. T. M. Kortner
4. Effects of dietary phosphatidylcholine and choline on intestinal lipid metabolism. A. K. G. Hansen

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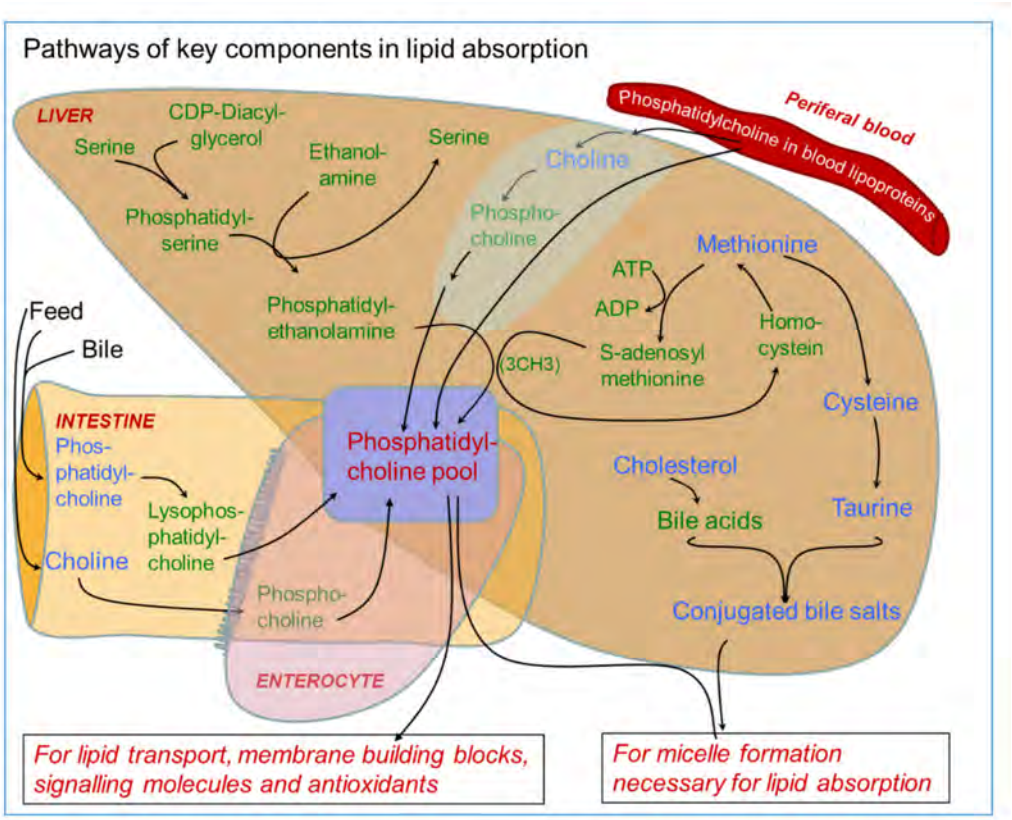

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IMPLEMENTATION OF PROGRAMMABLE STRUCTURES FOR MODEL BASED ANALYSIS OF RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

Recirculation Aquaculture Systems (RAS) became a commonly used solution to contribute food supply with the most productive utilization of limited land and water resources and, with the lowest possible load on environment. Regardless to the obvious advantages of RAS (e.g. Davidson et al., 2016), it is an almost closed technological system, involving livestock farming, (bio)technological and water treatment processes of high complexity in a recycle loop within a cyclically changing structure. Consequently, RAS is a typical example, where the design and operation of co-existing biological and technological processes require the application of dynamic simulation models and control engineering way of thinking.

Considering the challenges and the increasing importance of RAS, the modeling and control experts' attention has turned to this field for many years, and interesting works dealt with modeling and simulation supported design and operation. In line with the need to closing the loop, RAS modeling started intensively from the millennium. Several simulation and control models are introduced for RAS, e.g. (Wik and Lindén, 2004; Wik et al, 2009), (Schneider et al., 2007), (Barbu et al, 2016), (Yao, 2018), or for combined aquaponic systems (Karimanzira et al., 2016).

Materials and methods

In this work we investigated a pilot RAS, comprising 3x3 fish tanks with oxygen supply from an oxygen generator, a drum filte , and a biofilter for nitrification and COD removal from the recycling and emitted water. The investigate fish was *Cyprinus carpio*, fed with commercial carp feed (Aller Master –Aller Aqua Poland).

The applied non-conventional dynamic modeling methodology of Programmable Structure has developed by the process modelers of our team for the past years (Varga and Csukas, 2017a and 2017b; Varga et al, 2017). In this framework, process models can be automatically generated from one state and one transition meta-prototypes, as well as from the standardized description of the process network. The functionalities of the state and transition elements are declared by the local programs of the so-called prototype elements, which can also be derived from the general meta-prototypes. These prototype elements contain symbolic input, parameter and output variables, as well as an editable local program (e.g. equations, expressions, rules, constraints, etc.). The state and transition elements of the actual model can be parameterized and initialized concerning their case-specific prototypes. Next, the executable code of the model is automatically prepared and model is executed by a general purpose kernel program.

Results

First, we generated, parameterized and programmed the Programmable Structure based dynamic simulation model of the studied pilot RAS system. The model was prepared to follow the changing conditions. Accordingly, we utilized a mutual feedback between the pilot experiment and its model. Having compared the measured and calculated results we tuned the initially uncertain model parameters on the one hand, as well as we used the dynamic model for the prediction of the future behavior, supporting the defensive managerial control decisions in case of malfunctions (mainly caused by the applied drum filter). Regardless to the difficulties of simultaneous validation and model based control, this "predictor / corrector" like run tested the capabilities of the applied modeling methodology.

Based on the above experiences we prepared an improved model, and implemented it for the simulation-based analysis of a fictitious, but realistic system with typical parameters, adapted to the pilot unit (that helps to prepare the future experimentations). This model was applied for the simulation based sensitivity analysis of the investigated RAS process.

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Computational modeling supports building hypothetical models that help the effective problem solving with decreasing complexity. Actually, modeling of a single hypothetical fish tank with automatically increasing volume made possible to simulate the idealistic change of stocking density in preliminary design of a controlled multistage RAS process. The resulted change of volume may be derived from the simulated change of the volume. The Programmable Structures can easily be transformed into the State Space Model of control that helps the simulation based testing of various Multiple Input Multiple Output (MIMO) control strategies.

Discussion and conclusion

Considering the cost and time consumption of the experimental study of a realistic multistage RAS system, as well as the unavoidable malfunctions, dynamic simulation based analysis, design and control seems to be competitive and effective solution. In addition to the existing RAS simulation methods, the multiple time scales, the large complexity of the design space, as well as the highly interacting MIMO control are still challenging for the emerging non-conventional methodologies. Programmable Structures proved to be a possible candidate for model based analysis, design and operation of RAS. The structure, the data and the local programs can be reconfigured, conveniently. This flexibility supports also the utilization of the results, coming from non-ideal, erroneous experiments. Important issue is that the easily implementable, fictitious models can be used to simulate a single fish tank of hypothetically increasing volume with the prescribed change of stocking density. This make possible to reduce the complexity of design, because it supports to study the behavior and control of a multistage RAS in one single increasing volume. This supports also the simulation based testing of the various MIMO control strategies.

Acknowledgements

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PIRAB^{VP} TOXIN MEDIATES *IN VIVO* PATHOGENICITY OF *Vibrio parahaemolyticus* AHPND STRAIN: ROLE OF NOVEL PLANT-BASED HSP70 INDUCING COMPOUND IN PROTECTION AGAINST AHPND STRAIN IN SHRIMP SPECIES

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The halophilic aquatic bacterium, *Vibrio parahaemolyticus* is an important aquatic pathogen, also capable of causing acute hepatopancreatic necrosis disease (AHPND) in shrimp resulting in significant economic losses. The *V. parahaemolyticus* encodes deadly toxins (VP_{AHPND} toxins) that are responsible for shrimp mortality during AHPND. Therefore, there is an urgent need to understand the toxicity mechanism of *V. parahaemolyticus* AHPND strain and VP_{AHPND} toxins and develop anti-infective strategies to control AHPND. The gnotobiotic brine shrimp (*Artemia franciscana*) and freshwater shrimp (*Macrobrachium rosenbergii*) model was used to determine the toxicity of AHPND strain and VP_{AHPND} toxins. In addition, the effective role of phenolic compound phloroglucinol was examined *in vivo* against the AHPND strain *V. parahaemolyticus*. It was found that toxicity of *V. parahaemolyticus* AHPND strains is mediated by VP_{AHPND} extracellular proteins (ECP) comprised of $PirA^{VP}$ and $PirB^{VP}$ (mostly). Moreover, $PirB^{VP}$ is more toxic to brine shrimp larvae as compared to $PirA^{VP}$. Furthermore, the survival of brine shrimp larvae challenged with a mixture of $PirA^{VP}$ and $PirB^{VP}$ toxins decreased ~2-fold as compared to $PirB^{VP}$ toxin and ~3-fold as compared to $PirA^{VP}$ toxin, as anticipated, as these 2 toxins seem to form an active complex.

We also found that pretreatment with phloroglucinol, at an optimum concentration (30 µM), protects axenic brine shrimp larvae against *V. parahaemolyticus* infection and induced Hsp70 production (2 folds or more) as compared with the control. We further demonstrated that the *Vibrio*-protective effect of phloroglucinol was caused by its prooxidant effect and is linked to the induction of Hsp70. In addition, RNAi confirms that phloroglucinol-induced Hsp70 mediates the survival of brine shrimp larvae against *V. parahaemolyticus* infection. The study was validated in xenic *Artemia* model and in a *Macrobrachium rosenbergii* system. Pretreatment of xenic brine shrimp larvae (30 µM) and *Macrobrachium* larvae (5 µM) with phloroglucinol increases the survival of xenic brine shrimp and *Macrobrachium* larvae against subsequent *V. parahaemolyticus* challenge. Taken together, our study provides substantial evidence that the prooxidant activity of phloroglucinol induces Hsp70 production protecting brine shrimp, *A. franciscana* and freshwater shrimp, *M. rosenbergii* against the AHPND *V. parahaemolyticus* strain. Probably phloroglucinol treatment might become part of a holistic strategy to control AHPND in shrimp.

HYDRAULIC IMPACT ON FISH MIGRATION IN SARIAKANDHI FISH PASS OF BANGLADESH

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The importance of open water fish in our socio-economic regime has recently drawn the attention of the policy makers of the country. FCD/FCDI projects mainly serve the agricultural interests, but it interfere fish migration. This inevitably affects the open water fisheries sector as migratory routes. Nursing grounds of many species of fish are hampered and disturbed for these projects also. In order to permit fish migration in rivers, it is necessary to maintain conditions that help migrants reach their spawning grounds. To overcome obstacles, such as hydraulic structures, placed in the path of migrating fish, structures must be designed to assist the fish to pass them. The periodic and directed travel of fish mainly for feeding, breeding and over coming adverse climatic conditions is called migration. Fish passes are constructed to allow normal breeding migration and to ensure natural route of fish movement.

The concept of a fish passes is relatively new in Bangladesh. At present, two Fish passes and two fish friendly structures are constructed. These are Fish Pass in Jamuna to Bangali River at Sariakandi in Bogra, fish Pass in Kawadighi Haor of Monu river in Moulovibazar, fish friendly structure in Lohajong river of Tangail and fish friendly structure at Morichardanra in Chapainawabganj. Fish fry, spawning and hatchling movement from Jamuna to Bangali River was the main objective of Sariakandi Fish Pass Project. The Fish Pass Project of Sariakandi is necessary for the development of the dominant fishes like catfish and small fishes. The structures will also aid in efficient development of the carp fishes. Spawning migration, mainly in carp fish, in the study area was found to begin at the 2nd week of May and continue up to the 3rd week of July. Catfish migrations began at the last week of March and continue up to the nd week of June.

Fish fry and hatching movement from Jamuna to Bangali river was the main objective of Sariakandi fish pass project. The study also found that there were seven major category migratory species in the project area and the fish pass is contributing positively for growth of fishery resources in then study area. During the monsoon carp fish is the dominating migratory species. Carpfish migrates in a higher velocity, whereas, catfish migrates in a lower velocity. Some problems were found in the operation and management of fish pass.

COMBINING PHOSPHORUS REMOVAL BY ORGANIC FLOCCULANTS WITH WOODCHIP DENITRIFICATION IN RECIRCULATION AQUACULTURE SYSTEM (RAS)

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Introduction

RAS farms use and discharge a fraction of the water volume compared to traditional flow-through aquaculture. Concentrated effluent enables use of conventional wastewater treatment technologies such as phosphorus (P) removal by chemical treatment followed by mechanical separation. In recent years, improved nitrogen (N) removal by denitrifying systems such as woodchip bioreactors has been increasingly studied and also constructed in commercial scale under fresh water conditions. The current study focused on combining P and N removal using organic flocculants and woodchip bioreactor in and outflows of freshwater and brackish (7 ppt) water RAS. We hypothesized that carbon from organic flocculants would stimulate N removal in woodchip denitrification

Material and methods

Laboratory scale RAS run at 500 liters of replacement water per kg feed delivered the P-rich sludge and N-rich discharge water for the experiments. In the first phase, both anionic and cationic flocculants based on potato starch (Chemigate Oy, Finland) were screened by jar tests. In brief, 24 hour sludge from 100 gram of feeding was collected and diluted to 10 L, i.e., 100 L/kg feed. Polyaluminum chloride dosing was 50 mg/L and flocculants were typically in the range 10-500 mg/L of sludge.

Based on screening, combination of anionic and cationic flocculants was selected for the combined P and N removal. Supernatant of the sludge flocculation was stored in containers and pumped with discharge water into duplicate woodchip denitrification bioreactors using silver birch (*Betula pendula*). The selected organic flocculant usage (200 mg/L sludge) increased the inflow soluble COD on an average from 38 mg/L to 315 mg/L. The EBCT (empty-bed-contact-time = hydraulic retention time without the woodchips) was set to 24 hours in the woodchip bioreactors. In the control, discharge water without sludge supernatant was used. The trial was run for 6 weeks.

Results and discussion

The N-removal efficiency was improved in the freshwater woodchip bioreactors from 20 % to 30 %, when effluent from chemical water treatment was used (Table I.) Much higher improvement was observed in the brackish water RAS, from 14 % to 43 %, respectively. However, in the freshwater bioreactors using supernatant, the nitrogen was mostly discharged as a nitrite-N, suggesting an incomplete denitrification. The results suggest that the organic flocculants provided an extra carbon for denitrificati n process, which improved the N-removal process. However, the N_2O and N_2 were not directly monitored, thus the total fate of nitrogen end-products was not confirmed

Table I. Average inflow and outflow of different nitrogen compounds from woodchip bioreactors running with outflows of RAS (control) and outflows combined with supernatant of chemical water treatment (treatment).

	Fresh (g N m^{-3} d^{-1})		Brackish, 7 ppt (g N m^{-3} d^{-1})	
	Control	Treatment	Control	Treatment
N in	47	49	44	42
NO3-N out	38	34	37	23
NO2-N out	3	8	0	0
TAN out	1	2	1	2
N2/N2O out	5	5	6	17

ADVANCES IN POLYCHAETE CULTIVATION TECHNOLOGY – AN INDOOR SPACE EFFICIENT CULTIVATION SYSTEM

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Introduction

There is a growing interest in polychaetes, because of their superior biochemical composition and their role in maturation diets in shrimp cultivation. The annual trade of polychaetes is approximately 6000tons, of which ~5600tons are harvested from already depleted populations, whereas only ~400tons are cultivated in extensive systems in Holland, Wales and the USA (<4kg per m^2 pond area). Not all regions are suitable for pond culture due to available space, coast morphology or climate. Indoor cultivation might be a suitable alternative for extensive pond cultivation, allowing for full control of environmental parameters and year-round production. Moving cultivation indoors will inevitable increase production cost, therefore a need for space efficient and automated production arises to decrease cost and labou .

Materials & Methods

We designed a space efficient cultivation system which operates unattended over long periods of time, making the need of excessive manual monitoring obsolete. The system is comprised of shelf modules for polychaete cultivation units utilizing all three dimensions in order to increase space efficienc . The prototype accommodated 3 shelves (0.3 x 0.3m each) while the full-scale version is planned to be 1 x 1m with 10 shelves. Water was supplied in a recirculating manner and automatic feeding of suspended nutrients (e.g. salmon smolt sludge) was done using an actuated diaphragm valve controlled by the control unit. The control system was used to regulate water flow rates, light intensity and photoperiod. A concept sketch of the prototype is illustrated in Figure 1.

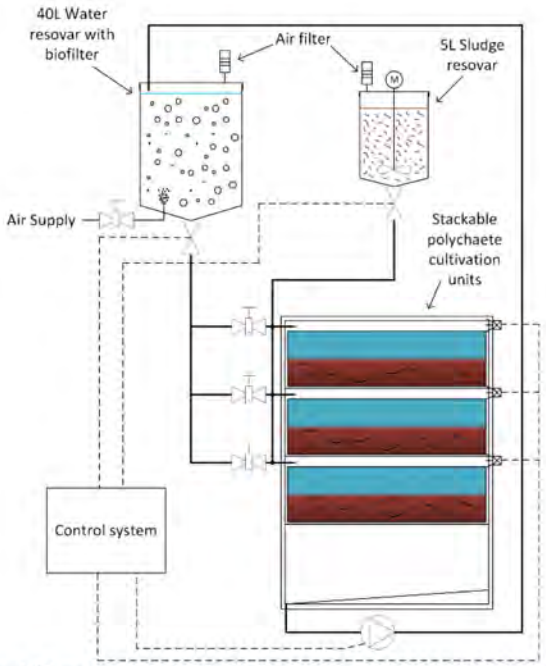

Figure 1 Conceptual outline of a production system designed for intensive cultivation of polychaetes

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Figure 2 Processed image of a polychaete where segments are marked with blue dots

A useful feature in this cultivation system is a machine vision-based system used for real-time monitoring of the worms coupled with automatic weight calculations based on L3-length. This feature allows for growth rate assessments without interfering with the worms in their burrows, monitoring worm densities and spatial distribution, and record mortality. We have experimented using state-of-art deep learning technology and computer imaging to automatically detect and count the number of segments in individual polychaetes, and the preliminary results look promising. Figure 2 shows an example of a processed image of a polychaete, where the blue dots indicate detected segments.

Future perspectives

The same technology used to detect segments is used to automatically measure L3 length (total length of prostomium, peristomium and first segment), a robust measure for worm size (Diaz-Jaramillo et al, 2011) when the worms come out of their burrows to feed, allowing to assess in situ growth rates in real time.

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RAS SHRIMPS POTENTIAL ON THE POMERANIAN MARKET

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Introduction

With increasing global demand for seafood and the limited capacity for wild capture fisheries to meet our need, aquaculture will continue its growth and diversification. Recirculating aquaculture system (RAS) is an ideal solution which enables the production of food while sustaining natural resources. New RAS farms are emerging not only producing fish but also shrimps. Still, Poland plays no role in the sector. To change the current situation the team from the Institute of Oceanography, University of Gdańsk, has taken a challenge to develop the potential of shrimp aquaculture focusing on a combination of new species and technologies. In the course of InnoAquaTech project, the first demonstration facility for shrimps production in RAS system has been established at the University of Gdańsk. Two white leg shrimps' breeding experiments were carried out together with a series of outreach activities and consumer perception and awareness research.

Materials and methods

Shrimps *Litopenaeus vannamei* were grown in the small-scale laboratory RAS culture in two cycles. The cultivation trials were accompanied by outreach activities: Innovation aquaculture workshop (March 2017) and In the direction of crustacean farming: Innovative aquaculture – white shrimp – *Litopenaeus vannamei* summer school (September 2018). Feedbacks from the stakeholders of both events were crucial for further studies. The events were directed to aquaculture entrepreneurs, potential investors and specialists interested in recirculation technology and crustacean farming. In total, over sixty participants took part in the events. Furthermore, questionnaires were directed to consumers (first trial April 2018, 180 consumers questioned, 6 different questionnaires directed to 30 respondents each). The second trial of questionnaires (composed after verification of the first trial questionnaires) will be carried out in May 2019. Questionnaires directed to restaurants (April 2019, 104 restaurants questioned) were carried out in the Pomeranian region on public perception and market analysis of shrimps on the Polish market.

Results

Market conditions for the domestic production of warm-water shrimps seem to be very promising according to the presented research and discussions among stakeholders of the InnoAquaTech outreach events. Results of the so far carried out questionnaires show consumers recognize crustacean species available on the market very well. Still, restaurants representatives are very often not aware of the species the restaurant offers. Around 60% of the respondents (inhabitants of the Pomerania region) consume shrimps. And shrimps are the most popular crustaceans of all available on the market in the Pomerania region. However, the shrimps are consumed very rarely, the most frequent answer is once a month. Shrimps are mostly bought in the supermarkets, and the most important factor determining their purchase is the price. Still, all asked restaurants have shrimps in their menu. Also, restaurants consider price as the most determining factor in the shrimps purchase. Respondents who do not consume shrimp indicate other dietary preferences most frequently as the main factor for their decision. Most consumers are not aware either of the country of origin or the cultivation/capture method, having no awareness if the shrimps were caught or farmed. The same applies to restaurants, which the majority is not aware of the area of origin of the delivered shrimps nor the way of capture/cultivation. Most of the restaurants have no awareness in the area of RAS production, however, when explained, they are very willing to try the product and consider locally produced shrimp as a very promising option, regardless on the price. The results of consumers perspective and product awareness will be verified by the second trial questionnaires (May 2019)

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Discussion and conclusion

Public awareness of the RAS shrimp production is still poor in the Pomerania region. More educational campaigns and trainings are needed as the outreach activities together with the results of the questionnaires carried out showed. More research is required to change customer perspective and raise awareness of the RAS farmed products. So far obtained results in the pilot trial shrimps are characterized by a similar nutritional value as the same species imported to the Polish market, still contained higher levels of polyunsaturated fatty acids. Since the facility is established at the University of Gdańsk and has been well tested, it is planned to be developed and used for further demonstrations within the coming international projects, for both the experimental and the educational purpose. It is worth engaging restaurant sector into the promotion of new products such as RAS shrimps. Innovative aquaculture based on recirculating systems as well as the introduction of new species, such as shrimp *Litopenaeus vannamei* may give a high potential for investors and market in Pomerania but requires broad educational activities among stakeholders.

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DIGITIZATION OF AQUACULTURE: REAL-TIME MONITORING AND PREDICTION FOR A SUSTAINABLE FUTURE

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Introduction

Aquaculture is a booming market since several decades and has surpassed fisheries worldwide (FAO, 2018). Like agriculture did, aquaculture needs to take the technological turn to be more simple, profitable and sustainable. The advancement of IoT will permits to answer to the digitization. In aquaculture, water quality is at the heart and its mastery is the key to success. Different parameters, biotic or not, such as pollutants, microbiological contamination, physico-chemical changes, coming to aquaculture can influence the quality of the breeding. These parameters can be disastrous for the farm and have dramatic consequences in the environment (Svobodová *et al*., 1993). The monitoring of water parameters is often long, expensive and gives a partial picture of the situation. The integration of sensors that we present in this paper, allow to measure water quality in real-time of a wide range of measurable parameters enabling a 3-dimensional view of the farm in real-time. To go further in this approach for aquaculture 4.0, it is now necessary to anticipate parameter changes that can influence the farms and more broadly the environment. The generation of large data sets coupled with big data analysis and machine learning now make it possible to anticipate the evolution of water parameters as the classical meteorology can do. The use and the control of the data leads to an improvement of the breeding techniques to anticipate risks from and to the farm. This transition to a connected aquaculture 4.0 will then allow to automate various processes towards a more profitable, sustainable and simplified management. This study was carried out to develop predictive algorithms to anticipate the evolution of dissolved oxygen in aquaculture farms.

Material and methods

The water parameters data were obtained by the Bioceanor products in an oyster farm in the Thau lagoon (Hérault, France), in an open environment. The water parameters were collected, using an autonomous energy measuring system coupled with a long-range radio wave transmission system (Aquabox, Bioceanor). This system allows the collection of water parameter data, processing and real-time visualization (less than 6s) on a computer interface (Aquareal, Bioceanor). The real-time analysis of the data enables to alert (by e-mail, sms) in case of exceeding of a critical threshold but also to store the data to access the history of measurements and for deeper analysis. Dissolved oxygen was managed every 20min during 21d, with 20d as training data and the 21th day as the test data. Data were aggregated to hourly level by arithmetic mean and imported in R Console for a 24h-ahead forecasting of dissolved oxygen (DO). The predictive model is based on an analysis of the DO signal by Complete Ensemble Empirical Mode Decomposition (CEEMD) proposed by Torres *et al.* in 2011. The resulting components were reduced into 4 components as recommended by Li *et al.* in 2018 and forecasted by Support Vector Regression (SVR) and Autoregressive Neural Network (ARNN). The 4 components were summed up to obtain the prediction of the DO signal.

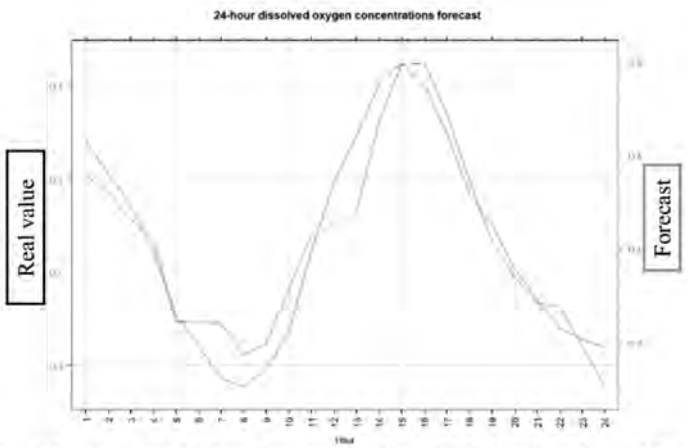

Fig. 1: 24h-ahead forecasting of DO concentrations (mg/L) with the CEEMD-SVR/ARNN model.

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Results

The results show an effective forecast of dissolved oxygen over a minimum of 24h. Based on the criteria of Mean Absolute Error equal to 3%, the predictive model tracked well the dynamics of DO, as shown in figure 1, overlapping the predicted (blue line) and the real (grey line) DO concentrations for 24h after 20d of data collection.

Discussion and conclusion

The complex model required a high computational cost, but it was able to predict the DO concentrations over 24h. Subsequently, the model can be used but with a lower stability. While all types of aquaculture are different and subject to several environmental impacts, the management and anticipation of input and output parameters are necessary for sustainable management. Therefore, integrating these parameters will greatly assist in improving the forecasts. Recently, the Convolution Neural Networks have proved successful (Ta and Wei, 2018). Theses algorithms thus provide an adaptability to various environments (such as fish/shrimp farms, open or closed like Recirculating Aquaculture Systems - RAS), and a global anticipation of the breeding. It will also make it possible to forecast parameters impossible to measure in real time, such as the bacterial microflora, reducing the need of long and expensive analyses. Correlative analysis coupled with forecasting algorithms could foretell risks of bacterial contamination of for example *Escherichia col*i or *Vibrio spp* in the field. Combining real-time measurements and forecasting systems in the aquacultures leads to a transition to aquaculture 4.0. Indeed, the opportunities of monitoring in real time providing a 3-dimensional view of farms coupled with prediction systems on the evolution of water parameters should revolutionize the management of aquaculture of tomorrow. These new systems assisted by the development of artificial intelligence will make it possible to automate many processes in aquaculture, for example aerator actuator in case of oxygen drop, the treatment or displacement of animals in case of risks contaminations, adjust the parameters of the water before they are harmful or even feeder actuator.

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IN-DEPTH GENOMIC CHARACTERIZATION OF A UNIQUE COLLECTION OF RAINBOW TROUT ISOGENIC LINES

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Introduction

A unique collection of 19 isogenic homozygous rainbow trout lines has been established and maintained in INRA fish experimental facilities. These lines have been characterized for a variety of traits and proved to be a relevant resource to investigate the molecular bases of complex traits at different biological scales (cell, tissue, organism). Genomic characterization of the lines is pivotal to realize the whole benefit of this resource in integrative approaches aimed at dissecting complex traits and understanding the "genome to phenome" mechanisms. Having access to the genomic variability among lines is essential in functional or QTL studies in order to identify polymorphism(s) responsible for the phenotypes of interest and their variation. The objective of this study was to carry out in-depth genomic characterization of the trout isogenic lines, by investigating both small genomic variations (SNPs and InDels) and structural variants (SVs). SVs are defined as genomic alterations that affect large DNA segments ≥ 50 nucleotides, thereby causing modifications in either DNA quantity (insertions, deletions and duplications) or DNA structure (inversions). Although SVs have received increasing interest in many species and were shown to be associated with several diseases and phenotypes, they are poorly documented in fish

Material and methods

All isogenic lines (one or two individuals per line) have been resequenced at a depth of coverage ranging from 10X to 32X, on an Illumina HiSeq X-Ten platform, in paired-end 2x150 bp configuration. Analysis of small genomic variants was performed according to the GATK Best Practices. The identification and characterization of SVs was done by using 3 different tools corresponding to two distinct but complementary approaches: i) Pindel and Delly (split-read approach); ii) BreakDancer (paired-end approach).

Results

Search for small genomic variations with GATK Haplotype Caller software resulted in the identification of 15 064 416 SNPs and 3 173 673 InDels. A total of 17 037 putative SVs were identified, corresponding to 13 271 deletions, 3 386 tandem duplications and 380 inversions. Analysis of the length distribution revealed that most deletions (98.3%) were less than 10Kb (27.8% between 51bp and 1Kb), whereas the vast majority of tandem duplications (95.1%) were larger than 1Kb. Analysis of chromosomal distribution was also performed (Figure 1). Annotation of the SVs revealed a total of 8 326 regions which contain either entire genes or parts of genes, among them 5 967 deletions, 2 091 tandem duplications and 268 inversions.

Discussion

The fine characterization of rainbow trout homozygous isogenic lines will allow not only production of the information necessary for a full exploitation of the ongoing and future studies taking advantage of the contrasted phenotypes and/or of the original genomic features of the lines, but also add to the overall knowledge on rainbow trout genomic structure and polymorphisms and provide a description of broad interest of the structural and functional organization of its genome.

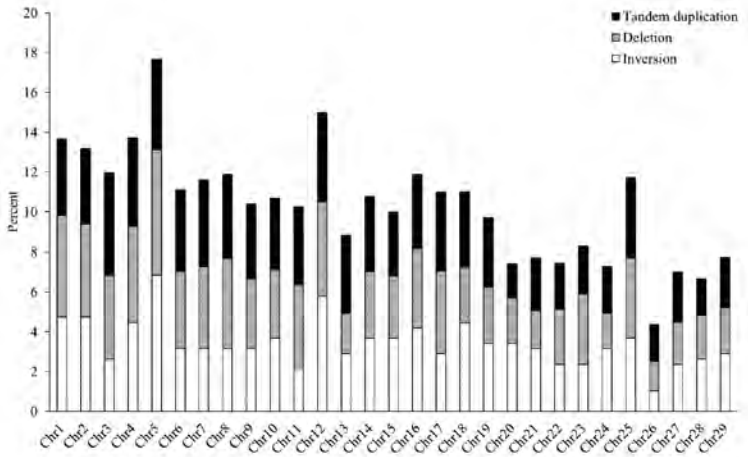

Fig. 1 Chromosomal distribution of SVs within the trout chromosomes.

ANALYSIS OF GENOMEWIDE PATTERNS OF DNA METHYLATION IN RESPONSE TO AN EARLY TEMPERATURE STRESS IN RAINBOW TROUT

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Introduction

We aim to investigate the potential role of epigenetic marks in the expression of phenotypes and their variability in fish, in particular to study whether the epigenetic marks established in response to an environmental stress depend on the genetic background. The environmental stress chosen here is temperature, a known induction factor of epigenetic marks in fish. In this context, rainbow trout isogenic lines are the material of choice. Within each line, all fish have the same genome i.e. there is no genetic variability. This allows the comparison of epigenetic marks among several individuals with the same genotype. The objective of this study was to test whether temperature regime experienced during early development leads to epigenetic modifications within and between lines

Material and methods

Six rainbow trout isogenic lines were chosen. For each line, half of the eggs were incubated at standard temperature (12°C) and the other half at high temperature (16°C), from eyed-stage to hatching. At eyed-stage just before hatching, analysis of HSP47 gene expression was performed by qPCR on 3 pools of 5 eggs per line and per incubation temperature. Also, genomewide patterns of DNA methylation were analysed by EpiRADseq on the same biological material. EpiRADseq is a reduced-representation library-based approach that has been recently developed and tested on a single clone of water fleas. The protocol was here modified to account for genetic variability and allow both within and between-lines comparisons

Results

An overexpression of HSP47 gene in the 16°C batches confirmed that the early temperature stress was successful. In total, 284 825 EpiRAD loci were defined, among which 102 354 were present in only one sample. In order to compare the lines, preliminary analysis was restricted to 57 129 loci that were common to the 6 lines. Globally, 325 loci spread across the genome (3 to 18 loci per chromosome) were differentially methylated between the two incubation temperatures, 169 loci being less methylated at 16°C compared to 12°C and 156 loci more methylated. This number differed between lines, ranging from 14 to 143 depending on the line (Fig. 1).

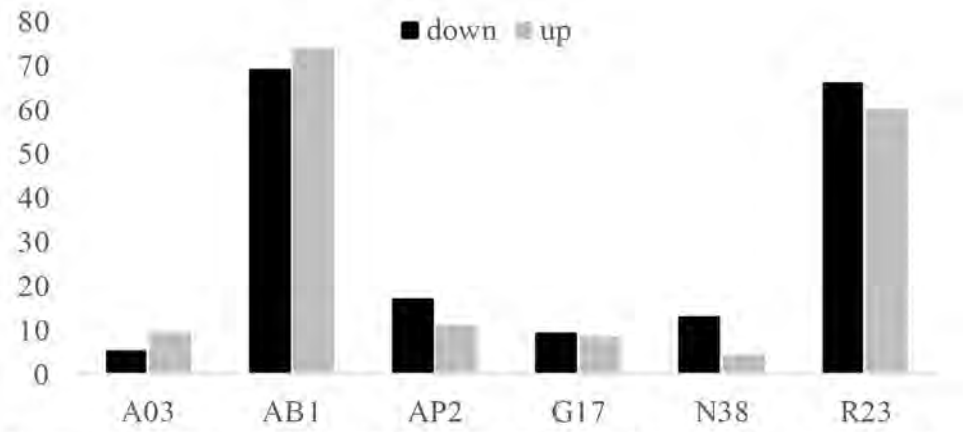

Figure 1. Number of differentially methylated loci between the two incubation temperatures (12°C vs. 16°C) for 6 rainbow trout isogenic lines. Down: less methylated at 16°C compared to 12°C; up: more methylated at 16°C compared to 12°C.

(Continued on next page)

Discussion

Rainbow trout isogenic lines are a unique biological model to study the interactions between genetics, epigenetics and environment. This study contributes to the understanding of the ability of organisms to cope with changing environmental conditions. Overall, the great majority of observed changes in methylation in response to an early temperature stress seem to be dependent on the genetic background. However, further studies are required. Preliminary analyses should be deepened by investigating the function of the genes located near differentially methylated loci. Analysis of expression of DNMT genes (DNA methyltransferase, involved in DNA methylation) could help to understand the establishment of differential methylation profiles during an early temperature stress. In the future, the impact of a longer exposure to high temperatures during early development could also be tested.

REPLACEMENT OF FISH MEAL USING ALTERNATIVE PROTEIN SOURCE IN FEEDS FOR PIKEPERCH *Sander lucioperca* (LINNAEUS 1758) DURING GROW OUT PHASE

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Introduction

With the increase in the interest and culture of pikeperch *Sander lucioperca* (Linnaeus 1758) in RAS, one of the most critical issues still has to do with feeding. Pikeperch is a carnivorous fish and therefore has a high protein requirement. Currently, few companies produce commercial diets for pikeperch with fish meal as the main protein source. Finding alternatives to fish meal as the main protein source in fish feeds is critical especially as we strive towards more sustainable aquaculture production. There are limited studies investigating alternative protein sources in feeds for pikeperch in the grow out phase. No study has investigated a combination of feather meal with poultry meat and bone meal as a potential substitute.

Materials and methods

To demonstrate the effect of the different levels of fish meal replacement on growth of pikeperch, an eight-week feeding trial is on -going at INAGRO aquaculture research facility (Rumbeke, Belgium). Pike perch of an initial average body weight of 115g are being fed iso-caloric and isonitrogenous diets containing four different replacement levels of fish meal using a combination of feather meal with poultry meat and bone meal (Treatment A, B, C and D). The experiment is carried out in triplicate in recirculating aquaculture systems (RAS) in a randomized block design. Fish are sampled fortnightly and water quality parameters monitored. At the end of the feeding trial, fish will be bulk weighed and returned to their respective tanks for collection of feces for apparent digestibility analysis. Feed, feces and fish samples will be analyzed for dry matter, crude protein, ash, moisture, crude lipid and phosphorous. Visceral somatic index (VSI) and Hepatosomatic Index (HIS) will also be determined. Results will be statistically analyzed.

Results

Preliminary results after the first four weeks indicated that the fish accepted all the experimental diets. In the case of feed performance specific growth rate (SGR) was 1.65 ± 0.07% in treatment A, 1.65 ± 0.08% in treatment B, 1.60 ± 0.10% in treatment C and 1.44 ± 0.03% in treatment D. Feed conversion ratio (FCR) was 0.79 ± 0.01, 0.80 ± 0.03, 0.81 ± 0.01, 0.97 ± 0.04 (increasing alphabetically from treatment A to D). Results of proximate analysis, apparent digestibility coefficient, VSI, HSI and whole-body analysis are not yet available.

Discussion and Conclusion

These preliminary results show that a combination of feather meal and poultry meat and bone meal may have the potential to replace fish meal to some extent without have severe negative impacts on pikeperch during grow out phase. It is expected that the results on proximate analysis and apparent digestibility coefficient will provide a clear evidence of this. Concrete discussion and conclusion will be provided at the end of the feeding trial.

REPLACEMENT OF FISHMEAL USING ALTERNATIVE PROTEIN SOURCES IN FEEDS FOR PIKE PERCH *Sander lucioperca* (LINNAEUS 1758)

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Introduction

Pike perch, *Sander lucioperca* (Linnaeus, 1758) is an emerging aquaculture species popular for its nutritional benefits, excellent flesh quality, good market value and as a sport fish. Pike perch is carnivorous and therefore has a high protein requirement usually met by fishmeal in commercial diets when cultured in recirculating aquaculture systems (RAS). The continued use of fishmeal is environmentally and economically unsustainable therefore, finding fishmeal alternatives is a priority. No study has assessed the potential of a poultry-based protein core comprising of poultry feather meal and poultry meat and bone meal as a fishmeal substitute in diets of pike perch.

Materials and methods

A 60-day growth trial was carried out to at Inagro aquaculture research facility (Rumbeke, Belgium). Four experimental diets were formulated in partnership with Odisee and Empro Europe (Dendermonde, Belgium). The positive control diet FM100 contained 55% of fishmeal as main protein source. In the 3 following diets i.e. FM80, FM60 and FM40, the percentage of fishmeal was gradually replaced by the poultry-based protein core. FM80, FM60 and FM40 contained 80%, 60% and 40% fishmeal compared to the FM100%, respectively. A total of 3000 fish with initial body weight (IBW) 113.09 ± 8.98 g were stocked in a total of 12 identical 1.8 m^3 circular RAS tanks in a randomized block design. A 12L:12D photo period was followed. The cut off biweekly feeding rates were 1.7, 1.5, 1.2 and 1.2% of total tank biomass. At the end of the feeding trial, feces were collected by dissection for apparent digestibility coefficient of protein (ADC) analysis. Proximate analysis of feed and whole fish carcass were carried out. Visceral somatic index (VSI) and Hepatosomatic index (HSI) were also determined.

Results

At the end of the study there were no statistically significant differences in feed intake between treatments. Feed conversion ratio (FCR) was significantly higher while final body weight (FBW), weight gain, final body length, protein efficiency ratio (PER) were significantly lower in fish in treatment FM40 in comparison to the other treatments ($p < 0.05$). There were no significant differences between treatments in condition factor (k), hepatosomatic index (HSI) and visceral somatic index (VSI). The ADC of protein decreased with increasing fishmeal replacement. The carcass of fish in FM40 had significantly higher ash content ($p < 0.05$) in comparison to the other treatments.

Discussion and Conclusion

The performance indicators observed in this study suggest that 40% of the dietary fishmeal could be replaced using a combination of poultry feather meal and poultry meat and bone meal in diets for pike perch without significantly affecting the growth and feed utilization.

Rhodomonas salina PRODUCTION: GOING FROM LAB TO SEMI-COMERCIAL SCALE

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Introduction

The microalgae species *Rhodomonas salina* is known to be an excellent diet for all kinds of aquaculture species. The species has been successfully used in the production of live feed organisms such as copepods and rotifers (Zhang et al. 2013), as a larval diet for all kinds of shellfish (Seixas et al. 2009) and as a specialty diet in the refinement of oysters (Van Houcke et al., 2016). *Rhodomonas* species however, have been reported to be difficult to cultivate (Bougaran et al. 2010, Peltomaa et. al., 2017). Renaud et al. (2002) and Guevara et al. (2016) reported low growth rates (0.35 d^{-1} and 0.6 d^{-1}). Furthermore it has been reported that *Rhodomonas* cultures are prone to contamination with other algal species and that is therefore hard to keep *Rhodomonas* in stable cultures (Wang et al. 2013). These above mentioned problems have resulted in the lack of adoption of Rhodomonas cultivation in many aquaculture companies.

The aim of our research was to show successful *Rhodomonas salina* cultivation on a (semi-) commercial scale.

Methodology

In several experiments the effect of cultivation media, temperature and light intensity on the growth rate, volumetric productivity and yield on light of *Rhodomonas salina* have been investigated. Experiments were done in Algaemist-S lab-scale photo bioreactors (400 ml). Media evaluated in the experiments were NHN, L1 and GB. The temperature range evaluated was 10-30˚C while the light intensity evaluated ranged from 200-1500 µmol $m^{-2}s^{-1}$.

Validation of these lab-scale experiments was done using three commercial 200l tubular photo bioreactors (L-Gem, the Netherlands). Results from the lab-scale experiments (medium and temperature) were used as settings in the 200 l trails. As the 200 l reactors are placed in a greenhouse at the HZ University of Applied Sciences (Vlissingen, the Netherlands) light intensity and photoperiod were variable during the trails. Growth rate, volumetric productivity and yield on light were recorded during the trails.

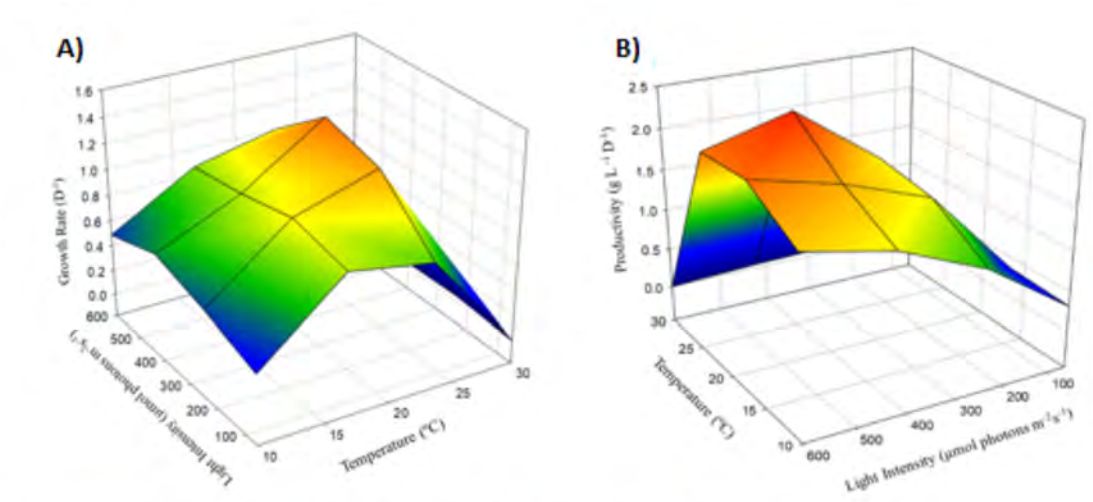

Figure 1: Optimization of Rhodomonas salina in lab scale experiments. Growth rate (A) and biomass productivity (B) for Rhodomonas salina. as a function of the supplied light intensity (µmol m-2s-1) and cultivation temperature (°C).

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Preliminary results and conclusion

The highest growth rate measured in lab scale experiments was 1.3 d^{-1} and maximum productivity was found to be 2.0 g l^{-1} d^{-1} with concentrated L1 medium. Optimal light intensity was 330 µmol $m^{-2}s^{-1}$ while optimal temperature was found to be 22-23˚C (Fig 1).

The commercial scale reactors were run in chemostat mode with a dilution rate of 0.35 d^{-1} and a biomass concentration varying from 2 to 5 million cells ml^{-1} depended on the natural light conditions.
The growth rate measured in the lab scale experiments is about twice the growth rate (1.3 d^{-1}) mentioned in literature. Growth rate was lower in the commercial scale trails, however this was due to a shorter photoperiod.

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LIPIDOMICS: SPOT ON LIPID METABOLISM DURING NURSERY PRODUCTION OF JUVENILE BLUE MUSSELS (*Mytilus edulis* L.) REARED UNDER DIFFERENT DIET REGIMES

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Introduction

Enhancing bivalve nursery technology is important for bivalve aquaculture, as deploying larger spat for on-growing has a significant effect on their survival (Cigarría and Fernández, 2000). However, spat have high nutritional requirements, in the order of 40% of live wet weight of the spat as diet dry weight (Helm and Bourne, 2004). Therefore, research efforts have been directed to develop cost-efficient and suitable diets for bivalve juveniles, including live or preserved microalgae, spray-dried microalgae, algae paste, artificial supplementation and single cell detritus (Hidalgo et al., 1994, Langdon and Onal, 1999, Caers et al., 2000, Nevejan et al., 2007, Carboni et al., 2016). Lipids represent the main energy reserve molecule in young spat (Holland and Hannant, 1974). To date, information available on bivalve lipid metabolism have been obtained through fatty acid or lipid class analyses. The application of these techniques has shown the importance of the essential fatty acid content in the diet for successful bivalve hatchery operations. However, such techniques can only target fractions or specific components of the lipidome (e.g. the totality of biological lipids). Recently, advancements in analytical platforms allow more holistic approaches, defined as untargeted lipidomics, to sample vast portions of the lipidome, providing detailed essential physiological information on organisms' biology and nutrition with minimal sample preparation work (Laudicella et al., 2019).

In this study, the effects of five diet are evaluated on growth performances (growth rate –GR and live weight increase – LWI) and the lipidome of recently settled *Mytilus edulis* (L.) spat. Four treatments included live single microalgae strains (*Cylindrotheca fusiformis* – CYL, *Isochrysis galbana* – ISO, *Monodopsis subterranean* – MONO and *Nannochloropsis oceanica* – NANNO), whilst the fifth being a commercial algae paste (InstantAlgae1800 – SP). The growth performances of lab-reared spat were compared with natural growth in outdoor deployed spats (OUT).

Materials and Methods

Recently settled Blue mussel (*M. edulis*) spat (shell length <5 mm) were placed in 10l tanks (static system, 20µm filtered seawater, aeration, 18^{0}C, 18:8 L:D). Diet was provided in a weekly ration of 0.4mgDW$_{diet}$ mgLW$_{spats}^{-1}$. Each treatment was replicated in three independent tanks with 30 spats per tank. The trial lasted for 4 weeks, at the end of which spats were measured and weighted before being snap frozen and stored (-80^{0}C). Diet fatty acid composition was analysed according to AOCS (2007). Untargeted lipidomics analyses were performed with a high-resolution LC-MS platform (Exactive, ThermoScientific) both in positive and negative ionisation modes. Acquired raw spectra data were processed with Progenesis QI (Nonlinear Diagnostics) and then analysed through the R package 'MetaboAnalystR' (Xia and Chong, 2018). Partial Least Square Discriminant Analysis (PLS-DA) was employed to identify the principal lipid responsible for groups clustering through variable of importance in projection (VIP) scores. These lipids were correlated (Spearman rank correlation) versus the spats' GR and LWI.

Results and Discussion

OUT deployed spat showed the best growth performances for GR and LWI. After OUT, the ranking order for GR and LWI in the lab reared spat sample groups was the following: ISO>NANNO/CYL>SP>MONO. All groups, apart MONO, resulted in significant increase in GR and LWI ($p<0.05$). PLS-DA analysis of untargeted lipidomics data evidenced the effect of diets on triacylglycerol (TG) accumulation in the spats, as poor performing groups (SP, MONO) resulted lower TG content ($p<0.05$). Furthermore, effects of the diet PUFA composition on spat phospholipids is also observed, with predominance of PC(38:6) in DHA rich diet (ISO), PC(36:5) in EPA rich diet (NANNO and CYL) and PC(36:4) in arachidonic acid (AA) rich diet (CYL). Several TG resulted positively correlated with GR and LWI across the different treatments (Spearman rank $R^2>0.91$ fdr-adj-$p<0.05$), suggesting their possible utilization as markers for spats growth performances. The results

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of this study show that ISO is a suitable diet for mussel spat, resulting in good GR and LWI. The elevate content of DHA in this diet is possibly one of the reasons for the good growth performances of spat fed with ISO. Diets lacking of DHA, but rich in other essential PUFAs (as EPA and AA), as CYL and NANNO, sustained spats growth, although with lower growth performances. SP, although balanced in the PUFA composition, resulted in minor growth, suggesting the use as partial substitutive for live algae. Fast growing spats are characterised by several TG species, thus lipidomics could represent a useful tool for identification of markers for mussel juvenile condition.

Acknowledgements

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PROOF-OF-CONCEPT APPLICATION OF THE BENTHIC BOUNDARY LAYER TRANSPORT (BBLT) MODEL TO SALMON AQUACULTURE WASTE DISPERSAL

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Dispersal from a fin-fis aquaculture facility in Jordan Bay, N.S., Canada was simulated using the Benthic Boundary Layer Transport model (BBLT). The currents and waves were measured during the fall of 2014 and along with calculated bottom shear stress were used as model inputs. Four waste classes were considered: fines, flocs, feca waste and feed pellets, each with distinct settling velocities and critical erosion shear stresses. BBLT was used to simulate a 46-day continuous release scenario. The resulting concentration of each class within the cage site varied by approximately 5 orders of magnitude. The four classes mean concentration in bottom 10 cm under the cage were 0.003, 0.082, 122.4 and 234.6 g/m^3 respectively. Spatial patterns reveal the concentration of the fines to be relatively uniform inside and within a few hundred meters of the cage, and to fall by 1/10 a few kilometers away. Floc concentrations fall to 1/10 within a 100 m of the cage and by 1/1000 within a few kilometers. For fecal waste, concentrations fall by 1/10 directly outside the cage and may be undetectable a few kilometers away. Feed Pellet removal is somewhat more complicated due to the episodic deposition and advection of the sediment patch. However, just like for the fecal waste the concentration drops by at least an order of magnitude directly outside the cage and by as much as 1/1000 within a few hundred meters. The general direction of travel is to the south. The higher concentrations of fines and flocs in the far field as compared to fecal material and pellets represent a transport mechanism for constituents such as trace metals, pesticides and organics which have an affinity for the large surface areas of fine-grain sediments

MODELLING DATA IN THE CONTEXT OF AQUACULTURE

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Introduction

Aquaculture is one of the fastest growing food-producing sector. It represents the farming of aquatic species like fish, crustaceans, molluscs, aquatic plants and micro-algae. Recently, the fish demand is increasing, being twice as fast as the population growth since the 1960's [1]. To meet the demand, aquaculture is exponentially growing all over the world.

In order to contribute to the food security and safety in an overpopulated, aquaculture needs to be sustainable and with the lowest impact on environment as possible. IMTA is a form of multi-species production that provides the by products including waste, from one aquatic species as inputs to another The equilibrium of this kind of aquaculture production is complex to reach, and a lot of parameters, including water quality, needs to be finely managed

For instance, monitoring the water quality in the farms is one of the crucial steps in the commercial aquaculture process. It consists mainly of controlling, physical, chemical and biological parameters, such as: oxygen, temperature and pH in water, on an on-going basis in order to maintain precise conditions and avoid undesirable situations that may lead to issues for the aquaculture environment. Currently, aquaculturists are depending on manual testing due to the lack of integration of smart connected devices in aquaculture farms.

Integrating IoT technologies in aquaculture farms will lead to a new era of connected, responsible and efficient aquaculture. This compels the development of new IoT oriented applications in order to collect and control data from heterogeneous sources, and organise it in an inter-operable way for rapid and autonomous decision-making processes.

Technologies, such as semantic Web [2] and AI [3] are proposing new capabilities to promote data interoperability and to develop around it new smart and autonomous applications. Integrating such technologies through IoT based solutions in aquaculture will improve the decision-making, production control and management for all fish aquaculture systems. In this context, we exploit in this paper the existing vocabularies and semantic Web technologies in order to develop an interoperable semantic model that covers the core concepts required for aquaculture management used for organizing and managing data in aquafarms and exploited by smart and AI services automatically.

Vocabularies and Standards Requirement for Designing an IMTA Model

The aquaculture domain cover several fields. The principle in data modelling is not to develop a new model from scratch but to summarise required topics and relevant standardised vocabularies. Requirements for modelling aquaculture data are categorised in four main parts detailed below with one example of existing vocabulary for each one.

- Fisheries and Marine Environments:

The Food and Agriculture Organization (FAO) of the United Nations[1] maintains the AGROVOC vocabulary, which contains terms for over 36k concepts covering all areas of the FAO's interest, in food, nutrition, agriculture, fisheries forestry developed by experts around the world. Parterres of the project are initiated contacts with AGROVOC community in order to add new terms.

- Physical, Chemical and Biological Parameters

Physical, chemical and biological parameters are widely covered in terms of data standards, but, being a highly diverse set of concepts, not necessarily in single vocabularies. Individual standards for parameters relevant to aquaculture include WaterML[2].

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• Sensors and Measurements

Sensors and measurements are useful in an extremely wide range of fields and there have been a number of approaches to the sharing of sensor readings. Several standardised ontologies for IoT are proposed: SAREF[3], WOT[4], SOSA/SSN[5]… They present efficient ways for modelling Io data.

• **General Purpose Concepts**

Individuals and organizations present in aquafarms are also modelled and thus we have to refer to the foaf ontology[6]. Other filed such as date, time… have to be also referenced

Conclusion and Discussion

The work in modelling data of the aquaculture is in progress. The presented Reference Data Model has is developed using the Simple Knowledge Organization System, SKOS[7] ontology. SKOS is designed as framework for modelling relationships between vocabularies and facilitates the relating data models. SKOS is expressed in RDF[8] – every valid SKOS document is a valid RDF document, and is interoperable with the full Semantic Web technology stack. The actual step, is about identifying all possible terms according to project uses cases. Then, all these terms are referenced and aligned with the presented vocabularies. The future work includes adopting a standardised API for a standardised data accessing enabling smart services such as machine learning exploiting data. The research leading to these results has been supported by the iFishIENCi project, which receives funding from the European Union's Horizon 2020 Research and Innovation Program under grant agreement No. 818036.

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EFFECT OF FISH FARM CONVERTED FROM RED LAVER FARM ON SEDIMENT GEOCHEMISTRY AND BENTHIC FORAMINIFERAL ASSEMBLAGE OF HWATEDO, SOUTH KOREA

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Introduction

Aquaculture continues to develop rapidly, especially through its growth in Asia. In marine farms, the inputs are juvenile fish, fish feed, medicines, disinfectants and anti-foulants, and the outputs (losses) are harvested fish, escaped fish, uneaten feed, faeces, excreted metabolic wastes, and effluent chemical species e.g. medicines (Mente et al., 2006). The accumulation of effluents released, without being filtered, from the cages cultures in aquaculture may cause a changes in the benthos communities, eutrophication, which affects the natural ecosystem (Mente et al., 2006; McKindsey et al., 2011; Vidović et al., 2014; Lee et al., 2016ab). The objective of this study is to define the effect of biodeposits discharged from fish farms which converted from red laver farm on geochemistry of sediment and benthic foraminiferal assemblages in the shallow sea.

Materials and methods

Hwatedo is located at the entrance area of Gamak Bay which lies midway of the south coast in Korea with type of Ria. A sediment core sample collected from the fish farm located closely at landward analyzed the geochemical elements, ^{210}Pb dating measurements and benthic foraminiferal assemblage. Sediment core sampling also carried out at control plot far away from fish farms to understand the spreading and e fect of biodeposits.

Results

The sediment is composed mainly of fine-grained mud facies with average silt and clay contents of 47.03% and 52.65%, respectively, and a mean size of 8.45Ø. The contents of total organic carbon with average 1.29%, total nitrogen with average 0.17% and total phosphate with average 0.18% are shown gradually increasing profile from 23 cm of core depth, respectively. The concentration of Cu with average 34.03mg/kg increased gradually from 25 cm of depth to uppermost layer. This increase pattern in concentration of Cu are also shown at Zn (average 92.7mg kg^{-1}), Cr (average 44.87mg kg^{-1}), As (average 3.51mg kg^{-1}) and Cd (average 0.18mg kg^{-1}). Seven benthic foraminiferal assemblages with the dominant species of *Cribroelphidium excavatum* composed of *C. excavatum-Epistominella naraensis-Elphidium advenum-Ammonia beccarii* (Ce-En-Ea-Ab), *C. excavatum-A. beccarii-C. subarcticum-E.naraensis* (Ce-Ab-Cs-En), *C. excavatum-A. beccarii-E.naraensis-C. subarcticum* (Ce-Ab-En-Cs), *C. subarcticum-A. beccarii-C. excavatum-E. tamana* (Cs-Ab-Ce-Et), *C. excavatum-A. beccarii-C. subarcticum-A. ketienziensis* (Ce-Ab-Cs-Ak), *C. excavatum-A. beccarii-A. ketienziensis-C. subarcticum* (Ce-Ab-Ak-Cs), *C. excavatum-C. subarcticum-A. ketienziensis-E. naraensis* (Ce-Cs-Ak-En) are characterized by high similarity index of 88.1%.

Discussion and conclusion

Geochemical effect of fish farming on sediment is pronounced in increase of silt content and sedimentation fluxes in Zn_{EX}, Cu_{EX}, As_{EX} and TP_{EX} contents after red laver farming. However, seven benthic foraminiferal assemblages with the dominant species of *C. excavatum* was not shown the remarkable variation of assemblage and sustained the very low abundance frequency indicating bad habitat condition. *C. excavatum-A. beccarii-A. ketienziensis-C. subarcticum* assemblage of fish farming distributed from 21cm of depth to uppermost layer, among assemblages, is characterized by increase of *A. beccarii*. These are caused by the accumulation of organic matter discharged consistently from fish farm after red laver farm. *A. beccarii* is thought to the tolerant species to fish farming. Effect of fish farming on sediment geochemistry at control plot about 160m from fish far may be caused by tidal current.

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FUNCTIONAL CLASSIFICATION AND EXPRESSION PATTERN ANALYSIS OF MEMBRANE PROTEIN GENES PRESENT IN IONOCYTES OF SKIN INCLUDING LATERAL LINE IN BRACKISH WATER EXPOSURE OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

The rainbow trout (*Oncorhynchus mykiss*) is one of the major freshwater aquaculture fish species widely consumed because it has excellent taste and growth and is highly nutritious. Until recently, researchers have been doing experiments on the seawater adaptation of the rainbow trout in an attempt to enlarge its body size and improve its taste. Gills and skin are the organs that are directly exposed to the external environment and are known to control the concentration of ions and molecules to overcome the high osmotic pressure of the water. There have been many studies on various aspects of seawater adaptation and osmotic control capacity of Salmonidae fish so far (Kim et al., 2016; Richards et al., 2003). However, such an analysis has rarely been reported that the expression patterns of various osmoregulation proteins in skin tissues including the lateral line are analyzed at one point using transcriptome RNA-seq. Therefore, this study aims to analyze the expression patterns of membrane protein genes in skin ionocytes including the lateral line while the rainbow trout are exposed to brackish water.

Materials and methods

Rainbow trout (average weight is 99g) were transferred from the Pyeongchang fish farm (South Korea) to the laboratory and stabilized at a temperature of 16 ± 0.5°C and a dissolved oxygen level of 8.0 ± 0.5mg/ l. Brackish water (50% seawater) was used after filtration, and the individuals acclimatized in freshwater were transferred to the brackish water for the 3-week-adaptation. Then, some of them were randomly sampled. Skin tissues including the lateral line were extracted for a total RNA purification, and an RNA-seq library was constructed. After obtaining the sequencing, the researchers mapped the raw data to the rainbow trout genome assembly. The expression patterns were analyzed using BLAST2GO, gene ontology (GO; level 5), InterPro, GO-Slin and EggNog.

Results

About 82 to 83 percent of the raw data obtained by RNA-seq were mapped to the rainbow trout genome assembly (Table I). The Go (level 5) analysis performed for functional annotation indicates that in biological process (BP), genes in charge of the cellular protein metabolic process account for the highest portion. Meanwhile, in molecular function (MF), genes related to DNA binding takes up the highest. As for the cellular component, genes concerning nucleus are expressed the most. In order to validate the expression patterns of RNA-seq, qRT-PCR was performed on membrane protein genes for osmoregulation (Table II). The result was similar to the expression patterns of RNA-seq.

Discussion and conclusion

The osmoregulation capability of various membrane proteins in skin ionocytes including the lateral line of rainbow trout was studied. When transferred from fresh water to brackish water, the rainbow trout showed various gene expression patterns including sodium/potassium-transporting ATPase subunit alpha-1 (ATP1A1), sodium/potassium-transporting ATPase subunit alpha-2 (ATP1A2), sodium/potassium-transporting ATPase subunit alpha-3 (ATP1A3), sodium/hydrogen exchanger 1 (NHE1), sodium/hydrogen exchanger 1 (NHE2), sodium/hydrogen exchanger 6 (NHE6) and aquaporin (AQP3) which are found in other Salmonidae fish (Choi et al., 2013; McCormick et al., 2009). Also, faced with stress, the rainbow trout expressed various genes to maintain homeostasis.

This study observed gene expression patterns of rainbow trout both in the fresh water environment and in the brackish water, and then categorized the osmoregulation membrane genes according to their functions. The findings of this study are expected to be the basis for future research on the osmoregulation capacity of other Salmonidae fish and their ability to maintain homeostasis.

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Table I. Mapping statistics of transcriptome reads to the *O. mykiss* genome assembly

Fresh water	**Number of sequences**	**Mapping rate (%)**
Reads mapped in pairs	55,012,194	73.52
Reads mapped in broken pairs	7,533,273	10.07
Reads not mapped	12,281,813	16.41
Total	74,827,280	100
Brackish water	**Number of sequences**	**Mapping rate (%)**
Reads mapped in pairs	58,206,628	73.31
Reads mapped in broken pairs	7,346,743	9.25
Reads not mapped	13,847,105	17.44
Total	79,400,476	100

Table II. The differential expression patterns of osmoregulation genes between the experiment and control groups

Gene	**Log□ fold change**	**Fold change**	**FDR p-value**	**Bonferroni**	**Accession #**
ATP1A1	-0.79786476	-1.73852614	0.013921481	1	XM_021570999.1
ATP1A2	4.46045952	22.0156803	0	0	XM_021588759.1
ATP1A3	-0.75394671	-1.68639993	3.25159E-07	0.000446444	XM_021595671.1
NHE1	-0.75450061	-1.68704752	9.09503E-06	0.017980871	XM_021595261.1
NHE2	-0.49550848	-1.40981756	0.008823663	1	NM_001130994.1
NHE6	-0.69720054	-1.6213556	0.003792481	1	XM_021591481.1
AQP3	-0.59179654	-1.50712235	1.17868E-05	0.024021515	XM_021605302.1

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DIETARY SUPPLEMENTATIONS OF *Bacillus* PROBIOTIC IMPROVE DIGESTIBILITY, INNATE IMMUNITY, WATER QUALITY AND GROWTH PERFORMANCE OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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Bacillus spp. was found to have great potentials as supplemental probiotics in diets for shrimps. A control was made without the probiotic supplementation and four other diets were prepared by inclusions of *B. subtilis* (*BS*) alone and a mixture of *BS* and *B. pumilus* (*BP*) at different levels (0.1 x 10^{10} *BS*, 0.2 x 10^{10} *BS*, 0.1 x 10^{10} *BS*/*BP*, 0.2 x 10^{10} *BS*/*BP* diets for control, *BS*0.1, *BS*0.2, *BS*/*BP*0.1 and *BS*/*BP*0.2, respectively). Quadruplicate groups of shrimp (average body weight, 0.14 g) per each diet were hand-fed the diets for 8 weeks. Shrimps fed *BS* containing diets showed significantly higher apparent digestibility coefficient of dry matter and protein than shrimp fed the control diet. Phagocytic activity of shrimp fed the probiotic diets except for *BS*0.1 was significantly increaed than shrimp fed the control diet. Shrimp fed the probiotic diets showed significantly increased phenoloxidase activity compared to the control diet. *BS*0.2 shrimp group showed significantly higher antiprotease activity than the control group. Glutathione peroxidase activity was significantly improved in shrimps fed the probiotic diets except for *BS/BP*0.2 diet. The growth was significantly improved in shrimp fed *BS*0.2 and *BS*/*BP*0.2 diets compared to that of shrimp fed the control diet. Significantly lower feed conversion ratio was obtained from shrimp fed the probiotic diets except for *BS*/*BP* 0.1 than that of shrimp fed the control diet. In a zero water exchange test, shrimp fed the probiotic diets resulted in lower total ammonia concentration than shrimp fed the control diet (Fig. 1). In conclusions, the tested *Bacillus* spp. can positively affect digestibility, innate immunity, water quality, growth performance and feed utilization efficiency of *L. vannamei*.

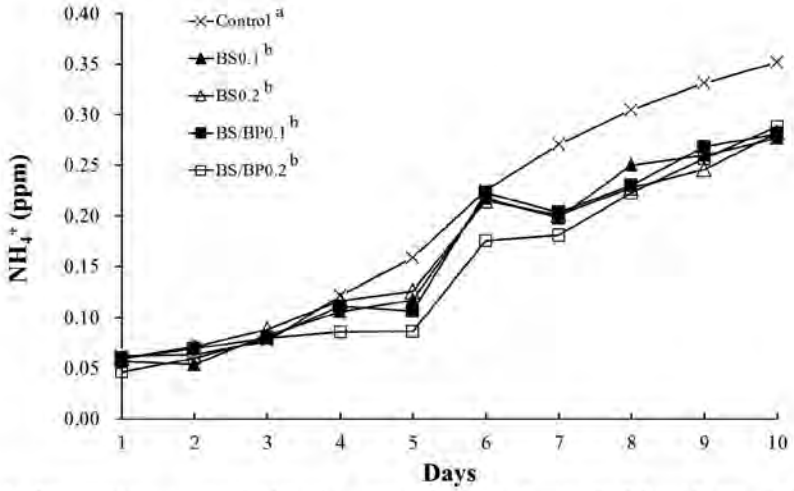

Figure 1. Ammonium concentration in a zero water exchange test for 10 days. Triplicate groups of shrimp were hand-fed with one of the test diets four times a day during the period.

ANIMAL WELLFARE AND HIGH YIELDS ARE NOT CONTRADICTORY

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Introduction

High stocking densities and poor water quality are stressors for fish in aquaculture causing decreasing growth performance, depressing immune system functions and leaving fish even more vulnerable to infections (Bly *et al.* 1997, Tort 2011). Environmental and human health concerns have resulted in a ban of most chemical control agents inside the EU (EG1272 2008).

"Functional Feeds" that increase natural defense mechanisms have been studied as alternative treatments (Hoseinifar *et al.* 2015, Azimirad *et al.* 2016); however, feed competition prevents equal distribution between all fish. Because of their large surface area, gills are often portal of entry for pathogens, but at the same time are prone to xenobiotics in the water. We therefore hypothesized that immunostimulation can be achieved by supplementing the water with additives.

Humic substances are natural products, used as feed supplements in agriculture because of their growth-enhancing, immune-stimulatory and stress-reducing properties (Steinberg 2003, Islam *et al.* 2005). Up to 95% of dissolved organic matter in aquatic ecosystems is humic substances (mainly fulvic acid), giving them a great potential to be used in aquaculture.

Material and Methods

Juvenile rainbow trout (*Oncorhynchus mykiss)* were exposed for 4 weeks in a flow through system to 5 and 50 mg C/L of a fulvic acid. After first sampling, remaining fish were captured and exposed to air for 30s. Ninety minutes post-stress, blood was sampled again in order to determine stress response and recovery. Remaining fish were sampled two days post-stress for effects of stress on immune parameters.

Morphometric parameters were measured prior and after exposure. Head kidney cells were isolated to measure phagocytosis with heat inactivated yeast cells and respiratory burst activity by nitro blue tetrazolium reduction (Crampe *et al.* 1998). Plasma samples were taken to determine stress and immune response; Cortisol was measured with an ELISA, lysozyme activity as reduction of absorbance of a *Micrococcus lysodeikticus* suspension (Sitjà-Bobadilla *et al.* 2008) and protein content following Bradford (1976). Furthermore, immune response of gills was determined by same methods in supernatant of gill homogenates.

Results

Relative growth rate was significantly increased in the 50mgC/L group (20% in length and 57% in weight) compared to the control group (15% in length and +47% in weight). A similar trend was also apparent in the 5mgC/L group. Feed conversion ratio was decreased from 1.04 to 0.79 in groups exposed to the high concentration of fulvic acid. Lysozyme activity in the gills of the fish exposed to 50mgC/L was significantly higher compared to the control fish (238U/mL and 190U/mL, respectively); however serum lysozyme activity was not affected. Phagocytosis rate of head kidney cells increased from 31.5% to 57.6% and 70.7% after exposure to 5 and 50mgC/L, respectively. Phagocytosis index increased from 1.8 to 2.2 and 2.3 ingested particles/phagocyte after exposure.

Discussion and Conclusion

Fulvic acid is a natural product that occurs in aquatic ecosystems. Our results show that fulvic acid can be used as additive in order to improve growth of juvenile rainbow trout and to stimulate the immune response. Growth was significantly improved after 4 weeks of exposure. In addition immune parameters were increased, especcially in the gills, which are an important portal of entry for pathogens. Furthermore, we showed that in general immunostimulation can be acchieved by addition of stimulatnts to the water body. Up to our knowledge, this is the first time increased gill-associated immunity was determined after exposure to a water additive. Although more research on the application of fulvic acid is needed, such as application to different life stages of fish and under facility conditions, it is evident, that it improves growth and stimulates the immune system and has the potential to reduce mortality by infections in aquaculture.

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DIETARY SUPPLEMENTATIONS OF *Bacillus* spp. IMPROVES INNATE IMMUNITY, GROWTH PERFORMANCE AND DISEASE RESISTANCE OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) AGAINST WHITE SPOT SYNDROME VIRUS AND ACUTE HEPATOPANCREATIC NECROSIS DISEASE

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Under the field conditions, multiple infections by more than one pathogen can cause much greater losses in shrimp culture than any single infection. White spot syndrome virus (WSSV) and acute hepatopancreatic necrosis disease (AHPND) infects shrimps and spread rapidly causing a serious loss of the shrimp production in the world. However, there is limited information available on the multiple disease infections. A control was prepared without probiotic supplementation and four other diets were prepared by inclusions of *B. subtilis* (BS) alone, and a mixture of *BS*, *B. pumilus* (BP) and *B. licheniformis*(BL) at different levels (0.2 x 10^9 BS, 0.2 x 10^{10} BS, 0.2 x 10^9 BS/BP/BL, 0.2 x 10^{10} BS/BP/BL diets for the control, $BS10^9$, $BS10^{10}$, $BS/BP/BL10^9$, $BS/BP/BL10^{10}$, respectively). Quadruplicate groups of shrimp (average body weight, 0.15 g) per diet were hand-fed the diets for 51 days. Shrimp fed the $BS/BP/BL10^9$ diet had significantly higher phagocytic activity than shrimp fed the control diet. Growth performance and feed utilization efficiency were not affected by the probiotics. After the feeding trial, the shrimp were challenged with WSSV and *V. parahaemolyticus* through an immersion method for 187h. Survival was numerically higher in shrimp fed the probiotic diets (except for $BS10^9$) than in shrimp fed the control diet (Fig. 1). The findings indicate that innate immune response and disease resistance of *L. vannamei* against WSSV and AHPND can be improved when they are fed the tested *Bacillus* spp.

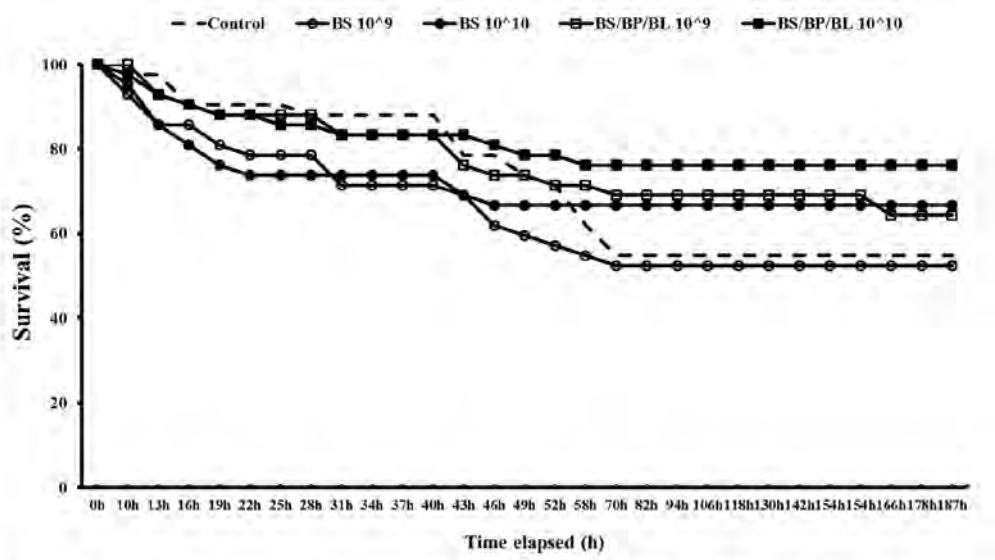

Figure 1. Survival rate of *L. vannamei* after challenged against WSSV and *V. parahaemolyticus*. Quadruplicate groups of shrimp were hand-fed with one of the test diets two times a day during the challenge period.

MOLECULAR CLONING, CHARACTERIZATION AND EXPRESSION OF OPSIN1 SUBFAMILY GENES FROM MUD LOACH (*Misgurnus mizolepis*)

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Introduction

The Mud loach (*Misgurnus mizolepis*) is a species that lives mainly in fresh water environment with shallow depths or changes in water level such as ponds, rice fields and waterways. They prefer dark places over bright ones and bury their bodies in the dirt. Mud loach can be found throughout Korea, but the number is gradually decreasing due to environmental changes, and much of it is supplied through aquaculture. In addition, mud loach farming systems have been conducted in a similar manner for a long time, and studies on growth, immunity, and induction of sexual maturation through various environmental conditions are less than those of other farmed fish. To date, various fish species have been studied for growth and immunity through food or administration, such as feed development research and functional peptide development. Recently, research has been published that improves the environmental conditions of fish as well as providing nutrition, resulting in increased growth and immunity. In particular, research has shown that it is possible to improve fish productivity by adjusting the type, intensity and exposure time of light sources. Therefore, the aim of this study was to identify the types of opsin protein genes that detect and absorb various light sources in retina tissues in mud loach eyes and to investigate their structure and expression characteristics.

Materials and methods

In-vivo experiments were conducted to investigate the effects of loach on light sources for each wavelength. The control group was set to two groups of natural light and non-light, and the experimental group was tested by exposing the respective wavelengths of LEDs (blue, green, red) to each tank. The eye and brain tissues were extracted and used for expression analysis of opsin genes. To identify mRNA structures of opsin genes, 3 ‘and 5’-RACE libraries were constructed and the genes were isolated using PCR amplification. In addition, opsin genes and amino acid structure comparison and phylogenetic analysis of other fish species were performed. We carried out qRT-PCR to investigate the effect of LEDs stimulation on the opsin genes in eye and brain tissues, and the expression of growth hormone and sex maturation genes.

Results and Discussion

As a result, opsin1 gene in mud loach retina was found to have short-wave-sensitive, long-wave-sensitive, and medium-wave-sensitive protein genes like other fish. Also within each opsin1 gene, various genes existed on the chromosome in the form of sub-isoforms. As a result of gene expression, various expression patterns were observed according to the LEDs stimulation of each wavelength in the eyes and brain, and the expression of growth hormone gene and sex maturation gene was also different according to the LEDs stimulation. The results of this study will be used as basic data for the study of protein in the fish eye and induction of growth and maturit .

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GROWTH PERFORMANCE AND GUT MICROBIOME OF JUVENILE ATLANTIC SALMON, *Salmo salar* FED DIETS REPLACING FISH MEAL AND PLANT PROTEIN BLEND WITH THE YEAST, *Candida utilis*

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Introduction

To meet the global food security needs of an expanding population, aquaculture production has grown rapidly Godfray et al. (2010). Atlantic Salmon *Salmo salar* are one of the most intensively farmed species in Europe and have a high dietary protein requirement. Traditionally, fish meal and plant proteins have been used to meet this dietary need, but the scale of aquaculture growth, negative environmental impacts and rising cost of sourcing large protein quantities hves driven a search for new protein sources Naylor et al. (2009). Single cell proteins, such as the protein-rich yeast, *Candida utilis*, represent a promising source of new material for future aquafeeds. It can be produced in industrial bioreactors using substrates derived from sustainable low-value feedstocks such as wood residues and has an amino acid profile appropriate for salmonid diets. Yeasts have historically been used in animal feeds as an additive to improve health, but cost and low protein content of standard production has limited applicability to *Salmo salar* diets Øverland et al. (2013). The juvenile stage of *Salmo salar* is a period of highest protein demand and fast growth and gut development, making it ideal to assess the potential of new protein sources NRC (2011).

Altering dietary protein can significantly change the gut microbiome of fish. This is important since the gut microbiome is linked to immune development, nutrient uptake and growth performance Ringø et al. (2016). The gut microbiome of juvenile salmonids is under studied and recently published literature highlights the challenging nature of characterizing the gut community Michl et al. (2019).

Candida utilis will be assessed in the diet of juvenile salmonids and this study aimed to investigate both the effect of increasing replacement of fish meal and plant protein blends meal on growth performance and the composition of the gut microbiome to inform the optimal development of future sustainable aquafeeds.

Materials and methods

Juvenile *Salmo salar*, of an average start weight of 1.14g were fed in quadruplicate tanks on 5 diets that increasingly replaced fish meal with *Candida utilis* at levels of 0%, 5%, 10%, 15%, and 20% and another 5 diets that increasingly replaced a plant protein blend with *Candida utilis* at levels of 0%, 5%, 10%, 15% and 20%. The *Candida utilis* cell biomass was produced in a 50-litre bioreactor using a hardwood carbohydrate hydrolysate as substrate and had a higher protein content than a standard industrial product at 52%. Growth performance of each diet was assessed by weighing all individuals before and after a 35-day feeding trial at 9.0±5°C in freshwater and statistically comparing results between feed

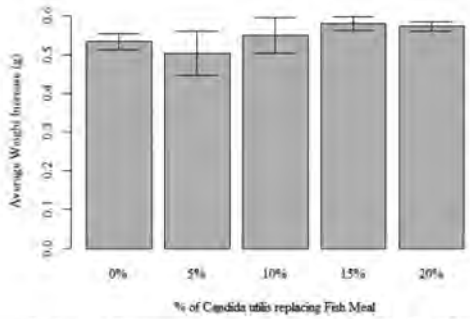

Fig. 1. Increase in weight (g) of *S.salar* fed diets where *C.utilis* replaced fish

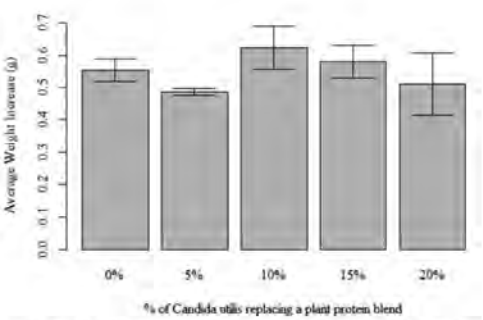

Fig. 2. Increase in weight (g) of *S.salar* fed diets where *C.utilis* replaced a plant protein blend.

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treatments. After a further 14-day feeding period 9 fish from each feed treatment were sampled for gut microbiome analysis of the mid and hind gut. Whole guts were processed using a novel DNA extraction protocol adapted from the QIAamp power fecal Pro kit by Qiagen with selective enrichment for prokaryotic DNA. Samples were then sequenced using the MiSeq Illumina platform to provide information about the bacterial community present for each feed treatment. Diversity indices and presence absence measures were statistically compared between feed treatments.

Results

Weight (g) of fish fed different feed treatments showed not significant difference after a 35-day growth period when either only fish meal was replaced at levels of (0%, 5%, 10%,15% or 20%) (figure 1), or when a plant protein blend replaced at levels of (0%, 5%, 10%,15% or 20%) (figure 2) by *Candida utilis*. The MiSeq Illumina sequence data from the gut microbiome of fish from different feed treatments will be presented at the European Aquaculture Symposium 2019 along with the statistical results from a comparative analysis of diversity indices of the gut communities.

Discussion and conclusion

Growth performance results suggest that the yeast *Candida utilis* can successfully replace both pure fish meal and a plant protein blend in the diets of juvenile *Salmo salar,* up to 20%, despite the high crude protein requirement of this growth stage NRC (2011). This result supports existing aquafeed research that suggests that many single cell proteins have comparable amino acid profile to fish meal, making them a promising replacement. The results from this study support the continued development of *Candida utilis* in salmonid diets and the results from sequencing will provide in-depth examination of how this protein alters the gut microbial community, which may have far reaching consequences.

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THE ROLE OF INTERNET OF THINGS FOR HEALTHY FISH AND ENVIRONMENT IN THE EUROPEAN AQUACULTURE

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Introduction

The overall objective of the project FutureEUAqua is to promote sustainable growth of environmental friendly organic and conventional aquaculture to meet future challenges of the growing consumer demand for high quality, nutritious and responsibly produced food. In WP5 of the FutureEUAqua project we are committed to develop and test a multiplatform tracking system for simultaneously monitoring the activity and physiology of fish, as well as the main parameters of the environment where they are farmed, by using a wireless communication system. The study of aquatic animals (e.g. fish behaviour, condition, physiology) and the farming environment presents unique challenges to scientists because of the physical characteristics of water. However, scientific studies and efforts have increasingly turned to the use of electronic sensors, which have enhanced our knowledge on the performances of the farmed fish and the impacts on the surrounding aquatic system. In their most basic form, electronic sensors and tags may include radio or acoustic beacons transmitting signals, which can bring specific codes to identify animals, and allow them to be tracked using receivers that detect the transmitted signals (Hazen et al. 2012). Basic archival tags must be, instead, physically recovered in order to obtain the data. Because the strength of radio signals rapidly attenuate in seawater, acoustic transmissions is preferred for fish tracking in marine environment (Lembo et al., 2002), while radio transmission is commonly used in freshwater environment. More advanced tags incorporate sensors that measure and record a suite of environmental and/or biological parameters of fish (Cooke et al. 2016). Simple biomass estimators and logging stations, installed on the feeding barge and/or on the cages, can give full control over all water parameters and provide the information required to monitor/expand the production. Flexible sensors systems are conceived to log oxygen, temperature, salinity, sea current, pH, wind and CO2. In addition, sensor and camera systems may provide also information for estimating the biomass in the cages and developing reliable fish feeding strategies. Indeed, electronic sensors are significantly improving our understanding of fish behaviour and are emerging as key sources of information for improving aquaculture management practices. The wireless communication system to monitor the large scale demonstration activities foreseen in the project FutureEUAqua will both facilitate effective study design and replication, increasing the accuracy of data standardization, processing and interpretation (e.g., Huveneers et al. 2016), providing industry with the information needed to facilitate health/welfare and optimal management practices.

State of the art

A first step activity carried out in WP5 was the review of the state of the art and future needs in terms of technologies for fish tracking, environmental monitoring and biomass assessing. The most relevant technologies and methodologies are described below. Accelerometer pressure tags transmit 3D acceleration of fish as they move within the receiver array and also transmit depth data. This acceleration value can be used as a measure of activity of a free ranging animal in nature or captivity. Applications may include measuring swimming speed via tail beat acceleration, feeding events, spawning activity, nocturnal/diurnal activity and responses to changing oxygen, salinity and temperature in the environment. Electromyogram (EMG) transmitters measure muscle activity using probes inserted into the musculature of the fish. They provide a powerful quantitative estimate of the energetic costs associated with physical activity of fish. EMG values can be calibrated in terms of fish oxygen consumptions measured over periods of spontaneous activity or over the same times in swims of selected speeds and durations, by using a swimming chamber. This in turn allows to obtain quantitative estimates of the metabolic costs of activity of farmed fish. The bioenergetics of the target species (muscular activity patterns) can be modelled, based on fish mass and swimming speed, to predict the mass-specific standard metabolic rate (SMR), the active metabolic rate (AMR) and the scope for activity (SFA). The difference between AMR and SMR indicates the energetic expenditure available to support all locomotor and physiological activities (SFA). The oxygen consumption rate (MO2) can be measured during exhaustive swimming trials (U_{crit}), carried out in swimming chambers, to estimate the energetic expenditure linked to the different swimming velocities. MO2 can be further calibrated with the signals transmitted by the tailbeat accelerometer tags, as well as with the EMGs signal received via two pairs of wire electrodes surgically implanted in both the red and white muscle (Zupa et al., 2015). The calibration gives the possibility to correlate each single swimming level of the fish to the oxygen consumption rate (e.g. Carbonara et al., 2014). In this way, the activity based energetic expenditure can be assessed (i.e. the fish physiological status), as well as the relative cost of living for fish in their environment. Fish detection and size measurement using acoustic signals has been used in research to measure fish

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size of farmed fish, although with severe limitation due to how inaccurate these measurements inherently are. Also infrared (IR) beam technology has been utilized in aquaculture to detect the presence of fish and biomass estimation. The concept utilizes an IR emitter on one side and an IR receiver on the opposite side. When the fish pass between the two then the beam is interrupted and the receiver will register the appropriate event. Moreover, mention should be made of underwater stereo-video systems, which are capable of making accurate, non-invasive measurements of fish length by analysing the pair of simultaneously captured images.

A real-time wireless communication system and sensor network

A real time wireless communication system and sensor network will be tested during the large scale demonstration activities. The system architecture includes a family of compact, submersible environmental sensors (e.g. dissolved oxygen, temperature, salinity, turbidity, tilt, etc.) with underwater and in air wireless communications. A cloud-based platform will allows to view and analyse data from the aquaculture sites in real time. The core of the system will be based on a hub supporting many telemetry protocols for cloud communications, including Cellular, Wi-Fi and Iridium. The wireless hub will supports also third-party sensors (e.g. accelerometer pressure tags, biomass monitoring systems, etc.) via its auxiliary sensor port. This technology will enable data-driven ocean farming where knowledge drives better decisions.

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BLACK SOLDIER MEAL *(Hermetia illucens)* INCLUSION BY SUBSTITUTING FISHMEAL AT 50% AND 100%, IN RAINBOW TROUT FEED *(Oncorhynchus mykiss)*

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In a continuous sustainability improvement process, the aquaculture sector has been interested for many years in various alternatives available in terms of marine products substitution. Innovative raw materials such as algae, microorganisms or insect meal are becoming more and more popular.

Following July 2017's authorization of insect meal in the fish and shellfish diet, Le Gouessant Aquaculture, a major player in aquaculture nutrition in France, has set up two comparative trials under experimental conditions and three trials on fish farms.

The target of those studies conducted between October 2018 and March 2019 was to evaluate black soldier fly meal (BSF, hermetia illucens) as a partial or total alternative to fishmeal. This insect meal influence on the rainbow trout (Oncorhynchus mykiss) zootechnical performances was observed during the fish growing stage with weights ranging from 120 g to 700 g.

In the first test set up in an experimental station, three diets were tested, containing respectively: 25% of fishmeal, 12.5% of fishmeal and 12.5% of BSF meal, meaning a 50% substitution, and a last one with 100% substitution by BSF meal. This test was conducted in 2 phases. In the second test, a control feed containing 23% fishmeal was compared to a feed where 50% of the marine protein was replaced by BSF meal, meaning 12.5% because the BSF meal is less protein-rich than fishmeal.

During these trials, two different suppliers of BSF meal were solicited. In the first trial, the two insect-containing feeds showed similar performance on the first part of the test, and then slightly lower than those of the control on the 2nd phase ($P < 0.001$). However, during the second test, the feed with insect meal induced a better growth than the control feed ($P < 0.001$). Tests conducted in fish farms showed trends equivalent to those obtained in experimental structures. Fish nutritional composition and organoleptic analyses were performed, but no differences in the measured parameters were statistically significant.

Overall, the results confirmed the interest of this innovative ingredient. In fact, it is conceivable to substitute partially or totally fish meal with insect meal in a rainbow trout diet. Those conclusions encourage the study of future promising leads: interests in other aquaculture species, or livestock health.

THYROID HORMONE RECEPTORS A NEW PLAYER IN LARVAL METAMORPHOSIS OF THE HARD-SHELLED MUSSEL

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Introduction

Many marine invertebrate larvae undergo a dramatic morphological and physiological transition from a planktonic larva to a benthic juvenile. Settlement occurs when larvae acquire "competence" and can respond to specific cues, that trigger a rapid metamorphosis and fixation to the substrate (Heyland et al., 2005). Metamorphosis in bivalves is correlated with morphological and physiological changes that include dramatic resorption and remodelling of larval and juvenile tissues. The regulation of this metamorphosis in bivalves remains enigmatic. The recent identification in bivalves of a thyroid hormone receptor (TR) gene raises the possibility that as occurs in vertebrate metamorphosis, TRs regulate this developmental process.

Materials and methods

Adult mussels were collected from Gouqi Island, (30↑72↑ N; 122↑77↑ E), Zhoushan, Zhejiang Province, China, in March 2017. Spawning and larval rearing followed the methods described by Yang et al. (2014). Swimming straight-hinge veliger larvae developed two days after fertilization and was maintained in 2 L glass beakers until they reached the pediveliger stage when siRNA transfection and metamorphosis assays were performed.

Results

An evolutionary study of TR receptors revealed they are ubiquitous in the molluscs. a single McTR gene was isolated from *M. coruscus*. An aa sequence alignment with vertebrate and invertebrate TR orthologues confirmed that McTR is a TR homologue. The BI tree showed that the TRs from invertebrates clustered separately from the deuterostomes and that McTR grouped closely with the other mussel TRs and they formed a specific cluster apart from the oysters TRs. Two TR genes (TRα and TRβ) were found in vertebrates but only a single TR gene was identified in invertebrates indicating that TR gene duplication occurred after the divergence of the vertebrates. Knock-down of the TR gene in the hard-shelled mussel, *Mytilus coruscus (Mc)*, using electroporation of siRNA significantly ($P < 0.01$) reduced TR gene expression. TR gene knock-down decreased pediveliger larval metamorphosis by 54% and was associated with a significant ($P < 0.01$) reduction in viability compared to control larvae.

Conclusions

TRs are ubiquitous across the bivalves. In the hard-shelled mussel TRs are essential regulatory factors for successful metamorphosis of the pediveliger larvae to a juvenile. The involvement of TRs during metamorphosis of bivalves suggests this function emerged early during evolution. It is revealed that electroporation is a practical approach for gene knock-down in minute marine invertebrate larvae.

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EFFECT OF ARTIFICIAL LIGHT ON THE GROWTH AND SERUM CORTISOL LEVEL OF JUVENILE CHINESE SOFT-SHELLED TURTLES (*Pelodiscus sinensis)*

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Introduction

It is well known that all living things depend on the sun for their growth. Therefore, light is essential to us and other animals and plants. Actually, light has been widely used in the aquaculture and plays an important role in larval(Suzer et al., 2006) and juvenile culture(Trippel and Neil, 2003), such as growth performance, survival, stress response. However, the influence of light on growth performance of fish differed among species. The Chinese soft-shelled turtle is an aquaculture species with a high nutritional and medicinal value which has been widely cultured in China and Southeast Asia. Juvenile soft-shelled turtles were usually cultured in the greenhouse without light for a year in order to increase the survival rate. However, it not only deprived the soft-shelled turtles the welfare of basking in the sun when cultured in the pond but also increased the aberration rates. The purpose of present study was to bring the sunlight into the greenhouse to improve the welfare of soft-turtles, meanwhile to investigate the effect of artificial light on the growth and serum cortisol level of juvenile soft-shelled turtles.

Materials and methods

Juvenile soft-shelled turtles (mean weight 3.0±0.1g) were cultured in an aquarium (1000*2000*300mm) which placed in a large concrete pond to keep the water temperature at about 30°C. There were a basking area, feeding table and water area in the aquarium. The stocking density was 60 individuals a square meter. There were two treatments in the study. One was without artificial light (WOL), and the other was with artificial light in the basking area (WL). Each treatment was with three replicates. The light source was from a sunlamp which contained UVB light, UVA light, visible light and some heat. The UVB light intensity was $0.25W \cdot m^{-2}$. The photoperiod was 12 h light and 12 h dark. The concentrations of ammonia nitrogen and nitrite nitrogen were maintained at a safe range. The concentration of dissolved oxygen was kept higher than 2mg/l.

Results

The parameters of growth performance and serum cortisol level were showed in table 1. The parameters of final weight and weight gain rate of soft-shelled turtles in the treatment with artificial light were higher than that without light. The serum cortisol level of turtles cultured in artificial light was lower than that in the treatment without light

Discussion and conclusion

The studies of light improving the growth of fish have been observed in various species. According to the results, the artificial light in the basking area increased the growth performance of juvenile soft-shelled turtles and decreased the level of the serum cortisol which was a parameter of stress response(Rtcsano J. SrneNcs and Scsnncx., 1978). It was possible that the components of artificial light were similar with sunlight which may comfort the juvenile soft-shelled turtles and improve the welfare for them as they had the preference of basking in the sun. Meanwhile, the UVB light could promote the skin to synthesis the vitamin D which can promote the absorption of blood calcium which may also enhance the growth performance of them.

In conclusion, the soft-shelled turtles cultured in greenhouse should be provided a basking area as it not only improved the welfare of soft-shelled turtles, but also increase the growth performance.

Table 1

Influence of artificial light on the growth performance and serum cortisol level of juvenile Chinese soft-shelled turtles at the end of the experiment (123 days). The data were presented by means ±S.D. (n=9).

Parameters	WOL	WL
Initial weight (g)	3.11±0.03	3.07±0.05
Final weight (g)	61.33±2.47	61.99±4.35
Weight gain rate (%)	1871.83±94.51	1921.10±168.61
Serum cortisol level ($ng.ml^{-1}$)	31.31±4.70	29.74±1.16

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GUT HEALTH AND MICROBIOTA IN POST-SMOLT ATLANTIC SALMON (*SALMO SALAR*) FED LARVAE MEAL FROM BLACK SOLDIER FLY (*Hermetia illucens*)

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Introduction

In salmon aquaculture, the limited availability of sustainable feed ingredients is a major obstacle. Insects, being part of the natural diet of salmonids, may become a sustainable resource and expand the raw material repertoire. In the "AquaFly" project, the potential of black soldier fly (*Hermetia illucens*) grown on low-quality organic matter as a source of sustainable feed ingredients for Atlantic salmon (*Salmo salar*) was assessed in one freshwater and one seawater feeding trial. Herein, we summarize data from the 16-week seawater feeding trial with salmon (initial body weight, 1.4kg) fed either a reference diet (REF) with a combination of fish meal, soy protein concentrate, pea protein concentrate, corn gluten and wheat gluten as protein source, or a test diet (IM) wherein all the fish meal and most of the pea protein concentrate were replaced by black soldier fly larvae meal

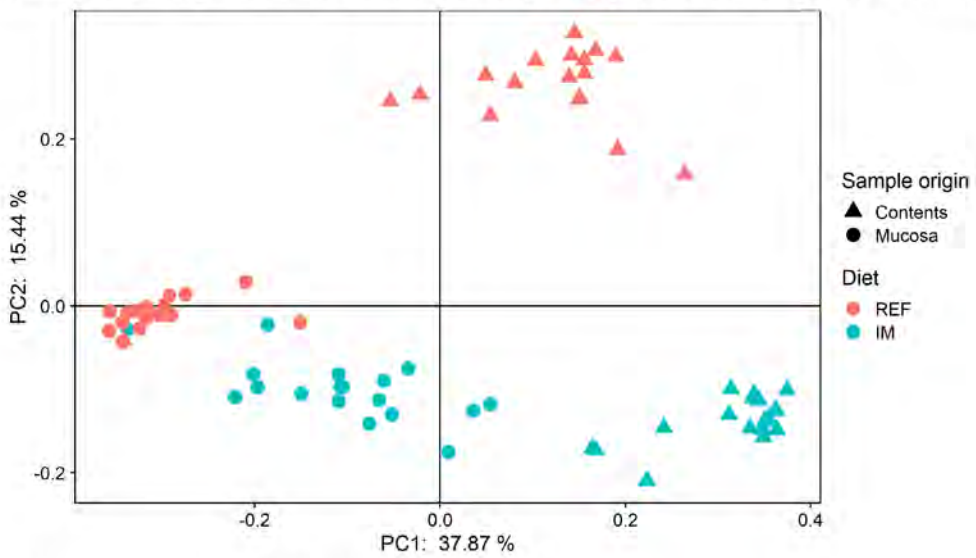
Materials and methods

The gut health of fish was evaluated using endpoints including organ and tissue indices, digestive enzyme activity, histopathological parameters and gene expression. In total, 36 genes under different functional categories were profiled via qPCR, including those indicative of lipid metabolism, immune responses, barrier functions and detoxification/stress responses. Microbiota in the distal intestine, both gut contents and mucosa associated, was analyzed via 16S rRNA gene sequencing.

Results

1. Histology. Hypervacuolization of enterocytes, suggestive of lipid accumulation, was observed in the proximal and mid intestine in both diet groups. It was, however, less severe in the proximal intestine of fish fed the insect meal diet ($p < 0.05$). Typical signs of enteritis commonly observed in salmonid intestine fed soybean meal diets were present in all the intestinal segments in both diet groups. A higher degree of submucosa cellularity was observed in the proximal intestine of fish fed the insect meal diet ($p < 0.05$).

Fig.1. Beta-diversity of distal intestine microbiota visualized by principle coordinate analysis (PCoA) of unweighted UniFrac distance. Each point represents one sample. The closer the points, the more similar their microbial communities are.



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2. Gene expression. Of the 36 genes profiled in the proximal and distal intestine, a few genes showed differential expressions. The only genes showing differential expressions were *mmp13* (matrix metalloproteinase 13) and *chk* (choline kinase), whose expression level was significantly lower in the proximal and distal intestine, respectively, in fish fed the insect meal diet ($p < 0.05$).

3. Gut microbiota. Both diet and sample origin (gut contents vs mucosa) affected microbiota in the distal intestine. All the measured alpha-diversity indices, including observed OTUs, Shannon index, Pielou's evenness and Faith's phylogenetic distance, showed higher values in samples exposed to insect meal diet or originated from gut contents ($p < 0.05$). For the beta-diversity, samples formed 4 distinctive clusters in the PCoA plot separated by the diet and sample origin (Fig.1), confirmed by permutational multivariate analysis of variance (*PERMANOVA*, $p < 0.05$). The diet showed a more profound effect on the samples originated from gut contents than those from mucosa.

Discussion and conclusion

In the present study, total replacement of fish meal with black soldier fly larvae meal showed no appreciable negative effects on the gut health and function of Atlantic salmon. In line with what we reported in the freshwater trial (Li et al., 2019), insect meal inclusion was associated with a lower degree of enterocyte hypervacuolization in the proximal intestine, which is suggestive of lower lipid accumulation. In the context of frequent occurrence of steatosis found in the proximal intestine of farmed salmon in field surveys (Chikwati et al., 2018), the potential beneficial effect of insect meal inclusion on reducing lipid deposition in the gut deserves further attention. In contrast to the previous finding in the freshwater trial that insect meal diet increased the expression of *foxp3* (Li et al., 2019), a transcription factor for the differentiation of naïve CD4 T-cells into regulatory T cells, no such effect was observed in the seawater trial. On the other hand, the present study showed increased submucosa cellularity in the proximal intestine. The effect of insect meal inclusion on the gut immune response is also worth of attention in future studies.

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LYSOZYME GENE FAMILY EVOLUTION AND FUNCTION DURING EARLY FISH DEVELOPMENT

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Introduction

The production of good quality fish eggs is influenced by environmental factors and aquaculture practices and development of biomarkers to assess egg viability is crucial (Migaud, Bell et al. 2013). Lysozymes (LYZ) are a group of non-specific innate immune enzymes. In vertebrates two main types exists C-type (LYZC) and G-type (LYZG) and they protect against bacterial invasion (Irwin, Biegel et al. 2011). In fish, where innate immunity prevails, LYZ studies are scarce. Multiple forms of LYZ exist in fish and they have been identified in the gills and skin (that are in direct contact with environment) but their role in fish immunity is surprisingly little understood (Saurabh and Sahoo 2008)Fish Hlth Management Div, Bhubaneswar 751002, Orissa, India</auth-address><titles><title>Lysozyme: an important defence molecule of fish innate immune system</title><secondary-title>Aquaculture Research</secondary-title><alt-title>Aquac Res</alt-title></titles><periodical><full-title>Aquaculture Research</full-title><abbr-1>Aquac Res</abbr-1></periodical><alt-periodical><full-title>Aquaculture Research</full-title><abbr-1>Aquac Res</abbr-1></alt-periodical><pages>223-239</pages><volume>39</volume><number>3</number><keywords><keyword>lysozyme</keyword><keyword>innate immunity</keyword><keyword>fish</keyword><keyword>immunostimulants</keyword><keyword>disease resistance</keyword><keyword>trout oncorhynchus-mykiss</keyword><keyword>carp cyprinus-carpio</keyword><keyword>goose-type lysozyme</keyword><keyword>indian major carp</keyword><keyword>dietary vitamin-c</keyword><keyword>flounder paralichthys-olivaceus</keyword><keyword>salmon salmo-salar</keyword><keyword>aeromonas-hydrophila infection</keyword><keyword>plaice pleuronectes-platessa</keyword><keyword>catfish clarias-batrachus</keyword></keywords><dates><year>2008</year><pub-dates><date>Feb 14</date></pub-dates></dates><isbn>1355-557x</isbn><accession-num>WOS:000252707800001</accession-num><urls><related-urls><url><Go to ISI>://WOS:000252707800001</url></related-urls></urls><electronic-resource-num>10.1111/j.1365-2109.2007.01883.x</electronic-resource-num><language>English</language></record></Cite></EndNote>. In this study we characterized LYZ gene evolution and function in fish. To assess the functional importance of LYZ in early developmental stages we determined enzyme activity and expression in important Mediterranean aquaculture species.

Materials and methods

In silico analysis: Homologues of human LYZG (LYZG1, ENSG00000144214: LYZG2, ENSG00000185674) and LYZC (ENSG00000090382) were retrieved from available fish databases (www.ensembl.org and https://www.ncbi.nlm.nih.gov/). Amino acid sequences were aligned and phylogenetic trees constructed using the BI (Bayesian Inference) method. *Functional studies*: Total RNA (tRNA) from sea bream (*Sparus aurata*) eggs (n=10 pools, Greece) was extracted and cDNA synthesized from 500ng genomic DNA-free tRNA and qRT-PCR reactions performed with the SsoFast EvaGreen Supermix (Bio-Rad, Portugal). LYZ activity was measured in egg protein extracts from: sea bream, sargo (*Diplodus sargus*) and meagre (*Argyrosomus regius*) (IPMA, Portugal). Total protein was extracted from 10 mg egg mixture (at least n=5 samples) from before hatch, hatch and after hatch with PBS pH 6.2 (0.25 mg of tissue $\cdot ul^{1}$ PBS). Assays were performed in duplicate using 20 µl of the protein extract diluted 1:2 in 0.05M PB, pH 6.2. Statistical analysis was performed using SPSS 25.0 two-way ANOVA.

Results

In fish LYZ gene members are variable and multiple members of the same family are present in some teleost species (Figure 1).

Phylogeny revealed that fish LYZ shared common ancestry with tetrapods but they evolved differently and species-specific gene duplicates exist suggesting functional divergence may have occurred. Only LYZG was detected in sea bream eggs by qRT-PCR. The overall LYZ enzyme activity was similar across the different egg stages but significant differences ($P < 0.05$) were found between the sea bream, sargo and meagre) (Table 1).

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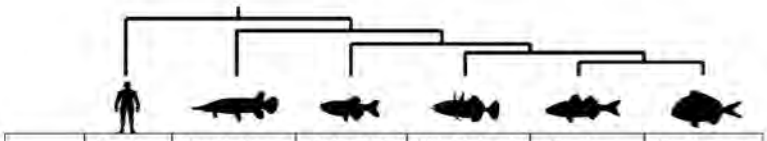

	Human	Spotted gar	Zebrafish	Stickleback	Sea bass	Sea bream
LYZC	1	1	2	1	2	1
LYZG	2	ni	3	1	3	4

Fig 1. LYZ members in ray finned fishes and human. ni: not identified.

pTable 1. Lysozyme activity (U/mg) during fish early development.

	Before hatch	Hatch	After hatch
Sargo[ab]	0.11±0.04	0.18±0.08	0.13±0.07
Meagre[c]	0.23±0.11	0.29±0.18	0.26±0.12
Sea bream[ac]	0.19±0.08	0.15±0.08	0.10±0.09

Discussion and conclusion

A diversity of LYZs members exist and gene mapping revealed that evolution was complex and resulted from lineage-specific and species-specific tandem gene duplications. In different stages of hatching LYZ gene expression varied. Egg LYZ activity differed between sea bream, sargo and meagre and overall our data appears to suggest that the functional importance of this enzyme complex is species-specific and may have been shaped by their habitat. The importance of egg lysozyme for egg quality is currently under investigation.

Acknowledgments: This work received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement no 727610 (PerformFISH) and Portuguese national funds from FCT - Foundation for Science and Technology through project UID/Multi/04326/2019.

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GENETIC PARAMETERS FOR WHITE SPOT SYNDROME VIRUS (WSSV) RESISTANCE IN *Litopenaeus Vannamei* SHRIMP

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Introduction

White spot syndrome virus (WSSV) is among the most damaging diseases in global shrimp aquaculture regarding production and economic losses (Sánchez-Paz, 2010). Resistance to WSSV in *L. vannamei* has previously been reported to have low but significant heritability in adult shrimp (Gitterle et al., 2006; Caballero-Zamora et al., 2015) and moderate heritability in juveniles (Trang et al, 2019). Selective breeding is therefore one method that is being used to increase WSSV resistance (Cock et al., 2015). Genomic selection has been predicted to be a powerful and accurate way of selecting among non-infected candidates based on challenge test results from relatives and estimated genomic relationships.

Two shrimp populations were used in this study, one line, "Tumaco", which had been exposed to WSSV infections for several generations and hence naturally selected for survival against WSSV, and a second line, "Cartagena", which had been kept in a pathogen free environment for generations and not exposed to or selected for WSSV resistance in the last 10 generations. The aim of this study was to compare purebred shrimp from the line already selected for resistance with crossbreds between the two lines, with regard to resistance and to estimate genetic parameters for WSSV resistance using genomic relationships.

Materials and Methods

A challenge test was performed with 751 pure Tumaco (T) and 696 crossbred Tumaco-Cartagene (TxC) shrimp randomly divided in two 4 ton tanks, using artificial seawater at 30 ppt salinity and 26^{o}C temperature. Juvenile shrimp of average weight 3g were infected with WSSV in a challenge initiated April 18th 2018, using minced infected tissue at 3 % biomass. Mortalities were recorded daily until May 10th. 12 days post infection, the shrimp were pooled into one single tank to avoid loss of infection pressure due to reduced shrimp density. Infection was confirmed by histopathology and PCR. All the shrimp in the study were genotyped for 18,643 SNPs. Based on the genotypes, a genomic relationship matrix between all the animals was estimated. Genetic parameters were estimated for survival, measured as a binary dead or alive trait during the trial and as days of survival. For the latter, all survivors were assumed to die the day after the trial ended. Genomic breeding values were estimated for both traits and a 10-fold random cross validation was used to estimate the accuracy of the genomic breeding values (correlation between estimated breeding values and phenotype divided by the square root of the heritability).

Results

The first mortality occurred April 19th, and peak mortality was observed from April 23rd through April 26th, approximately 5 days post challenge. Accumulated mortality for the whole experimental period was 888, corresponding to 61 % of the tested animals, 46 % of the purebreds and 76 % of the crossbreds.

Heritabilities of binary dead or alive and days survival traits were 0.30±0.05 and 0.55±0.05, respectively. Accuracy of genomic breeding values for the two traits were estimated to 0.69 and 0.64, respectively. The best linear unbiased estimated difference between the purebred T shrimp and the crossbred with regard to probability of surviving the test was 0.19, while the estimated advantage of purebred T in number of days until death was 2.71 days. Separate analysis of the purebred and crossbred animals did not reveal significant differences in genetic parameters between the purebred and the crossbred, and accuracy of selection decreased, probably because of the reduced size of the reference population when dividing the data in two.

Discussion and Conclusion

The heritability of WSSV resistance using genomic relationship data was shown to be moderate to high and genomic selection would therefore facilitate selective breeding for resistance. This holds regardless of whether resistance was measured as days survival or as a binary dead or alive trait. Accuracy of selection was high in both cases, indicating that the

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reference population, which consisted of 90 % of the animals in the cross validation test, was of sufficient size to estimate genomic breeding values for these traits. Selection of candidates based on information from challenge tested full sibs and genomic information is therefore expected to give genetic gain, dependent on selection intensity. The highest gain is expected if selection is based on days survival, because of the higher heritability of this trait.

The higher heritability found in this study as compared to other comparable studies (Gitterle et al., 2005; Trang et al., 2019) could be due to population characteristics or due to test characteristics. Mortality rate was lower and happened slower than in many other WSSV challenge test studies (Gitterle et al., 2006). This could be due to high WSSV resistance in this combined population, introduced by the Tumaco line. It could also be due to lower virulence or lower pathogen load in the present test as compared to the earlier trials.

The estimated difference in resistance between the purebred Tumaco line and the Tumaco x Cartagena crossbreds was significant, but relatively small, as compared to what should be expected based on the selection history of the respective lines and the heritability of resistance. This may suggest that the resistant genotype is partially dominant, causing crossbred animals to show a resistant phenotype. Alternatively, it could be that historical links between the populations has caused the resistant genotype to exist also in the Cartagena line, even without a selective advantage within this population or a heterosis effect from crossing the two populations. The similar genetic parameters obtained when analyzing purebred and crossbred animals separately (results not shown) indicates, however, that genetic variation for resistance has been maintained also in the purebred Tumaco line, despite a selection history with strong natural selection for increased WSSV resistance.

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FITNESS EFFECTS OF INTROGRESSION OF ESCAPED FARMED SALMON INTO WILD POPULATIONS

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Introduction

Every year, thousands of farmed salmon escape from Norwegian Aquaculture sites into the wild. These have been shown to have low spawning success and poor survival (Fleming et al., 1996), but still, extensive documentation exists that a fraction of the escaped salmon manages to interbreed with wild relatives and cause introgression of domesticated material into wild populations (Glover et al., 2013). Phenotypic changes due to introgression has been documented (Bolstad et al., 2017), which is likely to have negative impact on fitness and viability of the wild populations.

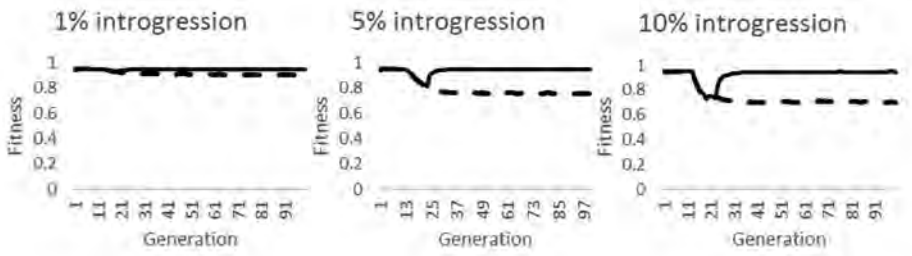
The aim of this study was to quantify the fitness consequences of introgression of farmed salmon into wild populations using stochastic simulations.

Materials and Methods

The software Nemo was used for stochastic simulations of individuals and their genomes. Fitness was modeled as a Gaussian function of one quantitative trait, which was controlled by 80 independent genes with Mendelian inheritance. A simple life cycle, consisting of mating and offspring generation, viability selection, straying and reduction to patch capacity was simulated for 2000 generations to obtain mutation-drift-selection balance. The wild population consisted of five subpopulations with 100-800 parents per generation in each subpopulation, 5% random straying between the subpopulations and slightly different environments between the subpopulations. Strength of natural selection was estimated based on the decline in wild survival for domesticated salmonids found in Araki et al. (2007). In addition, two levels of relaxed strength of selection were applied, to account for possible estimation errors due to confoundance between genetic and environmental effects and to test the sensitivity of the model for this parameter. Founders of the farmed population were sampled from all of the 5 subpopulations over two generations and domestication mimicked by running natural selection towards an extreme optimum. After 10 generations of domestication in the farmed population, farmed fish was migrated into the wild sub-populations to mimic escapees. Three levels of introgression, 1%, 5% and 10%, were tested, which corresponds to rivers with approximately 5%, 20% or 50% farmed salmon present. Introgression was then either kept constant for 85 generations, to study the long term effects of introgression that happens systematically over many generations or it was stopped after 10 generations to study the ability of natural selection to restore fitness in natural populations when introgression is stopped.

Results

Figure 1 shows the development in fitness assuming the strong natural selection as estimated from Araki et al (2007), and continuous introgression of 1%, 5% or 10%, respectively. The figure shows fitness for a river with 800 parents every generation, and results were very similar for smaller river sizes and different phenotypic optimum. Introgression started in generation 13 and lasted until generation 23 (solid line) or for ever (dotted line). The latter resulted in 5%, 20% or 30% loss of fitness in the wild populations, dependent on level of introgression (Fig 1). When introgression stopped after 10 generations, rapid recovery repaired 90% of the damage after 2 generations, but around 20 generations were needed for full recovery of all rivers. With relaxed natural selection (results not shown), rate of reduction in fitness was smaller but the asymptotic loss of fitness was higher (5%, 30% and 50%, respectively for the three levels of introgression). Rate of recovery was also slower with relaxed selection.



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Discussion and Conclusion

The results from the simulations support that systematic introgression over time reduces the fitness of the wild populations, and that the changes are reversible when introgression stops. The model used was not sensitive to river population size, fecundity or straying rate, including straying rates from 1% to 5% (results not shown).

The simulated life cycle was simple, with only one round of viability selection per generation. Density dependent or life stage specific selection, as well as non-genetic effects of farmed escapees were ignored. These limitation may cause under estimation of the damage caused by farmed escapees.

Regardless of strength of natural selection, 1% introgression per generation was shown to be negligible, but higher levels of introgression caused significantly reduced fitness. This is in concordance with findings from Castellani et al. (2015) who modeled a more complex life cycle using a different fitness and genetic model. However, Castellani et al. (2015) found that stronger natural selection, i.e. lower survival of escapees, led to a higher loss of fitness, which is opposite from other study, probably due to differences in model assumptions.

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AMINO ACIDS AS FEEDING STIMULANT IN THE DEVELOPMENT OF SOYBEAN-BASED DIET FOR JUVENILE GROUPER (*Epinephelus fuscoguttatus*)

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Introduction

Groupers are carnivorous species and require high dietary protein for optimum growth. Thus, the expensive fish meal (FM) based-compounded feeds have been commonly used to farm groupers. From previous studies, soybean meal (SBM) protein has been proven practical to partially replace the FM protein in juvenile groupers diets. However, high dietary SBM protein inclusion levels can reduce the diets palatability and causes poor intake and fish growth (Lim *et al*., 2014). Dietary supplementation of feeding stimulants (FS) can solve this problem but information on the suitable FS for groupers is limited. Amino acids are common FS for fish but fish taste preference for amino acids is species-specific (Kasumyan and Døving, 2003). Therefore, the present study was aimed to identify the amino acids which are preferable by the juvenile grouper *Epinephelus fuscoguttatus* through behavioral assays (video recording), and a feeding trial was conducted to evaluate the potential of the selected amino acids as FS for the fish.

Materials and methods

In behavioral assays, agar gel pellet was used as the medium to deliver 19 amino acids to the fish (BW 22.9 g). The pure agar gel (PAG) and feed extract (FE) pellets were used as the negative- and positive-control, respectively. From the recorded videos, two parameters were observed: (I) the pellet was consumed or rejected [A] – if consumed, recorded 1; if rejected, recorded 0, and (II) frequency of the pellet been captured before it was consumed or rejected and ignored [B], and the preference index was calculated through [A] /[B] (Lim *et al*., 2016). The amino acids with high preference index were then supplemented (1.0%) into the SBM diet to evaluate its potential as FS through an 8-week feeding trial. In this feeding trial, one hundred eighty three *E. fuscoguttatus* (BW 15.6 ± 0.2 g) were distributed evenly into 15 floating cylinder cages (12 individuals per cage) placed randomly in 2 fibe glass tanks (3 tonnes each) that were provided with constant flow-through sea water and aeration. Three dietary treatments were prepared, namely the Control (FM protein-based), SBM40 (40% FM protein replaced with SBM protein) and AAM10 (SBM40 supplemented with 1% selected amino acids mixture), and each diet was hand-fed to triplicate cages of fish twice daily (approximately 0800 and 1400) until apparent satiation level. The uneaten feed was counted and mortality was recorded daily. After 8-week, all fish were anesthetized and measured individually for their total length and body weight. The fish body weight gain (WG), survival rate (SR), and feed intake (FI), were calculated using the following formula: WG (%) = (Final – Initial fish weight)/ Initial fish weight ×100; SR (%) = (Final fish number/ Initial fish number) × 100; FI (g/ fish) = (Total given feed – Total uneaten feed)/ Fish number.

Results

In the behavioural assays, the PAG pellet was totally rejected by the fish (index's value = 0). Among the 18 amino acids tested, only 6 amino acids were found acceptable to fish (index's value ranged from 0.07 to 1.00, see Fig. 1). From the feeding trial, the FI of AAM10 was found significantly higher ($P < 0.05$) than that of SBM40 (Fig. 2a). Fish fed AAM10 diet also attained the higher WG and SR than SBM40 but no significant difference ($P > 0.05$) was noticed. However, the FI, WG and SR of fish fed AAM10 were not comparable to those fed with the Control diet.

Discussion and conclusion

The amino acid spectrum that suited the taste preference of the grouper was considered narrow as only 6 out of the 19 amino acids were accepted, and only 1 was fully ingested by them. In the previous study by Kasumyan and Døving (2003), brown trout (*Salmo trutta caspius*) accepted 6, tench (*Tinca tinca*) can accept 12, while chub (*Leuciscus cephalus*) rejected all of the 21 amino acids tested. Apparently, present study results were in agreement with the finding by Kasumyan and Døving (2003) that fish taste preference for amino acids is species-specific. From the feeding trial in the present study, although the FI, WG and SR of fish fed AAM10 were not comparable to those fed with the Control diet, the positive effective of the AAM as FS for the grouper was confirmed as AAM dietary supplementation has significantly improved the fish intake of SBM diet. In conclusion, amino acids are FS for the grouper and the selected one can be used to improve their feed intake of SBM diet. Further study should be conducted to analyze the cost of AAM supplementation in the diets for grouper juveniles.

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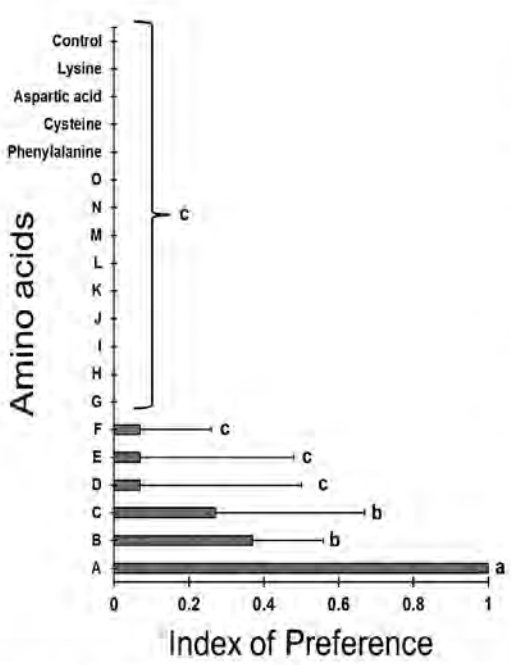

Fig. 1. Preferences of the *E. fuscoguttatus* for the 19 amino acids. Amino acids names were hidden (A – O) as this information has been protected as a trade secret of the university.

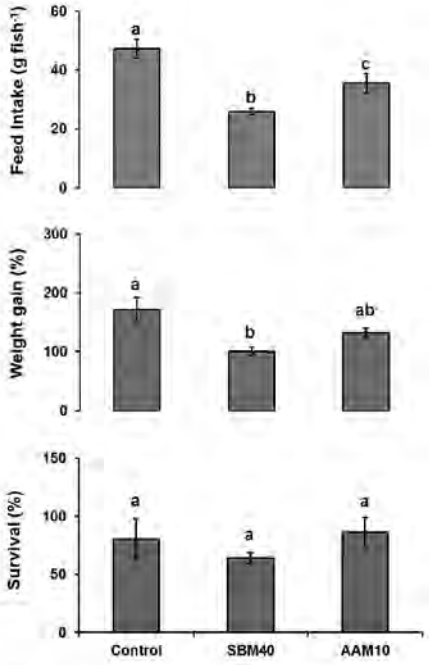

Fig. 2. (a) Feed intake, **(b)** weight gain, and **(c)** survival ratio of the *E. fuscoguttatus* juveniles fed with the experimental diets.

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CULTIVATION OF A GREEN MICROALGA *Tetraselmis sp.* IN THE OCEAN USING FLOATING PONDS WITH SEMI-PERMEABLE MATERIALS

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Introduction

Phosphorus, an essential element for microalgal biomass production, is a non-renewable, finite resource produced from underground reserves. For sustainable large-scale cultivation of microalgae, phosphorus should be provided from sustainable sources (Park and Lee, 2016). The ocean has a very large reserve of phosphorus along with other nutrients needed for microalgae. However, their concentrations are too low to use as a culture medium. We implemented semi-permeable materials (SPMs) that are permeable to nutrients in seawater but not to microalgae cells to enable microalgal cultivation utilizing nutrients in seawater. In the SPM culture systems, phosphorus supply rate was the major limiting factor for the biomass productivity (Kim et al., 2016a). In this study, we developed SPM culture systems with higher phosphorus supply rate to enhance microalgal biomass productivity.

Materials and methods

A green microalga, *Tetraselmis* sp. MBEyh01L, used in this study was locally isolated from the offshore ocean test-bed of Marine Bioenergy R&D Consortium located in the nearshore of Younghueng Island, Incheon, Korea. Various SPMs were designed and customized by the authors and constructed by local manufacturers. Lab-scale floating ponds could hold up to 12 L of algal culture, and ocean-scale floating ponds could hold up to 12 kL of algal culture volume with 16 m^2 of culture area. Lab-scale experiments were performed in raceway ponds containing 8 kL of seawater in a greenhouse, located on the land adjacent to the ocean test-bed. Ocean experiments were conducted in the ocean test-bed, consisted of cube-shaped pontoons for human access.

Results and Discussion

Properties of SPMs, cell and ion permeabilities, greatly varied by configurations of SPMs. By optimizing weaving pattern and configurations of SPMs, ion permeability was increased by 727%. The actual biomass productivities from SPM-equipped floating ponds were similar with the theoretical biomass productivity calculated using the phosphorus supply rate and average phosphorus content of *Tetraselmis* sp., indicating that phosphorus supply rate was still the productivity limiting factor (Fig. 1).

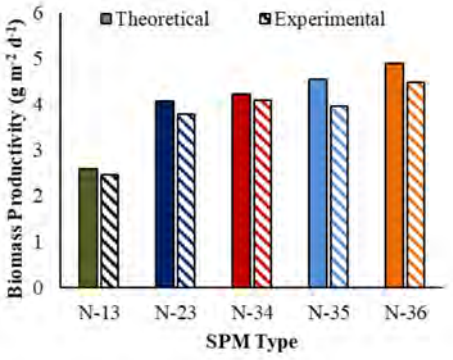

Fig. 1. Theoretical and Experimental Biomass Productivities by SPM Type

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Biomass productivity in the floating ponds equipped with the latest SPMs was improved by 971% compared with the early SPM culture systems used in the previous study (Kim et al., 2016b). In order to increase biomass productivity further from the level by passive diffusion, forced convection was implemented to the SPM culture systems, which could increase biomass productivity to 5.5-fold compared with the culture depended on diffusion. In the laboratory scale, up to 18.6 g m^{-2} d^{-1} of biomass productivity was achieved by supplying nutrients in the seawater to SPM Ponds. In the ocean scale, using floating SPMs ponds with forced convection, the SPMs ponds showed the similar level of biomass productivity to the cultures with concentrated nutrients, achieving up to 13.5 g m^{-2} d^{-1} of biomass productivity.

Conclusions

This study demonstrated that microalgal biomass could be produced using nutrients in seawater with SPM ponds. With enhancements in other aspects of microalgal cultivation, such as culture mixing by natural energy, pond design, operating parameters, *etc*. SPM culture technology would contribute to realization of sustainable mass production of microalgal biomass.

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WATER QUALITY IN RECIRCULATING AQUACULTURE SYSTEM WITH PASSIVE WATER TREATMENT RAISING RAINBOW TROUT *Oncorhynchus mykiss*

P. C. Lindholm-Lehto[a*], J. T. Pulkkinen[a,b], T. Kiuru[a], and J. Vielma[a]

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Introduction

In a conventional recirculating aquaculture system (RAS), the external water exchange is adjusted based on the concentration of nitrate in the system (Martins 2010). To reach a smaller inlet water requirement, the amount of nitrate must be reduced to acceptable level by denitrification. Denitrification is the process transforming nitrite and nitrate to reduced elemental nitrogen gas, but requires an external carbon source. Wood-based material is readily available as carbon source for denitrificati n (Schipper et al. 2010). As the water flows passively through the woodchip reactor, oxygen is removed due to bacterial metabolism, leading to anoxic environment suitable for denitrification (Warneke et al. 2011). However, woodchips can contain various compounds toxic to raised fish species (Billiard et al. 1999; Oikari et al. 2002). This study aims at evaluating a passive denitrification process in RAS, and developing analytical methods to quantify possibly toxic compounds towards the raised species.

Materials and methods

A new process design of a passive water treatment system was connected to a RAS. A more detailed description of the experimental RAS facility has been reported by Pulkkinen et al. (2018). In brief, the experimental RAS consisted of a 500 l fish tank, separate water treatment and water quality control systems. Two similar systems and side-loops with passive water treatment (small loop 25 l d^{-1}, large loop 40 l d^{-1}) included both a woodchip reactor filled with silver birch (*Betula pendula*) woodchips (< 5 cm), aiming at 95% denitrification efficiency with a 1.5 day delay, and a sand filter with an effective porosity of 0.35, 80% saturation, and delay of 3.5 days. Rainbow trout *Oncorhynchus mykiss* was fed at 0.1 kg d^{-1}, increasing in weight from 13g to 44g during the experiment. Denitrification was monitored by following nitrate levels after the side-loop reactors by a spectrophotometer (Nessler procedure 8038, DS 3900, Hach).

New analytical methods were developed and validated to study the fatty acid concentrations released from the woodchip reactor and to evaluate if they posed toxic effects towards the raised species. Fatty acids were extracted by liquid-liquid extraction (LLE) with MTBE, derivatized, and analyzed with GC-FID (Shimadzu GC-2010/FID). The analytes were identified by an Agilent 6890 series/5973 N GC/MSD under EI (70 eV) with [NIST] mass spectral library. For the elemental analyses, a microwave acidic digestion of the circulating water was performed according to US EPA 2008, method 3015. The analyses were conducted with a Perkin-Elmer (Optima 8300, Norwalk, CT, USA) ICPOES. Optimal analytical wavelengths were measured at (nm): Ca (315.887), K (766.490), Mg (279.077), and P (177.50).

Results

In the beginning of the experiment, denitrification rates ranged up to 85 %, but decreased later down to 40 %. This suggests that nitrogen load was excessive for the denitrification system and requires improved dimensioning. Certain fatty acids were detected in the circulating water, somewhat higher in the system with the large side-loop (Fig. 1). In the beginning, up to 2mg L^{-1} concentrations were found, but decreased rapidly to below 0.5mg L^{-1} levels. Similarly, increased levels of selected macro elements were found originating from the birch wood, especially potassium (Fig. 2). However, the concentrations settled rapidly to below 20mg L^{-1} for K and below 10mg L^{-1} for K, Mg, and P.

Discussion and conclusions

The results show that birch woodchips act as a carbon source for the denitrification. Overall, the concentrations of compounds accumulating into the system were low. Additionally, the developed analytical methods were shown suitable for analyses of the selected compounds or elements in aqueous matrices.

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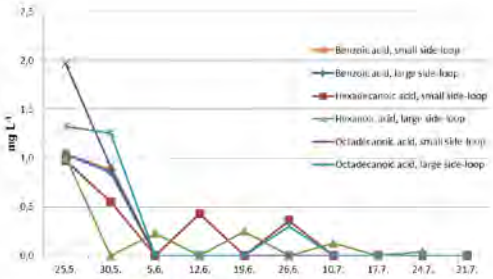

Fig. 1. Concentrations (mg L^{-1}, n=4) of benzoic acid, hexadecanoic acid, and octadecanoic acid in the circulation water (small and large side-loops) after the woodchip reactor during the 10 weeks of experiment.

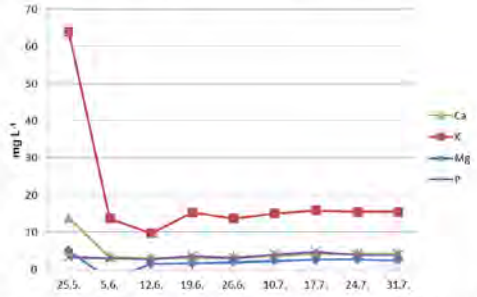

Fig. 2. Concentrations of calcium (Ca), magnesium (Mg), phosphorous (P), and potassium (K) (mg L^{-1}, n=4) in the circulating water after the woodchip reactor during the 10 weeks of experiment.

References

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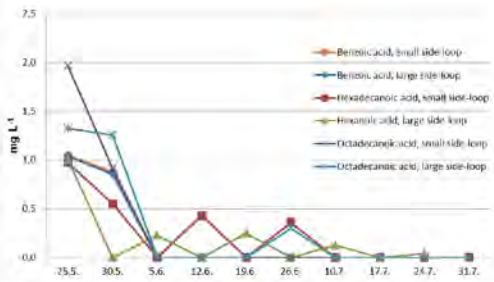

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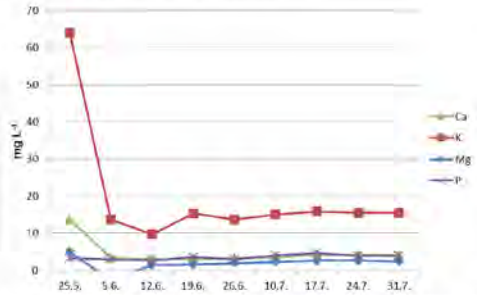

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TOXICITY OF PERACETIC ACID (PAA) PRODUCTS: IMPACT OF WATER QUALITY, PRODUCT COMPOSITION AND FISH SPECIES

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Background

Prophylaxis with peracetic acid (PAA) in aquaculture is a promising sustainable alternative. However, successful prophylaxis needs to consider composition of PAA products (ratio of PAA to hydrogen peroxide [H_2O_2]), fish production conditions (water parameters, culture system and management), cultured species and development stage of the fish. These parameters simultaneously determine an appropriate PAA product and PAA concentration to avoid harming the fish and to ensure prophylaxis success. The presentation will summarize three of our early-stage studies to address: 1) how water parameters affect the degradation of PAA, 2) how composition of PAA products affects their toxicity to aquatic animals and 3) how fish species di fer in their sensitivity to PAA.

Results

In study 1 we investigated the impacts of salinity, water hardness and dissolved organic carbon (DOC) on the degradation of 1 mg/L of three commercial Wofasteril® PAA products: E400, E250 and Lspez. The results showed that salinity and DOC stimulated PAA degradation, while water hardness had only minor impact.

In study 2 we performed 24-h toxicity tests using *Daphnia magna* with the aforementioned PAA formulations. Toxicity to *Daphnia* was greatest for Lspez (high total peroxide/PAA ratio), intermediate for E250 (intermediate total peroxide/PAA ratio), and lowest for E400 (total peroxide/PAA ratio) (Figure 1). An E400 + H_2O_2 mixture, which possessed a composition theoretically identical to the E250 formulation, had toxic effects and 24-h LC50 values similar to those of the E250 product. The results indicate that the toxicity of PAA formulations to *Daphnia* is due to the combined effect of both PAA and H_2O_2, namely the total peroxide.

In study 3 twelve species of fingerling fish that are important to aquaculture were exposed to PAA for 24-h static toxicity bioassays. Median lethal concentration (LC_{50}) values were estimated. The mean 24-h LC_{50} values were species dependent and ranged from 2.8 to 9.3 mg/L PAA. More importantly, the 24-h no-observed-effect concentration (NOEC) ranged from 1.9 to 5.8 mg/L PAA. PAA was more toxic in water with lower alkalinity and hardness, while a small increase of dissolved organic content had no effect on PAA toxicity.

Conclusion

Our early studies confirmed the complex influences of several biotic and abiotic factors on the toxicity of PAA. For this reason, strategies for successful prophylaxis with PAA in aquaculture facilities should be customized according to the onsite situation. In future studies we aim to quantify the impacts of these factors with a mathematic model and establish an easy-to-use tool for the customized use of PAA for prophylaxis in aquaculture systems.

Related publications

Liu, D., Steinberg, C.E.W., Straus, D.L., Pedersen, L.-F., Meinelt, T., 2014. Salinity, dissolved organic carbon and water hardness affect peracetic acid (PAA) degradation in aqueous solutions. Aquacultural Engineering 60: 35-40.

Liu, D., Straus, D.L., Pedersen, L.-F., Meinelt, T., 2015. Comparison of the toxicity of Wofasteril peracetic acid formulations E400, E250, and Lspez to *Daphnia magna*, with emphasis on the effect of hydrogen peroxide. North American Journal of Aquaculture 77: 128-135.

Straus, D. L., Meinelt, T., Liu, D. and Pedersen, L.-F., 2018. Toxicity of peracetic acid to fish: variation among species and impact of water chemistry. Journal of the World Aquaculture Society, 49: 715-724.

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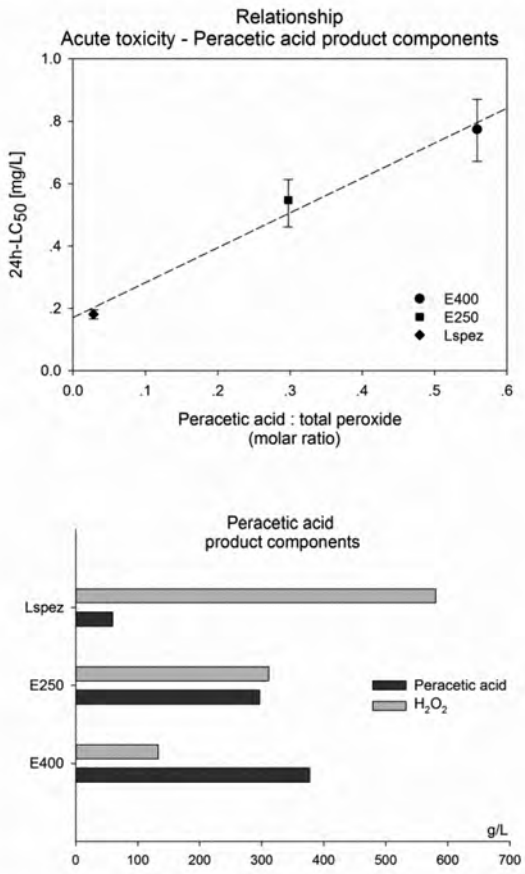

Figure 1 Relationship between the 24-h acute toxicity and the molar ratios of peracetic acid: total peroxide (peracetic acid and hydrogen peroxide) of three commercial PAA products (trade name: Lspez, E250 and E400).

MOLECULAR INSIGHTS AND IMMUNE RESPONSES OF BIG-BELLY SEAHORSE (*Hippocampus abdominalis*) SYNDECAN-2

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Department of Marine Life Sciences and Fish Vaccine Research Center, Jeju National University, Jeju Self Governing Province 63243, Republic of Korea.
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Introduction

Syndecans are the earliest family of transmembrane receptor proteoglycans. They can be found in the extracellular matrix, cell surface, tissue compartment, and nucleus. Proteoglycans essential for development and function of the immune system, central nervous system skeleton of vertebrates. Syndecans contain six domains; ectodomain, co-receptor binding domain, a transmembrane domain, conserved (C1 domain, C2 domain), and variable (V) domain. The soluble syndecan ectodomains are involved in inhibiting growth factor, enhance proteinase activities, inhibit cell proliferation, wound healing and inhibit mast cell migration in teleost syndecans.

Big-belly seahorse (*Hippocampus abdominalis*) is an essential seahorse species, and they were used in oriental medicine, ornamental purposes and as food. Hence studying of seahorse immunity upon various immune stimulants provide a better understanding of teleost immunity.

Materials and methods

Here we characterized syndecan-2 (HaSDC2) from seahorse transcriptomic database and analyzed the tissue-specific expression and temporal transcription in against poly I:C, Lipopolysaccharides, and *Edwardsiella tarda* and *Streptococcus iniae*.

Results and discussion

The coding sequence of HaSDC2 is 798 bp long and encode a protein sequence with 265 amino acids. HaSDC2 protein consisted of 28.9 kDa molecular weight and 4.13 theoretical isoelectric point. Further, HaSDC2 showed the highest identity with *Hippocampus comes* (94.34%). According to the tissue-specific expression, HaSDC2 highly expressed in the liver (Fig1) due to Syndecan-2 is one of the most important proteoglycans in the liver, and it normally expressed on the surface of hepatocytes. Further, blood HaSDC2 showed significant transcriptional modulation towards all the immune stimulants (Fig2). Because due to the Syndecan ectodomain released to the extracellular matrix to protect the cells by regulating growth factor binding (Chen et al., 2004), cell proliferation (Park et al., 2002) and wound healing (Worapamorn et al., 2002) growth factors and a variety of other effector molecules. Accordingly, these molecules play a central role in various aspects of cell-cell and cell-matrix interactions. To investigate the expression and distribution of the cell surface proteoglycans, syndecan-1 and -2, during periodontal wound healing, immunohistochemical analyses were carried out using monoclonal antibodies against syndecan-1, or -2 core proteins. Both syndecan-1 and -2 were expressed and distributed differentially at various stages of early inflam atory cell infiltration, granulation tissue formation, and tissue remodeling in periodontal wound healing. Expression of syndecan-1 was noted in inflam atory cells within and around the fibrin clots during the earliest stages of inflammatory cells infiltration. During granulation tissue formation it was noted in fibroblast-like cells and newly formed blood vessels. Syndecan-1 was not seen in newly formed bone or cementum matrix at any of the time periods studied. Syndecan-1 expression was generally less during the late stages of wound healing but was markedly expressed in cells that were close to the repairing junctional epithelium. In contrast, syndecan-2 expression and distribution was not evident at the early stages of inflammatory cell infiltration. During the formation of granulation tissue and subsequent tissue remodeling, syndecan-2 was expressed extracellularly in the newly formed fibrils which were oriented toward the root surface. Syndecan-2 was found to be significantly expressed on cells that were close to the root surface and within the matrix of repaired cementum covering root dentin as well as at the alveolar bone edge. These findings indicate that syndecan-1 and -2 may have distinctive functions during wound healing of the periodontium. The appearance of syndecan-1 may involve both cell-cell and cell-matrix interactions, while syndecan-2 showed a predilection to associate with cell-matrix interactions during hard tissue formation.”,”author”:[{“dropping-particle”:””,”family”:”Worapamorn”,”given”:” W”,”non-dropping-particle”:””,”parse-names”:false,”suffix :””},{“dropping-particle”:””,”family”:”Xiao”,”given”:”Y” ,”non-dropping-particle”:””,”parse-names”:false,”suffix :””},{“dropping-particle”:””,”family”:”Li”,”given”:”H”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:” oung”,”given”:”W G”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Bartold”,”given”:” M”,”non-

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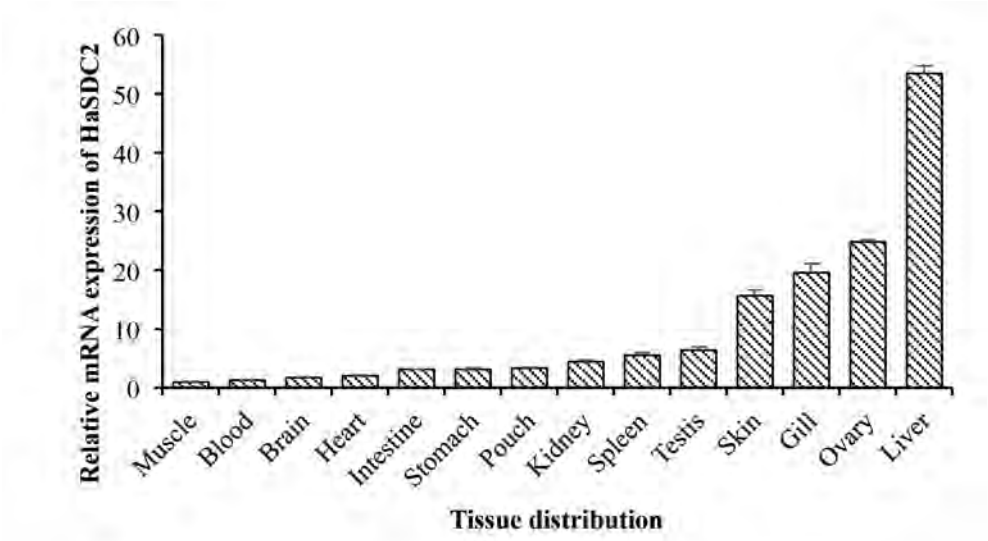

Fig1: Spatial expression profile of seahorse syndecan 2 in different tissues.

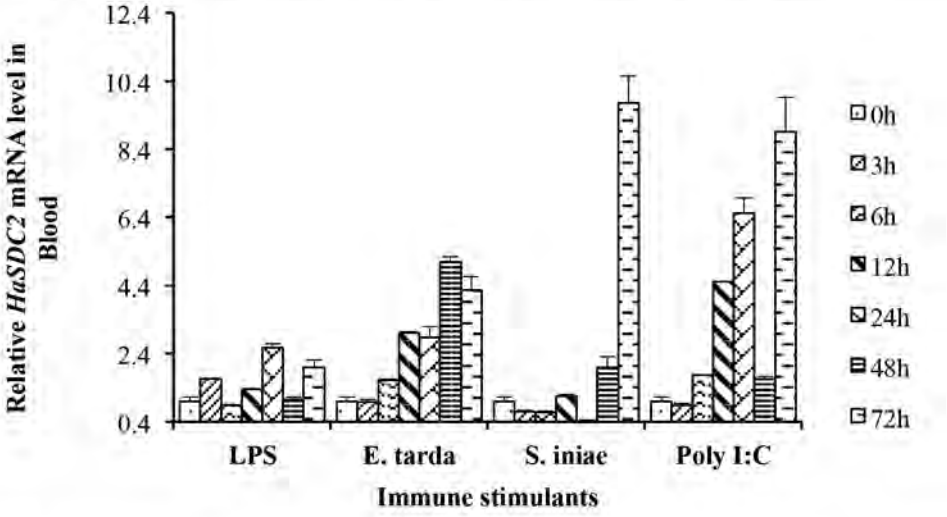

Fig2: Temporal mRNA expression of HaSDC2 in blood from seahorses challenged with different immune stimulants.

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Conclusion

According to the results it can be suggested that HaSDC2 is an immunologically important gene in seahorses.

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THE EFFECT OF DIFFERENT HORMONAL INDUCTION STRATEGIES ON PIKEPERCH (*Sander lucioperca* L.) GAMETE QUALITY OBTAINED AFTER OUT-OF-SEASON ARTIFICIAL REPRODUCTION

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Introduction

Seasonal artifici l reproduction is well documented in pikeperch and to date published protocols are applicable in hatchery practice all over the Europe (Zakęś and Demska-Zakęś 2009; Żarski et al. 2012). Nevertheless, hormonal induction in individuals which were induced to spawn in fully controlled conditions is still of unpredictable outcome. Therefore, the present study aimed to evaluate the effect of different hormonal preparations (gonadotropin vs. gonadoliberin) at different temperatures (5 °C vs 9 °C) on the spawning performance in males. Likewise, the effect of gonadoliberin dosage (5 µg vs. 50 µg) on the egg quality was additionally assessed.

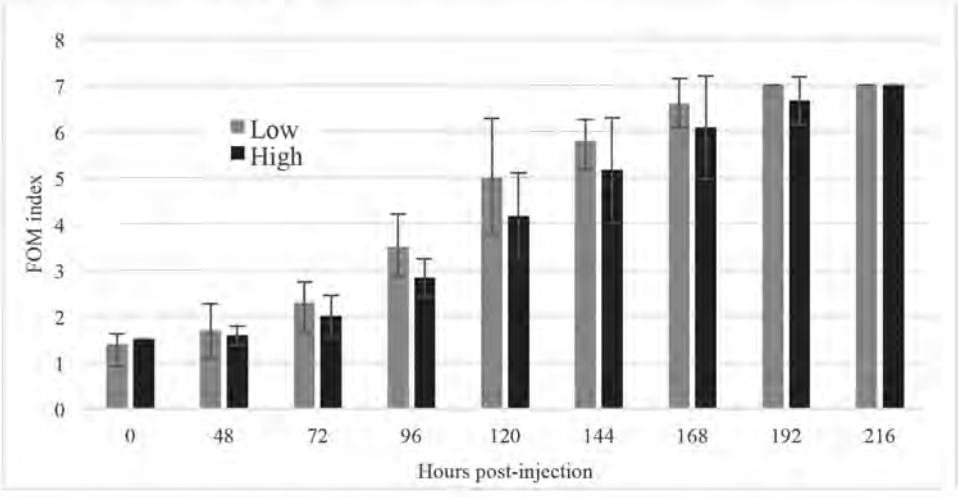
Materials and Methods

Following artificial reproduction, three-year-old-breeders were subjected to 5 months of grow-out, 4 months of cooling and 2 months of chilling and finally warmed in a week for one degree per day. At the start of warming week at 5 °C, two groups of males were injected with different hormonal preparations and at the end of warming week at 9 °C two additional groups of males were injected with same preparations as well as the control group which was injected with saline solution. A day after the second group of males two groups of females were hormonally treated at 10 °C. Each treatment group consisted of five individuals. Following the injection of females, water temperature was gradually increased to 12 °C in a week. Further artificial reproduction was performed as explained by Ljubobratović et al. (2018). Samples of sperm were taken 48 day-degrees following injection and their quality parameters were evaluated using a CASA system. Eggs of each female were fertilized with milt of two males and stocked into separate 2 L Zug jars for further evaluation of embryo survival, hatching and length of newly hatched larvae.

Results

Although no significant difference was found in males' reproductive performance, all evaluated parameters were highest in the group treated with gonadoliberin at higher temperature. In case of egg quality, significantly higher embryo survival (50.3 ± 7.3 % vs. 17.4 ± 29.1 %, $p = 0.048$) and hatching index (44.3 ± 0.5 % vs. 12.2 ± 20.4 %, $p = 0.031$) were found in females treated with higher dosage of salmon gonadoliberin analog. A more rapid progress of final oocyte maturation was observed in females treated with a lower dosage of salmon gonadoliberin analog (Figure 1).

Fig. 1. Final oocyte maturation (FOM) progress upon different dosage of hormonal injection (5 and 50 µg kg^{-1})



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Discussion and Conclusion

Salmon gonadoliberin administered at the higher temperature led to the highest spermiation response where 4 out of 5 injected males spermiated. In case of sperm quality parameters, these did not differ significantly among the groups, although all were in favor of the previously mentioned treatment. It appears that although some progress can be made in terms of sperm quality with different induction strategies, it remains rather tolerant to different preparation and temperatures of application. Unlike sperm, eggs seem to be rather vulnerable to hormonal application, namely the dosages. A previous study dedicated to the same issue (Żarski et al. 2019) found no such effect. However, using marginal dosages, the present study found the specific dose-dependent response of final oocyte maturation to the hormonal induction with salmon gonadoliberin. Application of salmon gonadoliberin at 9 °C can be suggested suitable to obtain high quality sperm in out-of-season pikeperch reproduction. Egg quality improvement can be foreseen with different hormonal strategies and the effect of temperature of application is to be considered for future studies.

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GROWTH RATE AND SURVIVAL IN JUVENILES OF GREY MULLET *MUGIL CEPHALUS* REARED UNDER THREE SALINITY LEVELS

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Introduction

Flathead grey mullet *Mugil cephalus* commonly inhabits tropical and temperate estuaries, where plays an important ecological role and supports fisheries, due to the high quality fish fillet, but also fish roe ("bottarga" in Italian). Mullets are highly euryhaline and tolerate a wide range of salinities (Whitfield et al., 2012), in fact they can be reared in brackish coastal lagoons or even raised in commercial freshwater ponds to improve yield (Cardona, 2000). Thanks to their euryhalinity, mullets have recently become a species of interest for worldwide aquaculture production. The influence of salinity on growth rate must be considered when developing its culture, in order to produce fish of the best quality at the most economical cost.

The growth of euryhaline species is often affected by salinity because the energy used for osmoregulation processes is not available for growth (Cardona, 2000). Therefore, they have an optimum salinity level at which growth rate is higher and osmoregulation cost is lower, which may affect fish distribution in the wild (Blaber, 1997). A study carried out in several inland estuaries of the Balearic Islands (Western Mediterranean) has shown that *M. cephalus* juveniles actively avoid polyhaline and euhaline waters, preferring freshwater and oligohaline waters (Cardona, 2000), even if laboratory experiences taking into account salinity and growth rate are often contradictory (De Silva and Perera, 1976; Rodriguez et al., 1993; Cardona, 2000). In the present work, the effect of three salinities on mullet growth and survival has been tested during the juvenile stage.

Materials and methods

The experiment was conducted in three separated recirculating aquaculture systems, each one composed by three circular, truncated, cone-shaped tanks (300 l volume each), equipped by biologic and mechanical filter and continuous aeration. Five-month old juveniles (N=540, 25±1mm length and 0.19±0.02g weight, mean±s.e.), from the same batch of eggs and reared in intensive conditions, were used in the experiment, 60 individuals per experimental replica. They were transferred into the rearing system containing seawater at 36ppt, and salinity was modified to the experimental salinity levels for one month before the onset of the experiment. The three salinity levels were 0ppt (freshwater, F), 18ppt (brackish water, B) and 35ppt (saltwater, S).

Water parameters such as salinity, dissolved oxygen, temperature were monitored daily. Ammonia, nitrite and nitrate were monitored once per week and 30% water exchange was performed when necessary. Fishes were fed *ad libitum* with artificial dry feed (Skretting).

Survival was monitored at the end of the experiment. Growth was assessed considering mean total length (TL, mm), mean body wet weight (BW, g), Growth Rate (GR, g day-

1), Specific Growth Rate (SGR, % day-1) and Condition Index (CI) for each treatment. Measurements were taken at the start and at the end of the experiment.

Results

Significant differences in terms of survival rate were observed among treatments, with similar survival in F (71±12%, mean±s.e.) and S (69±6%), higher than in B (33±3%). Therefore, F and S showed similar final fish densities. Both TL and BW were higher than the initial values, with no difference among treatments. GR for total length was similar among treatments. Samples from F and S showed similar GR for body weight (0.01±0.00g day-1) and SGR for total length (0.66±0.03 and 0.74±0.02% day-1) but were statistically different in terms of SGR for body weight (1.86±0.08 and 2.28±0.06% day-1). Final CI ranged from 2.95±0.07 in fishes reared in F to 2.55±0.13 in those reared in S.

Discussion

Fresh- and saltwater resulted the most suitable for juvenile survival. Both treatments showed similar growth (except for SGR for body weight). However, the Condition Index was slightly higher in juveniles reared in freshwater, suggesting that a longer trial period could result in a better growth than at higher salinity levels (i.e. marine waters).

Our results disagree with De Silva and Perera (1976), that observed maximum growth efficiency of wild *M. cephalus* juveniles (about 0.1g mean initial BW) at a salinity of 20ppt, and Rodriguez et al. (1993), that observed maximum survival and growth rate in wild juveniles (75-85mm mean initial TL) reared under salinities higher than 8ppt. Contrarily, the results we obtained are more in agreement with Cardona (2000), that described growth performance of wild juveniles (50mm mean initial TL) as negatively affected by salinity levels higher than 5ppt. Nevertheless, it should be considered that the mullet juveniles used in our experiment were born and reared in captivity, and tested during a different life stage compared to the specimens in previous studies.

Survival, growth and CI obtained at the salinity of 0ppt indicate that the rearing of mullet juveniles in freshwater is feasible. The rearing in freshwater could increase the number of suitable spaces available for mullet aquaculture, and could give a considerable boost to the development of mullet aquaculture techniques.

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ADULT ZEBRAFISH AS MODEL ANIMAL FOR THE EVALUATION OF POSSIBLE HEALTH EFFECTS OF SEAWEEDS IN THE DIET

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Introduction

Seaweeds are a renewable and non-traditional raw material that can be used as a feed ingredient to face some of the sustainability challenges in aquaculture production. Seaweeds are rich in nutrients and contain various bioactive compounds, which may have health-promoting effects in both humans and animals. Zebrafish (*Danio rerio*) is an attractive animal model for the investigation of immune and other health-related functions in animals, including fish as well as humans. The tools available for studying the biology of this model are numerous allowing thorough investigations of basic mechanisms. The intestinal wall morphology of zebrafish resembles that of other fish and mammals (Wallace et al. 2005), and most of the main immune cells have been described (Renshaw and Trede 2012). Feeding studies in zebrafish have however up until now mostly been performed in larvae or young individuals, which do not comprise a fully developed immune system and intestinal funtion. As part of a large collaborate project, PROMAC, adult zebrafish was used as animal model to evaluate possible health-promoting effects of two seaweeds: *Saccharina latissima* and *Palmaria palmata*.

Material and methods

Adult zebrafish were fed diets including seaweed products, and evaluated with regards to growth rates, intestinal immune stimulating effects and bacterial microbiota. Both whole products and protein concentrates of the seaweeds were applied and different inclusion levels of seaweed products were tested. As a dietary challenge, diet formulation also included high levels of soybean and purified soy saponin to induce an intestinal inflammatory response. In Atlantic salmon, anti-nutrients of soybean meal is known to induce a pronounced intestinal enteritis (van den Ingh et al. 1991; Krogdahl et al. 2015) and similar responses have also been reported in zebrafish larvae (Hedrera et al. 2013; Coronado et al. 2019). After four weeks of feeding, all fish were additionally challenged with a high cholesterol diet to trigger an inflammatory response based on previous experiment in rats and zebrafish (Progatzky et al. 2014; Arias-Jayo et al. 2018). Transcript levels of immune- and stress-related genes, enteritis markers and sterol metabolism genes were evaluated. Gut morphology was histologically evaluated and bacterial microbiome profile based on 16s rRNA amplicon sequencing was characterized. Possible effects of the seaweeds on tail fin regeneration were additionally approached with a fin clipping challenge. Fin regrowth lengths and regeneration stage was evaluated by macroscopic pictures and histology (Figure 1).

Results and conclusions

With this project, a model for feeding studies in adult zebrafish was established. Both soybean meal and cholesterol ingestion showed a significant effect on the expression of sterol metabolism genes in the intestine comparable to the lipid disorder observed in Atlantic salmon fed diets with high levels of soybean (Kortner et al. 2013). Interestingly, we could not demonstrate a pronounced intestinal inflammatory response after feeding with either soybean meal or cholesterol based on gene expression and histology evaluation. However, gene expression profiling demonstrated that both seaweed species were able to induce a mild modulation of stress and immune-related gene expression in the intestine. These findings suggest the presence of bioactive compounds in the seaweeds products evaluated. Preliminary results show no differences in intestinal microbiota profiles and tail fin regeneration between diet groups. Analyses are ongoing and more results will be presented at the conference.

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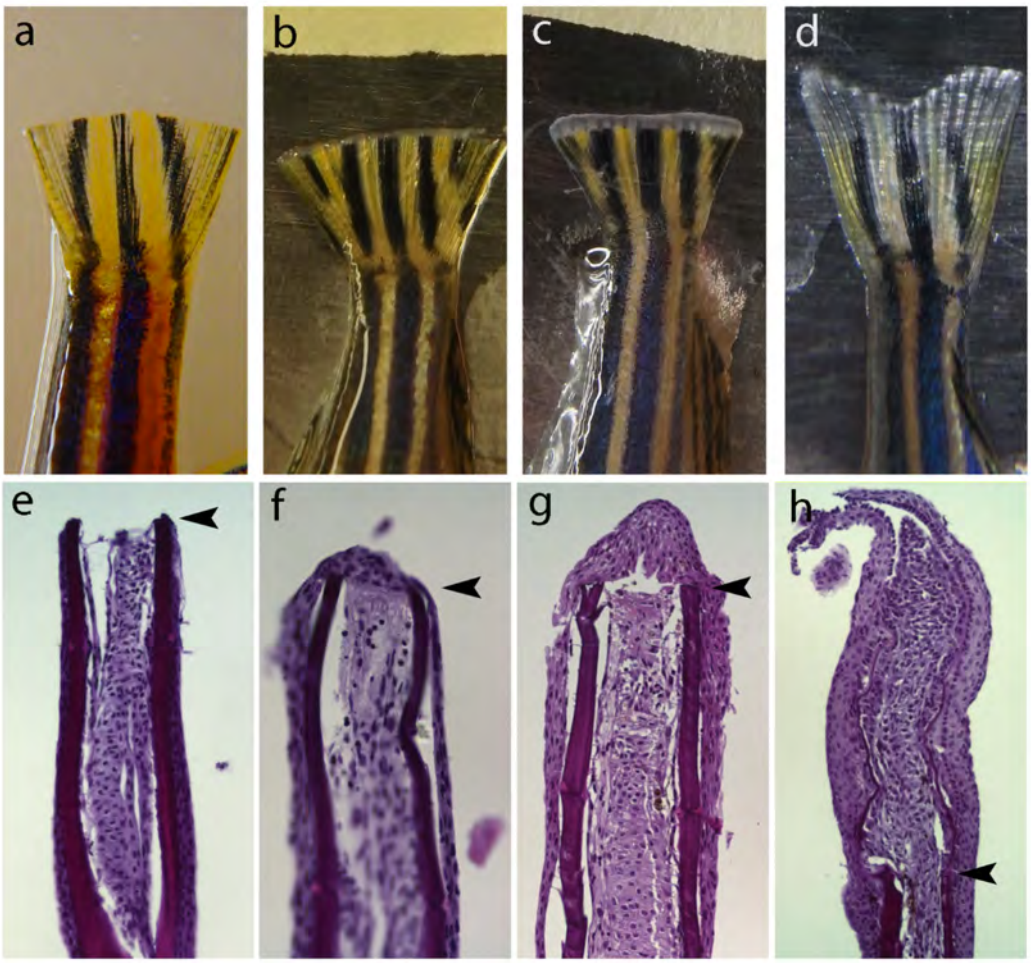

Figure 1: Macroscopic (a-d) and histological (e-h) appearances of tail fin tissue regeneration. Pictures, a and e: Freshly cut fin surface, b, f and g: early appearances of new fin regrowth, c and h: advanced fin regrowth, d: fully regrown fin tissue without pigmentation. Black arrowheads indicate the cutting line.

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MOLECULAR CHARACTERIZATION OF *Vibrio harveyi* STRAINS ASSOCIATED TO DISEASE IN REARED GREY MULLET (*Mugil cephalus*)

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Introduction

Several types of pathogens, embracing parasites, virus or bacteria,have been described in *Mugil cephalus*. Inside species of bacteria that have been described stand out: *Edwardsiella tarda*, *Eubacterium tarantellae*, *Lactococcus garvieae*, *Nocardia seriolae*, *Streptococcus agalactiae* and *Photobacterium damselae* (both subspecies). *Vibrio harveyi* is member of the common microbiota of many marine species, also known as opportunistic pathogen in marine invertebrates (specially inshrimp) and fish, including species of *Mugil* gender. In fact, *V. harvehi* has been described causing haemorrhagic septicaemia in *M. curema* (White mullet) (Alvarez et al. 1998). However, to our knowlegde, *V. harveyi* has not been previously described in *M. cephalus*. A disease outbreak with 20% mortality was observed in *Mugil cephalus* reared in IFAPA Centre Agua del Pino between May and October 2013. This study characterizes *V. harveyi* strains recovered from this disease outbreak.

Material and methods

Microbiological analyses were performed from ulcers, haemorrhagic sky and mouth, and kidney. A *V. harveyi* specific PCR protocol was carried out according Pang et al. (2006). Phylogenetic analyses were computed with MEGA6 software (Tamura et al. 2013) using partial sequences of 16S, *gyr*B, *rpo*D y *rpo*A genes. Fingerprinting of *V. harveyi* isolates was performed with enterobacteria repetitive intergenic consensus (ERIC) PCR and repetitive extragenic palindromic (REP) PCR methods (Rodríguez et al. 2006), including the type species of *V. harveyi*.

Results

Three isolates were recovered from grey mullet samples, namely isolate a385 (from external ulcer), a636 (haemorrhagic mouth) and a661 (kidney), these two last obtained in pure culture. Isolates were recovered in different moments during the outbreak (May, September and October 2013).

The result of the molecular diagnosis described by Pang et al. (2006) showed the expected band for the isolates a385, a636 and a661 and type strain of *V. harveyi*. The phylogenetic trees carried out with the four genes analysed locate the three mullet isolates clearly associated with *V. harveyi*. Multilocus sequence analysis (MLSA) using 16S, *gyr*B, *rpo*A and *rpo*D gene sequences (3701 bp) confirmed these results. Mullet isolates showed 99.48% similarity with *V. harveyi* type strain and 99.42-99.48% between them, but these values were bellow 98% with the type strains of other related *Vibrio* spp (*V. campbellii* 97.39%, *V. owensii* 96%, *V. hyugaensis* 94.92%, *V. rotiferianus*94.16%).

Molecular fingerprinting of the grey mullet isolatesshowed that they share quite similar profiles, but not identical (Dice coefficients were of 0.75-0.88 for REP-PCR and 0.71-0.76for ERIC-PCR). The profiles were also quite similar to that of *V. harveyi* type strain (0.72-0.88 REP-PCR and 0.66-0.90 ERIC-PCR).

Conclusion

In conclusion, this study present three isolates, a385, a 636 and a 661, identified as strains of *V. harveyi*, as the cause of a mortality outbreak in reared *Mugil cephalus*.

Acknowledgements

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INTEGRATED MULTITROPHIC AQUACULTURE APPLIED TO SHRIMP REARING IN A BIOFLOC SYSTEM WITH TILAPIA AND SARCOCORNIA

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Introduction

Shrimp farming has grown constantly in past years and Biofloc Technology (BFT) contribute with the intensification of Pacific white shrimp using minimum or zero water exchange, reducing the area and water resources use (Samocha et al., 2012). IMTA (integrated multitrophic aquaculture) is an aquaculture model which integrates different trophic levels in the same environmental system, resulting in a conversion of the culture residues of the main species into food, or fertilization, for the other species (Chopin et al., 2001).

This study aimed to evaluate the performance of an integrated multitrophic aquaculture (IMTA) system applied to shrimp rearing in biofloc technology (BFT).

Materials and methods

The IMTA system consisted of shrimp (*Litopenaeus vannamei*) in a rearing tank (800 L), a tilapia (*Oreochromis niloticus*) rearing tank (90 L), and a hydroponic bench with 0.33 m2 of planting space for *Sarcocornia ambigua* culture. A submerged pump constantly pumped water from the shrimp tank to the tilapia tank. Then, by gravity, water flowed through the sarcocornia hydroponic bench and returned to the shrimp tank. The hydroponic bench had enough capacity for 32 plants. Shrimp, tilapia and sarcocornia stock densities were 312 shrimp m-3 (250 shrimps per 800 L tank), 445 tilapia m-3 (40 tilapias per 90 L tank), and 97 sarcocornia plants m-2 (32 plant per system), respectively. The same experimental units were used in the control system which only differed by the absence of sarcocornia. The initial weight was 4.09±0.025 g, 1.16±0.02 g and 1.17±0.175 g for shrimp, fish and sarcocornia, respectively. Shrimps were fed according to the feed table, and the fishes were fed with 1% of fish biomass, stimulating tilapia to use biofloc as a food sourc

Table I: Total sludge produced, total suspendend solids (TSS), volatile suspended solids (VSS) and fixed suspended solids (FSS) for *Litopenaeus vannamei*, *Oreochromis niloticus* and *Sarcocornia ambigua* performance in an integrated biofloc culture system for 57 days.

	IMTA	Control	*p* t-test
Total sludge production (kg tank $^{-1}$)	0.18±0.02	0.35±0.055	0.039*
Total sludge removed by settling chamber (kg tank $^{-1}$)	120.27±11.04	194.18±14.13	0.028*
TSS (mg L^{-1})	437.9±5.7	484.4±7.35	0.002*
VSS (%)	50.9±0.5	51.1±0.5	0.227
FSS (%)	49.1±0.45	48.9±0.7	0.657

Data presented as mean ± standard error. * indicate statistical differences by the t-test ($p < 0.05$).

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Results

Results show no difference between shrimp and tilapia performance in either treatment. Only IMTA total yield (4.83±0.19 kg m-3) was significantly higher than that in the control system (3.99±0.045 kg m-3). Nitrate was higher in the control system (12.28±1.27 mg L-1) compared to the IMTA system (9.38±1.09 mg L-1). The total sludge production and the total suspended solids were reduced in the IMTA group compared with control group (table I).

Disscussion and conclusion

The IMTA system improves the total yield up to 21.5% by multitrophic integration of *L. vannamei*, *O. niloticus* and *S. ambigua* in a biofloc system. The increasing the sarcocornia area and/or tilapia stock density could improve this yield; however, this must be evaluated in future studies. Nitrate concentrations were lower in IMTA group probably by the preference absorption by halophytes plants for this N-source (Ventura and Sagi, 2013). The amount of sludge produced and consequently removed by the settling chambers, lower in IMTA group, could be related to a phytoremediation with microorganisms participation resulting in an organic suspended solids consume (Ansari et al., 2017) whit decrease of TSS and consequently sludge production. Experimental IMTA system with *L. vannamei*, *S. ambigua* and *O. niloticus* increased the total yield, reducing the nitrate levels, SST and sludge production that represents a significant ecological gain apart from the economic gain.

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MID AND HINDGUT TRANSCRIPTOME PROFILING ANAYSIS OF ATLANTIC SALMON (*Salmo salar*) UNDER UNPREDICTED CHRONIC STRESS

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Introduction
The gut barrier has several significant biological functions and in addition to the primary function of absorbing nutrients, it is also an essential barrier between the fish and the external environment, controlling loss of nutrients and preventing uptake of noxious substances. Furthermore, it also harbors an extensive microbiota that can aid in nutrient utilization and protect against pathogen agents. However, several factors can interfere with gut functionality, with serious implications for the health and welfare or the organism (Peterson and Artis 2014, Butt and Volkoff 2019). Combinations of stressors throughout an animal's life, especially in agriculture and aquaculture settings may affect the regular operativity of this organ with negative consequences for animal welfare (Jiminez et al. 2015, Niklasson et al. 2011, Rolf Erik Olsen et al. 2005, 2002, Segner et al. 2012, Sundh et al. 2010). In the current study we report the effects of a 3 week unpredictable chronic stress (UCS) period on the intestinal morphology and transcriptome response of Atlantic salmon *(Salmo salar)* parr, midgut and hindgut.

Material and methods
The experimental design is described in Madaro et al. 2015. Atlantic salmon parr in six different tanks were divided into two groups (n=3 replicates), of which one received a set of random stressors (unpredictable chronic stress (UCS)) while the other was left undisturbed (control group). The UCS group was stressed three times per day (08:30 h, 13:00 h and 17:00 h) using a total of eight types of stressors given in random and unpredictable order. On day 23, 2 fish were sampled from each tank subjected to UCS (n=6) and compared with 2 fish from each tank from the unstressed control group (n=6). Midgut and hindgut from both control and UCS fish were collected for histology and RNA-seq analysis to identify respective changes in the membrane structures and putative genes and pathways responding to UCS.

Results
Histological analysis did not show any significant effect on morphometric parameters. In the midgut, 1030 genes were differentially expressed following UCS, resulting in 279 genes which were involved in 13 metabolic pathways, including tissue repair pathways such as cell cycle, DNA replication, mismatch repair, nucleotide excision repair, base excision repair, ribosome and ribosome biogenesis, RNA transport, spliceosome, pyrimidine metabolism, proteasome, homologous recombination and oxidative phosphorylation. In the hindgut, following UCS, 591 differentially expressed genes were detected with 426 downregulated and 165 upregulated. A total of 53 genes were related to 3 pathways. Downregulated genes include cellular senescence pathways, p53 signaling and cytokine-cytokine receptor pathways.

Discussion and conclusion
The overall results corroborate that salmon parr were at least partly habituating to the UCS treatment, activating adaptive mechanism to ensure essential functions relating to cell/tissue integrity while the fish immunity decreased. In fact, in midgut, the main upregulation was related to cell growth and repair, while in the hindgut there were indications of activated apoptotic pathway, reduced cell repair, and inhibited immune /anti-inflammatory capacity. This may be the trade-off between habituating to UCS and health resilience. This study provides new insight into the understanding the integrated genetic regulatory mechanisms of Atlantic salmon parr to cope with UCS in a farmed environment.

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GENETIC EVALUATION FOR BODY WEIGHT USING SINGLE-STEP GENOMIC BEST LINEAR PREDICTION IN *Macrobrachium rosenbergii*

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Introduction

Unclear relationship inter- and intra-founder populations is a very tricky problem when constructing the based population in a selective breeding program. Moreover, there are often pedigree errors because of the lose of individual tag, the drift of visual implant elastomer fluorescent tag and incorrect records. The errors could lead to biased estimates of genetic parameters and predicted genetic gain using the conventional best linear unbiased prediction method with pedigree (pBLUP). The accuracy of estimated breeding value (EBV) can be improved through correcting the pedigree using high-throughput molecular markers. The single-step genomic BLUP (ssGBLUP) method has been proposed (Aguilar et al, 2010), which replaces the traditional additive genetic relationship matrix A with a more accurate relationship matrix H combining pedigree and genomic relatedness (Lourenco et al, 2014). The objective of this study was to estimate genetic parameters and predicted response to two-generation selection for body weight of the giant freshwater prawn *Macrobrachium rosenbergii* using ssGBLUP.

Materials and methods

The dataset for body weight included three generations and 50271 individuals which originated from four founder populations in *M. rosenbergii*. Variance components and EBVs for body weight were estimated by fitting the animal model which used the relationship matrix A (only pedigree) or matrix H (pedigree combined with genomic information of 215 genotyped parents with 41,960 SNP loci). The accuracy of prediction was calculated through the 10-fold cross validation in which each of the ten subpopulations was used exactly once as the validation population and then repeated 30 times.

Results

Correlation coefficient between lower triangular relationship matrix A and H of the 215 genotyped parents was 0.56, which implies that there might have been inacurrate estimates of kinship in the selection population. Heritabilities based on relationship matrix A and H were 0.18±0.03 and 0.21±0.03, respectively. Accuracies of EBV estimated by pBLUP and ssGBLUP were 0.65 and 0.67, respectively, with an increase of 3.1%when matrix H was used. The genetic gains after two-generation selections for body weight were 5.09g and 6.09g based on pBLUP and ssGBLUP, respectively (Table 1).

Discussion and conclusion

In this study, a higher prediction accuracy of EBVs was obtained through ssGBLUP compared to pBLUP although the number of genotyped parents is limited. Variance components and genetic gain obtained from relationship matrix A were biased compared to relationship matrix H. Desirable response to selection for body weight was obtained in this selective breeding program compared to other programs (Luan et al., 2012). Prediction accuracy of EBVs may be further improved with increase of the number of genotyped parents and the number of SNP loci in the next generations.

Table 1. Predicted genetic gain for body weight in *Macrobrachium rosenbergii*

Method	Generation	No. of Families	No. of individauls	Breeding Value (g)	Genetic gain (g)
pBLUP	G0	78	8361	0.12	-
	G1	120	15394	2.53	2.41
	G2	129	25059	5.21	5.09
ssGBLUP	G0	78	8361	0.36	-
	G1	120	15394	3.62	3.26
	G2	129	25059	6.45	6.09

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FISH WELFARE INDICATORS: LESSONS LEARNED FROM WELFARE STANDARDS IN TERRESTRIAL ANIMALS

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Introduction

There is substantial scientific evidence, that most animals, including fish, are sentient beings (Dawkins 1990; Manfrin et al., 2018). Accordingly, animal welfare has become an established scientific discipline with massive social support. The mass collection and analysis of data has led to a number of valid and reliable indicator to assess the status of animal welfare of almost every kind of terrestrial farm animal. However, the situation is somewhat more difficult in aquatic animals such as fish. The list of measurable parameters related to fish health and welfare easily exceeds several hundred items and largely varies between species, life-stage (Martins et al., 2012), and production system (van de Vis et al., 2012). As fish welfare is a comparatively novel discipline (Manfrin et al., 2018) and being confronted with this great number of measurable parameters it seems obvious to look into the development and application of terrestrial animal welfare indicators. This approach can help in order to develop appropriate strategies for the assessment of fish welfare and to avoid the repetition of errors.

Materials and methods

Today, a frequent corporate self-check of the state of welfare is required from every fish farmer. However, until now there is no universal set of indicators available to comprehensively implement this in an equivalent way. We reviewed the current guidelines for the assessment of welfare in terrestrial farm animals as well as the methodology used to develop these, in order to adapt a strategy for fish welfare indicators

Results

We found, that of a large number of measurable parameters only very few fulfill the necessary conditions in terms of reliability, objectivity, validity, and practicability (Figure 01). Additionally, such indicators need to be widely accepted by all, society, operators and farmers and need to be integrable into everyday routine work.

Figure 01: Two possible relationships between number of available parameters and practicability as welfare indicator

Discussion and Conclusion

Animal welfare is an ethical decision of society. Today more then ever, we have the understanding, knowledge, and evidence, that animals have the ability to suffer and experience distress. However, when compared to terrestrial animals, the evaluation of valid, reliable, and practicable indicators, to assess the state of welfare in farmed fish is yet at the beginning.

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SIMULATING FISH FEED STUDIES: LONG-TERM PREDICTION OF NOVEL FEED FORMULATIONS AND FEED INGREDIENTS

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Introduction

In aquaculture operations, feed may represent more than 50% of the total cost of operations (Rana et al. 2009). As the industry is expanding rapidly, the rise in global demand has led to an increased price for fishmeal of almost 300% since 2005 (Roberts et al., 2015). Similarly, the cost of major oils used in the aquafeed industry has increased by 250 percent (Rana et al. 2009). This global trend in major feed ingredient has increased the average prices for aquafeeds by 20-90%, depending on region and target species. Accordingly, there is a steady demand to reduce feed production price by finding novel and alternative feed ingredients and formulations, while maintaining feed efficiency and growth performance of the reared organisms at the same time.

Currently, it is common practice to test new ingredients and formulations in laboratory trials, mostly on juvenile fish. However, there is evidence, that results obtained in such trails cannot be transferred across different life stages (Lugert et al., 2019), and therefore contain little information regarding rearing time and harvest size.

Mathematical modeling is an important tool to describe the growth process of an animal across life stages, from juvenile to maturity. The method also allows to predict and evaluate the goals of trait specific breeding programs (Lugert et al., 2019). In the current study, we have successfully tested the possibilities, to model the long-term effects of different feed formulations on cultured rainbow trout.

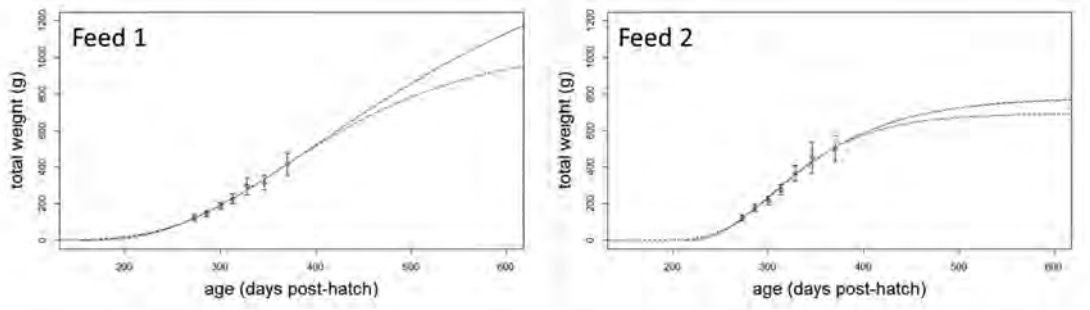

Figure 01: Simulation of long-term effects on growth trajectories, as a result of different feed formulations displayed by the same two models. Dotted line = model 1, dashed line= model 2.

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Materials and methods

The experiment was performed at a commercial farm. Fish (n=900) were acquired from the farms-own breeding program and were distributed randomly into nine tanks. Fish were fed with three different extruded pellets of different experimental formulations in triplicate groups. Rations were offered twice a day until apparent saturation. Several models of different complexity were tested against the data. The most suitable model was evaluated by a variety of statistical tests combined in a multi-criteria analisis.

Results

Despite the massive influence of the feed formulation on fish performance, the statistical analysis revealed the same mathematical relationships related to the growth process. This means, that different growth trajectories, triggered by different feed formulations and/or feed ingredients, can be described in the same manner and by the same function within the same species. This allows evaluation of a specific model and accordingly the prediction of the influence of specific feed formulations by a mathematical equation.

Discussion and Conclusion

The mathematical parameters estimated for the target species are in line with those from a variety of aquaculture studies. Accordingly, fish have grown in accordance with their biological attributes. This is important to justify the predictive capacities of the model. We could show, that it is possible to model the impact and long-term influences of varies feed formulations and ingredients on growth and rearing time in fish.

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PILOT SCALE SYSTEM FOR PHYCOREMEDIATION OF AQUACULTURE WASTEWATER USING THE MICROALGAE *Chlorella sorokiniana*

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Introduction

Microalgae has been tested widely as potential microorganisms to phycoremediate wastewater from different sources (WW), including aquaculture wastewater (AWW) (Ansari et al., 2017; Guldhe et al., 2017). The results are promising, as they proved that microalgae can remove even more than 90% of the pollutants from the WW; however, most of the experiments have been performed in lab scale (Guldhe et al., 2017). By growing microalgae in AWW the economic and environmental factors of aquaculture can be improved considerably, as the AWW is used to grow algae and produce valuable biomass. The algae products can be used as fish feed ingredients or other valuable compounds. Additionally, after harvesting the algae the water can be reused in the aquaculture system (Guldhe et al., 2017). Thus, given the great potential of using the nutrients from AWW for algae production and the lack of experiments in pilot or commercial scale, pilot experiments were carried out to phycoremediate AWW and optimize the biomass production of *Chlorella sorokiniana*.

Materials and methods

Nile tilapia (*Oreochromis niloticus*) was the fish grown in the aquaculture system. The effluent water from the aquaculture system was deposited in 50L photobioreactors (model: HXDYKZ-12) and used as growth media for the microalgae. The photobioreactors had external light-emitting diodes with a proportion of 3:1 of red and blue, and an input of air of 0.4vvm enriched with 0.1% of CO_2. The growth rate and biomass production of *C. sorokiniana* (CCAP 211/8K) were tested in batch cultures using filtered AWW and AWW with additional N and P. The growth rate and biomass production from this experiment were compared to the properties of *C. sorokiniana* when it is grown in synthetic growth media with the same content of nitrogen and phosphorous as the AWW experiements.

On the other hand, in order to evaluate the efficiency of *C. sorokiniana* to remediate AWW, the quality of the water before depositing it on the photobioreactors and after harvesting the microalgae was measured. Parameters such as pH, total nitrogen, total phosphorous, dissolved oxygen and chemical oxygen demand were used as indicators of the water quality (Mennaa et al., 2015).

Results

The microalgae *C. sorokiniana* was able to grow well in AWW. The cells grown in AWW were not phenotypically different compared to the cells grown in synthetic media, which indicates that the cells were not under stress conditions.

Regarding the analysis of the quality of the water, results revealed that the microalgae was able to lower considerably the nutrient content in the water. As well, the CO_2 concentration decreased considerably in the water, and the dissolved oxygen increased.

Discussion and conclusion

As the nutrient content of the AWW was lower than in the synthetic growth media normally used for algae production, the experiment was also conducted with additional nutrients to the AWW. The lower concentration of nutrients in AWW is related to water quality required in the aquaculture system in order to keep the fish in good conditions and have greater productivity. The limited quantity of nutrients affects directly the microalgae biomass productivity (Bohutskyi et al., 2017). Thus, the four tests included AWW with and without additional nutrients and two synthetic growth media for comparison with the same amount of N and P.

Ansari, et al. (2017) were able to reach a higher biomass productivity by adding nitrogen to the AWW. Moreover, the sludge contained in the AWW can lower the light scatter and penetration, resulting in a lower photosynthetic activity (Guldhe et al., 2017).

The use of AWW for algae production still require further improvements, but results indicate positive results and could provide an additional income for aquaculture companies as well as lower the ecological impacts generated by aquaculture.

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CONSERVATION OF EUROPEAN EEL *Anguilla anguilla* FEMALE GENETIC RESOURCES THROUGH GONADAL TISSUE CRYOPRESERVATION

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Introduction

European eel (*Anguilla anguilla*) is considered to be an economically important fish species. Overfishing, together with other anthropogenic factors and diseases suppress the revitalization of this species. Reproductive limitations in natural conditions are hindering the population regenerationand consequently, this species has been listed as a critically endangered species on the IUCN red list. Reproduction in captivity is considered as a possible solution to overcoming these problems. Although protocols for hormonal stimulation and *in vitro* fertilization are developed, there is still a serious problem in larval survival after hatching. Therefore, there is a need for new conservation strategies for genetic resources. Cryopreservation is a method that allows the preservation and storage of biological materials in liquid nitrogen for an indefinite time. Ovarian tissue banking is a novel strategy for the preservation of valuable female genetic resources (Lujić et al., 2017) and, accompanying with other methods, presents a promising method in overcoming reproductive difficulties in many fish species. The first step which can contribute to the development of new alternative conservation strategies for this species is the cryopreservation of early-stage germ cells such as oogonia. Further usage of obtained preserved cells might serve for subsequent manipulation, such as *in vitro* culture or germ cell transplantation.

Material and Methods

Fish were held in controlled conditions in a recirculating system of the Department of Aquaculture at Szent Istvan Univeristy in Hungary at a constant temperature of 16±1 °C without feeding. Individuals were euthanized, ovarian tissue was excised and immediately transferred into phosphate buffered saline (PBS) with 100 U/mL penicillin and 0.1 mg/ml streptomycin and placed on ice until further use. Tissue was cut in pieces which were subsequently used for cryopreservation. Fresh tissue was used as a control for assessment of OSC (oogonial stem cells) viability after thawing. Six sequential experiments were performed during optimization of the cryomedia and freezing protocol. Firstly, the effects of six cryoprotectants (dimethyl sulfoxide - Me_2SO, propylene glycol – PG, ethylene glycol – EG, glycerol – GLY, methanol – MeOH, 2-methoxyethanol – 2ME) on OSC viability were assessed. Then, different concentrations of the three best cryoprotectants from the firs experiment were tested followed by testing of sugar (glucose, sucrose and trehalose) and protein (bovine serum albumin – BSA and fetal bovine serum – FBS) supplementations. With the most suitable cryomedia, we proceed with testing the effects of different tissue pieces weight (25, 50 and 75 mg) and equilibration time (5, 15 and 30 min) in cryomenia prior to freezing to OSC viability. Then, six cooling rates (-0.5, -1, -5, -10, -20 and -40 °C/min) were examined using three thawing approaches (thawing in a 10 °C water bath, as well as at a controlled and reciprocal rate to the cooling rate). Lastly, three different plunging temperatures were assessed by cooling ovarian tissue pieces at -1 °C/min up to -40, -60 and -80 °C before the tissue was plunged into liquid nitrogen (lN_2).

In order to assess oogonia viability, all tissue pieces were weighed and dissociated in the solution of L-15, 2 mg/ml collagenase, 1.5 mg/ml trypsin and 60 µg/ml DNase I for one hour and terminated with adding the same volume of 10% Fetal bovine serum (FBS). The suspension was filtered, centrifuged and the supernatant was carefully removed since the pellet was resuspended by adding 50 µl of L-15. Verification of oogonia viability was conducted using the trypan blue exclusion test, where the viability was calculated as the proportion of the total number of obtained cells from cryopreserved tissue compared to the total number of cells obtained from the fresh dissociated tissue. Viability test was done using a Bürker-Türk hemocytometer.

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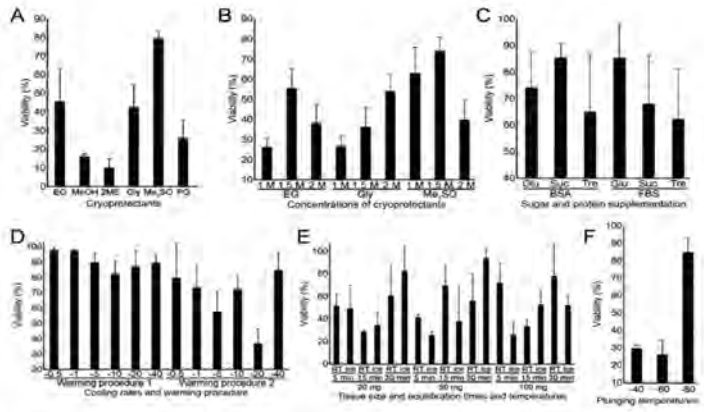

Figure 1. Optimisation of European eel ovarian tissue cryopreservation. Viability of oogonia after cryopreservation with A. 1.5 M of ethylene glycol (EG), methanol (MeOH), 2-metoxy ethanol (2ME), glycerol (Gly), dimethyl sulfoxide (Me_2SO) and propylene glycol (PG). B. 1, 1.5 and 2 M of EG, Gly and Me_2SO. C. different sugar (glucose (Glu), sucrose (Suc) and trehalose (Tre)) and protein (bovine serum albumin (BSA) and fetal bovine serum (FBS)) supplementations. D. different cooling rates (-0.5, -1, -5, -10, -20, -40 □/min) and warming procedures (warming procedure 1 (10 □ water bath), warming procedure 2 (reciprocal to the cooling rates)). E. tissue sizes (20, 50 and 100 mg), equilibration time (5, 15 and 30 min) and equilibration temperature (RT - 23 □ and on ice). F. plunging temperatures (-40, -60 and -80 □). All values are presented as mean ± SD. Different letters above the SD lines indicate statistical significance (Tukey's HSD, $p < 0.05$), while the lack of such letters indicates the lack of statistical significance.

Results and discussion

The first experiment (Fig 1.) displayed the superiority of three cryoprotectants: Me_2SO, EG and GLY. In the subsequent experiments, these three cryoprotectants were used in different molar concentrations (1 M, 1.5 M, and 2 M) and similar results were obtained where the highest OSC viability rates were observed when utilizing 1.5 M Me_2SO. Varying sugar and protein supplementation did not display significant differences, therefore 1.5 M Me_2SO supplemented with 0.1 M glucose and 1.5% BSA contributed to the best post-thawing survival rate. According to these results, the developed protocol was used to examine the influence of different tissue sizes, equilibration times and plunging temperatures. When comparing the effects of different tissue sizes, and equilibration techniques, equilibration of 50 mg tissue pieces for 30 min on ice resulted in the highest viability (93.8±5.8%). No significant differences were observed between the tested cooling rates when thawing in a 10 °C water bath, while reciprocal thawing rates yielded slightly lower viability rates. Lastly, the plunging temperature of -80 °C was superior to the other plunging rates.

Techniques developed in this study present the onset of the germline stem cell manipulation technologies in the European eel which will contribute to the development of new and improved conservation and management strategies for this species.

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TRIALS WITH THE SEA URCHIN *Paracentrotus lividus*: IMPROVING REPRODUCTION AND LARVAL REARING

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Introduction

Sea urchin gonads (roe) are a prize product and broadly recognized as a delicacy for human consumption. Due to the increasing demand for this product by Asian and European countries, overfishing is causing a sharp decline in the wild stocks of *Paracentotus lividus*. During the last decade a significant increase in efforts to develop cost-effective methods to culture sea urchins has been observed including research aiming to optimize the reproduction and juvenile production. The development and survival of larval planktonic stages is still one of the bottlenecks for the echinoculture development. This communication presents several trials aimed at improving existing hatchery methodologies and consolidate the early life stage culture of *P. lividus*.

Material and Methods

For this study, *P. lividus* adults, with test size superior of 35 mm, were collected from intertidal rock pools in the southern coast of Madeira island, Portugal. The rearing system consisted of four 200 L tanks at 22.05 ± 0.707 ºC and a 90 L/h of water renovation. Feeding was established at 5% weight.day^{-1} of macroalgae collected in the sampling site. The maturation stage of the population was analyzed *a priori* by determining the gonadal index (GI) and by histological analysis of the gonads of a subsample of 20 sea urchins. Following an initial evaluation of different spawning methods, the KCl 0.5M was chosen to induce spawning and to perform the artificial fertilization of sea urchin eggs. The viable echinopluteus were then fed with diets of different microalgae (*Dunaliella tertiolecta* and *Rhodomonas marina*) comparing larvae growth and survival, when competence for settlement was achieved. The larval experimental design consisted of three triplicated treatments, in 7 L cylindroconical incubators at 21 ºC. The microalgae rationing was established at 4000, 8000 and 16000 cells.ml^{-1} in treatment 1, 2 and 3, respectively for larvae (echinopluteus) with two pairs of arms. The microalgae concentration was doubled in all treatments with the increment of the 3rd and 4th pair of arms during the development of the echinopluteus.

Results

The first larval development experiments using a *R. marina* diet showed that the echinopluteus attain the settlement phase within 21 days. Higher survival rate in the treatment of lower microalgae concentration. Using *D. tertiolecta*, the echinopluteus attain the settlement phase within 15 days. Based on these initial results, further experiments are undergoing to allow for improvements in the larvae methods for *P. lividus*.

Acknowledgements: This study and Ricardo Luís research grant had the support of project ISLANDAP (Interreg MAC/1.1a/207).

INOCULATION OF PROBIOTIC *Bacillus licheniformis* BCR 4-3 IN THE REARING WATER ENHANCES SURVIVAL AND IMMUNE RESPONSE OF *Litopenaeus vannamei* CHALLENGED WITH *Vibrio parahaemolyticus*, THE CAUSATIVE AGENT OF AHPND

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Introduction

In the last decades, aquaculture has become the fastest growing food production sector with an average annual growth rate of 5.4% (Valenti et al., 2018). This growth has been achieved due to the technification and intensification of aquaculture production systems. However, the increase in stocking densities, excessive handling, suddenly changes in temperature, poor water quality, and nutritional deficiencies generate stress and weaken the immune system of organisms (Reverter et al., 2014). All these conditions favor the outbreak of infectious diseases such as the acute hepatopancreatic necrotizing disease (AHPND) caused by *Vibrio parahaemolyticus* in shrimp.

Materials and methods

To determine the protective effect of bacilli inoculated into the water in small shrimp, a bioassay was conducted during 15 days with healthy shrimp weighing 213.4 ± 5.0 mg. Bacteria was inoculated in water (1 x 10^6 CFU/L) every 3 d and added to commercial feed (1 x 10^6 CFU/g). The mean lethal concentration of *Vibrio* put in the water was previously determined (LC_{50}= 77,000 CFU/mL). Uneaten food and waste material were removed every 3 d until day 12. The determination of physicochemical parameters (dissolved oxygen, pH and salinity) were determined daily. Six shrimps were taken per treatment and each one was stored in 1 mL of RNALater. The relative expression of immune-related genes was determined by qRT-PCR before bacterial challenge at day 12. Reference genes were β-actin, 40S-S24 and EF1α. ANOVA and Tukey test were performed. Data are mean ± SE. Different letters indicate significant di ferences ($p < 0.05$).

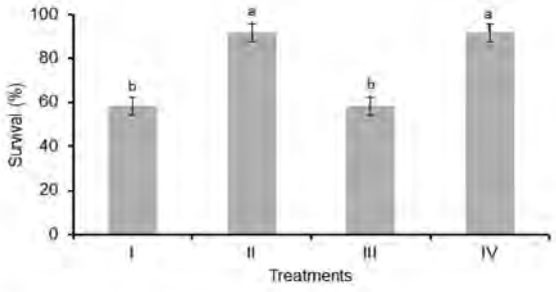

Figure 1. Survival of *L. vannamei o parahaemolyticus*. Treatments: **I)** Positive control, *Vibrio*; **II)** Bacilli (1 x 10^6 CFU/L) + *Vibrio*; **III)** Bacilli in feed (1 x 10^6 CFU/g) + *Vibrio*; **IV)** Bacilli (1 x 10^6 CFU/L, 1 x 10^6 CFU/g) + *Vibrio*.

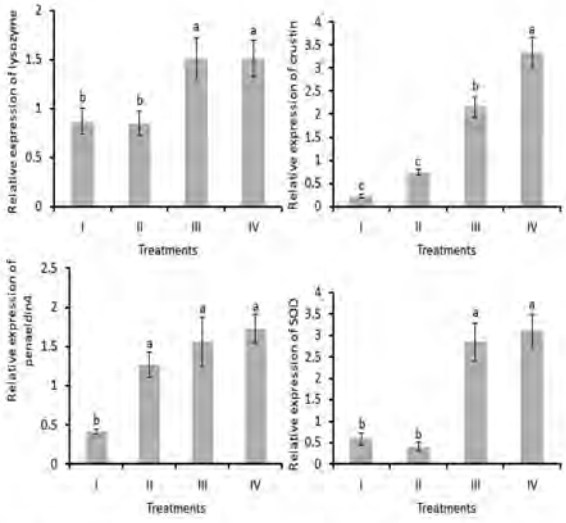

Figure 2. Gene expression in *L. vannamei*. Treatments: **I)** Positive control, *Vibrio*; **II)** Bacilli (1 x 10^6 CFU/L) + *Vibrio*; **III)** Bacilli in feed (1 x 10^6 CFU/g) + *Vibrio*; **IV)** Bacilli (1 x 10^6 CFU/L, 1 x 10^6 CFU/g) + *Vibrio*.

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Results

Survival was significantly higher ($p<0.05$) in the treatments with bacillus in the water as compared with the positive control and bacillus in the feed (Fig. 1). The expression of lysozyme, crustin, penaeidin4, and superoxide dismutase (SOD) genes was significantl up-regulated with bacillus in feed as compared with positive control and bacillus in water ($p<0.05$) (Fig. 2). *B. licheniformis* BCR4-3 in feed and water protects the white shrimp against AHPND and increases its immune response.

Discussion and conclusion

Bacillus licheniformis shown a positive overall effect when it was put in water during 15 d, efficiently increasing shrimp resistance to *V. parahaemolyticus* infection. Conversely, Zokaeifar et al. (2012) found that whiteleg shrimp treated with two strains (L10 and G1) of *B. subtilis*, added to feed during 8 weeks, improved disease resistance through an enhanced immune response. With respect to the immune system, the expression of the four immune-related genes studied was significantly up-regulated in treatments with bacilli in feed as compared with control group and shrimp of the treatment with bacilli in the water.

B. licheniformis BCR4-3 in feed and water protects the white shrimp against AHPND and increases its immune response.

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BLUE BIOMASS - A WAY FORWARD FOR MITIGATION MUSSELS

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Introduction

Blue Mussels (*Mytilus edulis*) have for decades been recognized as a potential mitigation tool to remove nutrients (Holmer et al. 2015, Petersen et al. 2019) while at the same time being a source of fatty acids and marine protein. Blue Biomass is a Danish company situated in the highly eutrophicated Limfjord in the Northwestern part of Denmark, aiming at producing 50,000 tons of blue mussels per year between 2020-2025. The farm now has 360 tube-net units (Smartfarm) with a total carrying capacity of 9000 - 12600 tons (25-35 tons/unit). The mussels are harvested twice a year and processed into animal feed and food for human consumption. Blue Biomass is the first large scale mussel farm in Denmark that through partnership with the feeds industry, has managed to open up for full scale production and processing of mitigation mussels. This study follows the biomass production and the effects from the farm on water transparency. Furthermore, the ecosystem service of nutrient removal provided by harvest is estimated.

Materials and Methods

The Blue Biomass mussel farm is situated in the western part of the Limfjord, in Northwest Denmark (56°34,266N, 8°34,372E). The mussel biomass, meat content, condition index (CI), and size distribution were monitored in diagonals from Southeast to Northwest in each farm area from 2018 - 2019. A one-way ANOVA was used to test for differences in CI of mussels between East, West and middle of farm areas. Additionally, profiles of fluorescence, salinity, temperature and oxygen (SeaBird, SBE19) was measured together with continuous surface fluorescence measurements, using a Turner Cyclops 7. In situ fluorescence was calibrated into in vivo chlorophyll from water samples using Turner designs (r^2 = 0.91). Mussel biomass was estimated from triplicate samples of areas of 25 x 25 cm from each unit (three units per area) and scaled up to full size (120 m long, three meters deep). Size distribution and meat content was measured on 100 mussels randomly selected from each sample. The CI was carried out on 10 mussels per sample and calculated as CI = dry $weight_{meat}$/$length^3$ according to standard methods. The monitoring was started with baseline measurements in May 2018.

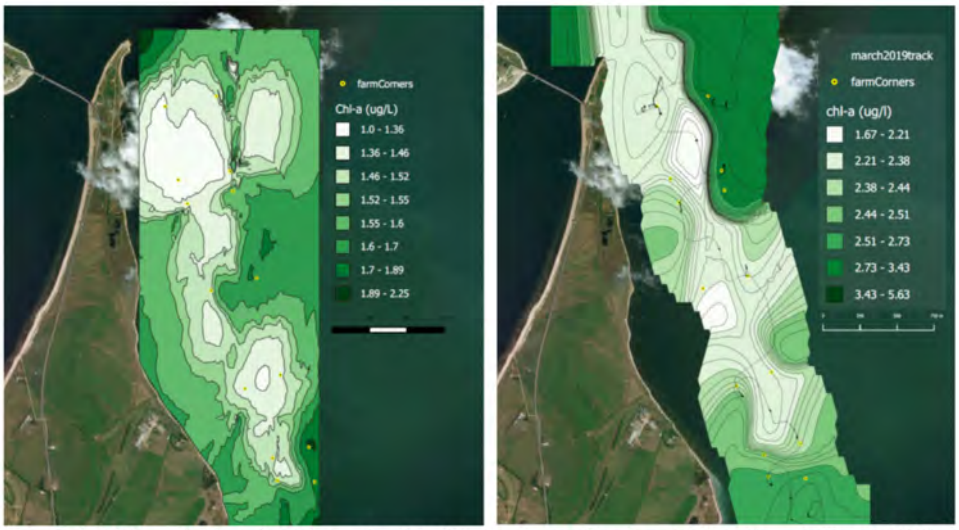

Figure 1. Chlorophyll (ug/l) at 2,5m depth in Limfjorden in October 2018 (left) and March 2019 (right). Farm corners are indicated with yellow dots. Current direction was NW on both days.

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Results

The settling of mussel larvae occurred in late May/early June and grew into an average biomass range from 6 – 34 tons per Smartfarm unit in December with an average value of 12.6 ± 7.7 tons, which was half of the estimated production capacity of the units due to loss of mussels caused by the extreme temperatures during the summer 2018. The average size of the mussels was from 39.2 ± 8.7 mm (sample size of 1200 mussels) in December. A complete harvest of the standing stock of mussels in December 2018 would remove 45.2 – 67.8 tons N and 2.2 – 3.1 tons P assuming the N- content of the mussels were from 1 – 1.5% and the P-content 0.05 - 0.07% (Petersen et al. 2014). Complete harvest of the full production capacity of 9000 - 12600 tons (25 - 35 tons per unit) would remove up to 189 tons of N and 8.8 tons of P per year from an area of 75 ha. The units reduced the phytoplankton concentration within the production area and downstream of the area, by more than 50 % (Figure 1). The middle of three out of four farm areas had the lowest average CI, and the Eastern part showed significantly higher CI than the middle and Western parts (one-way ANOVA, $p < 0.05$) indicating food depletion in the middle of the farm areas and a higher food availability in the eastern part of the farm areas. This indicates a maximum of carrying capacity for mussel density with regards to growth in the area.

Conclusion

The large-scale Blue Biomass mussel farm in Limfjorden, Denmark produces large amounts of marine protein and increases the visibility of the water. Measurements of growth parameters of the mussels from the middle, Eastern and Western part of the farm areas indicates food depletion despite the highly eutrophicated waters. A complete harvest of the standing stock of mussels in December 2018 would remove up to 67.8 tons of N which is equivalent to 3.2 % of the environmental reduction target of 2122.1 tons N yr^{-1} for the water body 156, as demanded by the EU water framework directive 1915-2021. The anticipated goal of 50,000 tons blue mussels per year harvested by Blue Biomass would cover 35.3 % of the reduction target and supply society with feed and food.

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SPATIAL MODELLING OF BLUE MUSSEL FARM PRODUCTION POTENTIAL IN THE WESTERN BALTIC SEA

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Introduction

Eutrophication of coastal water bodies by massive anthropogenic nutrient inputs is a serious global challenge. Suspended mitigation cultures of blue mussels have been suggested as a tool to remove nutrients through harvesting from eutrophic systems like the Western Baltic Sea (Petersen et al. 2014). The general idea of mitigation mussel farming is that the mussels remove nutrients contained in particles (mainly phytoplankton) directly from the water through their feeding activity and incorporate them into animal tissue during growth. Site-selection for marine mitigation aquaculture can be an important part of sustainable marine spatial planning considering both farm production, as well as environmental and socio-economic goals and interests. In the present study, mussel farm production potential was estimated for the Western Baltic Sea, which can provide input to a multi-criteria site selection tool in relation to marine spatial planning.

Materials and methods

We integrated data from field experiments and national monitoring programs within a sequence of numerical, statistical, and spatial models. Mussel individual growth was estimated by a dynamic Energy Budget (DEB) model (Maar et al. 2015) calibrated against observations from mussel long-lines and the environmental conditions. The DEB model was then applied to 59 monitoring sites with sufficient data and the results were used to make a more simple statistical model of mussel growth versus monthly data of temperature, salinity and chlorophyll-a (Chl *a*) concentrations. A spatial model estimated long-term (2008-2017) monthly means of environmental variables on 1km^2 scale based on monitoring data from Denmark, Sweden and Germany. The statistical growth model was imposed on the spatial environmental data and up-scaled to farm production taking bathymetry (depth of farm) and mussel densities within a standard farm into account.

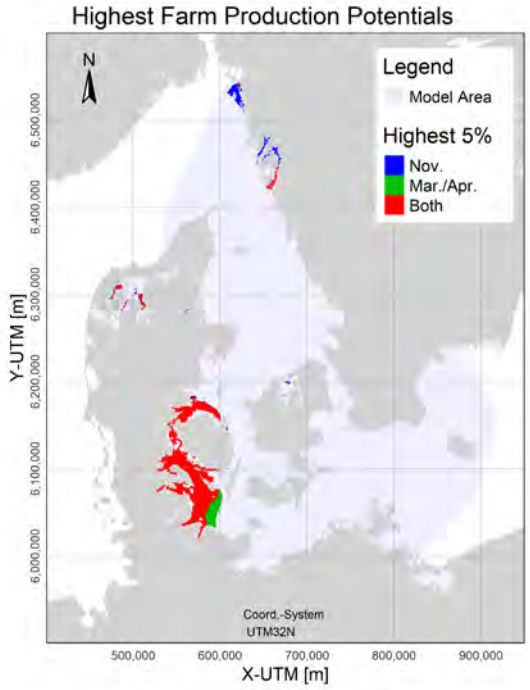

Fig. 1. Spatial model results of 5% highest farm production potentials in the W Baltic Sea indicated for harvest in November, March/April or both periods.

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Results and Discussion

We investigated model results for two growth seasons both starting in July and with harvest times in November or March/April. The modelled (long-term average) maximum biomass of individual mussels was 0.76g-DW and 1.7g-DW at harvest in November and March/April, respectively. Similarly, corresponding maximum farm production potential was 201t-DW and 302t-DW per standard farm. Water depths <4m was defined unsuitable for mussel farms. The smaller increase of maximum farm harvest compared to biomass of individual mussels is attributed to a functional decrease of mussel density on farm collector substrate with increasing mussel size. When selecting the 5% of sites with highest farm production potentials at harvest in November (163-201t-DW), Limfjord, Mariager Fjord, Isefjord, the whole area from the little Belt south to the Kieler Förde, and the north-west coast of Sweden are part of the selection (Fig. 1). The same selection at harvest in March/April (242-302t-DW) results in similar areas, except for the Isefjord. The impacts of different environmental variables on local farm harvest potentials are both of spatial and temporal nature. The typical temperature pattern is a major driver of seasonal dynamics of modelled mussel growth. Salinity expresses two major gradients: (1) There is a large-scale decrease of salinity from the Skagerrak area into the Western Baltic Sea; (2) numerous of the inner fjords show lower salinities. In our model, growth limitation by low salinity plays a major role south-east of the Danish islands Zealand, Falster, and Lolland between April and November. Chl-*a* expresses a strong seasonal pattern with a major peak in March, however, strong spatial heterogeneity is also observed throughout the year. Higher Chl-*a* concentrations are typically met within fjords and in near-coastal areas and especially along the northeast-coast of Germany. Low Chl-*a* concentrations limits mussel growth mainly in the open waters, as well as in the Sound and around the south-coast of Sweden. For the whole study area, water quality affects the growth of individual mussels in the following order of impact: salinity > Chl-*a* > temperature. Effects of bathymetry are super-imposed on the resulting growth of individual mussels, while upscaling to farm production potential. Within the applied spatial model, bathymetry limits the depth extent of mussel mitigation farms; therefore, shallow areas with depths <10m generally result in less farm production potential.

Conclusion and Outlook

Production potentials of mussel mitigation farms have been spatially modelled by integrating different modelling approaches. Results show that there are areas with likely good performance of this mitigation concept in the coastal waters of all three countries involved. In further steps, the results will be (1) validated against available independent mussel growth data from the study area, (2) evaluated with regard to model uncertainty and expected variability of mussel growth, and (3) overlayed with available spatial datasets relevant for marine spatial planning. Finally, a multi-criteria tool for optimal site selection of mussel farming will be developed to support sustainable marine spatial planning in the Western Baltic Sea.

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IMPACT OF NSP LEVEL AND ENZYMES (PHYTASE AND XYLANSE) ON NUTRIENT UTILIZATION, GROWTH PERFORMANCE IN NILE TILAPIA (*Oreochromis niloticus*)

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Introduction

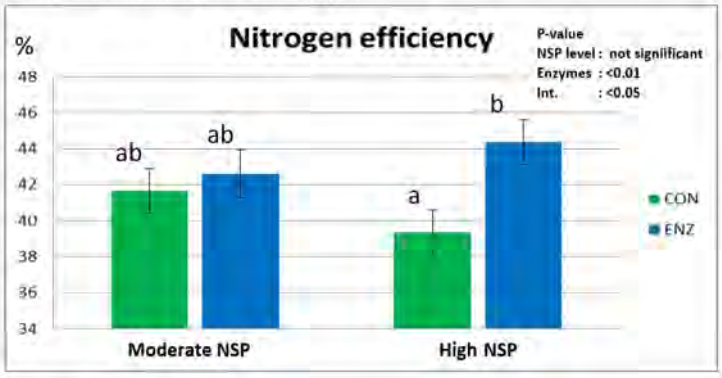
The expected future growth of the aquaculture sector increases the pressure of using more sustainable and novel feed ingredients in aqua feeds (Tacon & Metian 2015). However, with the inclusion of plant ingredients such as soybean and rapeseed meal, the content of non-starch polysaccharides (NSP) as well as phytate will increase in the fish feed. Both NSP and phytate are undesired in feed formulation due to their anti-nutritional properties. The NSP fraction generally remains undigested, as the enzymes to hydrolyse the glycosidic bonds are scarce or non-existing (Choct 1997; Francis *et al.* 2001; Sihna *et al.* 2011). Recently, the use of exogenous carbohydrase enzymes and phytase in aqua feeds is getting more attention. Studies showed that exogenous carbohydrase enzyme and phytase supplementation improved feed intake, growth rate and nutrient digestibility in fish (Castillo & Gatlin 2015; Goda *et al.* 2012).

The working hypothesis is that when NSP is degraded into less polymerised compounds enzymes, they can be further fermented by the gut microbiota into volatile fatty acids (VFA). By adding exogenous enzymes it is expected that the breakdown of NSP is increased and thus the production of VFA, thereby increasing the NSP digestibility. Differences in potential substrate (both composition and level) for gut fermentation is likely to influence the production of VFA. With an increasing breakdown of NSP, shift in microbiota can be expected as fermentation is through microbial anaerobic glycolysis. The main objective is to assess the impact of dietary NSP level and exogenous enzyme supplementation on the growth performance, nutrient utilization, gut microbiota and VFA production in Nile tilapia. This was tested using enzyme supplementation (yes vs. no) and two dietary NSP, to quantify the individual effects and the interaction between NSP level and enzyme supplementation.

Materials & Methods

Four experimental diets were formulated according to a 2 x 2 factorial arrangement, using enzymes (phytase and xylanase) and NSP level (moderate and high) as factor. For the enzymes a mix of phytase (from *Buttiauxella* at 1000 FTU/kg) and xylanase (from *Trichoderma ressei* at 5000 U/kg, both enzymes are provided by DuPont Animal Nutrition) was used (ENZ) versus no supplementation of enzymes (CON). A contrast in NSP level (moderate vs. high) was created by incorporating different levels of NSP rich ingredients into the diets. The first diet was formulated to have a NSP content of around 120 g/kg DM diet and is referred to as Moderate NSP diet (MOD), the second diet is formulated to have a NSP content of around 300 g/kg DM and is referred to as High NSP diet (HIGH). This resulted in a moderate NSP control diet (MOD-CON), moderate NSP enzyme diet (MOD-ENZ), high NSP control diet (HIGH-CON) and a high NSP enzyme diet (HIGH-ENZ). A recirculating aquaculture system was used, with common water supply and ensuring the same water quality for the inflow of each tank. In total 16 tanks (4 replicates/treatment) were used with 35 tilapia/tank and a mean

Figure 1. Effect of NSP level and enzyme supplementation on the nitrogen Efficiency in Nile tilapia.



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initial body weight of 33 g. Fish were restrictively fed (80% of expected satiation) twice daily with the experimental diets for 42 days. Growth rate, body composition, digestibility and the energy, nitrogen and phosphorus balances were measured. In addition, digesta in 4 different gut segments (stomach, proximal, middle and distal section) were taken and will be analysed for VFA concentration and composition and gut microbiota.

Results

The enzymes improved ($P<0.05$) the growth of the fish fed both diets. The interaction effect between enzymes and diet was present for FCR ($P<0.05$), with an improvement from 1.23 to 1.16 for the moderate NSP diet and from 1.33 to 1.19 for the high NSP diet (Figure 1). The enzymes improved the digestibility of the dry matter, carbohydrates, NSP, ash and the minerals P, Ca and Mg ($P<0.05$). In terms of growth (FCR and absolute) the diet high in NSP with enzymes was compatible with the moderate NSP diet without enzymes. The digestibility is not explanatory for the high growth of the fish fed the HIGH-ENZ diet (no interaction effects, $P<0.05$), with an ADC of 69.7% and 74.1% for energy and DM versus 80.7% and 84.4% for the MOD-CON diet. The nitrogen balance shows interaction effects ($P<0.05$) for the nitrogen retention, branchial nitrogen losses and nitrogen efficiency (Figure 1), with the highest nitrogen efficiency and retention for the HIGH-ENZ diet. Data on VFA production and gut microbiome are being analysed.

Conclusion

Supplementation of enzymes (*Buttiauxella* phytase and *Trichoderma* xylanase) showed to be an effective tool to improve nutrient digestibility and enhance growth in Nile tilapia at both dietary NSP levels. The improvement in growth was greater for the high NSP diet, with an interaction effect on the FCR. The higher improvement in growth for the high NSP diet can be explained by the higher nitrogen retention and efficienc . The data indicated that with the addition of the phytase and xylanase combination, diets can be formulated with increased level of high NSP by-products and maintaining the performance in tilapia.

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SOLVING THE PROBLEM OF SALT ACCUMULATION IN RECYCLED BREWERY EFFLUENT THROUGH THE INTEGRATION OF WATER TREATMENT, AGRICULTURE AND AQUACULTURE

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Introduction

Water scarcity in South Africa, and many other parts of the world, presents challenges for industries that depend on this natural resource (Arnell 2004). This makes it important to have responsible waster use, such as recycling and reusing water from food processing industries for example. Breweries are one of the major consumers of freshwater and produce nutrient rich wastewater which can be released to the environment or reused for downstream purposes aquaculture (Jones *et al*., 2014; Power and Jones 2015; Taylor *et al*., 2018). The Ibhayi Brewery (SAB Ltd) currently employs a combination of alternative, sustainable treatment processes such as anaerobic digestion, primary facultative ponds, high rate algal ponds and constructed wetlands to treat brewery effluent on an experimental-scale, and this treated effluent has been shown to have potential use in agriculture and aquaculture. However, salt in brewery effluent is a constraint for its downstream uses, because cleaning agents used at source contain salts that accumulate in the system as water evaporates during the recycle process, which makes it less suitable for reuse or downstream use in agriculture and aquaculture.

The objective of this study was therefore, to determine the salt removal efficiency of halophytes (*Salicornia*, *Atriplex*, *Sorghum bicolor*, *Spinacia oleracea*) by looking at (1) brewery effluent irrigation, (2) crop rotation and (3) soil amendment on soil structure and its effect on selected agricultural crop production and (4) the suitability of this treated effluent for use in downstream aquaculture.

Table I: Soil and leaf sodium level in various crops irrigated with brewery effluent.

	Salicornia	*Atriplex*	*Sorghum bicolor*	*Spinacia oleracea*
Soil sodium level (mg/L)	2 596.3	2 983.6	2 985.0	2 135.0
Leaf sodium level (mg/L)	105 800	64 790	147	76 050

Table II: Survival, specific growth rate (SGR) and feed conversion ratio (FCR) of fish grown in brewery effluent treated in an agricultural-wetland (Experimental) or from a conventional water source (Control).

	Clarias gariepinus (Experimental)	*Clarias gariepinus* (Control)	*Oreochromis mossambicus* (Experimental)	*Oreochromis mossambicus* (Control)
Survival rate (%)	70	100	20	100
SGR (% g/day)	0.84	1.53	-0.96	0.69
FCR	2.05	1.11	-0.22	3.80

(Continued on next page)

Materials and methods

Glasswort *Salicornia*, saltbush *Atriplex*, sweet sorghum *Sorghum bicolor*, and spinach *Spinacia oleracea* were grown in raised beds for four months and irrigated with treated brewery effluent. Soil samples were taken monthly for laboratory analysis and plant biomass was recorded. *Spinacia oleracea* was also grown in an artificial wetland to treat the brewery effluent, and the treated effluent from this wetland was used to culture the African catfish *Clarias gariepinus* and Mozambique tilapia *Oreochromis mossambicus*. The experimental treatments were compared to a fresh water source, as a control.

Results and discussion

Spinacia oleracea had the best growth rate and biomass production. Salicornia had the highest accumulation of sodium in the leaf, while *Sorghum bicolor* accumulated the least (ANOVA, $F_{3,16} = 3.23$, $p<0.001$; Table I). Crop rotation had no significant e fect on plant growth. Soil amendment had a positive effect on soil structure and crop production.

There were significant differences in survival and growth of *Clarias gariepinus* and *Oreochromis mossambicus* cultured in different water treatments. The highest mean survival rates were observed in the control groups for both fish (Table II). *Oreochromis mossambicus* showed low growth rates and below optimum feed utilization while *C. gariepinus* showed higher higher growth rates and better feed utilization (Table II).

Treated brewery effluent may be suitable for irrigation of salt tolerant plants. It may also be suitable for the culture of hardy fish species such as *C. gariepinus* but may not be suitable for tilapia culture. The application of these data in the development of methods to determine the risk of using treated effluent in agriculture and aquaculture will be discussed.

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EFFECT OF VARIANT OVARIAN FLUID ON SPERM PERFORMANCE AND EGG FERTILIZATION RATES OF ARCTIC CHARR (*Salvelinus alpinus L.)*

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Successful *in vitro* fertilization in aquaculture is highly dependent on the method used to handle or treat the eggs and milt. As such it is of paramount importance that timely evaluation of the methods is conducted to enhance hatchery productivity and reduce the cost of production. In this study, three methods of handling eggs of Arctic charr in Sweden were evaluated, with the objective of ascertaining the importance of retaining the ovarian fluid in the egg batch. Further, activation of fish sperms with a commercial activator, ActiFish^{TM}, was tested to compare the fertilization rates, in a bid to overcome the current low egg fertilization and hatching rates among farmed Arctic charr. Variation of the volumetric amount of the ovarian fluid did not yield dissimilar fertilization rates. As such, tempering with the volume of the ovarian fluid under the current study did not affect the performance of the sperms and consequent fertilization rates. Further, no differential fertilization rates were recorded for the sperm extender and freshwater. However, positive relationships were recorded for fertilization rate and sperm velocity (VCL). The study contends that high and successful fertilization rates are likely to be obtained with or without the ovarian fluids under *in-vitro* fertilization of Arctic charr eggs.

MANIPULATING MACRONUTRIENT CONCENTRATION (N, P, FE) TO ENSURE VEGETATIVE CULTIVATION OF KELP GAMETOPHYTES (*Saccharina latissima)* FOR THE AQUACULTURE INDUSTRY

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Introduction

European seaweed aquaculture requires reliable methods to produce high-volumes of kelp gametophytes as a seedstock. These can be maintained vegetatively in seedbanks for year-round access, negating the need for natural spore collection (Westermeier et al. 2006). Additionally, since kelps are a source of valuable bioactive compounds, these vegetative gametophyte could be an alternative source, reducing harvesting of natural beds.

A number of abiotic factors influence growth and gametogenesis of gametophytes. Commonly, red light is used to maintain their vegetative growth. Yet, their growth rate is not optimal under red light and faster growth can be achieved under white light, but this also stimulates reproduction. The aim of this study was to identify the conditions that inhibits gametogenesis and maintains vegetative growth under white light.

Material and Method

Oogenesis was studied through factorial experimental on the female gametophyte of *Saccharina latissima.* Cultures were maintained under either red or white light (at 10 , and 12:12 L:D photo period. The effect of concentration of phosphate and nitrate in culture media and the ratio (N:P range: 4.5:1 – 60:1) was investigated over 35 days. In a separate experiment the iron concentration in artific al seawater (GP2) was manipulated (range 0 -0.0117 mM) and availability of this iron modulated using the chelator EDTA.

Results

All cultures remained vegetative under red light. Under white light cultures with Nitrate at concentrations above remained vegetative, while increases in phosphate reduced oogenesis. The culture remained vegetative when iron was excluded from the culture medium. Additionally increases in concentration of the chelator EDTA reduced the oogenesis.

Discussion

The manipulation of macronutrient concentrations is an effective method to allow vegetative cultivation of gametophytes under white light. Alteration of the N:P ratio did not fully inhibit oogonia formation, yet high nitrate concentrations did; possibly due to toxicity.

Iron appears to be essential for gametogenesis, with similar results reported for other species of kelps (Lewis et al. 2013). Depleting the medium iron concentration may be an effective method to maintain vegetative gametophyte cultures as a seedstock for seaweed aquaculture.

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ADVANCING EUROPEAN AQUACULTURE BY GENOME FUNCTIONAL ANNOTATION: 'AQUA-FAANG'

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Introduction

Recent developments in genomics have greatly advanced research and commercial innovation in European aquaculture. In particular, the creation of reference quality genome sequences for all the major farmed finfis species has enabled the development of robust genotyping tools, allowing the identificati n of numerous QTL of commercial importance, alongside the routine application of genomic selection in many species. Despite this progress, our understanding of the functional genomic basis for commercially important phenotypic variation (i.e. growth rate, disease resistance, etc.) remains limited, limiting our ability to exploit the predictive ability of genetic information.

Our project, "AQUA-FAANG", funded by the European Commission H2020 program, is tackling this knowledge gap, and aims to deliver a step improvement in understanding of genome function and the exploitation of genotype-to-phenotype prediction in the six most important European farmed fish species, Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), turbot (*Scophthalmus maximus*) and common carp (*Cyprinus carpio*), which together account for >90% commercial finfis production in Europe.

The AQUA-FAANG project

Our poster communicates the aims, deliverables and progress of AQUA-FAANG, funded at €6-million over 48 months (start date: 1st May 2019). The consortium comprises an interdisciplinary team of academics and industry partners from eight European countries: Norway, the United Kingdom, France, the Netherlands, Spain, Italy, Greece and Poland. AQUA-FAANG is one of three projects funded under the same H2020 Research and Innovation Action, with the others focussed on livestock. All three projects will coordinate to ensure the outcomes align with the objectives and principles of the international 'Functional Annotation of Animal Genomes' (FAANG) initiative (Andersson et al. 2015; Giuffra et al. 2019). AQUA-FAANG will also coordinate with other international actions to promote knowledge exchange and standardization of functional annotation datasets, including DANIO-CODE (Tan et al. 2016) and FAASG (Macqueen et al. 2017).

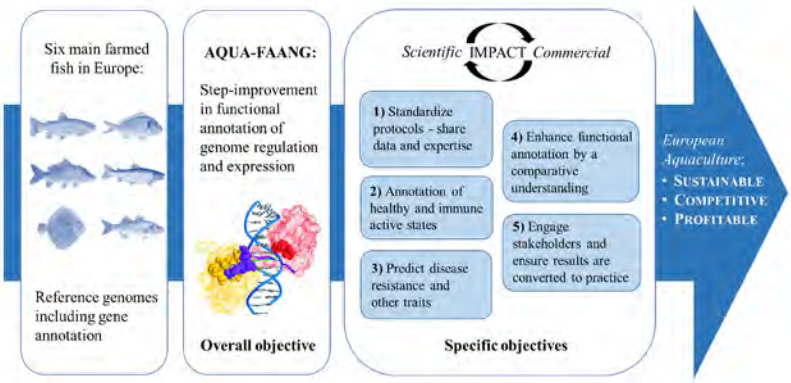

Fig. 1. Overall concept of the AQUA-FAANG project

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The AQUA-FAANG project (Fig. 1) will functionally annotate the genomes of the six targeted species, employing standardized assays and pipelines defined by FAANG. The goal is to document genome-wide functional and regulatory features under distinct biological conditions, including chromatin accessibility (by ATAC-Seq), enhancer and promoter activity (by ChIP-Seq), and protein and non-coding gene expression (by RNA-Seq). This will be done across distinct tissues and development stages in healthy animals, in addition to immune-activated and disease-challenged states, addressing the need to tackle the huge threat posed to aquaculture by infectious disease.

In total, ~28,000,000,000,000 basepairs of new sequencing data will be generated, which will be shared and managed through a dedicated centre (Harrison et al. 2018), and made publically available in an easy-to-visualize format via the Ensembl genome browser. Comparative analyses of the target species will enhance the quality of functional annotation by identifying conserved and lineage-specific features of genome functional regulation, which will provide transferable findings to other species and improve our understanding of genome functional evolution in fishes

The project includes applied research activities that aim to establish functional mechanisms underpinning resistance to infectious disease, and enhance the accuracy of genomic prediction of disease resistance. For example, marker panels enriched for prioritized causative genetic variants and novel statistical methods will be developed to improve the accuracy of genomic selection using functional annotation data.

To summarize, AQUA-FAANG is aimed at enhancing precision breeding, scientific innovation, and competitiveness within the aquaculture sector.

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OXIDATIVE STRESS STATUS AND MICROBIOTA OF GILTHEAD SEA BREAM (*Sparus aurata*) FED DIFFERENT DIETARY ARA/EPA/DHA RATIOS

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Introduction
The increasing fish oil substitution by vegetable oils in aquafeeds decreased dietary input of n-3 LC-PUFA. Marine carnivorous fish have limited capacity to produce LC-PUFA from C18-PUFA due to low enzymatic activity of fatty acid desaturases and elongases. Thus, dietary supply of arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 20:6n-3) are needed to fulfil animal's requirement for these essential fatty acids. A well-balanced dietary n-3/n-6 fatty acid ratio is also required to modulate fish health and welfare. The aim of this study was to evaluate different dietary ARA/EPA/DHA ratios on several oxidative stress indicators and microbial community of gilthead seabream juveniles.

Materials and methods
A feeding trial was performed with triplicate groups of gilthead seabream (IBW=15g), for 56 days at 23 °C. Four isoproteic (47%CP) and isolipidic (18%CL) plant-based diets (including 17.5% fish meal) were formulated containing a vegetable oil blend (20% rapeseed, 50% linseed, 30% palm) as main lipid source. Diets were supplemented with purified sources of fatty acids to obtain the following n3/n6 LC-PUFA levels: Diet A (2%ARA: 0.2%EPA: 0.1%DHA); Diet B (1.0%ARA: 0.4%EPA: 0.4%DHA); Diet C (0%ARA: 0.6%EPA: 0.6%DHA); Diet D (0%ARA: 0.3%EPA: 1.5%DHA).

Results
Diet composition did not affect growth performance of gilthead seabream. Total glutathione (tGSH), oxidized glutathione (GSSG), reduced glutathione (GSH), and oxidative stress index (OSI) in liver and intestine were not affected by dietary treatment (Table I). However, LPO was higher in fish fed diets C and D than diets A and B. GSSG, OSI, and LPO levels were higher in the intestine than in the liver, while GSH was higher in the liver (Table I). Glutathione reductase (GR) activity was higher in fish fed diet A than diets C and D. Catalase (CAT), glucose-6-phosphate dehydrogenase (G6PDH), superoxide dismutase (SOD), and glutathione peroxidase (GPX) activities were unaffected by dietary treatments (Table II). G6PDH and GPX activities were higher in the liver than in the intestine, while the opposite was true for CAT, GR, and SOD activities (Table II). In intestine mucosa, Bray-Curtis dendrogram showed that diets with and without ARA supplementation clustered separately (Fig.1). The pathogenic species *Edwardsiella tarda* was only isolated in the intestinal mucosa of fish fed diets with lower n-3 LC-PU A levels (diets A and B; Table III).

Conclusions
Overall, results indicate that diets with high EPA and DHA levels slightly increased lipid peroxidation but seemed to promote a healthier gut microbiota.

Acknowledgements
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Table I. Liver and intestine total glutathione (tGSH), oxidized glutathione (GSSG), reduced glutathione (GSH), oxidative stress index (OSI) and lipid peroxidation (LPO) levels of gilthead sea bream fed the experimental diets.

Organ	Liver					Intestine				
Diets	**A**	**B**	**C**	**D**	**SEM**	**A**	**B**	**C**	**D**	**SEM**
tGSH	1017	935	928	739	51.8	947	797	884	788	42.8
GSSG	39	29	36	28	3.8	111	114	135	133	7.5
GSH	977	906	893	711	49.0	837	683	748	655	42.3
OSI[1]	7.19	6.12	7.25	7.00	0.5	23.7	28.9	33.9	34.3	2.2
LPO	13.5	14.9	18.7	19.5	0.8	69.6	62.6	90.6	79.2	3.0

Two-way Anova

	Variance source			Diets			
	Diet	**Organ**	**Int**	**A**	**B**	**C**	**D**
tGSH	ns	ns	ns	-	-	-	-
GSSG	ns	0.000	ns	-	-	-	-
GSH	ns	0.043	ns	-	-	-	-
OSI[1]	ns	0.000	ns	-	-	-	-
LPO	0.000	0.000	ns	a	a	b	b

Values presented as means (n = 9 for liver and n = 6 for intestine)) and pooled standard error of the mean (SEM). LPO values expressed as nmols MDA g^{-1} tissue and GSH, tGSH, and GSSG as nmol g^{-1} tissue. Two-way ANOVA: ns: non-significant (P > 0.05).

Table II. Liver and intestine antioxidant enzymes activity of gilthead sea bream fed the experimental diets.

Organ	Liver					Intestine				
Diets	**A**	**B**	**C**	**D**	**SEM**	**A**	**B**	**C**	**D**	**SEM**
CAT	56.7	51.8	42.1	48.5	1.68	153.8	92.4	76.6	158.6	16.0
G6PDH	136.6	132.1	127.8	110.0	5.25	24.6	25.8	31.0	20.2	1.66
GR	8.8	7.3	7.0	6.6	0.28	20.7	15.9	14.9	16.1	0.96
SOD	106.9	126.9	110.3	108.5	6.59	536.6	488.1	493.0	371.6	25.3
GPX	54.2	36.4	38.5	35.5	3.22	10.2	9.6	9.6	9.0	0.64

Two-way Anova

	Variance source			Diets			
	Diet	**Organ**	**Int**	**A**	**B**	**C**	**D**
CAT	0.022	0.000	ns	-	-	-	-
G6PDH	ns	0.000	ns	-	-	-	-
GR	0.008	0.000	ns	b	ab	a	a
SOD	ns	0.000	ns	-	-	-	-
GPX	ns	0.000	ns	-	-	-	-

Values presented as means (n = 9 for liver and n = 6 for intestine) and pooled standard error of the mean (SEM). Enzyme activities expressed as mU mg $protein^{-1}$ for G6PDH, GR, and GPX and as U mg $protein^{-1}$ for CAT and SOD. Two-way ANOVA: ns: non-significant (P ≥ 0.05).

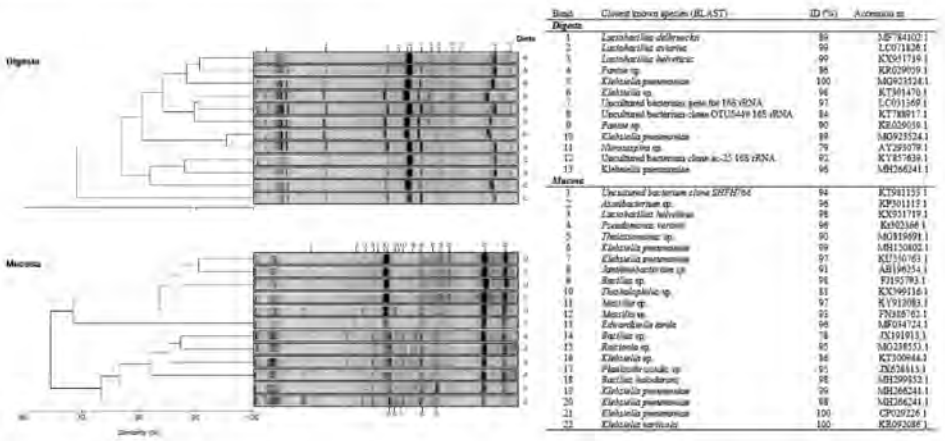

Fig. 1. Dendrograms and PCR-DGGE fingerprints of the allochthonous (digesta) and autochthonous (mucosa) intestinal microbiota of gilthead sea bream fed the experimental diets. Numbers (1 to 13 on digesta; 1 to 22 on mucosa) indicate bands excised for sequence analysis, identified on Table III.

DIETARY INCLUSION OF GLYCEROL IN RAINBOW TROUT (*Oncorhynchus mykiss*): EFFECTS ON GROWTH PERFORMANCE, DIGESTIBILITY AND PROTEIN EFFICIENCY

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Introduction

Glycerol is a readily available and inexpensive source of dietary energy proposed as a feed component to replace other ingredients currently included in animal feeds. Glycerol inclusion in feeds for terrestrial livestock has been investigated to some extent, although studies evaluating its potential use in fish feeds are scarce.

Rainbow trout is considered to be one of the most commonly exploited species in freshwater aquaculture worldwide. The provision of dietary energy in this carnivorous species derived from compounds other than from protein, such as glycerol, may reduce the use of dietary protein as an energy source through catabolism, decreasing the ammonia that is excreted to the environment by the fish

This study tested the effects of dietary glycerol inclusion on growth performance, nitrogen and energy balance, digestive capacity, as well as fillet quality in rainbow trout fed to apparent sa iation for 60 days.

Materials and methods

Rainbow trout were fed diets with 0 (control), 2.5, or 5 % glycerol inclusion (w/w, refined), all formulated to have an equal energy and protein content (21 kJ kg^{-1} and 49 % DM, respectively). The incorporation of cellulose at different levels in the formulation was used to create the dietary contrast for the addition of glycerol.

Table 1. Zootechnical and nitrogen balance parameters in rainbow trout fed diets with (2.5 and 5 %) or without (Control) glycerol inclusion.

	Control	2.5 %	5 %
IBW (g)	20.30 ± 0.06	20.14 ± 0.04	20.23 ± 0.07
FBW (g)	87.49 ± 2.09	85.27 ± 2.86	79.39 ± 1.39
SGR (% day^{-1})	2.43 ± 0.04	2.40 ± 0.05	2.28 ± 0.03
VFI (% BW day^{-1})	1.87 ± 0.04^{a}	1.87 ± 0.02^{a}	2.05 ± 0.03^{b}
FCR	0.90 ± 0.02^{a}	0.91 ± 0.02^{a}	1.04 ± 0.02^{b}
PE (%)	1.35 ± 0.07^{a}	1.30 ± 0.10^{a}	0.96 ± 0.04^{b}
RN (mg N $kg^{-0.7}$ day^{-1})	181.15 ± 7.12	177.35 ± 9.82	151.34 ± 4.45
TAN_{ER} (mg N $kg^{-0.7}$ day^{-1})	162.89 ± 4.80^{a}	175.57 ± 3.25^{a}	217.45 ± 7.22^{b}

Mean values ± SEM per tank (n=3). Letters indicate significant differences between treatments ($P < 0.05$). IBW and FBW, initial and final body weight; SGR, specific growth rate; VFI, Voluntary feed intake; FCR, Feed conversion ratio, PE, protein efficiency; RN, retained nitrogen; TAN_{ER}, total ammonia nitrogen excretion rate.

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Juvenile rainbow trout were reared at the UTAD facilities in freshwater. Fish were divided into groups of 25 fishes between nine tanks (300 l) connected to an open circulation water system (15 ºC). Experimental diets were randomly assigned to each tank in triplicates, and fish were hand-fed ad libitum daily (9:00 and 17:00 h). Total feed consumption and mortality data were daily recorded. Feed, faeces and whole-body samples were analysed for dry matter, ash, crude protein content crude fat content and gross energy. Digestive enzyme activities (α-amylase, lipase, trypsin, and chymotrypsin) were measured in whole intestinal tract including pyloric caeca at 6 or 24 h after feeding (n=8). A separate trial was conducted to measure the apparent digestibility (ADC) adding 1 % of Cr_2O_3 in each diet. Dietary and faecal Cr_2O_3 concentrations were determined after perchloric acid digestion. One hour after feeding, 6 fish (BW 112.4 ± 5.6 g) from each tank were transferred to 30 l boxes (triplicates) containing well-aerated freshwater. Water samples were obtained from each tank to calculate total ammonia nitrogen excretion rate (TAN_{ER}). Parameters were analysed by one-way ANOVA and Tukey's post-hoc tests were applied ($P<0.05$).

Results & Discussion

Refined glycerol was well digested (>99.7 %) in rainbow trout fed diets supplemented this compound up to 5 %. Amylase, but not proteases and lipase, was altered by glycerol inclusion, as this digestive enzyme activity increased 6 h post-feeding in trout fed 2.5 and 5 % diets. Growth performance was not altered by glycerol inclusion, although dietary inclusion of this compound at 5% induced an increase in voluntary feed intake (Table 1). Opposite to what it was expected, dietary inclusion with glycerol had a negative effect on protein efficienc . Trout fed the 5 % diet displayed increased energy intake, which was not reflected in a reduction of the TAN_{ER}, indicating that glycerol at 5 % does not spare the use of proteins as a metabolic fuel in trout. No differences were detected between dietary treatments in the fillet colour characteristics investigated. Despite of this increased catabolism of proteins when refined glycerol is included at 5 %, the inclusion of this compound at 2.5 % does not appears to had negative effects on growth performance and the nitrogen excretion rate, which may indicate that the inclusion of this ingredient could be possible in rainbow trout diets. This suggests that dietary inclusion with glycerol at this level could be a convenient source of metabolically available energy in rainbow trout.

Acknowledgements:

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THE SUBSTRATE SELECTION AND SPAWNING BEHAVIOR OF PIKEPERCH *Sander lucioperca* L. BROODSTOCK UNDER POND CONDITIONS

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Introduction

Artificial spawning substrates are widely used for supporting reproduction of percids (Čech et al., 2012; Crane & Farrell, 2013; Lehtonen et al., 2006). Investigation of spawning substrate preference could help to support natural pikeperch populations. The aim of this study was to determine spawning substrate preferences of pikeperch broodstock and to clarify some aspects natural spawning behavior. The secondary objective was to assess the suitability of the preferred substrates for egg incubation, hatching and subsequent larvae production in RAS (recirculating aquaculture system).

Material and methods

Mature pikeperch males (n=6) and females (n=6) with oocytes in first and second stages (Zarski et al., 2012) were randomly placed into each of three similar sized earthen ponds 10 m x 5 m x 1 m. To observe a natural pattern of spawning kinetics, spawning was not hormone-induced. Water temperature at one meter depth was measured at hourly intervals with an auto-recording thermometer (Minikin Tie, EMS Brno Ltd. Czech Republic) throughout the trial. Three types of substrate were used to make circular (Ø = 890 mm) spawning nests: soft brush (bottle brushes) with fiber length 100 mm, artificial turf of 35 mm length and a smooth plastic sheet (Fig. 1). The following parameters were observed: time from fish stocking to male occupation of a nest; time from stocking to spawning; and time from male occupation of nest to spawning. After detection of spawning, nests with attached eggs were moved to controlled conditions of RAS for evaluation of incubation success (hatching rate %).

Results

Fourteen days after the beginning of the experiment, 17 of 18 pikeperch pairs had spawned, and spawning success accounted 94.4%. A significantl higher preference ($p < .05$) was found for brush (61.1±9.6%) than for artificial turf (33.3± 0%). Throughout the experiment, 65% of spawning events occurred between 0700 and 1100 hr and 18% spawned between 1900 and 0700 hr. Spawning during the day was 12% and 6% for the periods from 1100 to 1500 hr and 1500 to 1900 hr, respectively. The time from broodstock stocking to male occupation was 96.5±45.5 hrs in brush and 98.5±17.9 hrs in turf. Time between the first male occupation of a nest and spawning was 79.5±77.1 hrs in brush and 48.7±52.2 hrs in turf nests. Time from stocking to spawning was 167.4±72.8 hrs in the brush nests and 147.2±46.3 hrs in turf. The general linear model showed higher water temperature to be significantly associated with spawning occurrence ($F_{1, 24} = 32.27$; $p < .05$) but not substrate preference. The preference for brush substrate was consistent throughout the experiment and was not influenced by water temperature or nest availability ($p > .05$).

Discussion

Availability of suitable spawning grounds is crucial for sustainability of wild population (Lehtonen et al., 2006). In this study pikeperch exhibited preference to thick rigid structure of the brush nest over soft artificial turf fibers, while smooth plastic has not been selected at all. Results confirmed opportunistic strategy of pikeperch spawning and emphasized the importance of suitable spawning sites to be available during reproduction period. Temperature was the only factor affecting spawning substrate selection. Fish was tending to select the most suitable type of spawning substrate even when number of available nest was decreasing. Despite the fact that significant difference in incubation success between two spawning substrate types was absent, there was a tendency to higher larvae production from brush nest compare to artificial turf. In the nature, pikeperch spawn on dense structures such as plant roots and branches (Lappalainen et al., 2003). Gravel or sand is used rarely, and according to current study could indicate lack of the suitable spawning grounds. Obtained data could be used for indication and supporting of spawning sites in natural water bodies and will help to increase the natural production of pikeperch populations. Adding of artificial substrate to shallow areas of open waters has been found supportive for several percid species (Crane & Farrell, 2013). In the same way, spawning opportunities for pikeperch in natural conditions could be increased by providing of artificial spawning substrates

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Figures

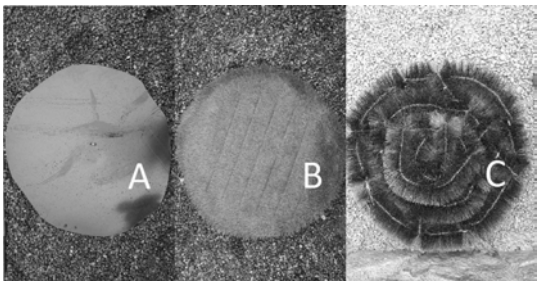

Figure 1. Photo of materials used for investigation of spawning substrate preference of pikeperch *Sander lucioperca.* A – smooth plastic; B – artificial turf; C – brush

PERSPECTIVES FOR INDOOR SEAWEED (*Ulva ohnoi*) CULTIVATION IN PHOTOBIOREACTORS

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Introduction

The potential of macroalgal biomass as feedstock for biorefinery is being are increasingly recognized worldwide (Bikker et al., 2016). Seaweed components have been shown to possess interesting properties for applications in functional feeds, for instance in aquaculture (Fernández-Díaz et al., 2017), and in the cosmeceutical and nutraceutical industries. However, to be able to guarantee constant quality and production, essential for the development of for instance human functional products, development of low-cost, high-efficient on-land cultivation systems is essential (Hafting et al., 2012)

Phytoplankton indoor cultivation is commonly carried out in photobioreactors (PBRs). Especially since the definite breakthrough of LED lightning, drastically decreasing energy costs of cultivation, this process has rapidly gained importance. First studies with *Ulva* sp. in PBRs under controlled climate conditions and fluorescent light (Rodríguez et al., 2012) and various seaweed species in outdoor PBRs aerated with CO_2-enriched air (Chemodanov et al., 2017) showed first potential of seaweed production using PBRs. In the framework of the European BIOSEA project, cost-effective cultivation technology of micro- and macroalgae is studied to obtain potentially valuable extracts through biorefinfer . Here, we explore the potential of PBRs under semi-controlled conditions for the year-round production of *Ulva* biomass.

Material and methods

The chlorophyte macroalga (*Ulva ohnoi*), isolated from a former salt evaporation pond in south Spain, was grown in 75 L polycarbonate photobioreactors (PBR) at room temperature and under constant aeration, receiving light from LED tubes (150 µmol photons.m^{-2}.s^{-1}, 16:8 L:D cycle). Nutrients (f/2 solution) were added following growth-dependent uptake predicted by a model. Temperature was recorded continuously. Trials were held in weeks with different temperatures to determine starting biomass for maximum yield. Seaweeds were restocked to starting biomass weekly, at which time yield and growth rate were calculated. Harvested algae were dried at room temperature for component extraction, analysis of proximate composition, and detailed analysis of amino acids, fatty acids and sugar composition. Cultivation periods were November 2017 – September 2018 and November 2018 until now.

Results

The algae generally grew well in the cylinders. Highest growth rates and yield were obtained between March and the end of July, with temperatures ranging from 16-17 to 24°C. Growth rates averaged 10% d^{-1} during this period (average yield 104g FW.PBR^{-1}). Optimum biomass yield could be obtained at an initial density of 60 – 70 g FW biomass per PBR. Growth was mainly determined by room temperature, which was limiting in the coldest months of the year, whereas continuously high temperatures (> 26 °C day and night) throughout summer caused collapse of the cultures by the end of September.

Preliminary results of proximate composition of samples taken at 2 month intervals indicate fairly constant carbohydrate, lipid and protein levels in the seaweeds. *U. Ohnoi* was rich in sugars with the polysaccharide ulvan and rhamnose, glucose and xylose as most dominant monosaccharides. Interestingly, lipid contents were consistently high (≥ 5% DW) compared to the literature median of 2.3% DW.

Discussion and conclusions

The results of this study show that it is possible to grow *Ulva* in indoor PBRs in the south of Spain during large part of the year with low energy consumption (LED light and aeration) and hence costs. The bottleneck for year-long growth under these circumstances is temperature. During winter months, growth is reduced but does not cease altogether. The collapse of the cultures in early autumn is most likely related to continuously high temperatures, especially at night. *U. Ohnoi* in the field from the same location have been shown to have highest growth rates in July, with water temperatures averaging ≥ 30°C, however, night temperatures always decreased below 25°C (Coste et al., 2018). This suggests that a better control of room temperature during the night (for instance through improved ventilation) might prevent collapse of the culture.

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Earlier studies have showed promising results of cultivation in PBRs for other smaller seaweed species, such as the red alga *Gracilaria gracilis* (Malta and Sánchez, 2012) and *Cladophora* sp., *Ulva compressa* and *U. rigida* (Chemodanov et al. 2017). As the method is entirely modular, it can be easily upscaled to larger surfaces, for instance in greenhouses. It is concluded that the PBR method combined with LED lighting shows great potential for the continuous production of seaweed biomass with a constant quality for production of valuable compounds through biorefiner .

Acknowledgements

The BIOSEA project (Innovative cost-effective technology for maximizing aquatic biomass-based molecules for food, feed and cosmetic applications) has received funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme under grant agreement No. 745622.

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TOWARDS A STANDARD MODEL FOR LAND-BASED IMTA: CASE STUDIES FROM EARTHEN PONDS IN SPAIN AND PORTUGAL

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Introduction

Integrated Multitrophic Aquaculture (IMTA) refers to the associated cultivation of aquatic species belonging to different trophic levels, in which only one level is externally fed. The other levels use the waste (debris from uneaten food and excreta) produced by the former, potentially increasing production efficiency and decreasing waste. IMTA is recognized worldwide as one of the models of sustainable aquaculture (Neori et al., 2004). The concept of IMTA has a long history of successful commercial applications in Asia, in particular. In Europe however, only few companies implement this system, while facing socio-economic, administrative and legal bottlenecks hindering the development of IMTA to its full potential (Kleitou et al. 2018). The European Project Integrate, funded by the ERDF through the Interreg Atlantic Area Programme, and running since 2017, is working on taking down these barriers in order to foster cooperation for industrial transition towards IMTA in the European Atlantic Area (Dove and Agraso, 2018). To identify the knowns and unknowns of IMTA, three pilot actions are being carried out within the project across the European Atlantic Area, representing forms of aquaculture in the participating countries.

Earthen pond IMTA pilot action

This pilot action intends to develop and consolidate a land-based IMTA standard model: fish (poly)culture-mollusks-invertebrates-salt tolerant plants or algae taking advantage of existing aquaculture earthen pond systems. Earthen pond aquaculture is commonly found in southern Spain and Portugal. The earthen ponds are salt evaporation ponds adapted for aquaculture production of fish, shrimps, bivalves and other species. These land-based production systems are ideal candidates for IMTA development due to the possibility of controlling the diversion of water through the different inputting and extracting compartments that comprise IMTA systems before final discharge (Cunha et al. 2019). However, there has been no major commercial development of pond IMTA due to the lack of clear standard models to enable upscaling.

Experimental pond IMTA systems have been set up for the Integrate project in an existing and operational fish/oyster farm (CTAQUA, Spain) and in a pilot scale experimental earthen pond system (EPPO-IPMA, Portugal) in which different cultures are grown simultaneously in the same water body. Cultures were started at the beginning of 2018 and have been ongoing throughout 2019. Different cultivation methodologies are being developed and tested and extensive sampling and monitoring of the different cultures is being carried out. For the biological compartments (fish, oysters, phytoplankton and seaweeds), this includes monitoring of growth and biomass development. Furthermore, the relevant water and environmental parameters are monitored.

In this poster, we present the first results of these pilot systems. Flow charts and conceptual models intend to trace the nutrient flows in the system and to show how and where these are interconnected. Data of the experimental systems, such as growth of the organisms, nutrient content of the water, the feed and the organisms form the basis of a numerical ecosystem model. The model is one way to determine the degree of interconnection of the cultures, alongside mixing models with stable nitrogen isotope levels. Results will be integrated in guidelines for good practice of a standard model for semi-extensive land-based IMTA.

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Acknowledgements
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BIOREMEDIATION OF AQUACULTURE AND BIOGAS SIDE STREAMS USING POLYCHAETES (*Hediste diversicolor*, O.F. MÜLLER, 1776). PART II: BIOCHEMICAL COMPOSITION

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Introduction

"*In a world with growing pressures on resources and the environment, the EU has no choice but to go for the transition to a resource-efficient and ultimately regenerative circular economy*" (EREP, 2014). Bio industries are especially well suited to spearhead circular principles, as biological processes *per se* rely on recycling and reuse of organic and inorganic compounds. Circular approaches can help making aquaculture operation greener, e.g. by producing high quality feed components rather than harvesting them. Polychaetes are a natural part of the diet of many aquatic animals and contains high levels of sought after biochemicals, such as omega-3-fatty acids and proteins. Further, they are detritivores, making them perfectly suited as recyclers of organic side streams. Sludge from RAS aquaculture is an obvious feed source for aquatic vermiculture, and has been shown to be promising (Bischoff, Fink, Waller, 2009; Pajand, Soltani, Bahmani, Kamali,

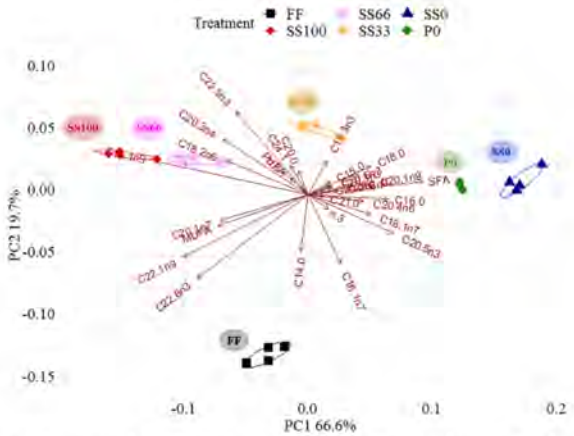

Figure 1: PCA of fatty acids (% of the total FA) of polychaete feeding with different feeds, the data was arcsine transformed, SFA, MUFA, PUFA, and TFA was included.

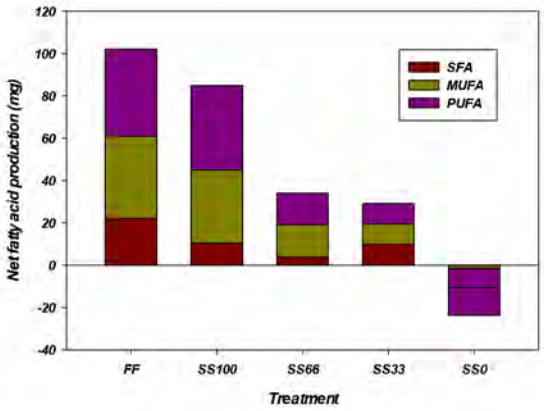

*Figure 2: Net production of different FA's (mg) in polychaetes (*H. diversicolor*) after 30 days of cultivation on fish feed (FF), solid biogas digestate (SS0), smolt sludge (SS100) or a 2:1 and 1:2 mixed ratio of smolt sludge and solid biogas digestate (SS66 and SS33, respectively).*

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2017). As an alternative, biogas production is on the rise, and the solid digestate after methanogenesis might also pose a promising substrate for worm faming.

Material & Methods

We reared the polychaete *Hediste diversicolor* along a gradient spanning from pure sludge from salmon smolt production to pure solid biogas digestate in 33% steps for 30 days. A group receiving fish feeds served as the positive control. Worms reared on these five different diets were analysed for growth, survival, (cf. part I of this study; Hagemann et al), and biochemical composition.

Results

The polychaetes accepted all diets and displayed positive growth. Their biochemical composition reflected their diets and the groups were clearly separated for considering fatty acids (Figure 1). Worms grown on fish feed showed the highest net fatty acid production, while there was a decrease along the gradient of solid biogas digestate addition (Figure 2).

Discussion & Conclusion

H. diversicolor is a promising candidate to produce high quality feed components from to-date-considered wastes. However, current regulations on an EU level do not allow to use animal products produced on aquaculture and biogas side streams as feed ingredients. Clearly, regulations have to be reviewed to unleash the full potential of circular approaches.

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IN VITRO MODELISATION TO DETREMINE THE EFFECT OF STANDARDIZED DRY GRAPE EXTRACT TO PROTECT RAINBOW TROUT (*Oncorhynchus mykiss)* RED BLOOD CELLS FROM OSMOTIC STRESS

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Introduction

Oxidative stress is the result of an imbalance between free radicals and antioxidants defenses. In aquaculture, this phenomenon can be induced by different factors, among which, the temperature, oxygen and salinity changes (Brinie-Gauvin *et al.*, 2017). To estimate the effect of treatments on the reduction of oxidative damage, red blood cells can be exposed to an artificial oxidative stress in order to activate the antioxidant defenses (Stocker *et al.*, 2003; Girard *et al.*, 2005; Lykkesfeldt *et al.*, 2007). Antioxidants from natural sources, especially grape polyphenols have been evidenced to reduce oxidative damage caused by free radicals (Bagchi *et al.*, 2000). The aim of this study was to evaluate the protective effect of standardized dry grape extract, as a source of antioxidants to protect red blood cells from hemolysis induce by osmotic stress in rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Red blood cells have been prepared from blood collected on rainbow trout at slaughter at the end of the production period then placed in heparin tubes. The blood samples were centrifuged at 3500 rpm for 12 minutes at room temperature. Supernatants were removed and the red blood cells were washed 3 times with a solution of NaCl (9mg/ml of distilled water). The solution of standardized dry grape extract (Nor-Grape® WS, Nor-Feed) were prepared at different concentrations, 2.5mg/ml, 5mg/ml and 7.5mg/ml in phosphate buffer saline (PBS, 1g/100ml of water, pH = 7.4).

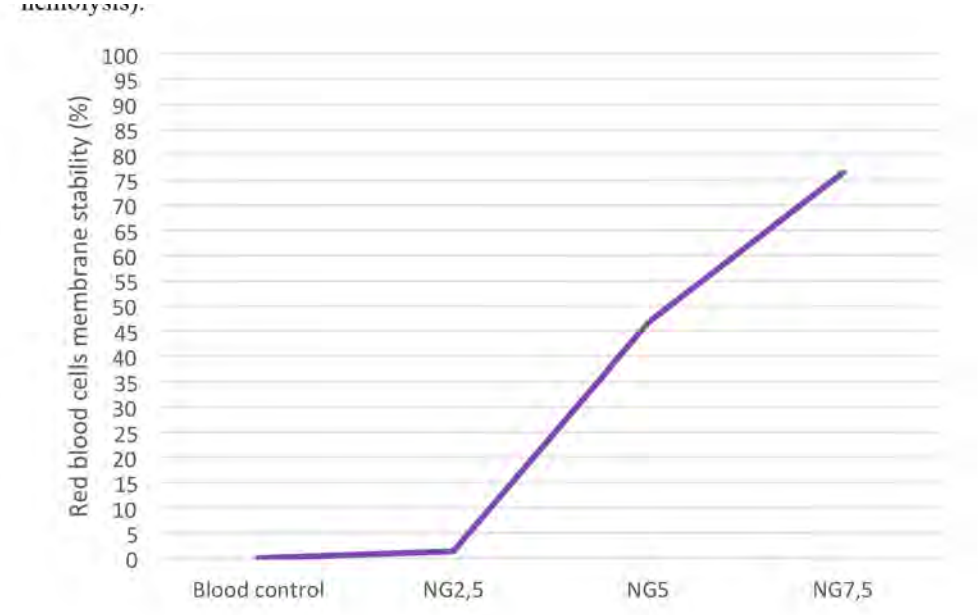

Figure 1. A standardized dry grape extract effect on red blood cells membrane stability

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The assay solutions (NG2.5 , NG5 and NG7.5) were composed of 1ml of PBS, 1ml of a solution of grape extract (2.5 mg/ml, 5 mg/ml or 7.5 mg/ml), 0.5ml of the red blood cell solution (RBCS) at 25% (v/v) and 2ml of NaCl solution at 2g/l in water, to induce an osmotic stress. In the blood control (BCTL), NG solutions were replaced by PBS while control solutions were also prepared (NGCTL), containing the NG solutions without RBCS (replaced by PBS). The solutions were incubated at 56°C for 30 minutes, to induce a hemolysis reaction and were then centrifuged at 5000 rpm for 10 minutes at room temperature. The released hemoglobin was measured by spectrophotometry at 560 nm. Stability of red blood cells was expressed as follow:

% stability = 100 – ((Abs assay solution – Abs NGCTL)/Abs BCTL) × 100
The absorbance value represented the mean of 3 consistent measures.

Result

Results evidenced that standardized dry grape extract at the two highest concentrations (5mg/ml and 7.5mg/ml) improved the membrane stability of red blood cells to 50% and 80% respectively (Figure 1.), compared to the lowest concentration (2.5mg/ml, 98% hemolysis).

Discussion and conclusion

This trial showed the positive impact of a standardized dry grape extract on the stability of red blood cells from rainbow trout. A dietary supplementation with a standardized dry grape extract in the fish diet could thus be an efficient solution to manage oxidative stress, induced by the fluctuation of salinity, in order to reduce oxidative damage fish cells. More research is required in order to evaluate the validity of this method on RBC from different situations.

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A STUDY OF THE EFFECT OF SAPONINS DIETARY SUPPLEMENTATION ON THE MANAGEMENT OF SHRIMP'S ENDOPARASITES INFESTATION (ESPECIALLY *Nematopsis sp*. AND *Cephalolobus sp*.) IN ECUADOR

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Introduction

One of the most common parasites in production of shrimp is an endoparasite, gregarines from gender *Nematopsis sp*. and *Cephalolobus sp*. These parasites represent one of the principal infectious agents of anterior, medium and posterior digestive tract in shrimp (Jones *et al.*, 1994; Zanguee *et al.*, 2011). They stay in the gut, reduce feed absorption and may induce mortality of the hosts (Lightner, 1933). The aim of this trial is to evidence the effect of a standardized saponins-containing plant extract used in animal nutrition on the reduction of gregarines from gender *Nematopsis sp*. and *Cephalolobus sp*. in shrimp in Ecuador.

Materials and methods

The trial was conducted in two pools heavily infested with gregarines (Pool 1 and 2). In these pools, the supplementation of a standardized saponins-containing plant extract (Norponin® XO2, Nor-Feed) was done during 3 days, incorporated at 2g/kg in the complete feed of shrimp. The measured parameter, count of shrimp intestinal parasites (gregarines trophozoite and adult forms), was analyzed before providing standardized saponins-containing plant extract and after 3 days of treatment. In the two pools, 10 alive shrimps were randomly collected before the inclusion of standardized saponins-containing plant extract, and 10 animals after 3 days of supplementation. Analysis of the shrimp intestine was made directly after their collection. This analysis consisted to get a homogeneous and representative sample of intestinal content of shrimp. The entire intestine was taken out the animal, placed with 10ml of lugol and the mixture was placed in test tubes. After the homogenization, a sample of 1ml of this mixture was placed under the microscope to count the parasites (gregarines trophozoite and adult forms).

Figure 1. Effect of standardized saponins-containing plant extract on the reduction of the intestinal endoparasites infestation in shrimp.

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Result

Analysis of the shrimp intestinal content (Figure 1) evidenced the positive effect of standardized saponins-containing plant extract inclusion at 2g per kg of shrimp complete feed during 3 days on the reduction of gregarines infestation by 64% in the pool 1 and 73% in the second, compared to animals before the treatment.

Discussion and conclusion

This trial evidenced the positive effect of the standardized saponins-containing plant extract in shrimp production to reduce infestation of the intestine by endoparasites, especially gregarines (*Nematopsis sp*. and *Cephalolobus sp*.). This treatment has to be tested in new trials in shrimp production to better understand the mode of action of the standardized saponins-containing plant extract.

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IMPACTS OF BOTTOM TRAWLING ON MARINE ECOSYSTEM

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Introduction

Trawling is a fishing technique in which a boat pulls a net through the water to trap and catch all kind of fishes. The net can be pulled anywhere in the water column of the ocean, including the midwater or bottom sections. Bottom trawling is a type of fishing in which a net or other collection device is dragged over the seabed to catch demersal fish, crustaceans and shellfishes. It is also referred to as 'dragging'. When the net is towed along the sea floo , the technique is called bottom trawling. There are two types of bottom trawling. They are benthic and demersal bottom trawling. Benthic trawling involves towing a net at the very bottom of the ocean, while demersal trawling is the process of towing the net just above the benthic zone.

Impacts on marine ecosystem

Bottom trawling causes several negative impacts on marine ecosystem which are leads to overfishing, bycatch, affect the seabed, damage coral ecosystem, affects benthic habitat and benthic flora and fauna. Large numbers of fish are caught through bottom trawling which adversely affect the fish stocks. The catch other than the target species is called by bycatch. Bottom trawling is entrap the many fish species, marine invertebrates, marine mammals, reptiles and even seabirds are caught in bottom trawling. The seafloor is a very stable and calmest part of the sea, where currents, temperature, and other natural conditions remain relatively undisturbed. Bottom trawling across the sea floor to scoop up fish, stirs up the sediment lying on the seabed, displaces or harms some marine species, causes pollutants to mix into plankton and move into the food chain and creates harmful algal blooms or oxygen deficient dead zones, resulting in a destabilization of the seaflo .

Coral reef is a habitat to many species. But, bottom trawling can uproot and kill coral colonies. Destruction of such corals adversely affects the species dependent on them. Bottom trawling cause negative effects on benthic habitats such as sandbanks, reefs and biogenic structures with their characteristic ecological communities and sensitive species. Bottom trawling operations uproot and displace marine flora and fauna living on the ocean floo . Sea anemones, sea pens, sponges, urchins and other fragile bodied marine fauna are destroyed during the trawling process.

Fishery management tools can be used to mitigate the effects of bottom trawling on seafloor habitats. Fishery management tools include fishing effort reduction, modification of fishing gear design or gear type and establishment of areas closed to fishing. Effort reduction is the cornerstone of managing the effects of fishing, including, but not limited to, the effects on habitat. The success of fishing effort reduction depends on the resilience and recovery potential of the habitat. Gear modifications will be most useful for finfis species that can be caught with gear that does not rely on disturbing the bottom to catch the fish. Closed areas are necessary to protect a range of representative habitats. Closures are particularly used for protecting areas with emergent epifauna (e.g., corals, bryozoans, hydroids, sponges) that are vulnerable to even low levels of fishing effort. The ultimate aim is to develop the alternative gear against bottom trawl net and create awareness to the fishermen s regarding the impacts on marine ecosystem

1-MONOGLYCERIDES OF SHORT- AND MEDIUM-CHAIN FATTY ACIDS PROVED TO BE EFFECTIVE IN DECREASING THE MORTALITY CAUSED BY *Flavobacterium psychrophilum* AND IN REDUCING THE ANTIBIOTIC TREATMENTS IN RAINBOW TROUT (*Oncorhynchus mykiss*), WITH FAVORABLE ECONOMIC RETURN FOR THE FISH PRODUCERS

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Introduction

Visceral flavobacteriosis is the most frequent cause of mortality in trout fry and fingerlings, involving economic losses for the trout producers. Flavobacteriosis outbreaks can occur up-to 10g of fish size. The standard approach to contrast this infection is the use of florfenicol. Despite the efficacy of the antibiotic in controlling the disease, the production losses can be severe if the antibiotic is not given promptly: the mortality caused by flavobacteriosis ranges, in standard farming conditions, between 10% and 30% during the whole cycle; it can reach peaks of 20-25% during a single outbreak. Another negative consequence deriving from the antibiotic usage is the photo-sensibilisation that can occur when the fish is treated in outside ponds and that can cause severe mortality. A specific composition of 1-Monoglycerides of short and medium chain fatty acids from C3:0 to C12:0, available on the market under the commercial name SILOhealth 108, inhibited the *F. psychrophilum* growth *in vitro*, at pH 6-7 (pH of the fish gastro-intestinal tract) at the concentration of 0.01%. In a previous trial, the 1-Monoglycerides composition modulated the fish intestinal microbiota by increasing the number of beneficial lactic bacteria and reducing several pathogenic bacteria (Rimoldi et al., 2018). In the present study the composition was tested for three years in two trout Italian farms with different environmental conditions and proved to be able to prevent flavobacteriosis outbreaks, to reduce the mortality and the antibiotic treatments in the hatcheries phase, with favourable economic return for the feed producers.

Table I. Characteristics of the farms

	FARM 1	FARM 2
Water source	Spring-water (or pumped water in emergency cases)	River
Geological features	Plain	Mountain
Yearly trout production in 2018	750 tons	450 tons
Stocked eggs/year	3 000 000	2 000 000

Table II. Direct yearly costs without or with 1-Monoglycerides in the hatchery period in two trout farms

Direct costs	Without 1-Monogl.	With 1-Monogl.	Without 1-Monogl.	With 1-Monogl.
	Farm 1	Farm 1	Farm 2	Farm 2
Antibiotic	990€	712€	519€	73€
Dead fish (*)	11 724€	6 224€	9 512€	2 182€
1-Monogl.	==	551€	==	248€
Total of costs	12 714€	7 478€	10 031€	2 503€
Saved amount		5 227		7 528

(*) The costs for the dead fish include the cost of the eggs, the feed cost and the general production costs.

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Field observations related to the control of infections caused by *Flavobacterium psychrophilum* in the trout farming practice with 1-Monoglycerides

The impact of the 1-Monoglycerides composition on the visceral flavobacteriosis incidence in the hatchery phase was evaluated in two trout farms located in North Italy for three years, from 2016 to 2018. The characteristics of the farms are reported in the table I.

The production parameters, some direct and indirect costs generated in the farms during the observation period were compared with the historical average data recorded in the previous three years. In the Farm 1, before the introduction of the 1-Monoglycerides composition, the flavobacterios outbreaks used to occur at the fry sizes of 0.6-0.7g, 1.8-2.2g and, sometime, at 3.5-4g, with mortality rates of 15%, 8% and 6% respectively per cycle. In the Farm 2 the flavobacterios outbreaks were observed at fry sizes of 0.55-0.65g, 1.5-2.1g and 3.0-3.8g, with mortality rates of 16%, 9% and 6% respectively per cycle. During the three years of observation the 1-Monoglycerides composition was administered at a dosage of 1% of the feed in both farms during the whole hatchery period. In the Farm 1 the treatments with florfenicol were reduced by 72% compared to the standard treatment programs implemented in the previous cycles, and the mortality was reduced in average by 65.2%. In the Farm 2 the treatments with florfenicol were reduced by 66% and the mortality by 71.6%. The 1-Monoglycerides composition prevented flavobact riosis in the majority of fingerlings, and only some tanks were treated with the antibiotic. The economic benefit for the producers derived from the reduction of the antibiotic costs and from the significantly higher survival rate which reduced the costs related to the dead fingerlings. The calculation factors are reported in the Table II. The costs incurred by the producers for the dead fingerlings were calculated by considering the biomass during each single flavobacteriosis outbreak, the mortality rate, the cost of the eggs and of the feed, the general production costs.

The direct costs resulted to be reduced by 41.1% in the Farm 1 and by 75.1% in the Farm 2. Other indirect costs like those related to the reduction of the final biomass to be sold on the market due to the mortality in the hatchery period were also considered in the final costs/benefits evaluation. Higher mortality in the hatchery involves less revenues for the trout producer at the end of the production cycle and higher incidence of the general production costs. As previously indicated, the mortality caused by flavobacteriosis in the hatchery period was reduced by 65.2% in the Farm 1 and by 71.6% in the Farm 2. The production costs at the end of the production cycle were reduced by 7% in the Farm 1, that is from 2.60€ to 2.45€/kg of produced fish, and by 6%, from 2.72€ to 2.55€/kg of produced fish in the Farm

Conclusions

The 1-Monoglycerides composition proved to be effective in decreasing the mortality caused by *F. psychrophilum* and in reducing the antibiotic treatments in the trout hatchery phase, with positive economic return for the fish producers. It can be taken into consideration as a valid and promising alternative approach, complying with the EU and global policy for antimicrobial resistance reduction.

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EVALUATING BARRAMUNDI (*Lates calcarifer*) SPERM QUALITY USING HIGH THROUGHPUT ADVANCED REPRODUCTIVE TOOLS

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Introduction

Barramundi, or Asian sea bass (*Lates calcarifer*), is an emerging species for aquaculture worldwide (ABARES, 2013). The growing demand for barramundi fillets necessitates intensification of barramundi production. Breeding practices in barramundi farming have not evolved since the 1980s with the introduction of hormonal therapy to trigger group-spawning events. This group mating strategy often leads to highly skewed paternity among offspring, resulting in a loss of valuable genetic diversity (Frost et al., 2006; Loughnan et al., 2013). With few studies available on male fertility and a lack of technology to quantify sperm quality in barramundi, efforts to develop reproductive tools to obtain such knowledge for this species is warranted. Thus, the aim of this study was to develop high throughput techniques to evaluate barramundi sperm quality in order to provide preliminary information on basic sperm biology that allows future investigation of male fertility.

Materials and methods

Spermatozoa were collected by catheterization on multiple occasions from n=15 mature male broodstock maintained in breeding condition at 30°C in 28,000 l tanks, with salinity at 30ppt on a 16h light: 8h dark cycle. Samples were diluted in marine Ringer's solution, adjusted to 400mOsm to mimic osmolality of barramundi seminal plasma, then held on ice. Sperm morphology was measured by ImageJ on SpermBlue-stained smears, and this data was used to calibrate and validate automated sperm counting and motility assessment by computer-assisted sperm analysis (CASA; Androvision, Minitube). Factors such as optimal sample dilution, the minimum number of fields, and the effect of motility were examined to determine sperm detection accuracy compared to manual haemocytometer methods. A Hoechst/propidium iodide (PI) viability assay was validated using 70°C heat-treated controls and a 5-point intact:damaged dilution curve of 0:100, 25:75, 50:50, 75:25 and 100:0% spermatozoa assessed by flow cytometry (≥100 000 cells; FACS Canto II, BD Biosciences). Lastly, a FITC/PI TUNEL DNA fragmentation assay (In situ cell death detection kit - fluorescein, Roche) was validated using DNase-treated sperm controls assessed by flow cytometry (≥100 000 cells). Once validated, the optimised assays were used to characterise baseline barramundi sperm quality.

Results

Barramundi spermatozoon has a structure, consisting of an ovoid head (length 2.44 ± 0.03µm; width 2.23 ± 0.03µm; ratio 1.11 ± 0.01), and a single flagellum (length 30.99 ± 0.49µm) connected by a short midpiece. Based on these measurements, a detection profile was created on the Androvision CASA system to identify barramundi spermatozoa. Accuracy of the automated sperm count was highly correlated with the manual counting method (r = 0.99, P < 0.001). Sample dilution at 1:1000 (across 5 dilutions ranging from 1:250, 1:500; 1:1000, 1:2500 and 1:5000) gave the most accurate automated sperm concentration when compared to manual haemocytometer (r = 0.87, P = 0.001, ICC = 0.99). Automated sperm concentration determined using three or more CASA fields of view have similar or improved precision (coefficient of variation ≤ 8.7%) when compared to manual haemocytometer (coefficient of variation = 8.0%). Moreover, detection accuracy was not affected by sperm motility since automated sperm concentration was highly correlated when using motile or immotile cells (r= 0.99, P < 0.001). Approximately 99.9 ± 0.07% of heat-treated spermatozoa were detected as dead Hoechst+/PI+ cells by flow cytometry. Moreover, sperm viability detected by Hoechst/PI assay was highly correlated with predicted viability using the 5-point dilution curve of intact:damaged spermatozoa (r= 0.98, P < 0.001). Lastly, 71.9 ± 4.4% of DNase-treated spermatozoa were detected as DNA damaged FITC+/PI+ cells by flow cytometry. Based on the validated assays and optimized conditions above, male barramundi broodstock exhibited baseline sperm quality of 15.1 ± 3.6 x 10^9 sperm.ml.-1 concentration, 52.8 ± 9.6% total and 13.1 ± 4.2% progressive motility, 64.2 ± 3.5% live, and 43.5 ± 6.0% DNA damaged spermatozoa.

Discussion and conclusion

Through several technical trials, we have validated the use of CASA, Hoechst/PI staining, TUNEL and flow cytometry for reliably assessing the concentration, motility, viability and DNA integrity of barramundi spermatozoa. Importantly, these assays permitted a rapid and accurate assessment of up to 100 000 sperm per fish; providing a comprehensive assessment of barramundi semen characteristics. Preliminary sperm quality data obtained in this study exhibited levels of cellular damage similar to that of frozen-thawed spermatozoa of other species (Zilli et al., 2003*;* Pérez-Cerezales *et al.*, 2010). Further research is necessary to identify the cause of such damage to determine whether it was artificially induced by sperm handling procedures (e.g. sub-optimal extender, prolonged storage), or was the result of poor quality spermatozoa in some individuals that could potentially explain the underlying mechanism for highly skewed paternity previously observed in offspring from captive bred barramundi.

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CRYOPRESERVATION OF AFRICAN CATFISH *Clarias gariepinus* SPERMATOGONIA

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Introduction

African catfish *Clarias gariepinus* is widely distributed throughout Africa, and has been considered one of the most important farm fish on that continent. During the last few decades, this species has been introduced into Europe, Asia and Latin America. The suitability of this species for aquaculture comes from its high growth rate, relatively low requirements for water quality, resistance to handling stress, ability to tolerate high stocking densities and having a good meat quality. Gamete manipulation during artificial propagation of these species presents several problems with the main one being that these species are oligospermic with the volume of sperm collected generally very low, even after hormonal treatment. Therefore, sperm needs to be extracted from the testes themselves, however, during this procedure males are either killed, or the testicular pieces are obtained through biopsy. As *in vitro* germ cell culture through which spermatogenesis can be recovered from a number of seeded SSCs can overcome some of the hurdles present in the artificial propagation of catfish species, the aim of this study was to optimize the spermatogonia cryopreservation procedure as the first step in the germline cell manipulation techniques, as well as to initiate *in vitro* spermatogonia proliferation from frozen/thawed samples.

Material and methods

African catfish used in this study were kept in a recirculation system (Sentimento Kft., Hungary) at the Department of Aquaculture, Szent István University, Hungary. Fish were housed under a 12 hr light/12 hr dark cycle at 26 ± 0.2 °C. Fish were sacrificed with a 2-phenoxyethanol overdose and testes were aseptically excised as previously described. Testes were kept in PBS on ice until further work (maximum 1 h). Tissue was cut into small pieces (~ 30 mg) which were subsequently used for cryopreservation.

Protocol for freezing of both European and African catfish spermatogonia was optimized in a single trial in which we tested the effects of two extenders and three cryoprotectants in three concentrations (a total of 18 test groups). Two tested extenders were (1) PBS supplemented with 1.5% BSA, 0.1 M trehalose and 20 mM HEPES and (2) the extender developed by Yoshizaki group (100% extender: 55.27 mM HEPES, 375.48 mM NaCl, 7.28 mM KCl, 23.10 mM KH_2PO_4, 3.82 mM Na_2HPO_4, 3.64 mM sodium pyruvate, 2.6 mM $CaCl_2 \cdot 2H_2O$ and 1.4 mM $MgCl_2 \cdot 6H_2O$, pH 7.88) used in zebrafish and trout species. Cryoprotectants tested were MeOH, Me_2SO and EG in 1, 2 and 3 M concentrations. Each piece was equilibrated for 30 min in 1 ml of cryomedium in 1.8 ml cryotubes. Samples were then frozen in CoolCell boxes in a -80 °C freezer (enabling a cooling rate of ~1 °C/min), while the thawing was conducted in a 26 °C water bath.

Optimization of the vitrification protocol was conducted in a single trial in which we tested the effects of two equilibration solutions and two vitrification solutions, as well as by testing the exposure time to the vitrification solutions. ES and VS contained different concentrations of MeOH, EG and Me_2SO (ES1: 1.5 M MeOH + 1.5 M M Me_2SO; ES2: 1.5 M PG + 1.5 M Me_2SO; VS1: 1.5 M MeOH + 5.5 M M Me_2SO; VS2: 3 M PG + 3 M M Me_2SO). The exposure to the equilibration solutions was 15 min, while the exposure to the vitrification solutions was either 1, 1.5 or 2 min. The extender consisted of L-15 supplemented with 10% FBS, 25 mM HEPES and 0.5 M trehalose. The NIV procedure was done following the protocol described above with ~20 mg tissue pieces.

In order to verify the functionality of SSCs after cryopreservation, testicular cells isolated from mature frozen/thawed African catfish testes were seeded into a 6-well plate, and cultured for three weeks. The culture medium consisted of L-15 medium supplemented with 10% FBS, 1% common carp serum, 100 U/ml penicillin, 100 mg/ml streptomycin, 2.5 µg/ml amphotericin B, 800 µM $CaCl_2$, 20 mM HEPES, 0.1 mM β-mercaptoethanol, 20 µg/ml L-proline, 20% ddH2O, 10 IU/ml hCG, 100 ng/ml EGF, 50 ng/ml 11-KT and 10 ng/ml DHP (pH = 7.4). Approximately 3 million cells were seeded in each well containing 3 ml of the culture medium. Cells were cultured at 26 °C in air. Medium was changed every two to three days; as the cells were cultured in a suspension culture, prior to every medium exchange cells were centrifuged at 200 ×g for 10 min to allow cells to sediment.

(Continued on next page)

Results

Cryoprotectants and their concentrations had a significant effect on post-thaw viability (three-factor ANOVA; $p < 0.01$), while the extenders did not have a significant effect. The highest SSC viability was observed when using 3 M Me_2SO with Yoshizaki extender and 3 M EG with PBS (Tukey's HSD, $p < 0.05$). Therefore, cryomedium containing 35.2% Yoshizaki extender and 3 M Me2SO was determined as the optimal reaching SSC survival rates of approximately 75%.

During NIV, all tested parameters displayed a significant effect on SSC viability (three-factor ANOVA; $p < 0.01$), while the statistical differences among individual groups were difficult to delineate (Tukey's HSD, $p > 0.05$). As the highest average post-warming viability was below 20%, freezing of catfish testicular pieces was evidently superior to vitrificatio

Individual cells that were seeded at the start of the culture started to form small spherical aggregates of approximately 15-20 cells during the first day. As the culture period progressed, the size of aggregates became larger which was indicative of cell proliferation. The size of aggregates grew from 28.1±4.9 µm on day 1 to 60.4±20.6 µm on day 7. At day 7, tails of spermatozoa started to emerge at the edges of aggregates. As all spermatozoa initially seeded were washed away during the initial two washes, spermatozoa observed after the first week were most likely produced from the seeded early-stage germ cells.

Discussion and conclusions

This study is the first to demonstrate successful freezing of African catfish SSCs. In addition, *in vitro* germ cell culture was adapted for the first time for African catfish SSCs and thus displayed that the cryopreserved early-stage germ cells are functional and are able to proliferate, differentiate and produce spermatozoa in culture. Technology developed in this study has the potential to overcome several hurdles present in catfish spawning. The main ones are the small volume of sperm produced by these species even after hormonal treatment, and the necessity to extract testicular sperm which can be of a lesser quality. Additionally, the need for extraction of testicular sperm inevitably evokes the need to either sacrifice the fish, or to conduct biopsy. In both cases, sperm extraction is laborious, and can lead to a reduction of brood fish, however the extracted sperm will most likely be used for only one spawning. A step towards improving this practice came in sperm cryopreservation, where the unused spermatozoa could be saved and stored for the next spawning.

The technique developed in this study presents the logical progression in the spawning technology. Beside the spermatozoa obtained through testicular samples, one can also obtain early-stage germ cells, including SSCs. These cells can be then seeded into culture, and spermatozoa can be produced through in vitro spermatogenesis. Later, such sperm can be used for fertilization and the production of the next generation. In addition to these techniques, an in vitro culture of SSC which can induce their self-renewal will enable a continuous availability of germ cells which can be later used for in vitro spermatogenesis, spermatozoa production, and subsequently in vitro fertilization and creation of offspring.

AN OPTIMIZED PROCEDURE FOR NUCLEAR EXTRACT PURIFICATION FROM MARINE FISH AND APPLICATION TO HISTONE ACETYLTRANSFERASE AND DEACETYLASE ACTIVITY EVALUATION IN GILTHEAD SEABREAM (*Sparus aurata*) EGGS

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Introduction

Gene expression regulation is an important mechanism to control the production of a given gene [Emerson and Li (2010)]. Changes in gene expression that does not involve alterations in the DNA sequence are, generally, designated by the term epigenetics and refers to the trait variations resulting from external or environmental factors that switch genes on and off [Moore (2015)].

Acetylation and deacetylation of lysine residues are common epigenetic modifications, mediated by histone acetyltransferases (HAT) and deacetylases (HDAC3), respectively.

In this work we aimed at the development and validation of an effective extraction method for an enriched nuclear protein fraction and evaluate how maintaining a viable portion of gilthead seabream Sparus aurata L. 1758 eggs in the sieve for a longer period of time (in this case 30 minutes) affects the histone acetyltransferase and deacetylase activities.

Material and methods

Gilthead seabream (*Sparus aurata*) eggs were obtained from the broodstock adapted to captivity, at the Aquaculture Research Station of the Portuguese Institute for the Sea and Atmosphere. Fish are kept in in a flow through water system with controlled temperature and constant aeration. Eggs were collected in triplicates at the beginning of the experiment (T0) and after were left exposed to air for 30min and new samples were collected. Protein extracts, enriched in the nuclear fraction were purified and used to determine the activity of histone acetytransferase and deacetilase using the Histone Acetyltransferase Activity Assay and Histone Deacetylase Activity Assay kits, respectively.

Results and discussion

Hereby, we describe a cost-effective methodology, which allows an enrichment of the protein extract in the histone-containing fraction and that have been successfully applied to marine species.

HAT and HDAC3 activities were measured and a significant decrease on the HAT activity (figure 1A) was observed, after the period of exposure (T30), suggesting a decrease on transcriptional activity [Bannister and Miska (2000)], fact that may have an important impact on the normal fish development. Early stages of vertebrate development are a critical period concerning epigenetic reprogramming with a consequent increase on the transcriptional activity and initial cellular differentiation [Bonnet-Garnier et al., (2018)].

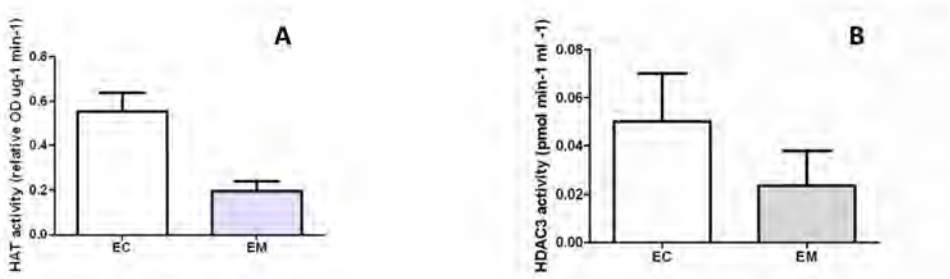

Fig. 1 Histone acetyltransferase (A) and deacetyltransferase (B) activities in control (EC) and manipulated (EM) situations. * indicate values significantly different (unpaired T-test; $P < 0.01$)

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Regarding the effect on deacetylase activity, it was not observed a significant change (figure 1B). The effect of histone acetylation is normally counterbalanced by histone deacetylases and gene knockout studies have shown that the four classes of histone deacetylases play important roles during vertebrate development [Haberland et al., (2009)]. Also, several studies suggest the use of deacetylase's-directed therapies for diabetes and cancer treatments, for example [Kawada et al., (2017)].

Since hatcheries daily routines could have a significant impact on organism's welfare and future development, handling procedures, should take these results into account in order to minimize as much as possible their negative effects on fish development.

Also, it would be interesting to use this methodology on the evaluation of the effect of other routine procedures (e.g. egg disinfection) and environmental changes (e.g. acidification) on HAT and HDAC3 activities and correlate the results with the level of expression of selected genes, to have some inference on the impact it may have on organism's welfare and development.

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ARTIFICIAL FERTILISATION IN SENEGALESE SOLE (*Solea senegalensis*): INDUCTION WITH GNRHa AND DETERMINATION OF EGG QUALITY

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Introduction

Currently, natural spawns from Senegalese sole (*Solea senegalensis*) in culture are only obtained from wild individuals adapted to captivity (Angus and Cañavate, 2005) or mixed broodstocks of wild males and cultured females (Personal observation). Spawning of cultured broodstock (F1 generation) has failed as fecundity was low and eggs were unfertilized (Guzman et al., 2008). This has been correlated with dysfunctions detected in both sexes, male and female cultured breeders exhibited reduced sperm production and low fecundity compared to wild breeders. Therefore, *in vitro* fertilisation is a possible solution to overcome these problems (Rasines et al., 2012; 2013). The most important aspects of an in vitro fertilisation protocol are to obtain high quality sperm and eggs at the same time. A commonly used strategy to stimulate ovulation for *in vitro* fertilisation in fish is by means of an exogenous treatment with gonadotropin-releasing hormone agonists (GnRHa). In the present study, the timing of ovulation, stripping and egg quality was determined when Senegalese sole (*Solea senegalensis*) females were administrated GnRHa.

Material and methods

Prior to hormone induction the female maturity status was determined using three different methods, abdominal swelling, ratio between total and gonad length and oocytes diameter. In addition, sperm parameters sperm motility (28.59 ± 22.18%) and velocity average path (VAP) (216.80 ± 55 μm/s) were determined with computer assisted sperm analysis (CASA - ImageJ). Optimal time between ovulation and stripping of eggs was determined by stripping induced females every three hours starting from 35 hours after the injection of GnRHa (25 μg/ kg). Each batch of eggs was fertilized with pool of sperm. Lastly preliminary trials were completed to examine the sperm to egg ratio (pure sperm, 1:2; 1:4; 1:8; 1:16; 1:32; 1:64) required to obtain high rates of fertilisation.

Results

The optimal stripping time was from 40 to 44 hours following GnRHa injection at 16ºC. The GnRHa induction had successful rates of fertilization of 37.81 ± 44.43 %. The period of egg viability after ovulation until eggs lost viability was three hours after the stripping 44 hours post injection. The three different methods: abdominal swelling, ratio between total and gonad length and oocytes diameter were useful for determining maturation status and were positively correlated. Highest rates of fertilisation were obtained with females that had abdominal swelling index 3, ratio between total and gonad length of 0.55 and oocytes diameters of 580 μm. High fertilisation rates (76%) were obtained with a spermatozoa to egg ratio of 75:1, however, this result should be treated with caution until confirmed with repetition

Discussion and conclusions

When *Solea senegalensis* was induced with a single injection of 25 μg/kg body weight of GnRHa at 16°C, the time of ovulation into the ovarian cavity was between 41 and 44 hours post induction (Rasines et al., 2012; 2013). There was a positive correlation between the different determinations of maturity status. According to oocytes diameter, the ideal stage for high fertilisation was the post vitellogenic stage. When the oocytes were in a hydration phase fertilisation was poor and it appeared that ovulation was earlier and eggs became overripe indicating that possibly it was not necessary to induce. In the case of abdominal swelling, stage 3, gave the higher fertilization rate. Ratio between gonad and total length was the least useful method to predict egg quality after GnRHa application.

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Sperm quality (VAP and motility) decreased during the experimental period. The percentage of initial motility was as others reported for sole (30-40%) (Chauvigne et al., 2017), nevertheless, there was a large difference with previous studies in pelagic fish. However, the VAP (µm/s) observed in this study, average values were 216 µm/s, was low compared with values obtained in previous studies. On the other hand, the successful fertilisation of eggs (76%) with a sperm to egg ratio of 75:1 was surprising and very different to other studies. In contrast, the values that were found in other reports about other species showed higher ratio of spermatozoa per egg.

The use of GnRHa to induce females successfully accelerated the oocytes develop to obtain good quantity and quality of eggs released. There was a positive correlation amongst the three different methods that were used to observe the maturity status. When broodstock were injected with GnRHa at 16ºC, females should be examined at 41 hours post induction. Good egg quality was maintained for three hours, between 41 and 44 hours post injection. High fertilisation rates (76%) were obtained with a spermatozoa to egg ratio of 75:1, however, this ratio should be treated with caution until confirmed with repetition.

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CELL GROWTH AND TOXIN PRODUCTION OF *Gambierdiscus* spp. STRAINS FROM THE MACARONESIAN REGION

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Introduction

Ciguatera poisoning is a food-borne illness mostly caused by consumption of fish contaminated with polyether toxins known as ciguatoxins (CTXs). Originally known as a tropical disease, Ciguatera is being increasingly reported from areas previously not considered endemic, namely in the North-Eastern Atlantic. The epi-benthic dinoflagellates *Gambierdiscus* and *Fukuyoa* are considered to be the causative agents of Ciguatera. Furthermore, these genera have recently been shown to have an increasing number of species, also discovered in areas where it had not been observed before, namely the Canary Islands. A total of 54 strains of the genus *Gambierdiscus* were recently isolated from field samples collected from the Macaronesian Region. The study presented here is part of the MIMAR project (MAC/4.6D/066) (2017-2020) aiming at the characterization of hitherto unidentified CTXs from the North-Eastern Atlantic Ocean.

Materials and methods

Two strains of *G. australes* (OCH85 and OCH141), one strain of *G. carolinianus* (OCH100) and two strains of *G. excentricus* (OCH45 and OCH92) originated from the Canary Islands were cultured using 150 mL of culture medium in 75 cm^2 culture flasks under the same culture conditions. Culture medium consisted of natural Atlantic seawater (salinity adjusted to 32), filtered at 0.2 µm and enriched with f/5 nutrients (Guillard and Ryther, 1962), with the addition of selenium and the exception of silica. Cultures were maintained in a thermostated incubator at 25 °C under full spectrum LED lights with an incident photon flux density of 60 µmol photons $^{-2}$ s^{-1} and a daily light-dark cycle of 12 h :12 h.

Culture experiments were conducted using a semi-continuous batch method to keep the cells into the log-phase growth, as previously described by Pisapia et al. (2017) and Litaker et al. (2017). Four different treatments of the bottom surface of the culture flasks (Corning®), i.e. Non-Treated (NT), TC-Treated (T), CellBind (CB) and Ultra-Low Attachment (ULA), were evaluated for their influence on the growth of *G. australes* strains. Cell concentration (cells mL^{-1}) was estimated once per week, using a 1 mL Sedgewick-Rafter counting chamber under a light microscope. Specific growth rate (µ, d^{-1}) was the slope calculated by the linear regression of the natural logarithm of the cell concentration versus time, after correcting for serial culture dilutions.

Gambierdiscus cells were harvested in the log-phase growth and toxins were extracted using MeOH (x2) and MeOH:H_2O (1:1, v/v) (x2). Crude extracts were adjusted to MeOH:H_2O (3:2, v/v) and partitioned twice with dichloromethane (DCM). The lipophilic CTXs were partitioned into the DCM soluble fraction (DSF). Triplicate DSF samples were used for screening their CTX-activity using the O/V neuro-2a (n2a) assay. The assay was carried out in 96-well flat-bottom plates with vacuum gas plasma

treatment for cell adhesion. Pacific ciguatoxin-1B (CTX1B) was provided by R. J. Lewis (Queensland University, Australia) and used as reference standard material. For each DSF sample, four 10-fold serial dilutions were tested in three separate experiments and three replicate wells for each dilution were run for each experiment.

Results

Specific growth rates (μ) of *G. australes* strains under ITC laboratory conditions ranged from 0.088 d^{-1} ± 9.2% (RSD) to 0.165 d^{-1} ± 3.3% (RSD). *G. australes* OCH141 exhibited slightly higher μ than *G. australes* OCH85 in all the conditions tested. Slight differences in μ were observed depending on the bottom surface tested. The fastest growth was observed when the cells were cultured in Corning® ULA flasks, and the slowest growth occurred in Corning® CB flasks. Estimation of μ of *G. carolinianus* and *G. excentricus* strains is currently ongoing.

A first screening of the neurotoxic activity of the strains revealed that *G. carolinianus* OCH100 and *G. australes* OCH141 showed no detectable CTX-activity at any of the concentrations tested. Some low CTX activity was observed in *G. australes* OCH85, at the highest concentration only. Both *G. excentricus* strains were clearly positive for the presence of CTXs.

Discussion and Conclusions

This study showed that *Gambierdiscus australes* strains behaved as slow growers under ITC laboratory conditions, with $\mu < 0.17$ d^{-1}, which appeared similarly low or somewhat lower than those reported in literature (Pisapia, 2017). All the different flasks employed for the culture experiments were suitable for cell growth, although some slight differences were observed in specific growth rates. Interestingly, the treatments for cell adhesion seem to disfavor *G. australes* growth. Still, statistical analyses are needed to determine whether the different substrates have an authentic impact on cell growth.

Preliminary results of the n2a assay suggest that *G. excentricus* species is likely to produce more CTXs than the others, in accordance with previous studies (Pisapia et al., 2017; Litaker et al., 2017). A serial dilution scheme with a narrower dilution factor is needed to trace sigmoidal dose-response curves, determine EC_{50} values and to quantify the amount of CTX1B eq per cell. For the other species, more concentrated samples are needed to clearly detect and quantify the low CTX amounts they are likely to produce – they are expected to produce CTXs in the range of fg $cell^{-1}$.

Further studies will focus on culture scale-up and bioguided fractionation of the most toxic strains in combination with high resolution mass spectrometry to pinpoint known and/or previously undescribed toxins.

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INFLUENCE OF DIETARY LIPIDS AND ENVIRONMENTAL SALINITY ON THE ω-3 LC-PUFA BIOSYNTHESIS CAPACITY OF SENEGALESE SOLE (*Solea senegalensis*)

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Introduction

The replacement of fish oils (FO) with vegetable oils (VO) in aquafeeds is a viable alternative for the sustainability of the aquaculture industry. However, it compromises the nutritional value of the species by decreasing the supply of long chain polyunsaturated fatty acids (LC-PUFA), such as 20:5n-3 (EPA) and 22:6n-3 (DHA). The ability of fish to biosynthesise LC-PUFA can be influenced by the environmental conditions, food habits, and phylogeny (Castro *et al*., 2016). It is well known that *Solea senegalensis* (SS), displays *fads2* (Δ4) activity which is involved in the biosynthesis of DHA from EPA, and its gene expression responds to dietary modulation (Morais *et al*., 2012, 2015). However, the combined effect of dietary and salinity modulation on the lipid metabolism of SS remains unknown. The aim of the present study was to assess the capacity of SS to biosynthesise LC-PUFA with different sources of dietary lipids (FO *vs* VO) as well as under different environmental salinities (35ppt *vs* 20ppt), analysing the flesh fatty acid composition and the expression of *fads2* (Δ4 desaturase) and *elovl5* (C_{18} & C_{20} elongation) in liver and foregut.

Material and methods

24 fishes of 491.0 ± 75g (mean ± SD) initial body weight were distributed into 4 tanks and cultivated for 9 weeks. Two tanks were fed with a commercial SS diet (FO) while the other two received an experimental diet (25% commercial SS diet and 75% commercial tilapia diet; VO). For each dietary treatment, a control and a low salinity were assayed (35ppt and 20ppt, respectively). For all treatments, fish were reared under natural photoperiod, at an average water temperature of 18.5 ± 0.4 ºC and dissolved oxygen above 5ppt. At the end of the experiment, the individuals were slaughtered by a sharp blow to the head, individually measured and weighed. Samples of blood, muscle and several tissues were taken for further biochemical analysis. In addition, samples of liver and intestine were collected in RNAlater® to measure the expression of the *fads2* and *elovl5* genes by qPCR (Morais *et al*., 2012).

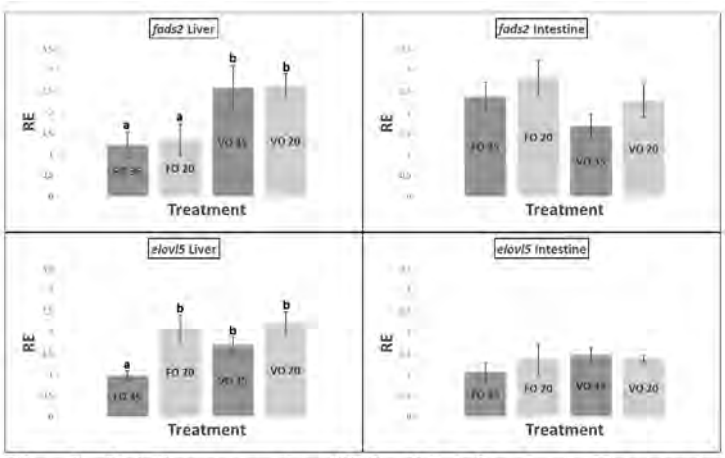

Fig. 1. Relative expression (RE) of *fads2* and *elovl5* genes in liver and intestine of *Solea senegalensis* (mean ± SE, n = 6). Different letters indicate significant differences (ANOVA, Duncan post-hoc test, P <0.05).

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Results

After 9 weeks of experiment, the greatest weight gain was registered in the FO-20 treatment (FO-20: 103g; FO-35: 54g; VO-20: 35g; VO-35: 33g). VO-based diets provided 43% less DHA and 42% less EPA than FO diets. Muscle of the FO-20 fish contained 18% less DH than that of FO-35.

The relative expression studies showed that liver *fads2* gene was upregulated in fish receiving the VO diet compared to FO-fed fish (Figure 1) whereas the *elovl5* gene was downregulated in fish fed the FO-35 treatment compared to the rest of treatments, indicating a trend for a higher expression at low salinity groups (FO-20 and VO-20). Intestinal *fasd2* and *elovl5* gene expression did not vary among treatments. However, for the same diet, *fads2* gene expression tend to be upregulated at lower salinity.

Discussion and conclusions

The greater growth of FO-20 fish could be associated with a lower energy expenditure for osmoregulation (lower salinity), in combination with a diet adapted to fulfill the nutritional requirements of *Solea senegalensis* (FO diet). On the other hand, although the VO diet provided less EPA and DHA, the composition of muscle fatty acids shows an important elongation and desaturation activity by converting significant amounts of E A into 22: 5n-3 and DHA, respectively.

The expression of *fads2* was upregulated in liver of fish fed the VO diet accordingly to Morais *et al.* (2015). This result confirms that lower dietary levels of ω-3 LC-PUFA, stimulates the capacity of biosynthesis in this species. Furthermore, Morais *et al.* (2012) observed that the enzymatic activity and the transcription of genes modulated by the diet were more evident in the liver than in the intestine, in agreement with our results. The liver has an important role in lipogenesis, showing more variable levels of gene expression than intestine. The partial substitution of FO by VO in combination with a decrease in salinity seems to be a more sustainable farming strategy in order to obtain SS specimens particularly rich in DHA.

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COMBINED EFFECT OF INCREASING TEMPERATURE AND FEED RESTRICTION IN GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES

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Introduction

Tolerance to environmental warming and persistence in the natural environment depends on the limited capacity to acclimate to changing temperatures. Temperature plays a key role among these environmental factors due to its direct impact on all physiological processes. Temperature has a direct impact on all physiological processes including oxidative damage, showing enormous influence in the physiological adaptation to the aquatic environment. Rises of temperature can increase growth rates and food conversion efficienc . For profitable operation of aquaculture facilities, it is necessary to feed fish preferably with the optimum feeding rate. The appetite and thus the optimum feeding rate increase with temperature. The food consumption continues to rise even if the temperature exceeds this limit, but the food is no longer completely digested. Overfeeding and excessive temperatures generate unnecessary costs; also decreasing the water quality and finally the profit. Therefore, in the present study we test the combined effect of three different temperatures (23, 25 and 27ºC) and two different ration sizes in growth performance and the response of biomarkers of oxidative stress and antioxidant enzymes within the nervous, branquial, intestinal and hepatic tissues and blood of gilthead seabream juveniles.

Materials and methods

288 alevins born in captivity (average weight of 6.2±1.15g and size 7.1±0.3cm) were randomly divided into 18 homogeneous groups of 16 fish each. The groups were maintained at the Instituto Español de Oceanografía (Tenerife, Spain) facilities in fibe glass tanks (1m^3 cylindrical) with a constant water exchange and aeration, under natural conditions of photoperiod, water salinity (37.5psu), and oxygen saturation (92.4 ± 4.8%). Fish groups were maintained at a temperature of either 23, 25 and 27ºC. Fish were fed a commercial pellet for seabream (Skretting Ltd 1.5) at 50% or 100% of optimum feeding rate resulting in 6 treatments by triplicate. Feed was supplied daily (6 meals day-1). Feed left uneaten was recovered from the bottom of the tank 30 minutes after its administration to quantify the daily feed intake (FI). The samplings were carried out over a period of 0, 15, 30, 45, 60 days. Growth performance (specific growth rate, SGR; coefficient of variation for weight, CV; condition factor, CF), Hepatosomatic index (HSI), survival (S) and feed intake (FI) were determined.

Blood was collected from the caudal vessels using heparinized syringes. Plasma samples were separated after centrifugation and levels of glucose, lactate sodium and potassium, were determined by standard spectrophotometric assays (Spinreact, Spain). Also classical stress biomarkers: reactive oxygen species (ROS) and lipid peroxidation and blood indicators were analyzed.

Results

Results in growth performance showed that significant main effects of feed restriction and temperature on specific growth rate (SGR) were detected. Also, there was a significant interaction between temperature and feed restriction. Overall, exposure to 27°C significantly increased SGR and feed-restriction significantly decreased SGR. Warming did not affect body size variations of fish (P>0.05). In contrast, hepatosomatic index (HSI) was lower in feed restriction and with temperature increased (P<0.05).

The condition factor index (CF) was similar during the experiment irrespective of the temperature tested. However, at 23º and 25ºC the fish under feed restriction showed a significantly lower CF (P<0.05). Regarding intake, an increase in total intake (g) was observed with the increase in temperature but % Feed Intake (% of biomass per day) was not affected by the effect of food restriction or temperature.

In relation to stress biomarkers and specifically reactive oxygen species, catalase, superoxide dismutase (SOD), glutathione peroxidase (GST) and lipid peroxidation were determined. Results on catalase showed that there was no significant main effect of feed restriction or temperature on catalase levels in gills, liver, intestine and brain tissues, nor was there a significant interaction between temperature and feed restriction.

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Furthermore, there was no significant effect of temperature, feed restriction or an interaction between temperature and feed restriction on SOD levels in brain and liver tissue
Other blood indicators were also analyzed, and again no significant differences were observed in haematocrit, glucose, lactate and sodium after 60 days of trial. Only levels of chloride were decreased in fish exposed to a 27°C compared to 23ºC.

Conclusions
Preliminary conclusions showed that higher temperatures promoted increased SGR and total intake regardless of the food restriction. No mortality was observed during the experiment and no significant effect of temperature increased on coefficient of variation for weight and condition factor was observed. Stress biomarkers and blood indicators were not significantly affected by temperature increased. These results suggest that the degree of thermal stress endured by these fish was not severe

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MODELLING THE IMPACTS OF CLIMATE CHANGE ON SHELLFISH AQUACULTURE

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A modelling approach was developed to analyse the potential direct (temperature and food availability) and indirect (pathogen) effects of climate change on cultivated shellfish species of commercial interest

Four species-location pairs were considered: Pacific oysters and blue mussels in the North Sea (Netherlands), blue mussels in the North Sea (Denmark), and Mediterranean mussels in the SW Atlantic coast of Europe (Portugal). The focus was on biological effects on shellfish production (growth and mortality), together with changes in environmental externalities related to water quality, and resulting socio-economic consequences.

The modelling work was supported by (i) multi-stressor laboratory experiments (temperature x food concentration x oxygen saturation) designed to parameterize the direct effects of climate change on bivalve growth; (ii) future climate predictions under several IPCC scenarios generated from downscaled physical and biogeochemical models. These predictions were used to drive individual growth models for the three cultivated shellfish species, applied to selected geographical areas where those species are presently cultured. The physiological models were used to drive two different local-scale models which provided outputs at the population level. One of these models (FARM[1]) focused on direct effects of climate change, such as temperature-driven changes in production, and the other (ABC[2]) provided results on host-pathogen interactions in a changing climate.

The results obtained for present-day conditions were compared with scenarios for the mid-twenty-first century (2040-2060), and the end of the century (2080-2100). For each of those scenarios, two different IPCC projections were considered: (i) a moderate situation (IPCC RCP 4.5); and (ii) an extreme situation (IPCC RCP 8.5).

We completed the study with the analyses of future economic drivers for grow-out production of the four species-location pairs under four socio-political scenarios studied in the EU Horizon 2020 project CERES. An established benchmarking approach was used to compare present-day economic performance of 'typical farms' with their future profitability under different climate change scenarios.

The modelling tools presented herein aim to increase awareness in the aquaculture sector about the potential effects of climate change, and help the industry respond appropriately. The results obtained will be used to support shellfish farmers in the adaptation of their farming activities to underlying biophysical and economic changes; such adaptations include the development of early warning methods, new operating procedures, infrastructures, location choices, and commercial markets.

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1 Farm Aquaculture Resource Management
2 Aquaculture Biosecurity and Carrying capacity

HOW WILL THE PRODUCTION OF AN ECONOMICALLY RELEVANT ALGA (*Gracilaria* SP.) VARY WITH TEMPERATURE RISE?

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Introduction

Red seaweeds belonging to the genus *Gracilaria* are within the most cultivated and valuable marine macrophytes in the world (Rocha et al., 2019). Their applications range from the nutraceutical and pharmaceutical industries to other emergent uses, such as dietary ingredients for fish or dairy cattle (Valente et al., 2015; Lima et al., 2019). In addition, *Gracilaria* spp. are present at coastal areas worldwide, being relevant to the function of these ecosystems, where along with other marine macrophytes, they act as nutrient sinks and carbon sequestration mediators (Duarte et al., 2018). However, since temperature is the most important range limiting factor for many marine macrophytes, ocean warming is expected to have significant impacts on the productivity of some species (Díez et al., 2012). Baring this in mind, our aim is to develop a numerical model for the production of *Gracilaria* sp. that can be both applied to natural systems and aquaculture farms, and that will be used to predict variations on *Gracilaria* growth, standing stock and production under climate change scenarios, especially, temperature rise within the ranges estimated by the IPCC (2014). Multiple-stressor simulations (e.g. temperature, salinity, sea level rise) are also run to check for interactions between different stressors and their impact on algal growth.

Materials and methods

The model accounts for the processes that control *Gracilaria* biomass through time (GPP, NPP, reproduction, decomposition, grazing) and the regulation exerted by environmental factors (temperature, PFD, external concentration of N and P, salinity). Along with *Gracilaria* biomass, the algal internal concentration of N and P are also described as state variables, due to the algae's capacity to store nutrients. Field, experimental and literature data are used to parameterize and test the model. All relationships are expressed as numerical formulations written in STELLA (ISEE systems) and R (R project). A simple conceptual diagram of the model is shown in figure 1

Long-term stability, verification, calibration and validation are performed to assess the model's accuracy and performance. Regression analysis is used to compare observations with simulations. Once the fitting level is statistically significant, scenario simulations accounting for temperature variation according to RCP scenarios (IPCC, 2014) and multiple-stressor scenarios are run, to check for the effects on *Gracilaria* production.

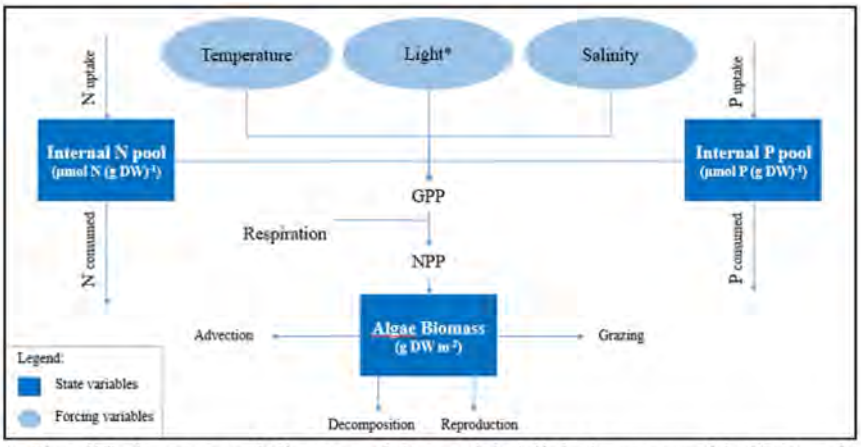

Fig. 1. Simplified conceptual diagram of the model. * light is measured in PFD – photon flux density (µmolE m^{-2} s^{-1}); GPP – Gross Primary Production (d^{-1}); NPP – Net Primary Production (d^{-1}); N – Nitrogen; P – Phosphorous

(Continued on next page)

Results

Results show that the model is able to reproduce the variation of *Gracilaria* sp. biomass and production through time accordingly to observations. Furthermore, while scenario simulations highlight the potential for a general enhancement of *Gracilaria* sp. biomass under temperature rise scenarios, multiple-stressor scenarios (e.g. temperature, sea level rise, DO) highlight the complexity inherent to stressors' interactions and the different responses of *Gracilaria* sp. to these.

Discussion and conclusion

The consistency found between the patterns of predicted and observed *Gracilaria* biomass variation validated the subsequent use of the model in checking the algal responses to single-stressor (temperature) and multiple-stressor scenarios (temperature, sea level rise, salinity). According to results, while temperature rise seems to benefit the production of *Gracilaria* sp., particularly in some parts of the growing season, the interactions between different climate stressors may be adverse to *Gracilaria* sp. production. This is in accordance with previous studies that show the existence of intrinsic interactions between the biological systems and climate change stressors, frequently resulting in synergistic or antagonistic interactions (Falkenberg et al. 2013). Thus, the present results should be acknowledged with caution. On the other hand, the model may be further improved e.g. by incorporating more accurate data. Nonetheless, the present model provides a comprehensive and supportive tool for sustainable management strategies, either aiming to harvest natural populations of *Gracilaria* sp or optimizing algae farming under a changing climate with therein improvements to the income of seaweed farmers.

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STRESS HORMONES EVOKE CHARACTERISTIC EXPRESSION PROFILES IN SALMONID HEAD KIDNEY CELLS UNDER IMMUNE STIMULATION

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Introduction

Under stressful conditions, the head kidney of teleosts plays a central role in the neuro-immune interactions. As an endocrine tissue, it releases cortisol and the catecholamines adrenaline and noradrenaline, but also fosters hematopoietic and lymph node functions (Wandelaar et al., 1997). Once released, cortisol and catecholamines will interfere with the immune response through the glucocorticoid and adrenergic receptors present on the different leukocyte populations (Cortés et al., 2013, Roy and Rai, 2008.). Using gene expression analysis and flow cytometry, we analysed the immune-cell lineages in the head kidney and their repertoire of glucocorticoid and adrenergic receptors. In the next step, we performed a series of in-vitro stimulations of head kidney leukocytes to study the expression of immune-relevant genes when stress hormones are present. This study aims at defining reliable markers for the stress-induced immune modulation, in order to evaluate the effects of stress on the immune system of maraena whitefish (*Coregonus maraena*), a 'newcomer species' in intensive aquaculture.

Materials and methods

First, a single-cell suspension obtained from *C.maraena* head kidney was analysed in the flow cytometer and sorted according to granularity and size. Subsequently, head kidney cells were cultured for defined periods between 1h and 48h and stimulated with adrenaline, noradrenaline or cortisol, either alone or in combination with pathogen-associated molecular patterns (PAMPs). A large set of specific primers was designed for reverse-transcription quantitative real-time PCR analyses, including marker genes for specific leukocyte populations, glucocorticoid and adrenergic receptors and markers for immune activation and stress response genes.

Results

Flow cytometry analysis of head kidney cells identified two groups with different cell characteristics. One group was less granular and of smaller size, located in the lower part of the forward and side scatter axis ($FS^{lo}SS^{lo}$). The other cell group showed higher granularity and bigger size, located in the higher part of the forward and side scatter axis ($FS^{hi}SS^{hi}$). $FS^{lo}SS^{lo}$ cells expressed a higher proportion of transcripts characteristic for lymphocytes, while $FS^{hi}SS^{hi}$ cells expressed higher levels of transcripts specific for the myeloid lineage. Gene markers for glucocorticoid and adrenergic receptors were equally expressed in both clusters. The in vitro experiments with primary cells stimulated with cortisol and PAMPs showed differential gene expression depending on incubation time, particularly with regard cytokine, acute phase and stress response genes. In essence, cortisol exerted a down-regulatory effect on immune-gene expression in the presence of PAMPs.

Discussion

The flow cytometry analysis together with cell-specific transcript markers corresponds to the known FS-SS distribution of myeloid and lymphocyte populations (Wilkerson, 2012). The even distribution of stress hormone receptors in both groups suggests potential stress-response targets, which might modulate leukocyte activity, updating the actual knowledge on adrenergic receptor gene expression in salmonid leukocytes (Flory et al., 1990). Our in-vitro experiment of stimulating head kidney cells with PAMPs and cortisol gave insights in the regulatory mechanisms behind the interaction of cortisol with activated leukocytes. On the one hand, our data indicates the constant upregulation of stress-related genes (HSP70) in the presence of either cortisol or PAMPs, confirming the interrelation of HSP70 with stress and immune system (Zhang et al., 2011a). On the other hand, we suggest a modulatory role for cortisol on cytokine gene expression, affecting their up-regulation when cells are confronted with PAMPs. This is in line with findings on the anti-inflammatory functions of a stress hormone (Barton et al., 1987).

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EFFECTS OF INGREDIENT INCLUSION LEVEL AND FECES COLLECTION METHOD ON THE APPARENT NUTRIENT AND ENERGY DIGESTIBILITY OF INGREDIENTS IN NILE TILAPIA

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Introduction

Formulating feed on a digestible nutrient basis is a key strategy on the success of profitable and environmental friendly farming. Several approaches have been taken in the past years to standardize methodology for determining nutrient digestibility of ingredients. In the digestibility study, test diets are normally produced by mixing a test ingredient with a reference diet at a ratio (weight basis) of 30:70 (test ingredient: reference diet) (NRC, 2011). Nutrient digestibility of reference and test diets are determined separately and used to calculate nutrient digestibility of ingredients. Recently, Carvalho et al. (2016) showed in shrimp that differences in the inclusion levels of a test ingredient in the reference diet affect the nutrient digestibility values of ingredients determined. This could be because certain ingredients at higher inclusion increases antinutritional factors and thus reduces the nutrient digestibility. On the other hand, at low inclusion of test ingredient, accuracy of nutrient ADC may be compromised. Another potential factor that can affect the measurement of nutrient ADC is feces collection method. Among different methods, Guelph system and its modified versions have been commonly adopted for several fish species. Although this system is known to produce reliable values, there is still an opportunity for nutrient leaching from feces. Another approach is called Choubert or INRA system where feces are continuously captured on a movable screen and then deposited in collection tray. With this approach, feces are removed from the tank almost immediately. However, this approach is not commonly practiced because of its practical difficult . The objective of this study was to evaluate the effect of feces collection methods (decantation vs. mechanical filtration) and the dietary inclusion level of test ingredients (10 and 30%) on the energy, fat, protein and amino acid digestibility of ingredients in Nile tilapia.

Material and Methods

The study was conducted at the experimental facilities of the University of Trás-os-Montes e Alto Douro (Vila Real, Portugal). Fish meal, meat and bone meal, soybean meal, rapeseed meal, and cotton seed meal were evaluated. A reference diet was formulated with fishmeal (15%) and several plant ingredients. None of the plant ingredient was included at more than 10% to avoid extreme levels when cumulated with the incorporation of the various test ingredients. Chromic oxide was added at 1% in the reference diet as an indigestible marker. All the diets were extruded at SPAROS Lda., Portugal.

Table 1: ADC of energy, protein and amino acids of various ingredients in Nile tilapia

Ingredients	Feces collection method	Energy	Protein	Lys	Met	Thr
Fish meal	Guelph	77.8	82.4	89.0	85.5	83.3
	INRA	78.9	84.6	89.7	85.7	83.8
Meat and bone meal	Guelph	73.8	75.3	72.3	61.4	69.7
	INRA	74.2	75.2	71.3	59.5	66.7
Soybean meal	Guelph	81.6	90.3	92.3	90.3	89.8
	INRA	81.7	91.3	92.6	90.1	89.7
Rapeseed meal	Guelph	63.5	80.2	83.1	84.8	81.2
	INRA	65.3	81.6	83.2	83.8	80.4
Cottonseed meal	Guelph	64.9	80.8	73.3	69.5	76.0
	INRA	65.7	81.5	76.5	72.2	78.7

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Ingredient inclusion level

Groups of 20 fish (body weight: 40-60 g) were stocked in 44 fibe glass tanks at a constant water temperature of 28 ± 1°C. Two sets of test diets for the five ingredients were prepared by mixing a test ingredient with the reference diet at a ratio of 10:90 or 30:70. Ten test diets and the reference diet were randomly allotted to the experimental tanks and all dietary treatments were tested in quadruplicate. Prior to initiation of feces collection, fish were subjected to a 12-day adaptation period to the rearing conditions and experimental feeds. Following this adaptation period, fish were fed once a day (9.00 h), by hand in slight excess. Upon a thorough cleaning of the tanks for any feed residues, feces were collected daily for 10-15 consecutive days in the feces decantation column (Guelph system). Feces were collected approximately 8 hours after the meal. After removal of excess water, daily feces were frozen at -20°C. Pooled feces from each group of fish were freeze-dried prior to subsequent analysis.

Feces collection method

Test diets for the five ingredients prepared at the ratio of 30:70 (test ingredient : reference diet) were used for evaluating the two feces collection methods. Data generated from the ingredient inclusion level (30:70) using the Guelph system were used for comparing the feces collection method. For the INRA system – outlet water filtration method, similar conditions were adopted as in the "Decantation method" except for the feces collection procedure. Feces were collected using a mechanical system by continuous filtration of feces in the outlet water (INR system).

Results and Discussion

Changes on the dietary incorporation level of the ingredient (10 or 30%) had little effects on the protein and amino acid ADC of fishmeal, and meat and bone meal. However, incorporation level of the test ingredient (10 or 30%) showed significant effect on the ADC of protein and amino acids for soybean meal, rapeseed meal and cottonseed meal. The ADC values for protein and most amino acids tended to be lower for the 10% ingredient inclusion relative those obtained for 30% inclusion level. In some ingredients, this reduction on ADC is quite marked and would require a further attention. However, inclusion of ingredients did not affect ADC of energy. Feces collection method (decantation vs. mechanical filtration of outlet water) had little effect on the nutrient and energy ADC of the various ingredients tested (Table 1). Results of this study have implications on determining digestibility values and formulating tilapia diets on digestible nutrient and energy basis.

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EFFECT OF DENSITY AND PHOTOPERIOD IN *Ulva ohnoi* PHOTOINHIBITION CULTURED WITH ARTIFICIAL ILLUMINATION

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Introduction

Photoinhibition is the light-induced reduction in the photosynthetic capacity of a photosynthetic organism which takes places when they are exposed to high irradiances that exceed its light energy requirement. Photinhibition includes photoprotection, to overcome the inefficiency in the utilization of light and photodamage, which takes place when light energy cannot be converted into chemical energy and cellular damage can appear.

Algae will be subjected to light conditions which can vary daily and also momentarily, either by the passage of clouds and the effect of waves in the open sea, or by the vertical movement of seaweeds when cultivated in aerated tanks. Effects on growth and photosynthesis have been attributed to both type of fluctuations: low frequency-fluctuations (photoperiod) (Fortes and Lüning 1980, Li et al. 2018) and high frequency-fluc uations (clouds, waves, and, in tanks, agitation) (Pang and Lüning 2004, Msuya and Neori 2008, Ben-Ari et al 2014).

In natural environments (under a sunlight regime) photosynthetic activity exhibits a symmetrical diurnal pattern, which inversely tracks instantaneous irradiance. A progressive decrease in the the effective quantum yield (ΔF/Fm') (which indicates that photoinhibition takes place) is observed from sunrise to midday and in the afternoon seaweed photosynthesis recovers and full recovery is reached during the night (Hanelt 1992). In seaweed cultured in tanks it has been observed that manage of densities and incident irradiance can aid to reduce the effects of photoinhibition.

The objective of this work was to study the photoinhibition of *Ulva ohnoi* submitted to sudden lighting in an IMTA-RAS system with artificial illumination. Experiments were made with four different photoperiods and with two stocking densities.

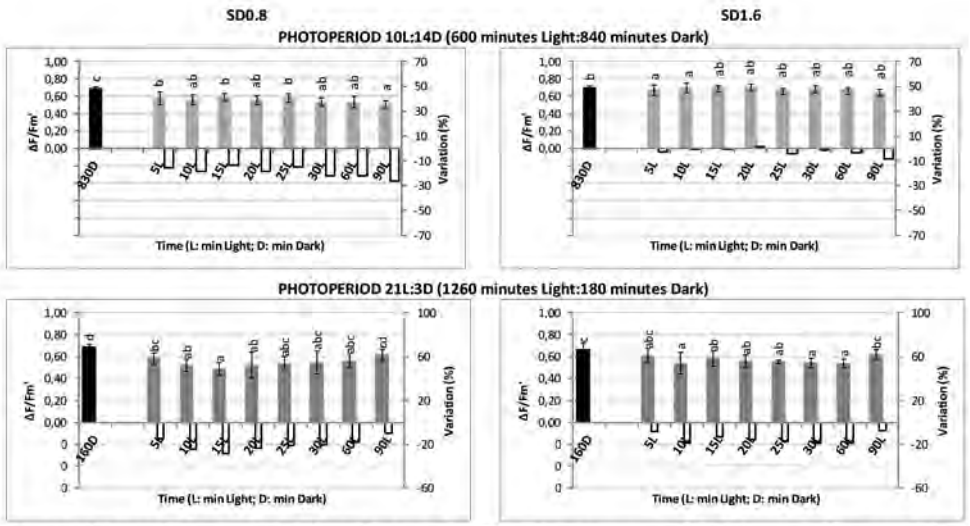

Figure 1: Evolution of ΔF/Fm' during the first 90 minutes of lighting in photoperiods 10L:14D (above) and 21L:3D (below) in SD0.8 and SD1.6 tanks (stocking density of 0.8 and 1.6 kg m^{-2}, respectively) Black bar: measurement in the dark period (10 min before lights on), Grey bars: measurements in the light period. Bars represent the average of five samples±SD. Different superscripts show significant differences (0.05 level, Fisher). White bars: difference in percentage respect the measurement in the dark period

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Material and methods

U. ohnoi tanks were integrated in a recirculating system (RAS) with one tank of sole (*Solea senegalensis*) and a nitrifying biofilte , and were cultured with bottom agitation at two stocking densities (0.8 and 1.6 kg m^{-2}, named SD0.8 and S1.6, respectively). Four photoperiods (10L:14D, 14L:10D, 18L:6D and 21L:3D) were tested; at each photoperiod after a complete cycle of light and darkness (24h) ΔF/Fm' was measured during the first 90 minutes of light (measurements were made at minutes 5, 10, 15, 20, 25, 30, 40, 50, 60 and 90 after the lights were on). Effective quantum yield of Photosystem II (ΔF/Fm', where ΔF=Fm'-Ft) was measured with a pulse-amplitude modulation fluorometer (PAM-2100, Walz, Germany).

Results

Photoinhibition, expressed as a decrease in ΔF/Fm', was observed in the first measurement made after lighting (after 5 minutes of light) and in general no changes were observed from then on (to 90 minutes) in all photoperiods (Figure 1). With 10 and 14 hours of light stocking density aids to reduce photoinhibition, but increasing lighting hours (photoperiods with 18 or 21 hours of light) photoinhibition effect was not different under the two stocking densities. Also this results confirm that under controlled photoperiod (artificial illumination) fluorescence parameters during light periods varied little (*U. rotundata*, Henley 1992) comparing with seaweeds submitted to sunlight.

These results together with the previous ones point out that photoprotection takes place in all photoperiods during the first minutes of lighting, and that photodamage appears from photoperiod of 18L:6D and persist even if after 18h of light, lighting hours were reduced to 14h (Masaló et al 2018).

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"ULVAPRO" COORDINATED PROJECT: OBJECTIVES AND PRELIMINARY RESULTS OF SUBPROJECT 2 "LIGHT MANAGEMENT STRATEGIES TO MAXIMIZE *Ulva* PRODUCTIVITY IN IMTA-RAS SYSTEMS AND PROMOTE EFFECTS INDUCED BY MICROBIOTA"

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Introduction

Among IMTA techniques, the integration of fish and seaweed cultures in recirculating systems (IMTA-RAS) is currently one of the most promising strategies, in order to achieve diversification and sustainability in aquaculture activities. Macroalgae of the genus *Ulva* (*Ulvales*, Chlorophyta) have shown to be especially suitable for use as biofilters in IMTA-RAS systems. Combining *Ulva* spp. with sole (*Solea senegalensis*) is based on the matching of ecophysiological requirements for both cultures, the simplicity of cultivation and the high growth rate of *Ulva*, as well as the commercial interest of sole, whose cultivation on an industrial level has been recently developed and mainly performed in inland facilities.

A crucial factor in the intensive cultivation of macroalgae is the light dose received by its thallus, which must be administered in the best possible way to obtain optimal growth, avoiding productive losses that could cause both light deficiencies and processes of photoinhibition. The determining factors to achieve this optimum growth are the incident light intensity, the photoperiod, the cultivation density and the rotational velocity of the thallus in the culture volume. The influence of culture conditions on the safety and nutritional quality of the obtained *Ulva* biomass is also a key factor for the viability of its commercialization. In this sense, the systems in recirculation can increase the nutritional quality of the algae and, in addition, eliminate the uncertainties due to possible microbiological contamination or heavy metals or other toxins that can occur in the crops of wild populations.

The main purpose of the **UlvaPro** coordinated project, funded by the Spanish government, is to find the most efficient design and the optimal management of the IMTA-RAS fish-macroalgae system, from the point of view of energy, quantity and quality of macroalgae production, biofiltration capacity and maximum probiotic effect of the epiphytic microbiota. Subproject 2 focuses on aspects related to the engineering of the IMTA-RAS fish-macroalga systems, emphasizing the adequate management of light in algae culture tanks to obtain the best macroalgae production, both from the quantitative and qualitative point of view, by managing the incident irradiance, the photoperiod, the cultivation densities and the rotating velocity of the algae. Here we show the main goals and activities of the project.

Material and methods

The optimization of *Ulva*'s culture conditions, emphasizing on the management of light, is approached from a multidisciplinary perspective, including different objectives and milestones (M):

Analyze the processes of photoinhibition and productivity in *Ulva* cultured in tanks according to the growing conditions: light intensity, stocking density, flow pattern and photoperiod

M: Establish the culture conditions (lirradiance, density and photoperiod) that optimize the growth of *Ulva* in an IMTA-RAS system of *Ulva-Solea senegalensis*

Determine the permanence of *Phaeobacter* in the IMTA-RAS system under the conditions selected

(Continued on next page)

M: Establish the most cost-effective combination of intensity and photoperiod for the optimization of *Ulva* growth and permanence of the *Phaeobacter* in the IMTA-RAS system

Analyze the influence f light intensity, stocking density and *Phaeobacter* colonization in the nutritional quality and food safety of *Ulva* grown in IMTA-RAS

M: Establish the nutritional quality and food safety of *Ulva* depending on the growing conditions in the IMTA-RAS system, as well as the influence of colonization with *Phaeobacter* on these parameters.

Results

The results of this subproject will help to identify the best algae management conditions for the most efficient use of light in order to maximize the productivity of the algae culture and, in turn, maintain a sufficient microbiota concentration to benefit of its probiotic effects. At the same time, the potential improvement of safety and nutritional quality of the algae produced in these systems will be evaluated.

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THE EFFECT OF FISHMEAL SUBSTITUTION WITH THREE DIFFERENT INSECT MEALS ON GROWTH PERFORMANCE, NUTRIENT UTILIZATION AND DIGESTIBILITY OF GILTHEAD SEA BREAM, *Sparus aurata*

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Introduction

Insects have been widely studied as a fishmeal substitute, due to their balanced amino acid profile, even comparable to fishmeal, and their ability to consume organic waste while producing high-quality protein and contributing to nutrient recycling and circular economy.

In gilthead sea bream (*Sparus aurata*), it has been found that *Tenebrio molitor* meal can substitute up to 33% of dietary fishmeal, resulting in improved growth performance and feed conversion (Piccolo et al. 2017). So far, most studies regarding the inclusion of insect meals in fish feed focus on one insect species individually. This study aims to compare the use of *Tenebrio molitor*, *Hermetia illucens* and *Musca domestica* meals in experimental diets and to examine their effect on growth performance, feed conversion, somatic indexes, whole-body composition, nutrient utilization and digestibility of gilthead sea bream.

Materials and methods

A growth and a digestibility experiment were conducted with juvenile sea bream. For the growth experiment, 360 fish (with an average individual weight of 29.5±0.7g) were divided into 12 indoor open-circulation 500 l tanks. Fish were fed with formulated diets (Table I), in which 30% of fishmeal was substituted by 30% insect meal (*Tenebio molitor*, *Hermetia illucens* or *Musca domestica*) for three months. By the end of the experiment growth and somatic indexes, as well as whole-body proximate compositions and nutrient productive values were determined. For the digestibility experiment, 180 fish (with an average individual weight of 49.9±0.7g) were divided into 250 l digestibility tanks. Fish were fed with the growth diets with the addition of 1% celite® as an inert marker. Feed, whole-body and faeces proximate compositions were determined according to (AOAC, 1990). Crude protein content was adjusted for the nitrogen linked to acid detergent fibe .

Results and Discussion

The experimental diets, in which 30% of fishmeal was substituted with insect meals, were well accepted from the fish and were rather palatable, as shown by the daily feed intake (1.4-1.5% of body weight day^{-1}, $p>0.05$). Numerically, the best FCR was achieved when fish were fed with FM and MD diets (1.0), though without statistical significance Growth performance was similar between fish fed the insect diets and the FM diet (1.5-1.6% day^{-1}). However, fishmeal substitution with TM led to statistically lower growth compared to substitution with HI. Fishmeal substitution did not affect viscerosomatic, hepatosomatic and mesenteric fat indexes. Gut length can be affected by the source of protein used in the diet and the diet digestibility. Fish fed with plant proteins appear to have longer intestines (Odedeyi et al. 2014), however, in the present study, fishmeal substitution with insect meals did not affect gut length. Piccolo et al. (2017) found that 33% fishmeal substitution with TM, improved growth and feed conversion and increased somatic indexes. Growth improvement was attributed to the presence of chitin, which in small amounts acts as a prebiotic while the increase in somatic indexes was attributed to the lower digestibility of the TM diet.

Table I: Composition of the experimental diets

Ingredients (%)	FM	TM	HI	MD
Fishmeal	65	45.5	45.5	45.5
Insect meal	0	19.5	19.5	19.5
Fish oil	9	5	9.7	6.3
Wheat	17.2	16.6	14.8	16.8
Wheat gluten	6	9	6	8.5
Premix	2.5	2.5	2.5	2.5
Methionine	0.3	0.7	0.7	0
Lysine	0	1.2	1.3	0.9
Proximate composition (% of dry weight)				
Crude Protein	55	55	55	55
Crude Fat	15.2	15.2	15.2	15.2
Ash	13.3	10.5	11.1	11.1
Crude fiber	1.7	2.5	3.9	2.9
ADF	6.2	5.6	8.8	7.1
Gross energy (Mj kg^{-1})	21.8	21.9	22.2	22.2

The diets containing insect larvae did not affect the digestibility of protein, energy and ADF in gilthead sea bream (90-92%, 81-85% and 48-61% respectively). Dry matter digestibility did not differ between the insect diets and the FM diet (66.5-73%); nonetheless the TM diet dry matter digestibility was significantly lower than the HI diet. Fat digestibility was lower for the diets containing TM (73.6%) compared to the other three diets (78.9-83.2%). Some studies have shown that

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chitin can interfere with fat and protein digestibility (Köprücü and Özdemir 2005; Kroeckel et al. 2012). Under the present experimental conditions, the ADF content of the diet appeared to be positively correlated with the digestibility of dry matter and energy of sea bream.

Sea bream whole-body dry matter, protein and ash were not affected by the fishmeal substitution (32.4-33.3%, 53-55% and 10.1-11% respectively). In addition, body fat of fish fed with TM and HI diets was similar to fish fed FM diets (31.9-35.7%), while fish fed the MD diet exhibited significantly lower body fat compared to the FM group. Finally, whole-body energy of the HI and MD (30.4Mj kg^{-1}) groups were significantly lower than the FM group (31.6Mj kg^{-1}), while no differences were observed between the three insect-fed groups. Even though significance occurred, the differences were minor, and it can be said that overall, the whole-body composition was not affected by the fishmeal substitution. Regarding the nutrients' utilization, fishmeal substitution did not affect protein retention (29.3-31.3%). Furthermore, the diets TM and MD led to similar dry matter, fat and energy retention to each other and the FM diet (32.5-33.8%, 69.2-74.2% and 37-39.1% respectively). On the contrary, the inclusion of HI in the diet resulted in significantly lower dry matter and energy retention compared to the FM group and lower fat retention than the rest of the dietary treatments. Our results showed a negative correlation between dietary fiber and whole-body fat and fat retention. Thus, the lower body fat and fat retention of the HI-fed fish could be attributed to the higher crude fiber an ADF of the HI diet.

In conclusion, under the present experimental conditions, the inclusion of *T. molitor*, *H. illucens* and *M. domestica* larvae meals had no adverse effects on growth performance and digestibility of gilthead sea bream and dietary fishmeal can be successfully substituted by these insect meals in 30%.

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TAURINE MODULATES PROTEIN TURNOVER IN SEVERAL TISSUES OF MEAGRE JUVENILES FED WITH PLANT-BASED DIETS

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Introduction

The sustainability of natural resources is a worldwide concerning issue, reflected in several production sectors such as aquaculture, where many efforts have being made. One of these efforts includes the substitution of fishmeal by proteins of vegetable origin in fish feeds. Grain and oilseed by-products are good sources of protein and energy for aquaculture feeds. However, high dietary inclusion levels of plant proteins generally reduce fish growth and feed efficienc . This poor growth performance commonly found in fish fed plant-protein rich diets is generally related to the lower biological value (essential amino acid imbalance, impaired phosphorus availability, presence of anti-nutritional factors, higher carbohydrate fraction) and lower palatability of the plant-protein sources, Gatlin III et al. (2007). Increasing evidence indicates that some marine fish have a conditional requirement for taurine, and its supplementation is often promoted in a fishmeal replacement scenario. Meagre is a very interesting species for aquaculture due to its fast growth rates and flesh quality. This fish species has high dietary protein requirement and a good tolerance to diets containing plant protein sources, Ribeiro et al. (2015). With this study, we intend to understand the mechanisms behind taurine and protein turnover in order to find an optimized vegetable fish diet formulation that reduces protein degradation and supports fish somatic growth

Materials and methods

Meagre juveniles (3.2±0.2g) were fed with a plant-based diet containing five different taurine concentrations (0%, 0.5%, 1.0%, 1.5% and 2.0%) for 38 days. The resulting growth parameters, protein expression and protein degradation levels in several tissues were analysed in order to evaluate the modulator potential of taurine in protein turnover of meagre fed with vegetable diets. Techniques like protein extraction, enzymatic activity and semi-quantitative protein expression were employed.

Results

Our results showed a significant correlation between the feed conversion ratio (FCR) of meagre and dietary taurine levels, indicating that as taurine concentration increases, juvenile meagre uses more efficiently feed to grow. Besides prompting significant growth rates, diet supplemented with 2.0% Taurine seems to induce the expression level of molecular chaperones and also to decrease protein degradation in several tissues, including muscle.

Discussion and conclusion

Somatic growth implies changes in protein turnover with slightly increase in protein synthesis over protein degradation. This phenomenon is a highly controlled process where several degradation systems assume their role, Seiliez et al. (2014). Because taurine deficiencies in fish fed with plant-based diets are often associated to depressed growth, Salze and Davis (2015), we hypothesized that this nutrient could be able to modulate protein turnover in meagre. In our study, meagre growth seems to be related to the increasing taurine concentration in plant-based feeds, which can be explained by an increase of protein turnover, Feidantsis et al. (2014) with a concomitant decrease of protein degradation in muscle.

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PRESENT BIOTECHNOLOGY OF AQUACULTURE OF THE SOUTH OF RUSSIA

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In the Southern federal district (SFD), the most suitable natural and climatic conditions are noted, which allow the development of all types of fish farming. The most promising areas are: pasture aquaculture; pond; industrial fish farming; recreational aquaculture; mariculture.

The volume of aquaculture in the southern regions of the country in 2018 amounted to more than 78,0 thousand tons, in total in Russia reached 268,65 thousand tons, an increase of 8 % (http://www.fish.go .ru). Leaders in the production of aquaculture in the southern Federal district are the Rostov region, Krasnodar region and Astrakhan region, their total share in 2018 amounted to 92.8 % (figure 1)

For the southern regions of Russia, it is most advisable to use intensive cultivation technologies that allow producing 17-24 c/ha of fish planting material and commercial fish. The main objects of pond fish farming are carp and herbivorous fish. In recent years, we added fish the native fish fauna (pike, perch, pike, tench, common catfish, crucian carp, and perch) and earlier species (paddlefish, channel catfish, haard , buffalo).

Special attention should be paid to the development and improvement of technologies used in the cultivation of hydrobionts in controlled conditions of installations with a closed water supply cycle. Scientific organizations have created and successfully implemented a number of scientific and technical solutions, new biotechnologies in sturgeon breeding on fish farms in the South of Russia. In order to obtain ecologically clean fish products of sturgeon, a complex biotechnology has been developed in the SSC RAS, which allows eliminating climate risks. “Green” technologies are widely introduced, which provide for general environmental management, that is, the utilization of production waste, the production of energy from renewable sources (solar energy, biofuels), the reduction of harmful emissions into the atmosphere, the fight against water and air pollution.

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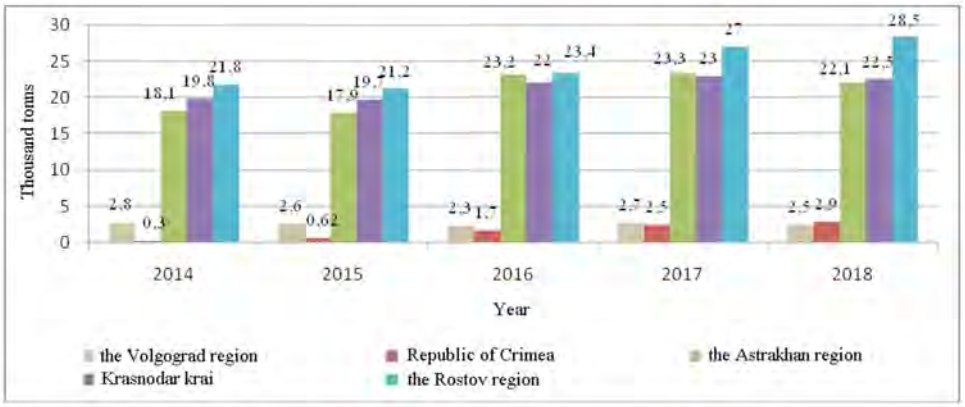

Fig. 1. Leaders in the production of commercial aquaculture in the Southern federal district

LIFE-CYCLE ASSESSMENT OF ANIMAL FEED INGREDIENTS: POULTRY FAT, POULTRY BY-PRODUCT MEAL AND HYDROLYZED FEATHER MEAL

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Introduction
Poultry production for human consumption results in large amounts of by-products, which can be rendered to produce hydrolyzed feather meal (HF), poultry by-product meal (PBM) and poultry fat (PF). These ingredients can be good protein (HF and PBM) and lipid sources (PF) for aqua feeds, mainly due to their high availability and relatively low price, reducing the need for imported feedstuffs. This work assesses the environmental life cycle impacts of rendering poultry by-products into PF, PBM and HF, based on their valorization as ingredients for the aqua feed industry, promoting a circular economy system.

Materials and Methods
Information regarding PF, PBM and HF production chains was collected directly from two by-product rendering units in Portugal and the following impact categories were analyzed: global warming (GW), abiotic depletion (AD), acidification (AC) and eutrophication (EUT), within the system boundaries, using the CML 2 baseline 2000 Life Cycle impact assessment method. A sensitivity analysis was conducted for the allocation method and for the type of fuel (fuel oil vs wood pellets) used to generate heat for the rendering process, aiming at impact reduction.

Results and Discussion
The results obtained show that, in both production systems, the poultry production is the main contributor for the impacts in AC and EUT categories, while the rendering process of the by-products is the main responsible for the generation of GW and AD impacts (Fig 1 and 2). Using exclusively wood pellets would reduce impacts in all categories except eutrophication. The sensitivity analysis conducted on the allocation method applied to the poultry by-products showed that it has a huge impact on the results obtained, being much higher for all impact categories when mass allocation is used.

Conclusions
The present results show that the poultry production phase is the main responsible for the acidification and eutrophication potential factors in the poultry fat and poultry by-product meal production system and in the feather meal production system. On the other hand, global warming and abiotic depletion factors are mainly influenced by the rendering process of the by-products, in which the main responsible is the fuel mix used in the boilers. A sensitivity analysis showed that a scenario where wood pellets were used exclusively as heat source in the boilers could reduce all the impact categories considered except eutrophication. A sensitivity analysis on the allocation method applied to the poultry by-products was also conducted and showed that the impacts of the life cycles analyzed depend greatly on the allocation method, being much higher for all impact categories when mass allocation is used. Overall, the production of these ingredients from poultry by-products has relatively low impacts for the categories analyzed and they could therefore be used as environmentally sustainable ingredients in animal feeds.

Acknowledgements
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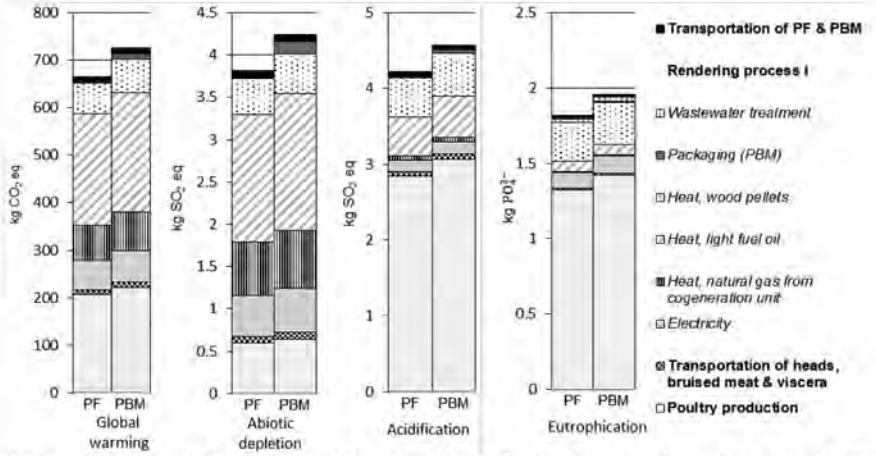

Figure 1. Life cycle impacts of poultry fat (PF) and poultry by-product meal (PBM; 1 t, economic allocation).

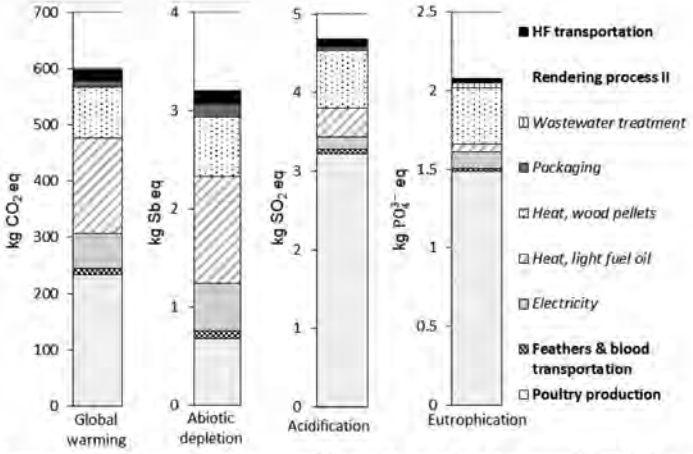

Figure 2. Life cycle impacts of hydrolyzed feather meal (HF; 1 t, economic allocation).

THE MICROALGA *Isochrysis* AS AN ENRICHMENT DIET FOR ROTIFERS: EFFECTS OF HARVEST TIMING ON ROTIFER VITALITY AND FATTY ACID PROFILES

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Introduction

Enrichment of docosahexaenoic acid (DHA) in the polar lipids (PL) of rotifers is an essential process in the larval rearing of marine finfish The microalga *Isochrysis* has the potential to be used in an enrichment diet due to its inclusion of DHA. However, little is known about the effects of the timing of *Isochrysis* harvest on its PL-DHA content and that of the rotifers to which it is fed. Concurrently, rotifer vitality should also be considered as a factor that can reduce later larviculture performance. In this study, we aimed to determine the optimum harvest timing of *Isochrysis* as an enrichment diet for rotifers which are essential for marine finfish larva

Materials and methods

Isochrysis sp. Tahiti strain was cultured in Guillard-*f* medium under irradiance of 150μmol photons m^{-2} s^{-1}. Changes in the concentrations of phosphate (P) and nitrate-nitrogen (N) in the medium were colorimetrically assessed. These measurements were used to harvest cells in P/N-sufficient, P-deficient, and P/N-deficient phases, and these cells were used for fatty acid analysis and rotifer enrichment. *Brachionus plicatilis* sp. complex was pre-cultured using commercial *Chlorella vulgaris* and fed *Isochrysis* of each phase for 12h. The population dynamics and fatty acid profiles of non-polar lipids (NL) and PL after the enrichment were measured. Swimming speed of the rotifers was also measured as a metric of individual vitality.

Results

During *Isochrysis* cultivation, P was depleted from the medium, followed by N starvation. At the transition from an N/P-sufficient phase to a P-deficient phase, *Isochrysis* exhibited a remarkable increase in DHA in its PLs, whereas DHA levels dropped in the P/N-deficient phase. Rotifers that fed on the P-deficient phase cells produced the highest content of DHA in PL (Table I).

The other highly unsaturated fatty acids (HUFA), arachidonic acid (ArA), and eicosapentaenoic acid (EPA) in rotifers were also enhanced by feeding P-deficient cells. The growth rate and egg ratio of rotifers was also the highest when they were fed with P-deficient phase cells. The same trend was observed with swimming speed.

Table I. Fatty acid contents (mg g dw^{-1}) in rotifer PL after the enrichment.

Rotifers fed cells of each phase			
P/N-sufficient phase		P-deficient phase	P/N-deficient phase
ArA	0.05 ± 0.03	0.12 ± 0.02	0.02 ± 0.01
EPA	0.06 ± 0.04	0.24 ± 0.03	UD.
DHA	0.07 ± 0.06	0.33 ± 0.06	UD.

Table I. Fatty acid contents (mg g dw^{-1}) in rotifer PL after the enrichment.

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	P/N-sufficient phase	P-deficient phase	P/N-deficient phase
ArA	0.05 ± 0.03	0.12 ± 0.02	0.02 ± 0.01
EPA	0.06 ± 0.04	0.24 ± 0.03	UD.
DHA	0.07 ± 0.06	0.33 ± 0.06	UD.

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Discussion and conclusion

Our results indicate that the harvest timing of *Isochrysis* is a crucial factor for enriching rotifers with PL-DHA. It has been reported that compared with microalgae with nutrient salt repletion, cells with nutrient salt deficiency showed lower proportion of HUFA in total lipids (Reitan et al., 1992). In this study, a similar trend was observed in PLs in the P/N-deficient phase, except for the phase with P deficiency alone. Accumulation of HUFAs in the PLs of microalgae under P starvation is possibly related to the dynamics of the betaine lipids (Iwai et al., 2015). From the perspective of fatty acid profiles and rotifer vitality, the recommended harvest timing for *Isochrysis* for later larviculture performance of marine finfish is the P-deficie phase.

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FATTY ACID PROFILE OF MUSCLE TISSUE OF MEAGRE *Argyrosomus regius* FED DIETS CONTAINING DIFFERENT LEVELS OF FISH OIL

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Introduction

Determination of the basic nutritional requirements of a species and, in particular, the protein/lipid ratio with optimal inclusion of fish oil, is an essential prerequisite for a well-balanced diet (Fountoulaki et al., 2017). Amongst other fatty acids, EPA and DHA are very important for human consumption because it can affect human health (Jobling and Leknes, 2010). The aim of the study was to evaluate the fatty acid profile of the muscle tissue of meagre (*Argyrosomus regius*) fed diets containing different levels of fish oil.

Material and methods

The feeding trial was performed in three feeding groups: A (CP=52.0; CF =21.0; FO=15%), B (CP=56.0; CF=18.0; FO=10%), C (CP=48.0; CF =16.0; FO=7%) with two repetitions in marine net cages (V = 225 m³) in the Adriatic Sea (44°01'28.6" N 15°13'11.2" E) during 15 months. Fish muscle tissues from each feeding group were sampled and stored at -80°C until analysis. The fatty acid analysis was performed by gas chromatography. ANOVA was used for data analysis with Fisher's LSD test ($p < 0.05$).

Results

The results of fatty acid profile of muscle tissue of meagre fed diets containing different levels of fish oil showed the fish fed diet A had the highest amount of C20:5 (n-3) (EPA) and C22:6 (n-3) DHA with significantly higher ω-3/ ω-6 ratio compared to diets B and C. Other results are presented in Table I.

Discussion and conclusions

The proportions of the fatty acids measured in the present experiment partly correspond to Hernandez et al. (2009) who mentioned higher levels of SFA and lower levels of MUFA in the same fish species. PUFA was found in higher proportions in all 3 diets. Evidence of similar levels of fish flesh n-3 in Grigorakis (2011) and Poli et al. (2003) implies that meagre retains n-3 PUFA well. This is also a good indication that EPA transforms into other n-3 fatty acids in fish flesh. The n-3/n-6 fatty acid ratio recommended by the WHO is 1:1 or above (Simopoulos, 1991), which emphasizes the significantly higher results of n-3/n-6 fatty acid ratio in diet A. In comparison to B and C, fish fed diet A had the promising results of LC-PUFA profile and -3/n-6 fatty acid ratio in terms of promotion of human health.

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Table I. Fatty acid profile of muscle tissue of meagre *A. regius* fed diets containing different levels of fish oil (% of total fatty acids)

	Feed mixtures						P level
	A		B		C		
Fatty acid	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
C14:0	3.03^{a}	0.68	2.80^{b}	0.70	1.97^{c}	0.413	***
C15:0	0.42^{a}	0.11	0.34^{b}	0.16	0.33^{b}	0.07	**
C16:3(n-3)	1.00^{ab}	0.39	1.21^{a}	0.35	0.79^{b}	0.20	***
C16:1(n-7)	4.10^{a}	0.89	3.95^{a}	0.93	3.24^{b}	0.51	**
C16:0	17.27^{a}	1.06	17.45^{a}	1.19	16.03^{b}	0.87	***
C17:1(n-9)	0.74	0.45	0.86	0.64	0.62	0.26	ns
C18:3(n-3)	2.20	1.18	3.72	2.11	3.05	2.24	ns
C18:2 (n -6)	5.86^{b}	2.18	7.76^{b}	2.23	10.48^{a}	2.62	*
C18:1	17.97^{ab}	3.00	17.43^{b}	2.80	21.05^{a}	4.77	*
C18:0	6.50^{b}	0.67	6.71^{a}	0.59	6.66^{b}	0.77	*
C20:4(n-6)_ARA	0.55^{a}	0.07	0.53^{a}	0.05	0.44^{b}	0.05	***
C20:5(n-3)_ EPA	11.65^{a}	1.56	11.21^{a}	1.10	9.34^{b}	1.10	***
C20:3(n-6)	1.40	0.29	1.38	0.32	1.34	0.18	ns
C20:1(n-7)	1.56^{a}	0.31	1.17^{b}	0.21	1.26^{b}	0.16	***
C20:0	0.27^{ab}	0.11	0.23^{b}	0.08	0.33^{a}	0.06	***
C22:6(n-3)_DHA	21.59^{a}	3.46	19.69^{a}	3.61	17.29^{b}	3.55	**
C22:4(n-6)	1.92^{b}	0.17	1.65^{a}	0.23	1.83^{b}	0.25	*
C22:1(n-9)	1.98^{a}	0.54	1.17^{b}	0.38	1.22^{b}	0.29	***
C22:0	0.23	0.12	0.15	0.018	0.19	0.043	ns
C24:1	0.68^{a}	0.22	0.49^{b}	0.13	0.46^{b}	0.09	***
SFA	27.49^{a}	1.25	27.61^{a}	1.50	25.43^{b}	1.35	***
UNSFA	72.51^{b}	1.25	72.39^{b}	1.50	74.57^{a}	1.35	***
MUFA	26.90	4.19	25.76	3.71	27.74	4.47	ns
PUFA	45.62	4.44	46.63	4.65	46.83	5.13	ns
ω-6	9.19^{b}	4.58	12.34^{ab}	4.42	14.50^{a}	7.16	**
ω-3	35.48^{a}	6.12	34.56^{a}	6.28	30.65^{b}	6.82	*
ω-3/ ω-6	3.86^{a}	1.54	2.80^{b}	1.13	2.11^{c}	0.86	**
DHA/EPA	1.96	0.40	1.70	0.28	1.83	0.22	ns
Total lipids	16.81	7.84	13.04	5.78	11.18	3.59	ns

A, Experimental feed for meagre; B, Commercial feed for sole; C, Commercial feed for meagre; SFA – Saturated Fatty Acids; UNSFA - Unsaturated Fatty Acids; MUFA – Monounsaturated Fatty Acids; PUFA-Polyunsaturated fatty acid; DHA-Docosahexaenoic acid; EPA-Eicosapentaenoic acid. Values represent mean ± SD of two repetitions; values within the same line with different superscripts are significantly different at the level: * P<0.05, ** P<0.01, *** P<0.001; ns P>0.05.

CLIMATE CHANGE AND EMERGING CHEMICAL CONTAMINANTS IN MARINE ORGANISMS: BIOACCUMULATION, ECOTOXICOLOGY AND PUBLIC HEALTH IMPACTS

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Introduction

Chemical contamination and climate change constitute two of the greatest environmental problems related with the increase of anthropogenic activities. Despite both factors acting alone can have negative effects at different levels of biological organization, as well as in seafood safety, the underlying interactions between them are still poorly understood. In this context, this work presents an overview of the main findings obtained within the framework of the FP7 project ECsafeSEAFOOD, to assess the combined effects of seawater warming and/or acidification on the bioaccumulation of different emerging chemical contaminants (ECCs) and ecotoxicological responses of farmed marine species from two distinct taxonomic groups (fish and bivalves).

Materials and Methods

The effects of the following ECCs were assessed: toxic metals – methylmercury (MeHg) and inorganic arsenic (iAs); pharmaceuticals and personal care products: diclofenac (DCF), venlafaxine (VFX), triclosan (TCS); flame retardants: dechloranes (Decs) 602, 603 and 604, and tetrabromobisphenol A (TBBPA); perfluorinated compounds - perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). Five marine species were used as biological models: European seabass (*Dicentrachus labrax*), meagre (*Argyrosomus regius*), white seabream (*Diplodus sargus*), Mediterranean mussel (*Mytilus galloprovincialis*) and Japanese carpet shell clam (*Ruditapes philippinarum*). Several *in vivo* trials were carried out between 2014 and 2018, simulating ECCs exposure, seawater warming (+5ºC) and acidification (-0.4 pH units; scenario RCP 8.5; IPCC, 2014). After a period of chronic exposure (minimum 20 days) to these three environmental stressors, marine organisms were sampled to determine ECCs' concentration (in different tissues), as well as the ecotoxicological responses at the behavioural (anxiety, activity, shoaling and lateralization), animal/organ condition (Fulton's condition, hepatossomatic and brain-to-body mass indexes), haematological (erythrocytes viability and nuclear abnormalities) and biochemical levels (antioxidant activity, protein repair and degradation, neurotoxicity and endocrine disruption; Maulvault et al., 2016, 2017, 2018abcd, 2019).

Results and Discussion

Warming promoted the bioaccumulation of lipophilic and persistent ECCs (e.g. MeHg, Decs and TBBPA), suggesting increased risks of human exposure to these compounds through the consumption of contaminated seafood in tomorrow's ocean (Maulvault et al., 2016, 2018a). On the other hand, warming and/or acidification elicited lower bioaccumulation of ionisable and/or less persistent compounds (e.g. iAs, VFX and TCS) (Maulvault et al., 2018ab, 2019a). Such trend may not necessarily represent lower human risks, as it may be associated with enhanced biotransformation of parental ECCs, potentially representing increased levels of metabolites for which the toxicological attributes (to both biota and humans) are still unknown. Within an ecotoxicological context, overall, the simultaneous exposure to ECCs, warming and acidification promoted more severe responses (at the biochemical, animal condition and behavioural levels) than those elicited when each stressor acted alone (Maulvault et al., 2018cd, 2019).

Conclusions

This work revealed that the exposure to ECCs within a climate change context will likely defy the resilience of marine organisms, particularly those farmed extensively and semi-intensively in coastal areas subjected to strong anthropogenic activities. Hence, climate change will greatly challenge the sustainability and management of aquaculture resources, thus, calling for urgent regulatory, mitigation and/or adaptive actions at a global scale.

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BROWN TROUT (*Salmo trutta*) FINGERLINGS FED WITH INSECT MEALS, AND THEIR EFFECT IN GROWTH PERFORMANCE

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Introduction

Insect meals have been emerging as a natural alternative to fishmeal replacement in fish nutrition, due to the fact that they are part of the fish diet in nature. In the last years, certain insect meal tests have been conducted in some Salmonidae species, such as *Hermetia illucens* in salmon (Belghit et al., 2019; Lock et al., 2015), and rainbow trout (Bruni et al., 2018) or *Tenebrio molitor* in rainbow trout (Belforti et al., 2015). Like the rest of Salmonidae species, brown trout (*Salmo trutta*) is a carnivore fish, and terrestrial and aquatic insects are part of their diet (Elliot, 1967). Considering these facts, the aim of this trial was to evaluate the growth performance and feed efficiency of brown trout fed with full-fat black soldier fly (*Hermetia illucens*) larvae.

Materials and methods

The test was done in the aquaculture laboratory, belonging to the Poznan University of Life Sciences. 225 brown trout fingerlings (*Salmo trutta*) of initial average weight of 48.88 ±2.19 g were fed with three diets: Control diet with 0% of BSF meal inclusion; 2nd diet with 5% of full-fat BSF meal; 3rd diet with 10% of full-fat BSF meal; and 4th diet with 20% of insect meal inclusion. Animals received their feed according to the size and water temperature. The fish were reared under the open-flow system. The trial lasted 58 days. During the experimental period, the water temperature was 14.7 ± 0.6°C, dissolved oxygen stayed constant at 7.5 ± 0.3 mg L^{-1} and the photoperiod was maintained at 16:8 (light: dark).

Results

At the end of the experimental period, there were no significant differences among treatments in the final growth performance as well as specific growth ratio (SGR) in fish fed with different levels of full-fat BSF meal inclusion. In the feed efficiency parameters, there were also no statistical differences among treatments.

In the case of survival rates during the experimental period, it was 100% in all the treatments.

Table I. feed efficiency and growth performance of brown trout fed different levels of full-fat BSF meal inclusion at the end of the experimental period.

	0%	5%	10%	20%	P-value
IBW (g)	49.34	47.51	47.71	48.79	0.6277
FBW (g)	111.30	100.82	99.40	102.14	0.1094
SGR	1.40	1.30	1.27	1.27	0.3236
FIR	1.85	2.03	2.07	2.10	0.2920
FCR	1.07	1.18	1.21	1.22	0.2813

Five replicates per treatments were used. Values in the same row having different superscript letters are significantly different at $P < 0.05$.
Specific growth rate (SGR) = [(ln final body weight (g) – ln initial body weight (g)) / number of days] × 100
Feed intake ratio (FIR) = [(feed intake (g) / total weight (g)) / number of days] × 100
Feed conversion ratio (FCR) = total feed supplied (g DM) / weight gain (g)
Protein efficiency ratio (PER) = [weight gain (g)] / total protein fed (g DM)

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Discussion and conclusion

Black soldier fly is one of the promising meals to replace fishmeal in farmed fish. In the case of juveniles brown trout, the inclusion up to 30% of BSF meal did not affect the growth and feed efficiency parameters, similar results were observed in total replacement of FM by BSF larva meal in salmon (Belghit et al., 2019). Also, Magalhães et al. (2017) did not find significant differences with the maximum inclusion of pre-pre-pupa BSF meal in seabass. Although, Dietz and Libert, (2018) found significant differences in SGR and feed efficiency parameters in Nila tilapia fed with the maximum level of inclusion in comparison to soybean concentrate. These differences in response could be due to the fish species habits due to sea trout, rainbow trout and salmon are carnivorous-insectivorous species, and in the case of Nile tilapia is an omnivorous species.

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LIFTING LARVAL MUSSEL YIELD BY BLOCKING HEAVY METALS

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Introduction

The chemical EDTA, is widely used for conditioning seawater for larval rearing in shellfish hatcheries around the world (Adams et al., 2009; Gale et al., 2016; Helm, 2004; Utting and Helm, 1985). The effectiveness of EDTA is widely attributed to its capacity to chelate heavy metals that would otherwise interfere with larval development, however, this mode of operation has not been confirmed to our knowledge. Alarmingly, EDTA is not readily biodegradable and will accumulate in the environment, which could result in significant changes in environmental metal distribution, and potential ecological impacts (Oviedo and Rodriguez, 2003). The present study was undertaken to determine the role of EDTA and a potential biodegradable alternative (EDDS) (Pinto et al., 2014), in improving larval yields in shellfish hatcheries. This was achieved by measuring the concentrations of heavy metals and how they are changed with treatment with the addition of chelating agents EDDS and EDTA, whilst rearing the New Zealand green-lipped mussel (*Perna canaliculus*) larvae.

Materials and methods

Fertilised *P. canaliculus* ova were incubated for 48 h in UV and 5 µm filtered seawater with five different treatments; control (no chelating agent added), 3 µM EDTA, 12 µM EDTA, 3 µM EDDS, and 12 µM EDDS. Each treatment had ten replicates, to account for between tank variability (50 tanks total). At the end of incubation, D-veliger larval yields in each tank were estimated using counts of three 10 µL samples under a microscope, and comparing these to the initial counts of ova for each tank. The tanks were drained through 40 µm nylon mesh, and D-veliger larvae were collected for analysis of metal content. All mussel larvae samples were analysed for concentrations of chromium, iron, cobalt, nickel, copper, zinc, arsenic, cadmium, mercury and lead with Inductively Coupled Plasma Mass Spectrometry (ICPMS). These data were statistically tested for significant differences among treatments using ANOVA. Mussel larvae samples were further analysed with synchrotron X-ray Fluorescence Microscopy (XFM), providing internal distributions of calcium, copper, zinc, iron, mercury and arsenic in larvae.

Results

There was a marked improvement (37 – 64%) in the yields of D-veliger larvae, with the addition of both chelating agents (Table 1). The yields were improved further at the higher concentration of chelating agent, i.e., 12 versus 3 µM**)**.

ICPMS results show a significant reduction in the concentrations of chromium, copper, zinc, cadmium and lead in D-larvae grown with EDTA ($P>0.05$) (Figure 1). However, they also show a significant increase in the concentrations of cobalt, arsenic, and mercury with EDTA ($P>0.05$) (Figure 1). XFM images demonstrate the distribution of six of the metals of interest for the first time in mussel larvae to the best of our knowledge, and allow for distribution comparisons between treatments. For example, XFM results indicated a change in the distribution of zinc between larvae grown with and without EDTA (Figure 2), with zinc more evenly distributed throughout mussel larvae, rather than localised in areas of high shell content.

Discussion and Conclusion

The results prove that EDDS is as effective as EDTA for improving D-veliger larval yields, and should be considered as a biodegradable alternative for aquaculture application. The concentrations of metals within mussel larvae are significantly different with the presence of 3 µM EDTA in the incubation water. Further work using XFM methods will allow us to investigate the chemical state of metals and their distribution within mussel larvae. This will allow us to determine if chelating agent affects the proportions of chemical states for each metal. For example, we could determine if the presence of chelating agent changes the concentration of protein bound metals in the larvae.

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Table 1. Percentage yields of *P. canaliculus* D-veliger larvae

	D-veliger Larval Yield (±S.E.)
Control	37.4 ± 9.7%
EDTA 3 µM	84.1 ± 11.1%
EDTA 12 µM	89.6 ± 12.9%
EDDS 3 µM	64.6 ± 12.6%
EDDS 12 µM	90.2 ± 9.59%

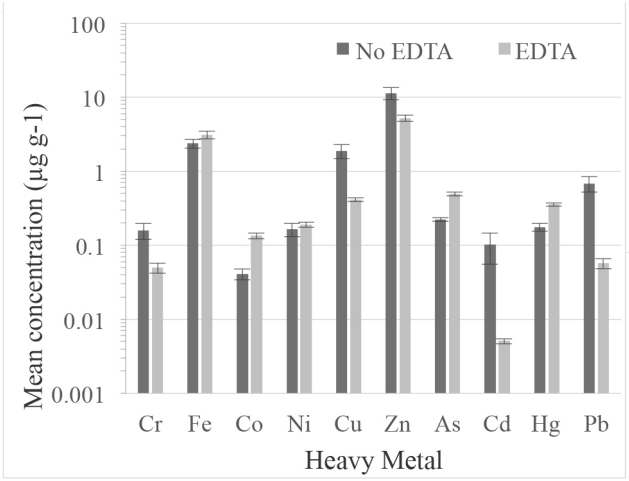

Figure 1. Mean concentrations (± S.E.) of heavy metals in control *P. canaliculus* larvae (reared in seawater without EDTA) and larvae reared in seawater with 3 µM EDTA added. Note: concentrations are on a log scale. Significant differences in the mean concentrations between larvae from control tanks and larvae from EDTA-treated tanks for Cr, Co, Cu, Zn, As, Cd, Hg and Pb ($P<0.05$), but no significant difference for Fe and Ni ($P>0.05$).

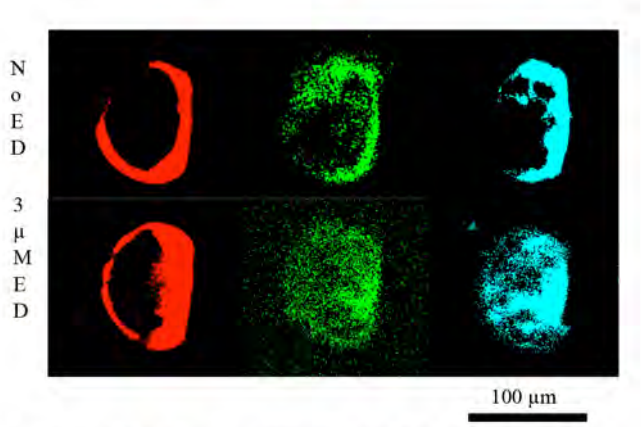

Figure 2. Distributions of calcium, copper and zinc within 2 day old *P. canaliculus* larvae raised with and without 3 µM EDTA added to incubation seawater. Pixels with the highest counts for the respective metal (10% of all counts) are displayed to visualize the distribution of metal concentrations without undue influence from measurement background.

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THE SHELLFISH GROWERS CLIMATE COALITION: A BUSINESS PARTNERSHIP OF 100+ COMPANIES TAKING ACTION TO SECURE A LOW CARBON FUTURE FOR THE BENEFIT OF SHELLFISH AND THE ENVIRONMENTS THEY DEPEND ON

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The Shellfish Growers Climate Coalition is a partnership launched in April 2018 by business owners who grow, harvest, prepare, and serve shellfish, in collaboration with The Nature Conservancy. Member businesses recognize that climate change threatens their businesses and food production for a rapidly growing human population. As of April 2019, more than 100 shellfish growers, hatchery operators, wholesalers, retailers, and restaurants from twenty U.S. states and Canada have joined the Coalition. The Coalition is dedicated to engaging consumers and policy makers to secure a low carbon future for the benefit of shellfish and the environments they depend o

Members of the Coalition recognize that human impact on the Earth's climate system is well-documented and it's happening right now. Carbon dioxide emissions are absorbed by the oceans, changing the chemical composition of the seawater. A decade ago the Pacific northwest shellfish industry experienced a crisis as production of oyster larvae failed in two of the major hatcheries and in the wild. Ocean chemistry altered by dissolved carbon dioxide (ocean acidification) was identified as the culprit. Acidification also impacted at least one hatchery in the Northeast at about the same time. Today, satisfactory larval production in these hatcheries depends on monitoring water chemistry and buffering the incoming seawater to ensure that the proper conditions exist for shellfish larvae to grow their shells. Some growers have shifted hatchery capacity to less impacted locations to avoid the effects of ocean acidification

Other climate-related impacts on our industry include:

- Increasing global temperatures have been strongly linked to growth in Vibrio bacteria abundance, resulting in rising costs of control measures to ensure shellfish safety.
- Storm damage from heightened winds and flooding, and storm tides exacerbated by rising sea levels, have severely damaged hatcheries and nurseries and can disrupt the transportation and sale of our products. Scientists increasingly can quantitatively attribute increased storm intensity to global warming.
- Increased stormwater runoff can mean lower pH water and costly harvest closures to ensure shellfish safety.
- Fluctuation in salinity levels can result in excessive shellfish mortalit .

All these impacts on shellfish farms add up to the need to take action to address climate change. Therefore, members of the Coalition agree that:

- Human impact on the Earth's climate system is well documented, scientifically understood and profound
- Taking action to address climate change is imperative to secure the viability of our businesses, our communities, and the natural resources they depend upon.
- Improving people's understanding of climate change and its impact on our businesses represents an important way to promote and enact climate policies that guide the U.S. to a low carbon future.
- Enacting policies that reduce carbon emissions and encourage low-carbon choices are crucial to a low-carbon future.

Based in these climate change impacts and agreed upon principles, the Coalition has taken actions with consumers and public policy makers to achieve its goal. These actions include educational events at food festivals, industry and scientific conferences, and online, and actively supporting policy measures at the state and federal level to address climate change.

GENERATION OF THE TAPAS TOOLBOX AND SUSTAINABILITY DECISION SUPPORT SYSTEM

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In response to the increasing global population and the resulting growth in demand for protein, aquaculture has been one of the main focus' of the EU's Blue Growth strategy. In comparison to other nations, Europe is a "small fish" in terms of overall production, with development hindered by stagnated growth and European and country-specific "gold-plated" regulatory obligations. In order to facilitate sustainable growth of the sector, an EU Horizon 2020 funded project called TAPAS (http://tapas-h2020.eu/) was established with the aim of promoting sustainable expansion of European aquaculture and identifying and resolving key bottlenecks.

In order to alleviate these regulatory bottlenecks a comprehensive consultation process was conducted with stakeholders all over Europe. The consultation method, consisting of a questionnaire and direct stakeholder interviews, identified the inefficiencies, burdens and faults within the licencing and regulatory processes.

The results of the consultation process allowed identificati n of key areas which required modifications such as; clarification of the consenting process; communication; simplifi ation and harmonisation of legislation; impact assessment and balancing risk; and facilitation of aquaculture zonation within the environment.

This poster presentation details the work done by the TAPAS working groups in finding resolutions to current issues, by designing new, flexible approaches to licensing and monitoring work to common standards. Constant testing, consultation with stakeholder and refining of technologies and ideas was continued throughout the duration of the project to deliver tools to support best practice and efficienc .

These novel ideas and methods were developed into the TAPAS Sustainability Toolbox and the Aquaculture Sustainability Decision Support System, to the acceptability and utility of the stakeholders, to support the development and implementation of spatial planning, and enable less costly, more transparent and efficient licensing the development of sustainable European aquaculture.

Here, brief overviews of the initial problem, followed by the developed method, guideline or integrated tools are given, along with case studies or examples of best practice, to demonstrate the work of the TAPAS groups. Tools discussed include "E-Licensing System", "One-Stop-Shop", "Public Information Platform", "Aquaculture Zones Guidance" and "Environmental Monitoring Database", amongst the many generated TAPAS Tools.

MATE FINDING OF *Ceratothoa oestroides* IN FISH FARMING CONDITIONS

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Introduction

An isopod, *Ceratothoa oestroides* (Risso, 1816) is a parasite in buccal cavity of farmed fish and is causing significant economic losses due to fish mortality and growth reduction (Šarušić, 1999, Horton and Okamura, 2001). Parasites live as a pair in fish buccal cavity and there is a challenge to find a mate in open sea conditions, where visual communication is very difficult. Males need to detect minor amounts of female pheromones and track down the female that usually remains stationary in fish host, while signaling (Phelan, 1997). Aim of the study was to evaluate the capacity of parasite to find a mate in farming condition.

Material and methods

The experiment was performed in two marine net cages (V = 225 m³) in Adriatic Sea near the island of Bisage (44° 01'28.6 "N 15° 13'11.2" E). The experiment was carried out from May 22 to July 4, 2018. The sea temperature during the experiment ranged from 19.7 to 24.0° C. Cage 1 represents infected fish, where female parasites were manually removed and male parasites were left in buccal cavity of fish. Cage 2 represents uninfected fish (i.e., control cage). At the end of the experiment visual control of all fish was carried out. *C. oestroides* were removed with tweezers. The total length (mm) was measured, sex, sexual maturity and fecundity of *C. oestroides* females was determined.

Results

At the end of the experiment, after 930 DD, 93.04% of fish in Cage 1 had a pair of parasites (both male and female). Conversely, in Cage 2 only 1.39% of fish had parasites in pairs. The results showed a statistically significant difference ($P < 0.05$) in the number of parasite pairs in fish between Cage 1 and 2 at the end of the experiment. There was no mature *C. oestroides* female found and therefore parasite fecundity was 0. In cage 1, average total lengths of female and male parasites were 1.89 ± 0.27 cm and 0.63 ± 0.22 cm, respectively.

Discussion and conclusions

Significantly higher number of parasite pairs in Cage 1 compared to the Cage 2 suggests that *C. oestroides* male turns into a female in the absence of females, and successfully found his male pair. Moreover, there were no mature female parasite found at 930 DD, which could indicate that maturity, and therefore fecundity of *C. oestroides* requires more degree days. Knowledge on *C. oestroides* mating behavior can be useful for future health management measures, especially in Mediterranean aquaculture.

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INCLUSION OF ENRICHED AND NON-ENRICHED INSECTMEAL IN FEED FOR RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Due to the fact that global food demand increases, the scientific community is searching for new sources of protein such as insects, both for humans and farm animals (Olsen and Hasan, 2012; Wan et al, 2018). Diets based on the most commonly researched insects have a similar amino acid profile to that of other viable diets (Iaconisi et al, 2017; Magalhaes et al, 2017) and, as well, fairly similar amino acid profiles to fishmeal (Barroso et al, 2014). But on the other hand, it is well known that using insectmeal to feed fish tends to lower the levels of n-3 fatty acids (FA) in their nutritional profile of the fillet, since these insects have a low concentration of these components (Belforti et al, 2015), being EPA and DHA content their major limitations. Nevertheless, some researchers already started looking for a solution to this problem, and there has already been described the possibility of improving the n-3 FA profile of insects by feeding them with a high n-3 FA diet during the last days of their raising period (Barroso et al, 2017). By this way, the FA profile in the fillet could be improved with the use of this enriched insectmeal. With this in mind, our experimental protocol aimed to use one enriched insectmeal to feed rainbow trout in order to, as a whole, prove the theoretical possibility of using an alternative protein source for fish without lowering its n-3 FA profile

Material and Methods

Different levels of fishmeal were replaced with three insectmeals (T: *Tenebrio molitor;* H: *Hermetia illucens*; H*: enriched with n-3 *Hermetia illucens*), leading to the following combinations of fishmeal replacement: 0% (control diet, C), 30% (H30), and 50% (T50, H50 and H*50). All three insectmeals were analysed to specify their proximate composition, FA and amino acid profiles before formulation. The FA profile showed a clear increase in the content of EPA (2.87%) and DHA (1%) of H* respect to T and non-enriched H, where these FA were not detected. For the growth essay, five isoproteic (~43%) and isolipidic (~17.5%) diets were formulated, and 600 rainbow trouts (*Oncorhynchus mykiss*) with an initial body weight

Table I. Effect on growth performance and protein utilization

	C	H30	H50	H*50	T50	SEM
Initial body weight (g)	14.33	14.28	14.82	14.73	14.68	0.22
Final body weight (g)	76.39^{b}	73.35^{ab}	69.36^{a}	75^{ab}	81.92^{b}	2.74
Weight gain (%)	415.03^{ab}	400.03^{ab}	354.75^{a}	387.87^{ab}	438.84^{b}	15.86
SGR (% day^{-1})	2.17^{ab}	2.12^{ab}	2^{a}	2.11^{ab}	2.23^{b}	0.04
DFI (g100g $fish^{-1}$ day^{-1})	1.57	1.59	1.62	1.54	1.57	0.02
FCR	0.98	0.98	1.03	1.02	0.91	0.03
ADCprot (%)	92.58^{b}	84.49^{a}	81.01^{a}	84.8^{a}	91.17^{b}	1.13

*SGR: Specific growth rate; DFI: Daily feed intake; FCR: Feed conversion ratio; ADCprot: Apparent Digestibility Coefficient of protein; SEM: Standard error of the mean. C: control diet (0% insectmeal); H30 and H50: 30% and 50 % fishmeal replacement with H. illucens; H*50: 50% fishmeal replacement with enriched H. illucens. T50: 50 % fishmeal replacement with T. molitor. [a, b]Indicate significant differences (P<0.05) between diets.*

(Continued on next page)

of ~15g were distributed randomly in 20 tanks of 500 l in a RAS system in optimal and controlled conditions. The growth performance shown (Table I) correspond to a 77 days trial, where they reached a final body weight of ~72.2g. At the end of the growth trial, apparent digestibility of the protein (ADCprot) and FA content in the fillet were analysed

Results and discussion

For the growth parameters studied (Table I) there was only one significant difference between diets C and H50, revealing the latter a lower growth. The growth rate (Weight gain and SGR) was significantly higher ($P<0.05$) in trout fed with T50 than H50. There was also a significant difference in the ADCprot, between diets C and T50, and those diets with H, being the H diets that maintained the lowest results. As a whole, these results could be positive in the way that diets with T50 are viable for rainbow trout growth, as well as H30 and H*50, even though these two have a lower ADCprot.

Samples of rainbow trout fillet will be analysed to quantify their proximate and FA composition, to determine the effect of the replacement of fishmeal by insect meal and if it is possible to modify the FA profile with the use of enriched insectmeal.

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PARENTAGE ASSIGNMENT IN THE WEDGE CLAM *Donax trunculus* USING MICROSATELLITES

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Introduction

The wedge clam *Donax trunculus* constitutes an important fishing resource in several countries, such as Spain. However, this bivalve is in regression in some Spanish natural beds (e.g. in Galicia). In order to increase the production of this resource hatchery-produced seed could be used to repopulate natural beds. The aim of this study is to carry out parentage analysis that could help improve supplementation strategies used in *D. trunculus* stock enhancement programmes.

Materials and methods

A total of 364 individuals from a wild population in Northern Spain (Vilarrube – Galicia) were used as breeders in the hatchery of Centro de Cultivos Mariños de Ribadeo. The seed obtained was maintained in the hatchery until they were at least 1500 µm and, after that, transferred to a raft in the Arousa estuary. Seed was divided into four groups for analysis (Table I).

DNA was extracted using the Chelex-100 based method (Walsh et al., 1991) and 11 microsatellites were amplified (Nantón et al., 2014). In order to reduce the number of amplifications, the previously optimized multiplex PCRs were redesigned. Multiplex 1 was used as previously described and four loci (Dtr47, Dtr90, Dtr108 and Dtr117) were optimized in a new multiplex PCR (Multiplex 2'). Parentage assignment was carried out using Cervus v3.0.3 (Kalinowski et al., 2007). This programme uses simulation of parentage analysis to evaluate the confidence in parentage assignment to the most likely candidate parent. This analysis is performed with the simulated genotypes as it is with real genotypes, but in the simulation the identity of the true parent is known for each offspring. Cervus compares the distribution of LOD or Delta scores for tests in which the most likely candidate parent is the true parent with that for tests in which the most likely candidate parent is not the true parent. Ten thousand cycles of simulated assignments were performed. Finally, all the offspring were assigned to the most likely candidate parent pair in the real parentage assignment. Both analysis, simulation and real assignment, were carried out using two confidence levels, strict and relaxed, with 95% and 80% of confidence, respective .

Table I. Characteristics of seed groups

Seed group	Number of individuals	Size (µm)	Days in hatchery	Days in raft
T1	400	>1000	~75	-
T2	100	>1500	~105	-
T3	100	>1500	~150	-
T4	99	3900 – 5900	~75	~165

Table II. Contribution of males and females to reproduction.

	Confidence level	N (%)	Mean	Variance	Range
Mothers	Relaxed (80%)	78 (45.88%)	4.91	52.39	1 - 41
(N=170)	Strict (95%)	43 (25.29%)	5.14	39.36	1 - 29
Fathers	Relaxed (80%)	97 (50.00%)	3.08	23.84	1 - 35
(N=194)	Strict (95%)	57 (29.38%)	3.16	26.81	1 - 30
Families	Relaxed (80%)	417 (1.26%)	1.27	0.56	1 - 7
(N*=32980)	Strict (95%)	176 (0.53%)	1.32	0.52	1 - 5

*: Number of potential families

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Results and discussion

Parentage assignment was carried out using 10 out of the 11 microsatellite loci amplified, given that Dtr126 locus showed high frequency of null alleles and was excluded from the analysis. Parentage simulation showed an assignment percentage of 55% and 84% for strict and relaxed levels of confidence, respectively. In order to carry out the real parentage assignment, a total of 1063 individuals were genotyped, including 699 seed individuals, 170 candidate females and 194 candidate males. Individuals genotyped for less than five microsatellites were excluded (6 individuals of seed and one candidate mother). Values obtained in the parentage assignment (33% and 76% for strict and relaxed confidence levels, respectively) were lower than those observed in the previous simulation. With respect to the seed groups, expected percentages of assignment were 55% and 84% for all cases. However, the real parentage assignments for strict and relaxed levels of confidence were: 35% and 76% for T1 group; 27% and 79% for T2 group; 46% and 87% for T3 group; 21% and 61% for T4 group, respectively. The decrease in the percentages of parentage assignment could be related to different factors that affect the power of assignment techniques (marker variation, null alleles, dependent segregation of the loci, mutation events and genotyping mistakes) (Castro et al., 2004; Dakin and Avise, 2004; Vandeputte et al., 2011; Karaket and Poompuang, 2012).

Table II shows the contribution of males and females to reproduction. Percentage of males and females that contributed with, at least, one descendant was approximately 50% considering a strict level of confidence and about 30% when a relaxed level of confidence was considered. The mean and variance of number of descendants were slightly higher in females, but the range of descendants was similar in both sexes. Regarding families, only 176 and 417 families were detected for strict and relaxed confidence levels, respectivel . The mean, variance and range of descendants were similar for both levels.

Conclusion

The present study constitutes the first work that evaluates the process of obtaining seed of *D. trunculus* in hatchery through parentage assignment. Parentage assignment percentages were lower than those expected. Moreover, a reduction in the number of males and females that contribute to the next generation was observed. However, further studies are necessary to provide genetic information for seed production in hatcheries.

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GENETIC PARAMETERS FOR RESILIENCE BASED ON FLUCTUATIONS IN BODY WEIGHT IN NILE TILAPIA GROWN IN AERATED AND NON-AERATED PONDS

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Introduction

The genetically improved farmed tilapia (GIFT) strain has been selected for increased body weight for 17 generations. The realized genetic gain in GIFT breeding program was between 10 to 15% per generation. The reported coefficient of variation (CV) for body weight in the GIFT strain ranges from 47 to 60% (Khaw et al. 2012, Ponzoni et al. 2005), which is relatively high compared to the CV reported for other Nile tilapia strains (18 to 34%; Gjerde et al. 2012, Omasaki et al. 2016). The GIFT strain has been selectively bred under optimal dissolved oxygen (DO) conditions, while most of the small-holder production still takes place in non-aerated ponds, where large diurnal DO fluctuations are apparent and DO levels can drop to almost zero during the night and early morning. Therefore, selecting for resilient fish for environments with large diurnal DO fluctuati ns is important. Berghof et al. (2019) defined resilience as the capacity of an animal to be minimally affected by disturbances or to rapidly return to the state pertained before exposure to a disturbance. Variance of deviations is one of the indicators of resilience. The objective of this study was to estimate heritability and genotype by environment interaction (GxE) between aerated (Ap) and non-aerated ponds (NAp) for resilience in Nile tilapia using the log-transformed variance of deviations (lnvar) of body weight measured five times during grow-out

Materials and methods

For this experiment, about 3000 fish were mass produced using 200 dams and 72 sires in four 30m^2 hapas. In each hapa 50 dams and 18 sires were stocked for 15 days. After 60 days of nursery period, the fingerlings were individually tagged using PIT tag. Next, the fingerlings were randomly divided between an Ap and NAp. The grow-out period was 218 days. Except for the use of aerator in one pond the feeding and pond management were the same in both ponds. Body weight was recorded five times: at stocking, at harvest and at three different times in between. Among 1500 stocked fish in each pond, 887 fish from the Ap and 799 fish from the NAp had body weight records for the five time points. The 1686 fish from both ponds with body weight records for the five time points were genotyped by sequencing (GBS). A genomic relationship matrix was built using 11,929 single nucleotide polymorphisms SNPs. To standardize the body weight deviations, first the mean body weight (WTg) and standard deviation (SDg) for fish belonging to the same nursery hapa, grow-out pond and sex were calculated. Subsequently, the standardized deviations of body weight were calculated by subtracting WTg from the body weight recorded and dividing by SDg. Finally, the variance of standardized deviations was calculated and log-transformed (lnvar) to estimate genetic and phenotypic parameters.

Results

Genetic parameters were estimated using a bivariate mixed animal model. The additive genetic and residual variances of lnvar for the Ap were 0.12±0.05 and 0.80±0.05, respectively, while for the NAp the values were 0.15±0.06 and 0.85±0.06. The h^2 for lnvar were 0.13±0.05 and 0.15±0.05 for the Ap and NAp, respectively, indicating substantial heritable variation for resilience. The r_g between the Ap and NAp for lnvar was 0.80±0.18. The r_g and r_p between lnvar and harvest weight were 0.42±0.30 and 0.11±0.04 in the Ap and 0.24±0.28 and 0.08±0.04 in the NAp.

Discussion

Variance of deviations is an indicator for resilience. Resilient fish are minimally affected by fluctuating DO and grow more uniformly. Uniformity in aquaculture is an important trait for various reasons. Resilient fish grow more uniformly, resulting in less feed loss and higher revenues. It allows harvesting the whole batch at the same time and avoids size grading, which means less stress for the fish and less labour cost. Finally, uniformly sized fish are easier to process. The additive genetic variances and residual variances of lnvar were higher for the NAp, likely indicating that genetic variation in resilience is more expressed due to greater fluctuations in DO. The estimated genetic variance for lnvar in the Ap and in the NAp were lower than in Marjanovic et al. (2016) and similar to Khaw et al. (2016). The heritability cannot be directly compared,

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because the heritability in Khaw et al. (2016) was defined at the group level and here at the individual level using five observations per fish. To the best of our knowledge, this is the first study on Nile tilapia resilience based on individual fish with repeated body weight measurements.

We found high positive r_g (0.80±0.18) between the Ap and the NAp pond for lnvar, indicating low GxE for resilience. This result shows that selecting for resilient fish in an aerated pond could also lead to a resilient fish in a non-aerated pond. The r_g between lnvar and harvest weight in both ponds were low, and come with high standard errors so caution is needed when interpreting these results. However, it seems that resilience and growth are different traits, and that both traits could be included in the breeding goal. In summary, our findings show the presence of substantial genetic variance in resilience and low or negligible GxE for resilience between the aerated and the non-aerated ponds.

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FEED AND NUTRITION IN ORGANIC AQUACULTURE

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Organic production is a system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes (Mente et al., 2019). Organic aquaculture reflects a specific production approach driven by the growing public interest in sustainable utilization of resources (Mente et al., 2011, 2012, 2019). When applying the principles of organic aquaculture production the following factors need to be addressed: production system design that assures ecosystem balance, biodiversity stewardship, and avoidance of environmental pollution; animal welfare and feeding requirements that respect the health of the organism, product quality, and the expectations of feeding requirements along the lines of similar organic systems; and certain challenges related to breeding (Lembo and Mente, 2019).

Organic fish feed is currently produced according to the EU regulations 834/2007, 889/2008 and 710/2009, 1358/2014, 673/2016, 848/2018. However, due to the limited options in available certified organic ingredients rich in essential nutrients needed to cover the dietary needs of farmed fish, it is challenging for the feed industry to achieve organic feeds of equal quality compared to the conventional ones. Thus, the farmers may experience reduced fish and feed performance leading to disproportionally higher costs in organic farming practices, both due to lower production volumes and to higher feed conversion ratios. Prolonged production cycles increase also the risk of losses due to diseases. Hence, replacing fishmeal and fish oil in high performing diets for organic fish farming is not straightforward. Another challenge related to organic aquaculture is maintaining in practice zero levels any undesirable compounds along the food chain, from ingredient to fork, as consumers often demand from organic products. Chemical antioxidants currently used in conventional aquaculture, in order to safeguard in particular marine ingredient quality, though some on the way out (such as ethoxyquin and its dimers), pesticides etc., find their way in the fish production line, and even if present in small amounts in the fillet, this may represent a risk for scandals and food scares (IFFO, 2015). However, the use of natural antioxidants is of great interest for organic aquaculture.

To safeguard biodiversity and sustainable exploitation of natural resources, the use of capture fisheries-based fish meal and fish oil needs to be limited in both organic and conventional fish feeds (Tacon and Metian, 2015; Lembo and Mente, 2019). However, fish performance, health status and final product quality (Kousoulaki et al., 2016) may be jeopardized when substituting dietary fishmeal by alternative ingredients of lower nutritional value. Thus, new fish aquafeeds and feeding strategies and the exploitation of the genetic potential of farmed fish by selective breeding in using and transforming more efficiently the dietary components to the necessary essential nutrients provides great potential and may allow safer larger steps in the progress of achieving sustainable and resilient fish farming practices

Moreover, though fish cannot synthesize several essential nutrients required for their metabolism and growth, and depend on the feed for their supply, certain animal groups can use nutrient-deficient diets, as they bear symbiotic microorganisms that can provide these compounds (Douglas, 2010). Thus, also aquatic animal's gut microbiota can in theory play critical role in obtaining sustainability in fish farming (Mente et al. 2016; Antonopoulou et al., 2019). Fish would obtain maximal benefits when the microbial supply of essential nutrients is scaled to its demand. Undersupply would limit fish growth while oversupply could be harmful due to allocation of resources to neutralise toxicity caused by non-required compounds. The extent to which the microbial function varies with fish demand and which are the underlying mechanisms are largely unknown.

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In terms of sustainable feed formulations, recent advances prove, among other low trophic level organisms the concept of the nutritional (Kousoulaki et al., 2017) and technical (Samuelsen et al., 2018) feasibility of substituting fish oil by heterotrophically produced microalgae in salmon feeds. Such organisms may be grown on byproducts and waste of other agricultural industrial practices. Nevertheless, achieving the desirable circular economy, which demands recycling of organic and inorganic nutrients, we unavoidably press the current set regulatory limits for undesirable compounds in raw materials and seafood products with potential risks for animal health, welfare, production performance and product safety for the consumers. Mapping occurrence and continuous monitoring and documentation on uptake and accumulation of contaminants on farmed animals in relation to the current levels in feed ingredients and diets is necessary to update and render regulations relevant.

The FutureEUAqua project through workpackage 2 will demonstrate sustainable and resilient nutritional solutions for highest possible fish performances, using common or specially selected fish population, in terms of growth, health, welfare and end product quality, progressing the current state of the art, already endorsed in laboratory or industrial relevant environments to operational environments. FutureEUAqua will design, produce and test commercially relevant, safe, of low ecological footprint, species specific nutritionally adequate or currently inadequate (for non-selected families) innovative feeds for organic aquaculture using feeding protocols, that match the natural feeding behaviour of farmed fish. Both already existing and new candidate ingredients will be documented. Pilot (tanks and cages) and large scale (tanks/ cages) fish performance results will be validated via innovative nutrient retention biomarkers (gut microbiome evaluated by 16S rRNA NGS), welfare indicators (e.g. mucosal mapping), quality indicators (nutritional value, taste and texture) and bioenergetics modelling. The ongoing positive growth trend of the aquaculture industry is expected to continue, reflecting the rising demand for healthy human food products. Hence, since 2000 there has been an increasing demand for seafood that has been farmed according to certified organic standards, notably in European countries. It follows, therefore, that like the terrestrial organic livestock production sector, the onus is on the aquatic nutritionist to formulate an organic feed close to the feed that each fish and shrimp species are consuming in their natural environment

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COMPARISON OF SEASONAL PHYCOTOXIN PROFILES OF CULTIVATED MUSSELS FROM THE BLACK SEA, BULGARIA

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Introduction

Phycotoxins are potent marine toxins produced by certain photo- or mixotrophic microalgae. Being accumulated and sometimes metabolized by shellfish these compounds find their way to human diet (Lewitus, et al., 2012). If contaminated seafood is consumed by humans, severe intoxications can occur (Hégaret and Shumway, 2009). Depending on their chemical structure phycotoxins are divided in hydrophilic (e.g. saxitoxin and analogues such as gonyautoxins (GTX), domoic acid (DA) etc.) and lipophilic toxins (e.g. diarrheic toxins, yessotoxin (YTX), pectenotoxin-2 (PTX-2) etc.)

Investigation on occurrence of phycotoxins in shellfish has become important since the Bulgarian coast developed into a spawning area for mussels.

The aim of this study was to compare seasonal phycotoxin profiles of cultivated mussels collected from the South Bulgarian coast.

Materials and Methods

Cultivated mussels were sampled at the main farming areas situated on the South Bulgarian coast. Sampling campaigns include harvesting seasons in 2017 and 2018.

Aliquots of mussel hepatopancreas homogenates were subjected to extraction with acetic acid and subsequently analyzed for paralytic shellfish toxin (PSP) analysis by ion pair chromatography coupled to post-column derivatization and fluorescence detection (Krock, Seguel and Cembella 2007). Extraction with methanol followed by LC-MS/MS analysis (Krock, et al. 2008) was performed for DA and lipophilic toxins.

Results

In total 41 farmed mussel samples collected in four harvesting seasons – spring 2017, summer-fall 2017 and winter-spring 2018 were studied for the presence of hydrophilic and lipophilic toxins.

Results showed significant variations in phycotoxin content between seasons. 60% of the samples from spring 2017 (N=15) were positive for DA. YTX was registered in 27% of the samples, PTX-2 in 13% and the PSP toxin - GTX-2 in 7% (one sample). Other lipophilic toxins were not detected. In the samples from summer-fall 2017 (N = 15) GTX-2 was again detected in only one sample. YTX was registered in 60% of the samples. Domoic acid and other lipophilic toxins were not detected. In the winter-spring 2018 samples (N=11) 64% were positive for PTX-2sa/ epi-PTX-2sa.

Table I. Concentration range of the detected toxins in investigated period (nd – not detected)

Harvesting season	Detected toxins, positive concentration range				
	GTX-2, $ng.g^{-1}$	DA, $ng.g^{-1}$	YTX, $ng.g^{-1}$	PTX-2, $ng.g^{-1}$	PTX-2sa/ epi-PTX-2sa, ng PTX-2 $eq.g^{-1}$
Spring 2017	nd-1.75	108.3 – 618.9	0.009 – 24.6	0.6- 1.8	nd
Summer-fall 2017	nd - 2.59	nd	2.2 – 14.8	nd	nd
Winter-spring 2018	nd	nd	nd	nd	3.1-7.1

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Abundances of the determined phycotoxins (Table I) showed that DA level increased up to six times and PTX-2 up to three times in spring 2017, approaching highest values in April 2017. YTX is reaching its highest value in May 2017. Detected GTX-2 levels in the two samples from spring and summer- fall 2017 were similar. PTX-2sa/ epi-PTX-2sa appeared in winter-spring 2018 when their abundances increased more than 2 times in this season.

Discussion and conclusion

Phycotoxins regulated in EU - DA, YTX, PTX-2 and GTX-2 as well as the non-regulated epimeric pair PTX-2sa/ epi-PTX-2sa were detected in the samples. Richest variety of marine toxins was estimated in spring samples from 2017 whereas DA, YTX, PTX-2 and GTX-2 were determined. Interestingly, DA and PTX-2 were determined in the samples until beginning of May, while YTX is detected throughout the whole season. DA being the predominant toxin in the phycotoxin profile, is the toxin that is achieving highest level (619 $ng.g^{-1}$) for the whole investigated period.

The phycotoxin profile of summer-fall 2017 is more uniform containing YTX and GTX-2. In general, the presence of GTX-2 in samples from both seasons is scarce. YTX appears in the samples from two subsequent seasons – spring and summer-fall 2017. Its positive concentration range in spring 2017 is much wider than in summer-fall 2017.

Surprisingly, in the third studied period- winter-spring 2018, none of the already registered phycotoxins were detected and a new toxin emerged in the samples - the epimeric pair PTX-2sa/ epi-PTX-2sa. As there is evidence of PTX-2 presence in plankton samples from other investigation seasons (Peteva, et al. 2018) and conversion of PTX-2 into PTX-2sa is well documented in the literature (Ciminiello, et al. 2010), it is reasonable to assume that PTX-2sa/ epi-PTX-2sa also result from PTX-2 through metabolic conversion in mussels.

Since the comparison of seasonal phycotoxin profiles showed the presence of DA, YTX, PTX-2, GTX-2 and PTX-2sa/ epi-PTX-2sa in farmed mussels at low but significantly varying levels in time, further surveillance on phycotoxins content in cultivated mussels is required in order to protect consumers ’health.

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ANNUAL CHANGES IN DIETARY BIOACTIVE LIPIDS IN AQUACULTURE MUSSELS (*Mytilus galloprovincialis*) FROM BULGARIA

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Introduction

The Mediterranean mussel (*Mytilus galloprovincialis*) is the most important marine aquaculture species in Bulgaria with high socio-economic value in the Black Sea region (Stoykov and Petrova, 2010). The aim of this study was to determine seasonal changes in proximate and lipid composition in market ready mussels from the northern part of the Bulgarian Black Sea coast.

Materials and methods

Proximate composition (carbohydrates, crude protein and total lipid) was determined using standard procedures. Lipids were extracted from mussel tissue by solvent extraction and purified by chromatographic techniques. Gas chromatography–mass spectrometry (GC-MS) was used for the analysis of sterols (St) and fatty acid (FA) composition of phospholipid and neutral lipid fractions. The non-saponifiable lipids were identified by high-performance liquid chromatography (HPLC) with ultraviolet (UV) and fluorescence (FL) detectors

Results and discussion

Highest protein (19.90g.100g^{-1}) and lipid (2.34g.100g^{-1}) contents were found in spring, carbohydrates in autumn (2.73g.100g^{-1}), whereas lowest values were detected in summer samples: protein (16.60g.100g^{-1}), carbohydrates (2.40g.100g^{-1}) and lipids (1.45g.100g^{-1}). Results for lipid classes and FA distribution are presented in Table I.

Although there were significant seasonal variations in lipid classes and fatty acids profiles of studied mussels, lipid classes followed the same pattern: PL > NL > St in all seasons. FA groups showed similar distribution in TL and PL (PUFA > SFA > MUFA) fractions, while in NL: PUFA ≈ SFA > MUFA in spring and summer seasons. NL classes presented notable annual variations (MUFA and SFA decreased from spring to autumn), since their FA composition reflects the dietary sources (Balzano et al., 2017). The sum of EPA (eicosopentaenoic acid, C20:5n-3) and DHA (docosahexaenoic acid, C22:6n-3) were higher in spring and autumn, despite 100 g edible portion (EP) provides more than 100% of RDI (EFSA, 2012). Well-balanced n-6/n-3 ratios were observed in all lipid fractions. The main sterols identified were cholesterol (43.4-52.0% of total sterol fraction) and brassicasterol (24.3-34.0%). Other minor sterols (<10%) were campesterol, β-sitosterol and Δ^5 – avenasterol. In this study, significant annual change in analyzed fat-soluble vitamins (A, D_3 and E) and carotenoids (β-carotene and astaxanthin) was observed. Highest vitamin A content (59.3μg.100g^{-1} EP) and vitamin E (5.9mg.100g^{-1} EP) were found in autumn samples. Studied Black Sea shellfish could be regarded as a good source of vitamin D_3 (from 5.53μg.100g^{-1} EP in summer to 14.7μg.100g^{-1} EP in spring). There was inverse correlation in the astaxanthin and β-carotene amounts in the studied seasons. Astaxanthin content was highest in summer (50μg.100g^{-1} EP) and lowest in spring and autumn (23.9 and 26.4μg.100g^{-1} EP, respectively), while β-carotene levels were lowest in spring (318μg.100g^{-1} EP) and increased to 950 and 760μg.100g^{-1} EP (in spring and autumn, respectively).

Lipid classes, % of TL	Spring			Summer			Autumn		
NL	33.8			40.0			30.3		
PL	52.7			42.5			59.7		
St	2.1			4.7			6.9		
Other	11.4			12.8			3.1		
FA composition, g.100g^{-1} EP	TL	NL	PL	TL	NL	PL	TL	NL	PL
SFA	0.51	0.25	0.23	0.36	0.12	0.10	0.37	0.10	0.20
MUFA	0.36	0.20	0.15	0.11	0.03	0.02	0.09	0.02	0.04
PUFA	1.06	0.21	0.62	0.62	0.14	0.20	0.83	0.21	0.42
EPA + DHA	0.79	0.14	0.57	0.56	0.13	0.18	0.78	0.19	0.36
n-6/n-3	0.10	0.17	0.14	0.08	0.10	0.11	0.05	0.16	0.18

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Conclusions

There were significant seasonal variations in proximate and lipid composition of aquaculture *Mytilus galloprovincialis*. The present study characterized Black Sea mussels as high in proteins and low in carbohydrates and lipids, regardless of season. The tendency of decreasing levels of macronutrients, cholesterol and β-carotene in the summer season correlates with the reproductive cycle of the Black Sea mussels. Although low total lipid content, mussel meat showed appreciable amounts of long-chain PUFA. Beneficial levels of EPA+DHA (mean 0.71g.100g^{-1} EP) were found during the whole study period, supplying 142% of RDI for these FAs. In addition, more than 50% of these n-3 PUFA were bonded to PL, which increases their bioavailability.

Presented results illustrate well the high potential of Black Sea aquaculture mussels as healthy food, regardless of season. Although proximate and lipid composition of mussel meat are strongly dependent on biotic and abiotic environmental factors, we can summarize that their consumption could promote dietary recommendations for the consumption of low-saturated fat, high n-3 PUFA and vitamin D_3 food.

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THE USE OF COMET ASSAY TO DETECT DNA DAMAGE IN *TILAPIA* ERYTHROCYTES TREATED WITH MALATHION

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Pollution of environmental waters is a serious problem all over the world. Fish accumulate pollutants directly from contaminated water and indirectly by ingestion of the contaminated organisms. Genotoxic pollutants induce DNA strand breaks and mutagenic lesions that proposed as genotoxicity biomarkers monitoring the fresh water environment.

These lesions are believed to induce chemical carcinogenesis. In the present communication, the alkaline single cell gel electrophoresis; comet assay which is a rapid ,simple and sensitive technique measuring DNA strand breaks in individual cells was used. The genotoxic potential of the widely used pesticide (Malathion) was evaluated in *tilapia* erythrocytes. The cells were exposed to three sub-lethal concentrations of malathion (0.04, 0.5 and1.0 µ g/ml).
Alkaline comet assay was performed on nucleated erythrocytes after 24,48 and 72hrs. The amount of DNA damage in cells was estimated from comet tail length as the extent of migration of the DNA damaged.

DNA damage was observed to be increased with the increase of the concentration compared to the control ($p<0.05$).
The comet tail showed a concentration – time relationship. The maximum tail length at highest concentration and longest time was (8.62µ m). The present study indicated that comet assay applied on fish erythrocytes is a useful tool in estimating genotoxicity of chemical pollutants, such as malathion .

OPTIMIZING OXIDATIVE STABILITY OF EXTRUDED FEED

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Lipid autoxidation is a free radical-mediated chain reaction in which oxygen binds to unsaturated lipids under mild conditions. Fish meal, fish oil, and vegetable oils are rich sources of polyunsaturated lipids, making them highly susceptible to autoxidation. Lipid peroxides are the primary oxidation products and when these are decomposed, secondary oxidation products are formed that result in a rancid or fishy smell. Lipid oxidation products can be taken up in the body through the diet and affect cellular integrity by reacting with proteins, phospholipids and DNA. Fish have protection mechanisms against free radicals, including endogenous antioxidants like vitamins E and C, and endogenous enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Exogenous antioxidants can also be obtained through the diet. An imbalance between uptake of oxidation products and the animal's defense system has a negative impact on the health and the growth performance of the animal. This has been demonstrated in different species when oxidized lipids were used in test diets. In some but not all of these cases supplementation with vitamins E or C could reduce this negative impact. To ensure optimal health and growth, it is necessary that the feed is low in oxidation products. Application of antioxidants can be used to prevent oxidation of feed.

Extrusion is a continuous process that uses high shear and high temperature to cook and shape feed. During this process free radicals are generated. The oxidative quality of extruded feed is impacted by the quality of the raw materials, the formulation and processing of the feed. All sensitive ingredients should be of good quality and be treated with antioxidants as early as possible. Using antioxidant treated protein meal results in a more stable extruded feed compared to non-treated protein meals (Figure 1).

During extrusion and drying, high oxidative stress is put on the feed. Antioxidants are added to the dry mix to protect the sensitive raw materials during this process. At the end of the process, a significant part of the added antioxidants is sacrificed (Table I). The final feed is protected against oxidation by further coating the extrudate with a good quality oil containing enough antioxidants.

By applying a comprehensive program to control the quality of raw materials, protection of the feed during processing and optimizing the feed characteristics, oxidation can be limited. This reduces the potential production loss associated with the consumption of oxidized feed.

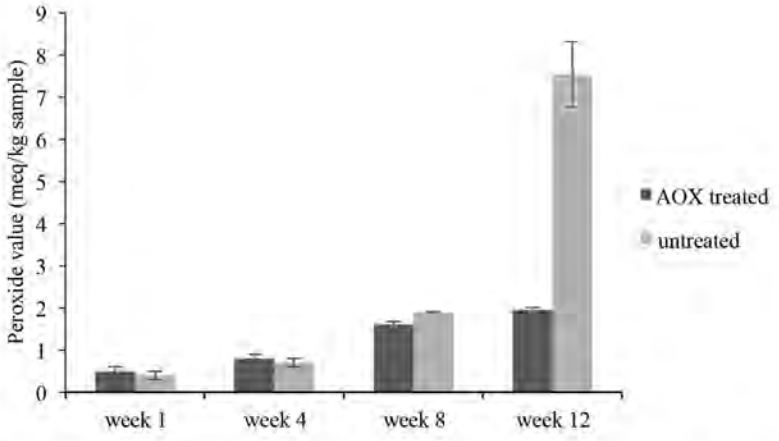

Figure 1. Using antioxidant (AOX) treated protein meals in extrusion improves stability of the extruded feed.

Table I : Antioxidant sacrificace due to impact of processing conditions

	Low	High
Extrusion	15%	35%
Drying	20%	45%
Total	32%	64%

SPAWNING KINETICS OF GREATER AMBERJACK *Seriola dumerili* IN RESPONSE TO DIFFERENT DOSES OF GnRHa IMPLANTS

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Introduction

The greater amberjack (*Seriola dumerili*) is a prominent species for aquaculture diversification due to its high growth rate, late reproductive maturation, excellent flesh quality and worldwide consumer acceptability. One of the main bottlenecks for its aquaculture production is the lack of reliable reproduction, which has been recently overcome using agonists of gonadotropin-releasing hormone (GnRHa). The use of implant-delivered GnRHa was proved to be more effective than injections (Fakriadis et al., 2018). In the present study two different doses of GnRHa were evaluated in terms of egg production and quality.

Materials and methods

Twenty wild-captured breeders (10 females mean weight ± SD, 23.0±2.2 kg and 10 males 18.4±1.9 kg, respectively) were kept at Argosaronikos Fishfarm S.A. in a 1000 m^3 cage throughout the year and were fed with a broodstock diet (Skretting, Vitalis Cal, 22 mm). The spawning trial was conducted between June 7 and July 5, 2017. Females were treated with an Ethylen-Vinyl Acetate copolymer (EVAc) GnRHa implant of either ~25 μg GnRHa kg^{-1} ("LOW") or ~75 μg GnRHa kg^{-1} ("HIGH") (Mylonas and Zohar, 2001). To enhance spermiation and ensure adequate sperm production, all males were treated on the first day with a GnRHa implant at a dose of 58.3±17.7 μg GnRHa kg^{-1}. After being implanted, fish were transferred to an inland facility into four 23-m^3 flow-through round tanks (n=2-3 females in each), in a 1:1 sex ratio. Females and males were given a second treatment of the same dose two weeks later. Tank overflow egg collectors were examined three times a day, and fecundity and fertilization success were estimated immediately after egg collection. Egg and larval quality parameters were estimated using the microtiter plate method (Panini et al., 2001) with some modifications

Results and discussion

In both treatment doses, spawning started one day after the 1st application as some females had oocytes in maturation stage at the time of treatment. Fish spawned for 7-9 times after the 1st treatment and only 5 times after the 2nd treatment. The highest egg production was observed in the LOW group with 33826 eggs kg^{-1} 2 days after the 1st treatment, while egg production in the HIGH was 30206 eggs kg^{-1} on the same day (Fig. 1).

Mean daily relative fecundity was not significantly different between the two groups. The LOW group produced 17801±4127 eggs $kg^{-1}day^{-1}$ and the HIGH group 14648±2285 eggs $kg^{-1}day^{-1}$ after the 1st treatment. After the 2nd treatment, the LOW group spawned 13,373±3,022 eggs $kg^{-1}day^{-1}$ and the HIGH group 8,484±3,228 eggs $kg^{-1}day^{-1}$. Fertilization success was significantly higher in LOW group after the 2nd treatment ($p=0.015$). No statistical differences were observed for the 24 h embryo survival, hatching and 5d larval survival between the different doses and treatment number.

Conclusions

As no significant differences were observed between the LOW and HIGH GnRHa doses, it is preferable to use the LOW dose due to its cost-effectiveness. It should be noted that in a different experiment where a MEDIUM GnRHa dose (50 μg kg^{-1}) and exactly the same setup were used (Fakriadis et al., 2018), the fecundity was higher compared to both LOW and HIGH doses.

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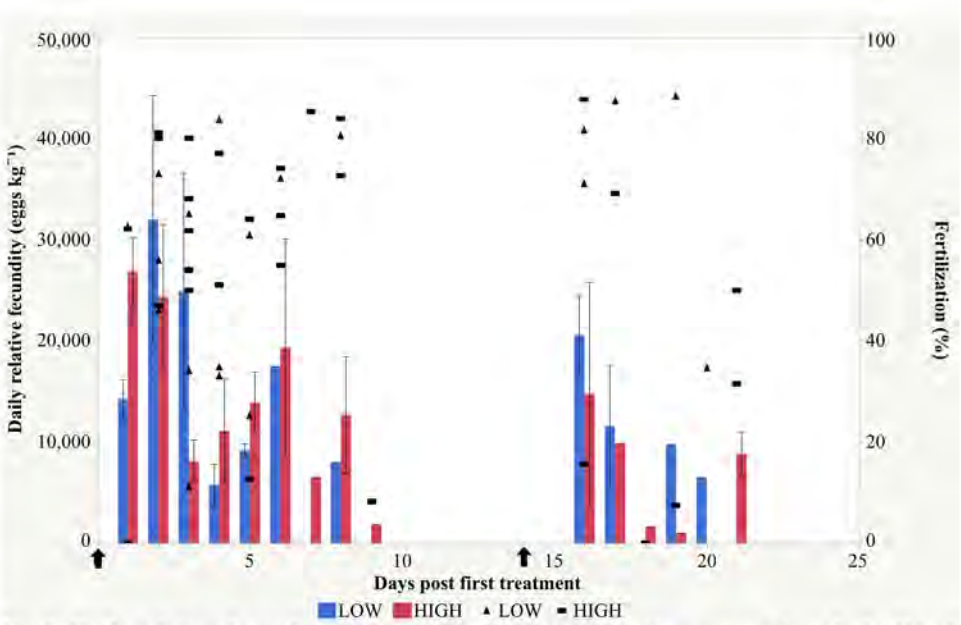

Fig.1. Daily relative fecundity (bars, eggs kg^{-1}) and fertilization success (symbols, %) of the LOW (blue bars) and HIGH (red bars) GnRHa groups of greater amberjack implanted with different doses of GnRHa. Arrows indicate the time of treatment. The first application was done on June 7, 2017.

Acknowledgments

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Acknowledgments

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TOWARDS AN INTELLIGENT MANAGEMENT SYSTEM FOR INTREGRATED MULTI-TROPHIC AQUACULTURE (IMPAQT)

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Aquaculture is growing rapidly in response to increasing protein demands and limited resources from wild fisheries. At the same time, the sector continues to intensify and diversify by modifying systems and practices in place as well as introducing new cultivated species.

Here, integrated multi-trophic aquaculture (IMTA; i.e. co-cultivation of fed species (finfish and extractive species such as seaweeds and/or shellfish), offers a promising approach to improving the sustainable development of the sector by providing environmental benefits, spatial optimisation and increased productivity for cultivation sites. The efficient development of the sector will thereby largely depend on improved monitoring and management practices offered to the producers, while paving the way to a more environmentally friendly, efficient and high-yielding industry in the future

The project IMPAQT (Intelligent Management Systems for Integrated Multi-Trophic Aquaculture) was funded under the EU H2020 call for 'Sustainable Food Security' to specifically address these needs, bringing together 21 partners from research, technology, and industry; all experts in their respective fields and businesses. Our mission is to develop and validate in-situ a *multi-purpose* (inland, coastal, offshore production), *multi-sensing* (heterogeneous sensors and new technologies) and *multi-functional* (advanced monitoring, modelling, analytics and decision making) management platform for sustainable aquaculture/IMTA production.

Using six pilot sites located inside and outside of Europe, which differ in farm scale, design and species cultured, IMPAQT adopts a holistic approach combining i) autonomous real-time data acquisition and communication, ii) an advanced IMTA model and iii) an integrated management system. IMPAQT outputs provide monitoring and management guidance to producers specificall , and inform and promote the eco-intensification of EU aquaculture in general.

SPECIES COMPOSITION, INTROGRESSION AND SHELL PLASTICITY IN FARMED MYTILUS SPP. ON THE WEST COAST OF SCOTLAND

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Northern Hemisphere populations of mussels belonging to the *Mytilus edulis* species-complex (*M. edulis*, *M. galloprovincialis*, and *M. trossulus*) often occur in sympatry, facilitating allele introgression and the formation of hybridization zones. Mussel aquaculture practices can favour hybridisation and, depending on the species present, can lead to an increased occurrence of individuals with unviable traits for cultivation; e.g. weak-shelled and low meat-yielding mussels, as observed at several farm sites in Scotland.

We combined multi-dimensional genetic analyses (monolocus genotyping, developed SNP panel diagnostic for *M. trossulus*) and shell morphometric analyses (shell shape and shell strength) to assess the genetic and phenotypic variability in naturally occurring mixed-species stocks at Scottish mussel farms on a vertical scale - along the cultivation rope.

The genetic composition and shell morphology of the stock differed across culture depth. Although *M. edulis* was the predominant genotype, every sixth individual carried alleles of *M. trossulus*, but the level of introgression was overall low. However, highly-introgressed mussels (*M. trossulus* allele frequency $\geq$ 75%) presented distinct shell morphology with significantly lower shell strength and a more elongated shell shape, and originated predominantly from shallow culture depths. This phenotype distinguished them from their congeners and further allowed for their identification based on shell characters.

Our data highlight the variability as well as complex relationships between the environment of culture, and the genetic and morphological make-up of the stock. In addition, our findings suggest that a combination of shell strength and shape assessments provide a promising approach for the identification of *M. trossulus* mussels on site, which would support the prevention of a further spread of this commercially damaging species for Scotland.

ANTI-INFLAMMATORY EFFECTS OF BETA-1,3/1,6 GLUCAN SUPPLEMENTED FEED IN ATLANTIC SALMON (*Salmo salar*)

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In two recent studies, evidence was found that orally administered ß-1,3/1,6 glucans possess anti-inflammatory effects in fish. In the first trial, groups of seawater adapted Atlantic salmon post-smolts were fed a commercial salmon diet without (control) or with supplementation of 0,1% of a beta-1,3/1,6 glucan derived from *Saccharomyces cervisiae* (Macrogard – MG diet) After 4 weeks of priming, the study groups were vaccinated intraperitoneally using a multivalent, oil adjuvanted salmon vaccine. This and similar vaccine formulations are known to induce intra-abdominal inflammatory reactions in the fish during the subsequent period

Periodically after immunisation, the lymphoid organs of both feed groups were sampled and the expression of inflammation associated cytokine genes was assessed. In head kidney and spleen there was a weak upregulation of IL-1β in both groups shortly after vaccination. However, at several time points thereafter, a clear down-regulation of the pro-inflammatory cytokines TNF-α and IL-17a in head kidney and spleen of the MG group compared to the control fish was observed. The consistency of the outcome patterns indicates that the effects were systemic.

To confirm these results, a second vaccination study of similar design was initiated, this time using salmon smolts reared in freshwater at higher temperature. Again, a downregulation in the expression of several inflammation-associated genes in the MG dietary group was observed during the post-vaccination period.

The strong and systemic effects seen in fish receiving the MG supplemented feed suggests that oral administration of beta-glucans may reduce inflammato y damage caused by viral infections of major relevance to salmonid farming. Examples of such clinical applications will be discussed.

NUCLEIC ACID VACCINES ARE EFFECTIVE FOR CONTROL OF VIRAL INFECTIONS AND DISEASE IN SALMONID AQUACULTURE

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Inducing transient production of vaccine antigens in the recipient's own cells by delivery of naked DNA or RNA is one of the most recent developments in disease prophylaxis and therapy. DNA vaccines were first licensed for use in animals 2005, one of which conferred protection against infectious hematopoietic necrosis (IHN), a viral disease of wild salmonids of the Pacific regions of North America. The disease is currently also endemic in many parts of international salmonid culture including Europe. For humans, several DNA vaccines (the majority targeting HIV and influenza) are still in the clinical development phase, while the same technology is even more intensively employed for experimental immunotherapy against cancer.

The field experiences with use of the North American DNA vaccine against IHN have been remarkably positive, as the historic pattern of clinical disease outbreaks associated with the annual migration of wild carrier populations has discontinued since mass vaccination of smolts were implemented by the local salmonid farming industry.

Although over many years very successful research on DNA vaccination of rainbow trout against viral hemorrhagic septicemia (VHS) that is caused by another virus closely related to IHN virus has been carried out by European researchers, there has been no attempts for commercializing vaccine in Europe.

Then in 2017, the first nucleic acid vaccine for Europe was licensed for prophylaxis against Pancreas Disease (PD) in Norwegian salmon farming. The vaccine has been on the Norwegian market since mid-2018. PD is caused by several genotypes of salmonid alphavirus (SAV), and occurs in Norway, Scotland and both parts of Ireland. In European freshwater pond culture of rainbow trout, SAV genotype 2 causes Sleeping Disease (SD).

In controlled challenge trials, DNA vaccination was highly protective against PD clinical symptoms and tissue pathology for at least 12 months and allowed the challenged fish to experience a normal weight gain.

Recent further information and results emerging from the use of DNA vaccines against IHN, PD and other viral infections of salmonids in clinical trials and in the field will be summarized and discussed. All of the viral infections mentioned above are widely spread in European aquaculture, yet the transfer of highly successful research into vaccination practice is slow or non-existent. The reasons for and potential ways to remedy this situation will be discussed.

INFLUENCES OF ALTERNATIVE FEEDS BASED ON *Arthrospira platensis* AND *Hermetia illucens* ON INTESTINAL HEALTH OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Genetic adaptation to new environmental conditions, such as changing diets, has always played a key role in the evolution of animals. Due to the fishmeal intensified feeding of carnivorous fish species, not only sustainably managed and overfished fish stocks have to face a growing problem. This is one of the reasons why novel sources of protein substituted fish feeds are of increasing interest in aquaculture. However, plant-based protein sources in the diet of carnivorous fish, may lead to inferior feed conversion and hence poorer growth, as well as negative animal health and welfare effects. An inflammation of the small intestine as well as immune suppression due to various amounts of soybean supplementation within the ration was observed in a number of species, such as rainbow trout *(Oncorhynchus mykiss)*, carp (*Cyprinus carpio carpio*), zebrafish (*Danio rerio*) and salmon (*Salmo salar*) (Burrells et al., 1999; Hedrera et al., 2013; Krogdahl et al., 2003; Urán et al., 2008; Venold et al., 2012). Also, studies on red sea bream (*Pagrus major*) suggest that high levels of soybean meal can lead to increased stress and reduction of immunoglobulins (Khosravi et al., 2015). The cooperative project "Sustainalbe Trout Aquaculture Intensificati n – SusTAIn" aims at making use of the genetic variability of trout in order to gain new insights into the adaptability to innovative raw materials and thus to pave the way for a sustainable and intensified, as well as animal and environmentally friendly aquaculture.

Material and methods

Trout of a commercially available origin were compared to a regional trout strain under the influence of three different feeds that included a conventional fishmeal-based trout feed and two feeds in which the fish-meal was partly substituted with *Hermetia illucens* or *Arthrospira platensis*. Overall, the research included 54 almost one year old animals at a weight of 75-124g and a length of 18-22cm. Three animals per feeding group in three replicates (n=9) were sampled. All animals were examined macroscopically for fin and skin lesions and deviations from physiological traits were recorded. Sections of the anterior and the posterior intestine were prepared for histological examinations using HE and PAS staining. Histologically the total number, the color and the fill level of goblet cells were examined. Furthermore, the thickness of tunica muscularis, stratum granulosum and stratum compactum were measured. Inflammatory cell infiltration was analyzed on the basis of a predetermined score from 0 (absent) to 3 (high grade infiltration of inflammatory cells)

Results

Macroscopically, the muscles of the *Arthrospira platensis* fed groups showed a yellowish coloration. In addition, in some animals from this group, and in some fish fed with *Hermetia illucens*, a dull and whitish appearing foregut was noted. Almost 50% of the *Arthrospira platensis* fed group showed a dilatation of the stomach with injected blood vessels. Those alterations could not be observed in the control group. The lamina epithelialis was densely populated with goblet cells in trouts from all feeding groups. Per 100µm in the *Arthrospira* fed group 8.0 goblet cells, in the *Hermetia* fed group 8.6 goblet cells and in the control group 9.7 goblet cells were counted. The filling and color of the goblet cells turned out to be independent of genetic lineage and feeding. Initial results also indicate that in approximately 44% of the examined animals in the lamina epithelialis mucosae of the foregut, vacuoles occured in varying degrees, which overlaps predominantly with the macroscopically findings

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Discussion

In the current work we describe the histological changes of rainbow trout. As a part of the research on intestinal health and welfare, it can only be considered as such and provides a first impression of the animals studied. No clear inflammatory response could be detected in the small intestine of rainbow trout of different genetic origins and in relation to substitution of *Arthrospira platensis* or *Hermetia illucens* to the feed. This is in contrast to other studies (Hedrera et al., 2013). Our results might show that possibly an adaptation of the fish to the novel feed occurred. Subsequent gene expression of various inflammatory markers, anti-microbial peptides and transmembrane proteins on internal organs of the test animals should bring deeper insights. Noticeable however, was the formation of fat vacuoles in the foregut in 44% of the animals, which largely coincided with the slightly yellowish muscle and blunt consistency of the foregut in the feeding groups with *Arthrospira platensis* as well as in parts of the *Hermetia illucens* fed groups. These changes suggest a feeding-related influence, whose relevance for intestinal health and animal welfare requires further research

Conclusion

Initial results suggest that feeding based on substitution of fishmeal with *Arthrospira platensis* and *Hermetia illucens* does not result in increased inflamma ory infiltration of the intestines, which is an essential parameter in terms of gut health and animal welfare. Nevertheless, further investigations are needed, such as the gene expression of inflammatory markers and anti-microbial peptides.

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GOOD ATTRIBUTES OF SUSTAINABLE AGRICULTURE DEVELOPMENT WITH FOCUS ON CAP REFORM AND WATER FRAMEWORK DIRECTIVE

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This paper focuses the attention on contribution of the diversity of production of farms on the economy scale and the related social impact for the environment process. Farms in Italian regions are growing fast and are developing new paradigms on sustainable agriculture for development and eco-friendly products for the future of farming. On behalf of CAP reform 2020 and the Sustainable Development Goals, Agriculture is one of pointed sector and discussed for preservation of environment and the welfare of the future generations. This article will focus on evaluation of the three pilasters of sustainability, in economic, social and environmental reality of Italian regions. The methodology of this research is a quantitative model on using data from ISTAT and EUROSTAT, elaborating them and find out new paradigms and methods on the sustainability of water use on farm organization for more sustainable agriculture development and revenue.

AQU@TEACH: INNOVATIVE EDUCATIONAL TOOLS TO PROMOTE LEARNING AMONG EUROPEAN STUDENTS USING AQUAPONICS

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Introduction
Aquaponic food production is complex and requires a broad spectrum of knowledge in order to understand and manage the processes involved. If aquaponics is to develop its potential in Europe, it will require an appropriately trained workforce. AQU@TEACH is the first aquaponics curriculum to be developed specifically for university level students. Devised in collaboration as an Erasmus+ Strategic Partnership in Higher Education, the multidisciplinary curriculum covers the basics of aquaponics with a focus on transferable and entrepreneurial skills.

Aquaponics curriculum
The aquaponics curriculum (150 hours, 5 ECTS) can be taught either using blended learning – combining digital media and the Internet with classroom formats that require the physical co-presence of the teacher and students – or as an e-learning course. The fifteen modules were designed using the Learning Designer software, which assists with achieving a balance between the six types of learning – acquisition, inquiry, practice, production, discussion and collaboration, and employ a toolbox of innovative didactic techniques suited to blended learning and e-learning, such as workshops, wikis, discussion forums, social bookmarking, and e-portfolios. The curriculum was pilot tested as an e-learning course with five students from each of the partner institutions, and then optimised on the basis of a combination of student feedback and self-evaluation by the tutors.

The project also afforded a valuable opportunity for the students to take part in a practical experiment which was run concurrently at the partner institutions. The aim of the experiment was to investigate the growth of the same vegetable cultivars in different conditions (system configuration, climate), in order to explore the factors responsible for differences in yield and elemental composition of the dry biomass.

Entrepreneurial skills curriculum
The supplementary entrepreneurial skills curriculum (60 hours, 2 ECTS) was devised on the basis of two surveys: 1) of aquaponics companies around the world, in order to ascertain which entrepreneurial skills are particular to aquaponics; and 2) of European Higher Education institutions which teach courses where aquaponics could be incorporated in the curriculum – such as aquaculture, horticulture, sanitary engineering, ecological engineering, landscape architecture, agronomy, etc. – in order to ascertain which entrepreneurial skills are particular to those disciplines.

The curriculum follows the lean start up methodology, and includes different types of business model, capital and financing, customer development, marketing, and start up pitches. It was piloted during a summer school in Slovenia with students from the four participating countries, and then added to the e-learning modules.

Teaching aquaponics: best practice guide
The best practice guide provides teachers and trainers with fresh ideas for achieving the best possible educational results in teaching interdisciplinary subjects such as aquaponics, which require high-quality knowledge in diverse fields as well as the specifics of interdisciplinary integration. It also encourages them to introduce their students to technical and business skills, and to develop their own specific professional and transversal competences through the use of innovative teaching methods and tools.

All of the outputs – the e-learning course, textbook, module guide, curriculum guide, teaching materials, best practice guide and toolbox of innovative didactic techniques – will be publicly and freely available at the end of the project in March 2020.

DEVELOPMENT OF A *Scrombidae* MODEL MARINE FISH FOR DISEASE ECOLOGY, PACIFIC CHUB MACKEREL - *Scomber japonicus*

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Offshore marine aquaculture has one of the greatest opportunities for expansion, yet many questions remain about the environmental and economic sustainability of such operations. Sustainable growth in mariculture production is limited by technical challenges such as hatchery mortalities, growout morbidities from infections, and public concern regarding environmental degradation from disease outbreaks and waste pollution, while proper colonization and development of mucosal microbial communities during early life events are critical for health across vertebrates. Changing ocean conditions driven by anthropogenic activity may have a negative impact on fisheries and aquaculture production by increasing stress and disease with the mucosal microbiome as a potentially important intermediate role. Evaluating and testing hypotheses on disease transfer within marine aquaculture net pens and to and from wild populations remains challenging especially for highly valued species like tunas. Therefore, we have developed a new marine model fish which can be used to test hypotheses on disease ecology including population transfer and effects of abiotic and biological stressors. Using long read (Oxford Nanopore) and short read (Illumina) sequencing we have assembled a draft genome of Scomber japonicus with an N50 of 3.0 Mb. To investigate natural (abiotic and biological) impacts on the fish mucosal microbiome, we sampled 229 individuals surveying five body sites (gill, skin, digesta, GI, and pyloric caeca) collected across 38 time points spanning one year from the Scripps Institution of Oceanography Pier, making this the largest and longest wild marine fish microbiome survey. The skin and gill was explained primarily by sea surface temperature, chlorophyll a, and fish age, consistent with an exposure gradient relationship. A cosmopolitan pathogen, *Photobacterium damselae*, was prevalent across multiple body sites, but highest in the skin, GI, and digesta between June and September (warm water and low nutrients). To validate similarity between the mackerel microbiome to a tuna species microbiome, we sampled 72 Southern Bluefin Tuna samples from South Australia and compared the gill, skin, and digesta communities. This study demonstrates the importance of sampling wild fish to understand underlying disease distribution particularly prior to installation of fish farms. We demonstrate how environmental factors such as temperature influences the mucosal microbial ecology. With the successful establishment the natural microbial variation and genome assembly, we can now begin testing effects of increasing temperature stressors on parasite or bacterial infections as a model for tuna health.

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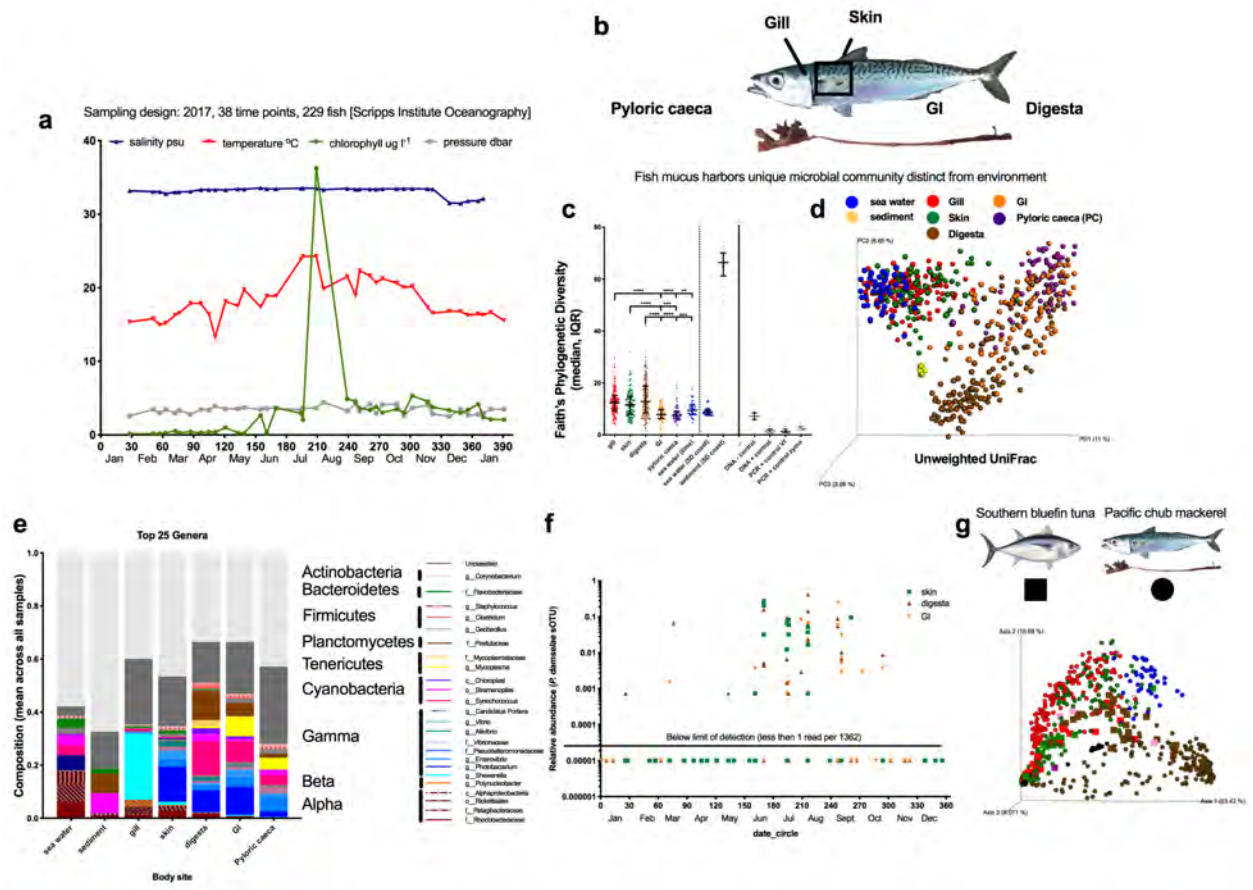

Figure 1. Microbial ecology of mucosal microbiome from *Scomber japonicus*. (a) Fish were sampled on 38 time points during 2017 from SIO pier with water parameters measured by in situ probes. (b) Five body sites were sampled from each of the 229 fish. (c) Alpha diversity as measured by Faith's Phylogenetic diversity was calculated and compared across mucosal sites with the gill, skin, and digesta samples having the highest diversity (Kruskal-wallis test). (d) PCoA plot of microbial communities (Unweighted UniFrac) from fish mucus and environment reveals skin and gill communities most similar to environment (water). (e) Top 25 genera across all sample types reveals high prevalence of Photobacterium sp. in skin, digesta, and GI communities. (f) Prevalence and relative abundance of species level resolution (150 bp sOTU from 16S rRNA V4 region) of *Photobacterium damselae* (fish pathogen) across body sites and sampling time points indicates elevated presence during warm water periods. (g) Microbiome comparison of mucosal sites (gill, skin, digesta) of Pacific chub mackerel (229 individuals) to Southern Bluefin Tuna (72 individuals).

CONSIDERATIONS FOR MICROBIOME RESEARCH IN AQUACULTURE: OVERCOMING CHALLENGES WITH LOW BIOMASS ENVIRONMENTS, REDUCING COSTS, ADDRESSSING TECHNICAL NOISE, AND META-ANLAYSES

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The microbial ecology or microbiome of both the organism's body sites along with the environment is important for evaluating and maintaining animal health. Rapidly identification and monitoring of disease outbreaks in clinical cleanroom environments such as hatcheries is essential for preserving life and preventing catastrophic economic loss due to massive deaths. One of the primary challenges with microbiome research is understanding and choosing the current protocols as the variety of methods in continually expanding. Microbiome processing and analyses of low-biomass samples are challenging because of contamination and inefficiencies, leading many investigators to employ low-throughput methods with minimal controls. We introduce a high-throughput low-biomass pipeline (Katharoseq) that reveals the whole bacterial community from inputs as little as 500 cells using automated robotic platforms which we determined by comparing samples processed by 5 different methods (n=300). We apply this method to both the 16S and metagenomics analysis of five unique built environments including a NASA spacecraft assembly facility, a Neonatal Intensive Care Unit, a critically endangered abalone rearing facility, an Atlantic salmon hatchery, and a yellowtail kingfish hatchery revealing spatially resolved, distinct microbiomes, reproducible across hundreds of samples. We have furthered optimized this method to reduce well-to-well contamination during DNA extraction while reducing costs over 20x to a final library prep cost of $1.4 per sample.

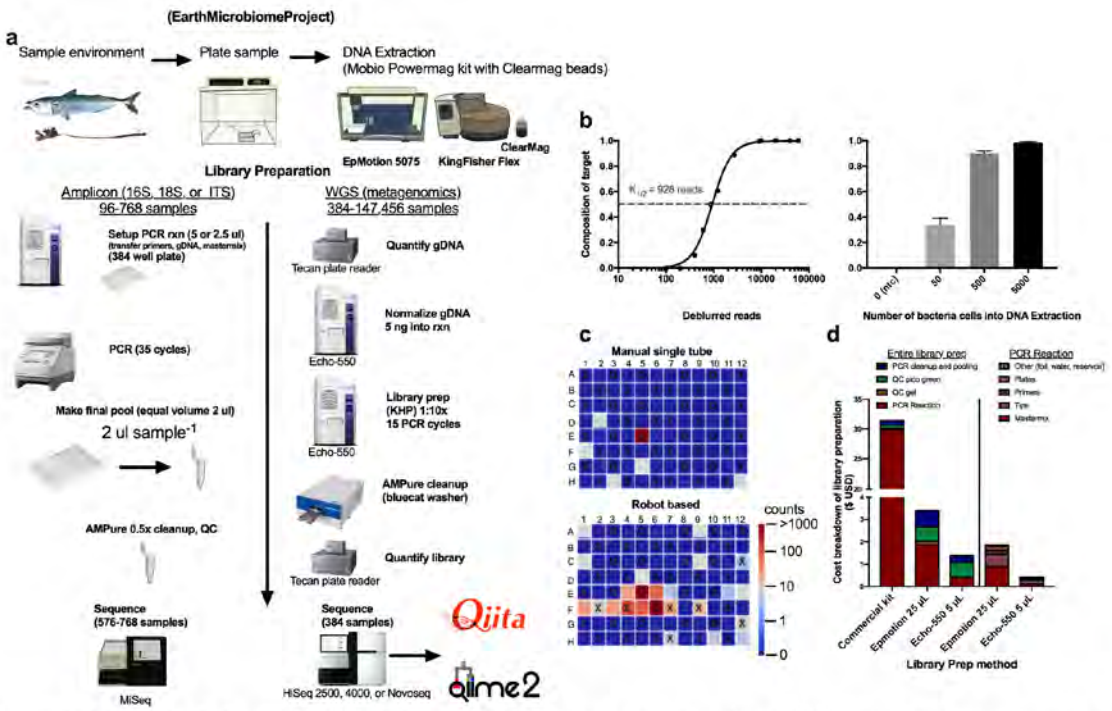

Figure 1: (a) Methods for High-Throughput (96-384 samples), low noise, and low cost metagenomic sample processing of DNA extraction and library preparation (amplicon & metagenomics sequencing) [EarthMicrobiomeProject]. (b) Protocol enables low biomass detection down of 500 cells of bacterial material at 92% accuracy. (c) Well-to-well contamination noise occurs primarily during DNA extraction thus requires a hybrid approach of single-tube and magnetic bead cleanup. (d) Cost reduction enables 5 ul amplicon library prep reactions at $1.4 per sample.

HATCHERY SYSTEM (RAS VS FT) EFFECTS ON FISH MICROBIOTA FOR ATLANTIC SALMON, *Salmo salar*, AND YELLOWTAIL KINGFISH, *Seriola lalandi*

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Successful rearing of fish in hatcheries is critical for conservation, recreational fishing, commercial fish through wild stock enhancements, and aquaculture production. Hatcheries however, can have a negative impact on the environment by requiring large volumes of water from local streams or rivers and in turn producing large volumes of effluent waste. The recent development of engineered, enclosed Recirculating-Aquaculture-Systems (RAS) enables up to 99% of water recycling thus significantly reducing environmental impacts. One major concern for RAS systems over traditional flow through (FT) systems is a negative impact on fish health which is in part thought to be due to microbial dysbiosis either to the fish or the environment (water). In this study we evaluated how the built environment of a hatchery is influenced by the hatchery type from three Atlantic salmon (*Salmo salar*) hatcheries (RAS n=2, FT n=1). For Atl salmon, six fish (300 days post hatch - parr) were sampled from three independent tanks in each of the three hatcheries for a total of 54 unique fish. Water and a swab of the tank side biofilm was collected from each of the nine tanks to evaluate the environmental microbiome. To assess the entire mucosal microbiome, three body sites including the gill, skin, and digesta were swabbed from each fish and processed for 16S rRNA analysis using the EarthMicrobiomeProject protocols. The water and tank biofilm communities, especially from the RAS systems, generally had a higher microbial richness compared to the fish mucus. The three body sites each had unique microbial communities ($P<0.001$) and thus were analyzed independently. Within each body site (gill, skin, and digesta), microbiomes were uniquely driven by the various hatchery systems ($P<0.001$) with both RAS systems being more similar. We next investigated the relationship between the tank system and the fish mucus and found that both the water and tank biofilm richness was positively correlated with skin and digesta richness with the biofilm association being slightly stronger. We next tested whether fish mucosal microbiomes were more similar to their origin tank compared to the similarity to other hatchery tanks. Strikingly, the gill, skin and digesta communities were more similar to the origin tank biofilm vs. all other experimental tanks suggesting that the tank biofilm has a direct influence on the fish communities. For water samples, the skin and digesta were more similar to the origin tank compared to all other tanks. Furthermore, the gill and skin communities were always more similar to tank water and tank biofilm than the digesta samples. To determine a biological effect, we compared the gill, skin, and digesta microbial communities to health measures from histopathology analyses and found that both digesta and skin microbiomes were associated with skin mucus cell levels. Lastly, we also repeated this analysis on a marine fish hatchery, Yellowtail kingfish (*Seriola lalandi*). Specificall , we sampled 12 fish (130 days post hatch) each from three FT tanks, and two RAS systems and found similar environmental associations to fish mucosal communities. The results from this study provide evidence for the first time an association and link between the tank microbiome and the fish microbiome. We show how the skin microbiome of the fish is most influenced by the environmental community which in turn is also related to skin mucus health.

FISH FARMING IN TANZANIA AND THE AVAILABILITY AND NUTRITIVE VALUE OF LOCAL FEED INGREDIENTS

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Introduction

Aquaculture industry is one of the fast food-growing activities in Tanzania (URT, 2015). Its expansion has been constrained for decades by limited availability of good quality feeds at affordable prices (Al-Sayed, 2006). Hence, majority of tilapia farmers opt for locally available feed ingredients in the country (Kaliba *et al.,* 2006). However, there is a lack of data on locally available feed ingredients in different regions of Tanzania and their nutritional content. Therefore, an investigative surveyed study was performed from January 2017 to February 2018 to gather baseline data on their availability and nutritive values content.

Materials and methods

The present study was carried out in nine regions of mainland Tanzania and Zanzibar Island during the period of January 2017-February 2018. A structured questionnaire form comprising questions concerning with type of local feed ingredients used and other issues relating to aquaculture was used to collect data. In addition, some local feed ingredients of interest were collected at four different geographical locations were analysed for proximate analysis (Dry matter, Crude protein, Crude fibe , ash, Crude lipid) and their mineral content at Sokoine University of Agriculture according to methods described by AOAC (1990). Moreover, amino acids analysis were also done on an interested feed samples (n=17) at Tanzania Veterinary Laboratory Agency (Dar-es-Salaam) by near-infrared reflectance spectrophotometry (NIRS) according to the manufacturer's instructions.

Results

In total, 202 fish farmers were interviewed and 30 different ingredients were collected from the study sites for chemical and other nutritive values analysis. The findings showed that, majority of respondents involved in aquaculture operations were males (82.7%), with a high proportion (55.0%) in the age group 40-60 years. Most commonly cultured fish species (82.2%) was tilapia, mostly (87.6%) raised semi-intensively at a stocking density of 2-3 fish/ ^{2}. Over 80% of respondents relied on local feed ingredients as supplement diets for cultured fish. The most common feed ingredients were maize bran (28%), Lake Victoria sardines (11%) and sunflower seed cake (11%). Crude protein content in most analysed local ingredients was medium-high, while Crude fat content was high in some animal and agricultural by-products, but was medium-low in other ingredients. In general, agricultural by-products, aquatic plants and industrial by-products had a medium-high content of nitrogen-free extract, while there were major differences in ash content between the feed ingredients analysed. According to mineral content, the results showed a wide range of mineral concentrations in the local feed ingredients used by tilapia fish farmers in Tanzania. On other hand, marine shrimps, freshwater shrimps and prawn head waste were high in tryptophan, lysine and methionine plus cysteine compared to other tested ingredients.

Discussion and Conclusion

There was a great variation in the size of fishponds, tanks and cages between fish farming systems within regions, and between regions, confirming earlier findings for Tanzania (Kaliba et al., 2006; Mwaijande & Lugendo, 2015). Earthen ponds were the most common fish farming system in all regions except Dar-es-Salaam, the findings was similar to previous study done by Kaliba et al. (2006). Moreover, tilapia was the most commonly cultured fish species that was coherent to findings reported by Kaliba et al. (2006). Moreover, the present study found that more than 80% of respondents relied on locally available feed ingredients as a major feed supplement for their cultured fish, which was comparable to data for eight regions of Tanzania reported by Mwaijande & Lugendo (2015). According to the chemical analysis, the results showed that the chemical composition of collected common feed ingredients was generally within the range reported by others (Chiba, 2009). However, there were great differences in the ash content of feed ingredients analysed in the present study, with very high values found in many fishery by-products, but also in some agricultural by-products, plant leaves and aquatic plants. The variation in similar feed ingredients between studies can be due to many factors, such as animal species, contamination, processing, handling and storage, climate conditions, production season, geographical zone, soil type and stage of maturity at harvest. We also found that animal by-products, except for Nile perch fish frames, were

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high in lysine, tryptophan and methionine plus cysteine. In some agricultural by-products (i.e. soybean), plant leaves and weeds, aquatic plants (i.e. azolla) and spent brewer's yeast were intermediate in lysine and methionine plus cysteine, but high in tryptophan (0.2-1.5%). This is consistent with findings reported in other studies (Kibiriza et al., 2016). According to El-Sayed (2004), the essential amino acid (EAA) requirement for tilapia species is in the range 1.43-1.62% for lysine, 0.17-0.6% for tryptophan, 0.53-1.13% for methionine and 0.53-2.1% for cysteine. Therefore, the integration of more than two locally available ingredients in fish diets has provided the balanced fish diets that meet amino acid requirements in a farmed tilapia species.

These data provide a good platform for development of feeding strategies for cultured tilapia based on current culture systems, availability of local feed ingredients in Tanzania and their nutritional content.

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ADMINISTRATION OF GALACTOOLIGOSACCHARIDE AND *Pediococcus acidilactici* ENHANCED CUTANEOUS AFFECTS MUCUS IMMUNE PARAMETERS, HUMORAL IMMUNE RESPONSES AND IMMUNE RELATED GENES EXPRESSION IN COMMON CARP (*Cyprinus carpio*) FINGERLINGS

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Introduction

One of the major challenges in intensive carp farms is related to stressful situation, and subsequently, diseases and destruction of environmental conditions might be happened (Esteban, 2012). To resolve the issue, antibiotics have been usually used as a preventative means of bacterial control (Balcazar et al., 2007).The application of antibacterial agents can induce food and environmental pollution, development of antibiotics resistant bacterial strains, toxic accumulation in edible tissues of fish and suppression of immune system (Cerezuela et al., 2016). The present study was performed to investigated the effects of singular or combined administration of galactooligosaccharide (GOS) and *Pediococcus acidilactici* on cutaneous mucus immune parameters, humeral immune responses and immune related genes expression in common carp (*Cyprinus carpio*) fingerlings

Materials and methods

Carps were fed with experimental diets for 8 weeks as follows: non-supplemented (Control), prebiotic diet (10 g/kg GOS), probiotic diet (1g/kg [0.9×10^7 CFU] lyophilized *P. acidilactici*) and synbiotic diet (10 GOS in combination with 1 g/kg [0.9×10^7 CFU] lyophilized *P. acidilactici*). The blood samples were obtained from three fish per tank and serums were isolated (Zou et al., 2016). Skin mucus samples were obtained based on the modified protocol of Subramanian et al. (2007) and serum immunity parameters were then measured (Demers et al., 1997). The mRNA levels of immune related genes (LYZ, IL1b and TNF-a) were analyzed in intestine tissue using Real-Time PCR (Cerezuela et al., 2016).

Results

Unlike skin mucus, the serum lysozyme activity showed no significant difference between carps fed supplemented or control diets. Remarkable elevation of serum ACH50 activity was noticed in carps fed supplemented diet (pro-, pre- and symbiotic diet) compared control group. Besides, feeding on pro-, pre- and synbiotic supplemented diet significantly increased serum and skin mucus total Ig levels. However, no significant difference was observed between treatments and control group in case of skin mucus proteases activity. There was no significant difference between expression levels of intestinal genes of LYZ and IL1b in fish fed on pre- and synbiotic, compared to the control. However, evaluation of TNF-alpha gene expression in the intestine of carps revealed remarkable down-regulation in treated groups ($P < 0.05$).

Discussion and conclusion

There was no available information about comparative study of pre-, pro and synbiotic on common carp innate immune parameters. However, in accordance with our findings, Hoseinifar et al. (2015) reported that serum ACH50 and lysozyme activity significantly increased in rainbow trout fingerlings following administration of the same pre-, pro- and synbiotic as ours. . Furthermore, Ye et al. (2011) demonstrated that combined administration of dietary fructooligosaccharide (FOS), mannanoligosaccharide (MOS) and *Bacillus clausii* as synbiotic significantly increased innate immune parameters in Japanese flounder (*Paralichthys olivaceus*). However combined dietary supplementation with *Bacillus subtilis* and chitosan or *B. subtilis* and FOS (Geng et al., 2011) did not remarkably elevated immune parameters of cobia (*Rachycentron canadum*) and yellow croaker (*Larimichthys crocea*), respectively. The discrepancies observed in different studies might be in part due to differences in fish species, life stage and the type and dosage of administered pre- or probiotic as well (Hoseinifar et al., 2016).

The present results showed positive effect of supplementation of carp diet with GOS and *P. acidilactici* on some mucosal or serum immune parameters. However, these feed additives down-regulated immune related genes expression in intestine. The present study encourages further studies on different aspect of synbiotic application in carp aquaculture.

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DETERMINATION OF HEAVY METALS FROM ALOE VERA BY_PRODUCT IN GOLDEN MULLET (*Liza aurata)*; HEALTH RISK ASSESSMENT FOR CONSUMERS

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Introduction
Heavy metals have become subject of concern in the recent years because of its potency to cause cardiovascular diseases and other toxic health effects (Alissa et al. 2011). Furthermore, Spain is, after Japan, the world's second fish-consuming country, at over 30 kg per person per year, and consumption is expected to rise in the future (Jose´ Usero et al. 2003). Since a long time, the use of fish as an indicator of heavy metals contamination has been increased which represent a principal health hazard on human health through the food chain and Dietary intake. Therefore, this research was assumed to investigate the level of toxicity in terms of heavy metals accumulation in the fish samples and its benefits and risk for human consumers health and also evaluate the partial replacement of plant sources by canarian Aloe vera diets as a pure product or by-product on growth and toxicological effects on the golden mullet (*Liza aurata*) fillet and whole body and compare it with control.

Materials and Methods
A total of 240 juveniles were chosen for the experiment with an average weight and size of 8.93 ± 1.88 g and 10.08 ± 0.95 cm respectively, which were randomly assigned in 15 tanks (tripled by treatment and fed for 91 days with substitution of plant sources by 5 diets were made with 0% of aloe inclusion (control diet), 2% of pure form of aloe (pure product) (diet P2), and 2, 4 and 6% of Aloe vera by-product (diets BP2, BP4 and BP6). Heavy metal concentration determined using the ICP-MS Agilent 7900 (Agilent Technologies, Tokyo, Japón).

Results
The results showed that fishes doubled their initial weight after three months from the start of the test, resulting in an average of 18.64 ± 4.02 g of weight and 12.38 ± 0.96 cm of full size and there are no statistically significant differences were found in any of the calculated indices. All heavy metal levels in the fish tissue and diets were below the safe limits for consumption which confirmed by various international standards such as European Union (EU) and World Health Organization (WHO). In case of diets it is obvious that the level of metals in both product and by_product was lower than the control in most of metals. However, in whole fish results proved that the diet with by_product 2 % have the highest levels of accumulation of metals compared with the other diets and the accumulation of this metals depend on the fish species, diet and metal concentration.

Discussion and conclusion
Concerning growth parameters, both the specific growth rate and the feeding efficiency were significantly superior to those described for this species by Karapanagiotidis et al. (2014).

The analysis of contaminants represents that all metals were below the safety limits. While some articles used (*Liza aurata*) as an indicator of contaminant levels in the environment and there are no references to heavy metals have been found in the animal's entire body. To our knowledge, there is no articles discussed the topic of determination of heavy metals in fish and by product which feeded on with different concentration as the present study.

It should also be taken into consideration, that muscle tissue, being the less active metabolically, accumulates metals in lower levels than other tissues (Renieri et al., 2014; Squadrone et al., 2016 Nasyitah Sobihah et al., 2018;) however, metals are transported to muscles through other tissues and muscles can serve as indicators of an implemented chronic exposure (Kalantzi et al., 2016).

Thus, it can be concluded that Aloe vera product and by_product were in safety limits for fish and also for humans through the food chain.

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CHANGES IN PROTEIN, FAT, ASH AND DRY MATTER IN DIFFERENT WEIGHTS OF COMMON CARP

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Introduction

The body composition of fish in different species may vary under different conditions.This difference is due to different nutritional conditions, different water quality, maturity and sex. Also, the amount of protein, fat, carbohydrate and ash in aquatic muscle varies in different species (Javaid et al., 1992; Ali et al., 2005; Saliu et al., 2007; Sharifian, 2014). The amount of chemical composition in the aquatic body depends on the type of nutrition, living environment, age and sex of the living creature (Kochekian Sabour and Yasimi, 2011: Velayat- Zadeh, 2013). However, these indices vary significantly between and among species, size, sex, nutrition season, and physical activity (Ali et al., 2005). Therefore, in the present study, changes in the composition of common carp meat, which is a valuable bone fish that is bred in many parts of the world, were studied at different weights.

Materials and Methods

For this research, 2 year old carp were used in 5 weight groups as follows: Group A: Average weight 500 gr, Group B: Average weight 700 gr. Group C: Average weight 1000 gr. Group D: Average weight 1300 g and Group E: Average weight 1600 g. To evaluate the quality of carp meat, 5 fish were separated from each weight group after checking for health and abnormal symptoms and disease in the fish. After biometrics and measurement of fish length and weight, 100 grams of muscle was isolated from each fish sample to evaluate meat quality. The AOAC (1990) method was then used in the Food Analysis Laboratory to evaluate and analyze meat quality. SPSS 22 software was used for data analysis.

Results and Discussion

Table 1 shows changes in protein, fat, ash and dry matter quality of common carp in different weight groups. Results showed no significant changes in protein content in different groups and no significant difference was observed between different weight groups (P> 0.05). Fat changes of common carp showed the lowest fat content in the high weight group (1600 g) and the highest fat content in the lower weight group (500 g), but the results showed a decreasing pattern in the meat fat change process with increasing fish weight. But overall results showed that there was no statistically significant difference between different weight groups in fat (P> 0.05). The results of meat ash changes, in spite of the lowest ash content in the 1600 g group and the highest in the 700 g fish, indicated no regular decreasing or increasing pattern in the different weight groups. The results also showed that there was no statistically significant difference in ash content between different weight groups (P> 0.05). Dry matter variations showed no significant difference (P> 0.05) between mean dry matter groups. In general, the results showed that there was no significant difference between different weight groups of common carp in terms of protein, fat, ash and meat dry matter (P> 0.05). The results of this study show that weight gain in a typical carp does not make a difference in fish meat quality, and this is inconsistent with the findings of researchers who have reported that fish meat quality changes in weight and size

Table 1: Changes in protein, fat, ash and dry matter at different weights of common carp

Factor (%)	Protein	Fat	Ash	Dry matter
500 (g)	10.176 ± 3.878 [a]	9.624 ± 1.971 [a]	1.547 ± 0.293 [a]	21.347 ± 2.20 [a]
700 (g)	10.105 ± 1.641 [a]	9.957 ± 0.432 [a]	1.565 ± 0.034 [a]	21.596 ± 1.246 [a]
1000 (g)	9.22 ± 0.841 [a]	9.287 ± 0.134 [a]	1.362 ± 0.071 [a]	19.821 ± 0.43 [a]
1300 (g)	10.837 ± 2.998 [a]	9.399 ± 1.104 [a]	1.296 ± 0.245 [a]	21.533 ± 2.304 [a]
1600 (g)	10.129 ± 1.468 [a]	8.645 ± 0.643 [a]	1.294 ± 0.064 [a]	20.068 ± 0.76 [a]
Average	10.209 ± 2.106	9.429 ± 0.937	1.407 ± 0.198	21.03 ± 1.62

*The small Latin letters show that there are significant differences among different groups

(Continued on next page)

Fish tissue protein is a favorable combination of amino acids as well as a rich source of vitamin B and rich in vitamins A and D (Zmijewski et al., 2006). Based on the results of this study, the average protein of common carp was 10.209 ± 2.106 %, which is higher than that of *Liza dussmieri* (10.13%) (Aberomand, 2012) and much less than fish such as golden mullet (*Liza auratus*) (17.69%), *Hemisynodontis membranacea* (20.26 %), *Clupea harengus* (18.45), *Tilapia zilli* (18.80 %) (Olagunju et al., 2012), *Orcynopsis unicolor* (22%), *Euthynnus affinis* (24%) (Aberomand, 2012), *Otolithes ruber* (19.64 %), *Scomberomorus guttatus* (19.9%), *Scomberomorus commerson* (19.5%) (Velayat-Zadeh and Askari Sari, 2013). Fat is a component of the chemical composition of the muscle that represents the largest difference in the amount of fish muscle (Askari Sari et al., 2016). The average fat of common carp in this study was 9.429 ± 0.937 %, which was higher than the amount of fat in *Liza auratus* (0.74%), *Liza dussmieri* (0.25%) (Aberoumand, 2012), *Otolithes ruber* (1.23 %), *Scomberomorus guttatus* (2.1%), *Scomberomorus commerson* (3.4 %) (Velayat-Zadeh and Askari Sari, 2013) and much less than fish such as *Clupea harengus* (11.14 %), *Scomber scombrus* (12.33 %) (Olagunju et al., 2012), *Tilapia zilli* (18.80%) (Olagunju et al., 2012), *Orcynopsis unicolor* (16 %), *Euthynnus affinis* (14 %) (Aberoumand, 2012).The average ash of common carp in this study was 1.407 ± 0.198 %, which was higher than *Liza abu* (1.36%), *Liza auratus* (1.37%) (Askari Sari et al., 2016), *Liza dussmieri* (1.36 %), (Abromond 2012), *Otolithes ruber* (1.32 %), *Scomberomorus guttatus* (1.13 %) (Velayat-Zadeh and Askari Sari, 2013) and much less than fish such as *Liza macrolepis* (2.6%), *Liza klunzingeri* (2.63%) (Askari Sari et al., 2016), *Scomber scombrus* (1.79 %) (Olagunju et al., 2012), *Scomberomorus commerson* (3.4 %), (Velayat-Zadeh and Askari Sari, 2013), *Clupea harenguss* (1.6%) (Olagunju et al., 2012).

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COMPARATIVE STUDY OF THE REPRODUCTIVE PERFORMANCES OF FIVE POPULATIONS OF *Oreochromis niloticus* (Linnaeus, 1758) FROM THE FIRST GENERATION OF WILD BROODSTOCK COLLECTED IN THE MONO, NIGER, OUÉMÉ WATERSHED IN BENIN

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In order to investigate the reproductive performance of five populations of *Oreochromis niloticus* from F1 wild populations collected in Nangbéto, Sohoumè and Togbadji in the Mono watershed, Gobé in Ouémé and Gbassa in Niger, tests were conducted for ten weeks. After selection of breeding stock from different populations, males and females were kept in different ponds and fed for two weeks before mating. All populations were monitored once a week, the laying females were identified, the lay eggs were collected, weighed, photographed, and incubated up to 10 Dpf where the larvae were counted. Overall, for all the populations considered, the rate of females having laid (66.7% to 91.7%), the rate of females having had more than one laying (33.3% to 100%), the average total weight of the clutches (4.1 g ± 1.5 to 7.1 g ± 4.6), gonadosomatic index (2.5% ± 1.9 to 4.8% ± 2.8), average absolute fertility (616.9 ± 380 eggs at 987.8 ± 548.9 eggs), average fertilization rate (89.3% ± 14.4 to 99.3% ± 1.2) and the average hatching rate (58.9% ± 20.3 to 89.1% ± 10.4) varied significantly ($P < 0.05$) with the Sohoumé, Togbadji and Gobé populations who had the best reproduction performance.

SUSTAINING A MOBILE APPLICATION FOR FISH FARMERS IN UGANDA: POLICY CONTRADICTIONS, TECHNICAL CAPABILITIES, AND USER NEEDS

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Introduction

Mobile phones have a rapid diffusion rate and facilitate farmers' access to information, helping increase their bargaining power, control over external events, develop new skills and grow revenues (Myhr and Nordstrom 2008). For instance, in Tanzania the arrival of mobile phones, transformed agricultural business performance at all points by augmenting farmers' access to education and vital market information (Timuray 2014). Matuha (2015) found that fish farmers use mobile phones to access technical guidance from intermediary farmers, obtain market information, accomplish mobile banking and receiving, contact family members and make plans for procurement of fish farming inputs. Factors that seemed to discourage mobile phone use included: lack of electricity, poor network coverage, high calling credit and maintenance costs, lack of awareness and promotion. On the other hand, information regarding stocking and harvesting, feeding management, pond construction and management, disease management, water quality management, broodstock management and market prices were information topics most needed by fish farmers. Several different business models have emerged in efforts to provide technical support to African farmers with cell phones. Each varies in the level of public sector control, business model, cost, and flexibilit . One commercial model invites farmers to subscribe to a fish-focused network of producers managed by a service provider who moderates the transactions and may be compensated by subscription fees, transaction fees, or commissions. The entrepreneur firm builds and supports a network of suppliers, producers, and buyers whose transaction costs support the network. The source of technical information may be uncertain, but the responsiveness to technical questions may be rapid because the entrepreneur is motivated to keep and grow the number of participants. This is the approach we take in Uganda. The purpose of this paper is to describe the implementation of a mobile-based application for fish farmers, participation processes, and services provided. The conclusion considers how ICT advances food security and development by empowering farmers and linking them to each other, extension, and input suppliers.

Materials and methods

We examine trends in mobile phone utilization, subscription, and deployment of mobile applications as support mechanisms for training and aquacultural development. We also examine the structure of licensing fees, SMS charges, and mobile time as barriers to the expansion and impact of mobile applications for fish farmers. We discuss the results of focused group discussion with fish farmers conducted during the process of developing and launching the application.

Results

The data outline some of the barriers and opportunities to the spread and augmentation of mobile applications for fish farmers.

Discussion and conclusions

The Uganda policy environment presents significant challenges for mobile phone applications in Uganda. Annual charges for mobile short codes far exceed others in Africa. The mobile network is capable, but the affordability of smart phones is a barrier to many. The Android system is widely used due to its affordability. Government needs to harmonise the ICT policy and the agricultural policy that aims at establishing a knowledge based management and information system for improving agricultural extension service delivery.

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THERMAL TREATMENT AND LESIONS ON ATLANTIC SALMON (*Salmo salar*)

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Introduction

Thermal treatment is widely used in Norwegian aquaculture for delousing of Atlantic salmon (*Salmo salar*) (Overton et al. 2018). The treatment procedure is to crowd the fish in the sea cage and pump them past a dewatering strainer into a treatment chamber where they are exposed to seawater at a temperature of 28–34°C for 20–30 seconds (Holan et al. 2017).

High mortality and various lesions (e.g. skin wounds, scale losses, degeneration of nasal mucosa, and bleedings in eyes, palate, gills, thymus, and brain) have been reported on Atlantic salmon after industrial thermal treatments (Hjeltnes et al. 2018, Overton et al. 2018, Poppe et al. 2018, Stien et al. 2018). Clear causal relationships for the mortality and pathological findings, as well as the prevalence of the various lesions, are, howeve , not documented.

The objective of this study was to reveal whether the thermal component of an industrially relevant thermal treatment inflicts lesions on Atlantic salmon.

Materials and Methods

- The study included 60 post-smolt Atlantic salmon ($\overline{WW}$ = 1117 ± 250g) of which 40 got thermal treatment ($\overline{TT}$ = 34.0 ± 0.1ºC) and 20 got control treatment ($\overline{TT}$ = 9.2 ± 0.2ºC) for 30 seconds.
- The fish were collectively sedated (level 1, Iversen et al. 2003) in the stock tank before being individually anaesthetised (level 3) in a bucket and treated in a submerged bag of non-slip fabric.
- After treatment, the fish were immediately euthanised, welfare scored (Noble et al. 2008), and sampled for later histopathological examination. Samples from 28 thermally treated fish and 14 control fish were examine
- The study included skin, fins, eyes, snout, nasal pits/mucosa, palate, gills, thymus, pseudobranch, brain, heart, liver, kidney, pyloric caeca, pancreas, and spleen.

Results and Conclusion

Thermal treatment at 34°C for 30 seconds did not inflict any statistically significant acute lesions on Atlantic salmon except caudal, dorsal, and right pelvic fin injuries (Fisher's Exact Test, p = 0.002, 0.002, and 0.014, respectively). As the fish displayed a strong behavioural reaction in the treatment bag when exposed to warm water, the fin injuries may have been self-inflicted

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NON-INVASIVE SEX DETERMINATION IN LUMPFISH (*Cyclopterus lumpus* L.) USING ULTRASOUND TECHNOLOGY

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Introduction

The lumpfish (*Cyclopterus lumpus* L.) is used as a cleaner fish in the Atlantic salmon farming industry providing a non-medicinal treatment against sea lice infection. Lumpfish production relies on the capture of wild broodstock which may not be sustainable in the future, limits year-round production, and the possibility to select for desired traits such as robustness and sea lice grazing efficienc . As most teleost fish, lumpfish develop secondary sexual characteristic when becoming sexually mature and display a sex-dependent growth dimorphism (Davenport 1985). Mature males are smaller, are richly colored and possess a relatively larger suction disc compared to mature females (Davenport and Thorsteinsson 1990). However, small immature fish of either sex cannot be easily distinguished outside breeding season and have similar sized suction discs. Both sexes loose color and separation of the sexes by external characteristics becomes less easy at equivalent weights. The knowledge of lumpfish reproductive biology in captivity is very limited and the lack of non-invasive techniques to identify gender and track maturity stages are common management handicaps in captive broodstock management. In most sexually reproducing species, the gender ratio tends to be close to 1:1. However, due to the high sperm density in males, in commercial broodfish production a ratio of 1 male to 10 females is considered most cost-efficient. It is also unknown if some of the desired lumpfish traits, such as sea lice grazing efficiency or swimming ability differ between sexes. Our goals was therefore to test the use of ultrasound as a non-invasive method for sex determination in lumpfish of commercial size and during broodfish production

Material and Methods

A total of 85 juvenile, commercial size lumpfish were netted randomly from Atlantic salmon sea cages at Hemne fjord, Norway, in September, October and December 2017. At Nofima s Center for Marine Research in Tromsø, Norway, 419 lumpfish produced to become broodfish were examined at increasing sizes during twelve samplings between 30.01.2018 and 14.03.2019. A MyLab Alpha (Esaote, Italy) ultrasound machine was used to determine the sex in all lumpfish. Individuals where an ovary was observed were categorized as females. In individuals were no ovaries could be observed, were categorised as males. After ultrasound examination, body weight and length were registered, and sex was verified by dissection. Ovaries were dissected out and weighed for calculation of gonado-somatic index (GSI).

Results and discussion

In juvenile, commercial sized lumpfish (48 ± 14 to 72 ± 16 g), sex was correctly determined by ultrasound examination in 87 to 100 % of lumpfish, with highest accuracy in lumpfish of 72 ± 16 g. In larger lumpfish (192 ± 53 g to 742 ± 310 g), sex determination accuracy varied between 69 to 100 % for both sexes. In females, an accuracy of 100 % was achieved at a smaller size (392 ± 141 g) compared to males (742 ± 310 g). In all larger lumpfish (up to 1438 ± 872 g), sex was correctly determined and was related to an increase in GSI. Male coloration became visible in males at 392 ± 141 g and increased from 44 % to 96 % during the last examination at 1438 ±872 g. The sex of immature males lacking coloration was determined with 100 % accuracy using ultrasound in 742 ± 310 g and larger males. Ultrasound seems to be will suited as a non-invasive method for sex determination in lumpfish. We will further compare our observations to external morphological measurements of lumpfish and test how the handling during ultrasound examination influences survival

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NUTRITIONAL EVALUATION OF SOME ECONOMICALLY IMPORTANT MUSSELS AND SNAILS OF BANGLADESH

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Introduction

Mussels, snails and oysters are the most important resources among all the non-conventional food items in Bangladesh. Even though mussels and snails are not traditional food items in Bangladesh, they have great importance in terms of nutritional evaluation especially for tribal communities. In contrast of meat, mussels and snails are regarded as high quality food items because of having high protein, important vitamins, different minerals and omega-3 (EPA, eicosapentaenoic acid and DHA, docosahexaenoic acid) and omega-6 (LA, lenoleic acid and ALA, alpha-linolenic acid) fatty acids.

Materials and methods

In this study, fresh mussel and snail samples were collected from freshwater, coastal region and marine environment of Bangladesh and stored at -20 ^{0}C. Prior to the proximate analyses, muscle portion of each samples were collected for further nutritional studies of micro-nutrients like amino acids, fatty acids, vitamins and minerals as well as heavy metals.

Results

The results of the proximate analyses revealed that 65.3% crude protein, 11.2% crude lipid, 13.8% ash and 88.4% moisture are present in oyster (*Saccostrea cucullata*). Whereas, freshwater apple snail (*Pila globosa*) contained 49.6% crude protein, 3% crude lipid, 16.8% ash and 83.4% moisture. In addition, freshwater mussels, *Lamellidens marginalis* and *L. corrianus* contained 36.9-40.9% protein, 4.4-4.8% lipid, 11.4-13.1% ash and 84.8-85.4% moisture. The results also show that micro nutrients like amino acids profile and fatty acids profile are enriched in oyster in comparison to freshwater mussels and snails. All the amino acid contents are higher in oyster than the mussels and snails. The fatty acid contents especially omega-3 (EPA and DHA) fatty acids are higher in oyster than those in freshwater mussels and snails. However, omega-6 fatty acids like lenoleic acid (LA) and alpha-linolenic (ALA) acids are higher in freshwater mussels and snails than in the oyster. Overall, the results indicate that oyster can be a good source of high quality protein and lipid especially EPA and DHA. On the other hand, freshwater mussels and snails also could be good sources of protein and LA and ALA but scarcity of EPA and DHA.

ENRICHMENT OF *Brachionus* SPP. AND *Artemia* SP. WITH NEW MICROALGAE STRAINS

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Microalgae are considered a potential source of nutrient in aquaculture and food industries due their high content and quality of lipid, protein and vitamins. They also have antifungal an antibacterial activities. Focusing on their nutritional value and therapeutic activities, they can play an important role on aquaculture, especially in fish larvae production based on the transference of nutrient through food chain. Trying new microalgal strains is important for improving the feasibility of microalgae in aquaculture. For this propose, a protocol to enrich *Brachionus* spp. and *Artemia* sp. with novel (*Tetraselmis* sp. IMP3, *Chlamydomonas reinhardtii* and a chlorophyte PT FAR14E) and traditional (*Nannochloropsis oculata*, IPMA algae collection) microalgal strains was developed to evaluate the value of fatty acid transference between them. The enrichment took place in 3 l tanks during 24 hours with air supplement for *Artemia* and oxygen for *Brachionus*, and culture density were 200 and 800 ind.mL^{-1} respectively. Samples were collected after 12 and 24 hours of cultivation. Microalgae consumption was evaluated by cell concentration in the culture tank. *Brachionus* and *Artemia* survival performance were obtained by counting the number of live individuals. Results indicate both organisms can graze on that microalgal species (Table I) and they promote *Artemia* and *Brachionus* survival rate (100%). Samples were also collected and preserved to fatty acid analyses.

Table I - Consumption of different microalgae, *Nannochloropsis oculata*, IMP3, *Chlamydomonas reinhardtii* and PTFAR14E (mean ± standard deviation), by *Brachionus* spp. and *Artemia* sp., in terms of cell concentration (n° cell. 10^5.mL^{-1}) variation between samplings 0-12 (Δ T0, T12) and 12-24 hours (Δ T12, T24).

	***Brachionus* spp.**		***Artemia* sp.**	
	Δ(T0,T12)	**Δ(T12,T24)**	**Δ(T0,T12)**	**Δ(T12,T24)**
N. oculata	3.58 ± 0.02	1.38 ± 0.01	2.98 ± 0.02	2.52 ± 0.02
IMP3	2.92 ± 0,02	1.38 ± 0.02	1.57 ± 0.01	3.50 ± 0.07
C. reinhardtii	5.09 ± 0.02	6.81 ± 0.12	3.11 ± 0.01	2.55 ± 0.02
PTFAR14E	24.5 ± 0.01	1.25 ± 0.01	1.83 ± 0.01	10.8 ± 0.01

BACTERIAL OUTER MEMBRANE VESICLES - TARGET AND CARRIER FOR FISH ORAL VACCINATION AND CHARACTERIZATION OF THE SYSTEMIC AND MUCOSAL INNATE AND ADAPTIVE IMMUNE RESPONSE

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The role of the peritoneum as a model for infection has been described previously in rainbow trout (*Oncorhynchus mykiss*), however, most pathogens infect fish via mucosal surfaces such as skin, gills or the mouth oftentimes ends up in the gut. Therefore, we established an oral stimulation model with bacterial antigens to analyze the mucosal and systemic immune response as a model for salmonid fish

Methods: Fish were treated with a bacterial vaccine formulation either by intraperitoneal or oral vaccination. At different time points after vaccination peritoneal, blood, spleen and head kidney leukocytes were isolated and comparatively characterized by flow cytometry in target immune organs (gut, peritoneum) and effector immune organs (spleen and head kidney) labelled with a cocktail of lineage marker specific antibodies. Moreover, using the lineage marker specific antibodies the recognized cell populations were sorted at function related time points after stimulation. Finally, the mRNA was prepared form these isolated cell populations to characterize their mRNA profile

Results: A comparable kinetics of distinct leukocyte populations was seen after both oral as well as intraperitoneal administration especially in the targets immune organs.

Conclusion: The data indicates a similar stimulation of immune cells either after intraperitoneal and after oral vaccination and therefore the potential of the oral vaccination approach.

LETTUCE (*Lactuca sativa*, VARIETY SALANOVA) PRODUCTION IN DECOUPLED AQUAPONIC SYSTEMS

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Introduction

Decoupled aquaponic systems (Kloas et al. 2015, Monsees et al. 2017) have the potential to become one of the most effective sustainable production systems for the combined production of animal protein and plant crops. Here, recirculating aquaculture systems for fish production are combined with hydroponic systems for soilless plant production allowing individual management of each single compartment thereby recycling dissolved nutrients derived from metabolism of the fish. The aim of the present study was to compare a conventional hydroponic lettuce production with decoupled aquaponics using the nutrient rich fish water as basis for the nutrient solution being supplemented with missing nutrients. In addition, one aquaponic treatment became disinfected in order to assess any occurring benefits of the aquaponics derived fish water.

Material & methods

The experiments were conducted in a 75 m^2 compartment of an experimental Venlo-type greenhouse located at the Humboldt University, Berlin, Germany.

Briefl , three different treatments were applied in triplicates (Fig 1). Two nutrient solutions were prepared with fish waste water obtained from a recirculating aquaculture system rearing Nile tilapia (*Oreochromis niloticus*) and supplemental fertilizer. One nutrient solution was used directly for the experiments, whereas the other was disinfected before application. The hydroponic control was prepared with fresh tap water and rain water (50:50, v/v) and mineral fertilizer.

Results

The use of aquaponic fish water saved 62.8% mineral fertilizer and fully substituted the required fresh water for the nutrient solution in comparison to the control. No significant difference in growth of green open butterhead lettuce was observed. Additionally, the content of DOC/TOC as well as RAS derived microorganisms had no obvious positive/beneficial e fects on the growth and phenolic compounds of lettuce.

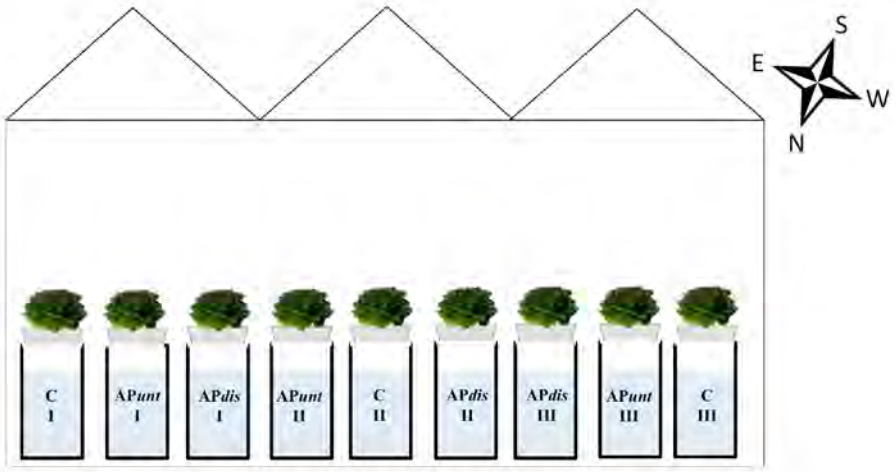

Fig. 1. Schematic description of the arrangement of the experiments. C = control, fresh water based nutrient solution; AP*unt* - untreatend aquaponics, fish water based nutrient solution with supplemented nutrients; AP*dis* - like AP*unt*, but fish water was disinfected before use. The three different treatments were applied in triplicates, each with ten individual lettuce plants.

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Discussion

The benefits of nutrient and water recycling caused by a combined production of fish and plants in a decoupled aquaponic approach were clearly shown in this study. The recycling of fish water resulted in a reduction of fertilizer application of nearly 63% and a complete substitution of freshwater which underpins the effective resource utilization in decoupled aquaponic systems. The post adjustment of the nutrient profile using fish water for professional hydroponic application was more challenging as in conventional hydroponics (with rain water or tap water) but it was demonstrated that for most nutrients the set points were reasonable close to the recommended nutrient concentrations as it was also shown in other studies (Suhl et al. 2016, Goddek and Vermeulen 2018). With ongoing professionalization and standardization of practices in decoupled aquaponic technology it is assumable that optimal nutrient profiles in decoupled aquaponic applications will be met with reasonable effort and comparable yields to conventional hydroponic production can be expected.

Additionally, the reduced fertilizer demand using decoupled aquaponics can contribute to reduce greenhouse gas emissions of an annual lettuce production site per ha by 72% due to saving the energy for fertilizer production.

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ANTIBACTERIAL AND ANTIVIRAL ACTIVITIES FROM MACRO- AND MICROALGAE EXTRACTS

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Introduction

During the last years, attention has been focused on marine organisms as a source of substances of therapeutic interest for the prevention and prophylaxis of farmed fish diseases, augmenting host defense mechanisms (Harikrishnan et al., 2011). For this purpose, the ability of algae to produce secondary metabolites of potential interest as immunotherapeutics has been documented (Reverter et al., 2014). Thus, the present research intends to explore novel bioactive compounds produced by macro- and microalgae, assessing antibacterial and antiviral activities against the main bacterial and viral pathogens in aquaculture.

Material and methods

Three macroalgae species were evaluated, *Fucus vesiculosus*, *Gracilaria* sp. and *Ulva rigida*, and two microalgae species, *Nannochloropsis gaditana* and *Chlorella* sp. Algal extracts were prepared from freeze-dried biomass in four binary solvent systems: methanol/water, 80:20 v/v (M80:20); methanol/water, 50:50 v/v (M50:50); ethanol/water, 80:20 (E80:20) and ethanol/water, 50:50 v/v (E50:50).

The antibacterial capacity of algal extracts against the main bacterial pathogens in aquaculture was evaluated using the standard disk diffusion agar method. The most promising algal extracts were selected to determine their possible citotoxicity and antiviral activity. Citotoxity of 10 selected extracts was determined based on cellular morphologic alterations in established fish cell lines, epithelioma papulosum cyprini (EPC) and rainbow trout gonad (RTG-2). Based on the maximum non-toxic concentration (MNTC), antiviral activity of the previous selected extracts against two pathogenic virus, the Viral Hemorrhagic Septicemia Virus (VHSV) and the Infectious Pancreatic Necrosis Virus (IPNV), was assessed by the reduction of virus titers measuring the using Median Tissue Culture Infectious Dose (TCID50).

Results

Overall, *Ulva rigida* provided stronger extracts with broader activity, which suggests it may be richer in antibacterial compounds than the remaining macro- and microalgae covered in this study. However, *Fucus vesiculosus* provided the strongest extracts against *Photobacterium damselae* subsp. *pisicida*, one of the main pathogens in aquaculture.

The MNTC of the extracts in EPC ranged from 6250 μg ml^{-1} to 24 μg ml^{-1}. The least cytotoxic extracts were *Chlorella* sp. E80:20 and *Ulva rigida* M50:50, requiring higher concentrations to induce cytopathic effects. On the other hand, E80:20 extract of *Fucus vesiculosus* was the most harmful against this cell line. Regarding to RTG-2 cell line, the MNTC of the extracts ranged from 6250 μg $ml^{-1}$to 98 μg ml^{-1}. *Ulva rigida* M50:50 and *Chlorella* sp. M80:20 and E80:20 extracts were tolerated at higher concentrations, while *Fucus vesiculosus* E80:20 and M50:50 extracts were the most cytotoxic.

Regarding antiviral activity, *Fucus vesiculosus* E80:20 and M50:50 were the most efficient extracts against VHSV while *Gracilaria* sp. and *Nannochloropsis gaditana* were the most efficient extracts against IPNV. Although M50:50 *Fucus vesiculosus* extract was active at a seven-times lower concentration, it only weakly inhibited IPNV replication.

Discussion and conclusions

Results obtained from this study demonstrated that ethanolic and methanolic extracts of macro- and microalgae showed antibacterial activities against fish pathogenic microorganisms. In particular, algae extracted with M50:50 exhibited stronger antibacterial activity suggesting that the methanol as solvent is capable to extract more compounds with antibacterial action. In fact, organic solvents have been reported to provide a higher efficiency in extracting compounds with antibacterial activities compared to water-based methods (Biswas et al., 2013).

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Regarding antiviral activity, the totality of the extracts was active by pre-incubation with EPC or RTG-2 cells before infection, which suggests they may interact with or persist locally on the cell surfaces, preventing the virus replicative cycle. A similar profile of antiviral activity has been found with the rabbit antimicrobial peptide defensin 1 (NP-1), which protected cells *in vitro* from infection by herpes simplex virus type 2 (HSV-2) (Sinha et al., 2003).

This study has shown that macro- and microalgae have a great potential for further development as a promising natural source of bioactive compounds in aquaculture industry, since their potential viral and bacterial inhibitory effects against the main fish pathogens have been demonstrated

Acknowledgements

The authors would like to thank BUGGYPOWER for the kind supply of the *Nannochloropsis gaditana* and *Chlorella* sp. strains used in this study. M. Monteiro was supported by grant SFRH/BD/114995/2016, from FCT – Foundation for Science and Technology, under the POCI program. A. Couto, C.R. Serra and P. Enes are supported by national funds through FCT. This research was partially supported by the Strategic Funding to UID/Multi/04423/2019 (POCI-01-0145-FEDER-007621) through national funds provided by FCT under the project PTDC/CVT-WEL/5207/2014 (POCI-01-0145-FEDER-016797).

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AQUABIOTECH GROUP - ABT INNOVIA
EXCELLENCE THROUGH QUALITY AND INNOVATION

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Introduction

The expanding aquaculture industry is essential as a source of food for a growing population. Whilst the industry is growing at a fast pace, it continues to face numerous challenges which impact on production potential and overall sustainability. Such challenges include provision of sustainable ingredients for feed production, tackling of diseases and reduction of environmental impacts. Solutions to these challenges typically require the performance of scientifically designed experiments having specific objectives. It can take multiple trials, sometimes over periods of years, before a solution can be provided for a particular problem.

Providing facilities to industry

ABT Innovia (ABTI), the research division of AquaBioTech Group, has been carrying out contracted research for internationally established clients for over sixteen years. The scope of the trials carried out have been very diverse, from the use of live-feed enrichment products for marine larvae to vaccine development, safety and potency (GMP Certification) for major farmed species.

ABTI continues to carry out nutrition trials involving fish feed ingredients, micro-ingredients and supplements, health associated trials related to vaccine development as well as dietary functional ingredients, and ecotoxicology (GLP certification)

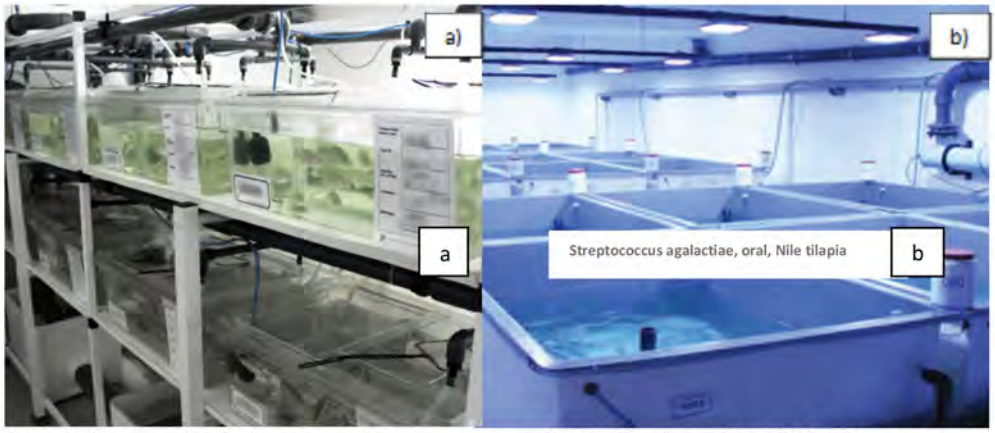

Plate 1: Two recirculating aquaculture systems at ABT Innovia. a) 16 x 40L tanks; b) 15 x 670L tanks.

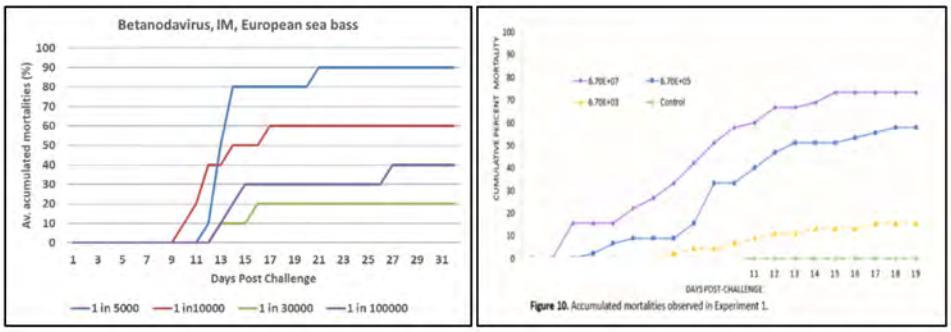

Fig. 1. Results of two challenge models established at ABT Innovia. a) VNN, European sea bass; b) *Streptococcus agalactiae,* Nile tilapia

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The majority of the trials carried out are based on recirculating aquaculture systems (RAS), with tanks having capacities ranging from 10L to 1500L (Plate 1). Over 40 individual, biosecure RAS units are available as well as a number of GLP and GMP certified labs used for microbiology ecolog , and ecotoxicology.

Both fresh water (e.g. rainbow trout, *Pangasius*, Nile tilapia, Salmon, barramundi) and sea water species (e.g. gilthead sea bream, European sea bass, Turbot) can be tested and the RAS can be cooled and heated as required for the species being tested. Environmental parameters in the tanks and systems are checked continuously by a monitoring system.

Standard operating procedures are applied during the handling of test organisms in the trials and for any samples taken for subsequent analysis. At the same time, ABTI continuously works to develop new procedures and models for use by companies (Figure 1), whatever the end application, be it research and development, GMP or GLP.

Providing solutions for industry

Through the use of ABT Innovia's facilities, many companies have been able to develop and commercialise solutions to specific problems in the industry. This has been the case for a number of diseases, feed formulation challenges and others.

ABT Innovia will continue to play an important role in industry research and development, more so as it expands further its tank and dry lab facilities and develops new procedures and models.

DEVELOPMENT OF FISH FEEDS AND FEEDING STRATEGIES FOR GENETICALLY SUPERIOR FISH FROM BREEDING PROGRAMMES

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Introduction

Selective breeding means that individuals that are genetically superior in respect to economic, ecological and ethical breeding objectives are used as parents for the next generation. Several traits that may change genetically during breeding, such as growth, body composition and feed efficienc , also influence nutritional requirements of the fish, which ultimately determine feed formulations. The objective of the present study was twofold: **1)** To provide a conceptual framework, and its practical implications, for developing novel feeds and feeding practices for genetically improved fish originating from breeding programmes, and **2)** to review the degree of evidence that fish traits related to nutrition are expected to change due to breeding programmes.

Selective breeding changes fish traits related to nutrition and feeding

A fish trait can change genetically either when it is directly selected, or if it is genetically correlated with the selected trait. In both cases, the trait needs to exhibit genetic variation. Table 1 illustrates, for groups of traits related to feed utilisation, their potential to be changed by selective breeding.

How this impacts formulation of feeds?

The evidence shows that due to breeding programmes, genetic changes occur and are expected to occur in the fish traits that influence the nutritional requirements and the way feeds are formulated to obtain better growth and healthy fish. This should lead to reciprocal changes in the feed formulations and feeding practices (Figure 1).

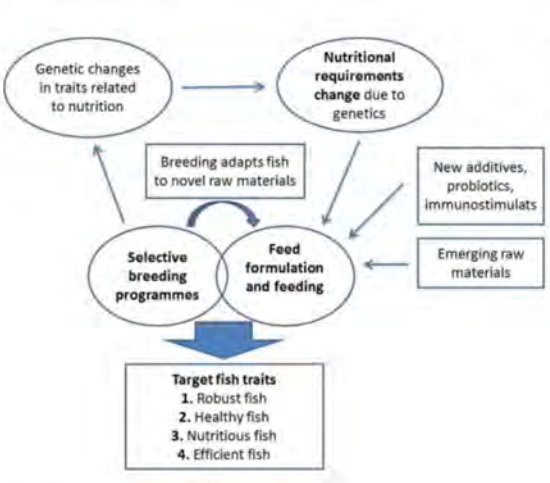

Fig. 1. The logic of how breeding programmes influence feed formulation.

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Four examples are highlighted here:
• When growth rate is genetically improved, fish can be reared for a shorter time period to obtain the same body weight. This requires a modified feed and/or feeding practices that account also for limiting factors at farms (e.g. oxygen concentration).
• Selection for growth, fillet yield, or lipid% itself changes lipid% of the fish body, and hence the amount of energy a fis needs for building tissues such as fillet is changed. Energy content of one g of lipid is 39.5 kJ/g, the cost of retention is 1.1-1.4 kJ / kJ, and the energy cost of depositing is 43.5-55.3 kJ/g lipid (Emmans 1994).
• The concentrations of fatty acids in fillets may reduce due to selection rapid growth, or increased lipid%, which may need to be counterbalanced by ingredients in feeds.
• Selection for better performance on plant-based diets permits formulation of more sustainable diets and more efficien utilization of novel dietary ingredients.

Implications for industry

Feed, feeding and genetics do not function independently but influence each other in a way that is important for aquaculture industry:
• Genetically improved fish may need tailo -made feeds that differ from the feeds for non-selected fish
• Feed development that utilizes modern fish material from breeding programmes ensure the synergetic effects of feeds and genetics for the benefit of farmers
• Advanced feeds do not provide the expected advantage for a farmer, if fed to inferior fish material not adapted to the feed.
• Selective breeding adapts the fish to the prevailing feeds and raw materials used. In the long-term, deficiencies in feed can be partly counterbalanced by selective breeding.

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EFFICACY OF THE ADDITION OF TWO ADJUVANTS TO AN INACTIVATED VACCINE AGAINST *Streptococcus agalactiae* AND THEIR EFFECT OVER HEMATOLOGICAL PARAMETERS IN NILE TILAPIA FINGERLINGS

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Introduction

Vaccination is a widely accepted and effective method to prevent *S. agalactiae* infection and mass mortalities in Nile tilapia, *Oreochromis niloticus* (Liu et al., 2016). Inactivated vaccines represent the majority of commercial vaccines currently used, with formaldehyde as the most common inactivation method (Munang'andu, 2014). Recently, the use of hydrogen peroxide as inactivating agent was tested in others species showing an improvement in the humoral and cellular response (Fan et al., 2019). In addition, adjuvants are often needed to increase the vaccine performance. Freund's incomplete adjuvant (FIA) and aluminum hydroxide (AH) are Signal 1 and Signal 2 type adjuvants respectively and are commonly used in fish vaccines (Tafalla et al., 2013). Thus, the objective of this study was to evaluate the effect of two adjuvants (FIA and AH) over the efficacy of an inactivated vaccine against *S. agalactiae* (inactivated with H_2O_2) and to determine their effects over hematological parameters in Nile tilapia fingerlings

Material and methods

Nile tilapia, *O. niloticus*, from the same spawn (20 g) were randomly distributed into six experimental groups (n=20), in triplicate. The experimental groups were designed as it follows: T1: vaccinated with inactivated *S. agalactiae*. T2: inactivated vaccine + AH. T3: inactivated vaccine + FIA. T4: AH. T5: FIA and T6: control group, none vaccinated (sham vaccinated with sterile PBS). Inactivation was performed with H_2O_2 following Fan et al., (2019) scheme and the adjuvant was added in 1:1 proportion. Tilapias were acclimatized for two months before the vaccination trial. On day zero, fish were vaccinated by intracoelomic injection (0.2 mL) with each vaccine type. Two and four weeks after vaccination (WAV), seven fish from one of the replicates were used to assess hematological parameters (red blood cells number, hematocrit, hemoglobin, and hematemetric values). Later, at 30 days post-vaccination, the remaining replicates (2) were challenged with *S. agalactiae* by intracoelomic injection of 0.2 mL from a bacterial solution containing 1.65×10^6 CFU ml^{-1}. Mortality after challenge was evaluated daily for 21 days, and relative percent survival (RPS) was calculated. Differences on hematological parameters between groups and sampling times were evaluated by one way ANOVA with Tukey's post test or Kruskall Wallis with Dunn's post test.

Results and discussion

Hematological parameters are shown in Table I. Significant differences between treatments were found for hemoglobin (T1-T2), and for MCHC (T1-T2, and T2-T5), in both cases at two WAV. Statistical differences between sampling times were found for hemoglobin (T2, T3 and T5), and MCHC (T2-T6). The RPS for the inactivated vaccine without adjuvant was 40.74%, while for the vaccine + AH was 59.26% and for the vaccine + FIA 77.78% (Table II). The results proved that the T3 vaccine was considered efficient (RPS>70%), while the other two were considered deficient (RPS<70%). The adjuvants alone conferred no protection; nevertheless higher protection was achieved when added to the inactivated antigens.

Discussion

Hematological alterations were mild and related to the vaccine + AH group, mild toxicity for AH has been related in some fish species and my explain these findings (He et al., 2014) as well as the lowest RPS obtained for the vaccine + AH when compared to vaccine + FIA.

Table I. Hematological parameters (mean ± standard deviation) of Nile tilapia two and four weeks after intracoelomic vaccination against *S. agalactiae*.

Group	T1	T2	T3	T4	T5	T6
Two weeks after vaccination						
Hemoglobin (g dL^{-1})	6.97±1.04A	5.57±0.60Bb	5.96±0.70ABb	6.46±0.48AB	6.34±0.63bAB	6.64±0.46AB
Hematocrit (%)	33.42±5.45	34.60±6.83	30.80±4.30	33.50±1.47	28.86±7.55	31.57±4.32
Erythrocyte (x10^6 uL^{-1})	1.52±0.51	1.61±0.39	2.25±0.84	1.91±0.18	1.67±0.93	2.11±0.35
MCV (fL)	254.87±154.38	216.44±21.15	147.83±42.17	175.87±10.23	228.83±120.42	159.93±32.93
MCH (pg)	40.61±10.81	35.31±4.20	28.46±7.21	34.06±4.53	39.88±16.07	33.46±3.31
MCHC (g dL^{-1})	21.73±5.73AB	16.37±1.87Bb	19.43±1.30AB	19.33±1.92AB	23.63±2.70A	19.60±1.09ABb
4 weeks after vaccination						
Hemoglobin (g dL^{-1})	7.97±2.05	6.85±0.84a	7.32±0.61a	7.25±0.71	7.43±0.46a	7.53±0.85
Hematocrit (%)	34.50±2.55	33.86±2.48	35.79±4.85	35.50±3.57	32.64±1.75	33.67±2.62
Erythrocyte (x10^6 uL^{-1})	1.87±0.43	2.22±0.54	1.89±0.51	2.01±0.46	2.04±0.40	1.97±0.32
MCV (fL)	191.23±38.38	158.94±34.08	202.16±59.83	178.57±40.31	165.98± 35.77	173.41± 21.12
MCH (pg)	43.79±11.23	31.92±6.39	41.37±12.01	36.91±9.23	37.57±7.21	38.71±4.92
MCHC (g dL^{-1})	23.13±5.98	20.18±1.32a	20.77±3.44	20.53±0.94	22.77±1.33	22.35±1.51a

Different capital letters indicate significant differences between treatments and different small letters indicate differences between times (P<0.05).

Table II. Experimental design, mortality rates, percent survival and RPS in Nile tilapia fingerlings vaccinated with *S. agalactiae* inactivated with hydrogen peroxide, two different adjuvants and their combinations.

Treatment group	Challenge dose (CFU mL^{-1})	n	Fish mortality	Percent survival (%)	RPS (%)
T1	1,65x10^6	40	16	60.0	40.74
T2	1,65x10^6	40	11	72.5	59.26
T3	1,65x10^6	40	06	85.0	77.78
T4	1,65x10^6	40	29	27.5	-
T5	1,65x10^6	40	31	22.5	-
T6	1,65x10^6	40	27	32.5	-

A high RPS was achieved for the hydrogen peroxide inactivated vaccine + FIA (77.78%) similar to a *S. agalactiae* subunit + FIA injection vaccine (Zhang et al., 2017), but still below a recombinant vaccine + FIA (90%) (He at al., 2014). The combination vaccine + FIA proved to induce higher protection than vaccine + AH in agreement with previous results for *S. agalactiae* recombinant injection vaccines (He at al., 2014). Also, our results did not reach the efficacy found for a formaldehyde inactivated vaccine + oil adjuvant (Wang et al., 2018). Hydrogen peroxide inactivated vaccine + FIA induce high protection against *in vivo* challenge with *S. agalactiae*, though protection levels can still be improved. Hematological alterations induced by vaccination at early stages were not clearly evidenced, yet more research is needed to clarify the vaccine effects over other physiological parameters.

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TASTE SENSING AND GUT FEELING: POSSIBLE INVOLVEMENT OF TASTE RECEPTOR TYPE 1 FAMILY IN SEABREAM *Sparus aurata*

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Introduction

Taste perception of sweet and umami stimuli in vertebrates is largely controlled by a family of G-protein-coupled receptors, the taste receptor type 1 (T1R) family, with three members that combine in heterodimers to form the taste receptors. The mammalian T1R1/T1R3 receptor responds to umami compounds such as amino acids, whereas T1R2/T1R3 is activated by sweet substances. In mammals, it has been well established that taste sensing in the oral cavity (taste buds) not only guides the consumption of foods, but the presence of taste receptors in specialized cells of the gastrointestinal tract (enteroendocrine cells) can also regulate digestive, absorptive and metabolic functions through gut sensing mechanisms (Alpers, 2010; Depoortere, 2014). In fish, however, very little information is available, particularly in species of aquaculture interest. We have previously cloned the full-length cDNA of five T1R genes in *Sparus aurata* (*sa*), including the specific heterodimer subunit of the umami taste (*sa*T1R1), three novel sweet taste duplicate genes (*sa*T1R2x, *sa*T1R2y, *sa*T1R2z) and the *sa*T1R3 gene common to both umami and sweet taste heterodimers. The objective of this study was to further characterize their functional properties and patterns of expression in both larval and adult seabream tissues.

Materials and methods

In order to functionally characterize this family of receptors we developed a stable Ca2+/reporter gene activity cell line assay (NFAT-Luc-HEK-293) to specifically examine the responses of the multiple *sa*T1R(s) heterodimers to amino acid ligands. On the other hand, gene expression of four T1R genes (*sa*T1R2x is not expressed, being likely a pseudogene) was examined through qPCR on different adult tissues to assess possible differences in the profile of tissue distribution. A qPCR analysis was also performed in whole larval tissues from 1 until 12 days post-hatching (dph), spanning life-stage transition from yolk-sac sustenance to exogenous feeding (initiated at 9 dph). To establish the tissue profile of gene expression in larvae, whole-mount *in situ* hybridization (WISH) was also performed to localize the expression of the four genes in pre- and post-exogenous feeding larvae (5 and 12 dph, respectively).

Results and discussion

Cotransfections of the reporter cell line with *sa*T1R1/R3, *sa*T1R2z/R3 and *sa*T1R2y/R3 heterodimers showed a dose-response activation of the putative (mammalian-homologous) umami and the two sweet receptors by several amino acid stimuli suggesting that, unlike mammals but similarly to what was previously reported in zebrafish and medaka (Oike et al., 2007), the three taste subunits may equally serve to transduce umami/amino acid taste sensations. Furthermore, the *in vitro* assays evidenced a fairly promiscuous activation profile of the three heterodimers, although with some differences in the sensitivity to some amino acids (not shown).

Gene expression in adult tissues (figure 1) showed a predominant expression in oral tissues including lips (L), tongue (T) and oral cavity (OC) for all genes, and in particular for *sa*T1R3, in agreement with its common involvement in the three receptor types. However, all the subunits were also found in gills (G), stomach (ST), foregut (FG), midgut (MG), hindgut (HG), forebrain (FB), midbrain (MB) and hindbrain (HB) tissues, even if with different profiles that might indicate a functional specialization.

Quantitative *sa*T1R(s) mRNA expression in whole seabream larvae (figure 2) showed that the four transcripts are stably expressed from 1 dph onwards and greatly increase shortly after exogenous feeding (initiated at 9 dph). WISH (figure 3) reveals the lack of expression of *sa*T1R(s) in oral tissues of both pre- (5 dph) and post- (12 dph) exogenous feeding stages, with the genes being expressed exclusively in the gastrointestinal tract.

These findings indicate an adaptive evolution of *sa*T1R gene repertoire towards amino acid perception, and provide a molecular/cell basis for both oral and gut chemoreception in seabream. Surprisingly, however, results in young larvae suggest only gut sensing capabilities. Studies are underway to determine the ontogenetic onset of *sa*T1R expression in oral taste sensing tissues, by examining later larval stages.

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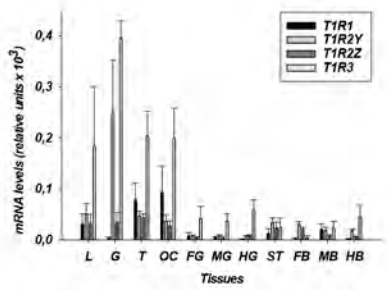

Fig. 1. Tissue distribution of the four functional *sa*T1R transcripts, assessed by qPCR.

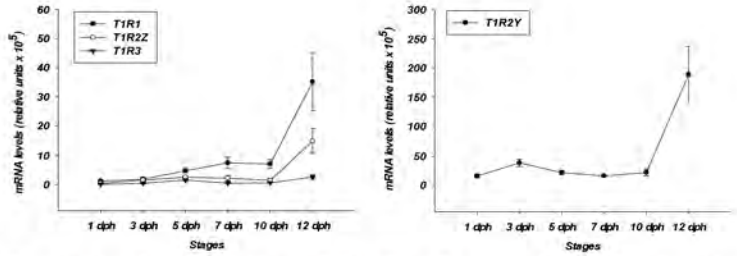

Fig. 2. Changes in the expression of the four functional *sa*T1R transcripts in whole larvae tissues during early ontogeny, assessed by qPCR.

Fig. 3. WISH expression patterns of *sa*T1R(s) at 5 dph (images at 12 dph showed the same pattern). Stomach (ST); Foregut (FG); Midgut (MG); Hindgut (HG).

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EVALUATING FEED DISCRIMINATION IN SEABREAM *Sparus aurata* USING A DUAL-CHOICE SELF-FEEDING SYSTEM

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Introduction

Feed intake is a critical variable in aquaculture that limits growth and survival of reared animals. The inclusion of new raw materials in fish diets to meet cost-efficient production and sustainability goals may compromise the organoleptic quality of diets and, by extension, fish growth (Yaghoubi et al., 2016). Therefore, it seems reasonable to evaluate the fish s discriminatory capacity towards feed in order to discern the organoleptic preferences of aquacultured species. The goal of this study was to develop an experimental model to test feed discrimination in the gilthead seabream (*Sparus aurata*), an important species for Mediterranean seawater aquaculture.

Materials and methods

We set up a dual-choice feeding system using self-feeders activated by a string sensor placed 3cm below the water surface. Feeders were connected to a computer system that recorded the date, time and tank from which each feed demand originated (Leal et al., 2009). The feed reward per sensor activation was set at approximately 1g/demand. Initially, 500 juvenile gilthead sea bream of approximately 10g were maintained in two 2500 l tanks provided with self-feeding systems during eight months for accommodation and learning. Subsequently, animals (body weight around 100g) were transferred to eleven 500 l experimental tanks (n=10/tank) provided with a dual choice feeding system consisting of two string sensors, each activating a different self-feeder. During 28 days, all feeders were provided with a control diet (44% CP and 18% CF, containing 12.5% fishmeal) for accommodation to the experimental tanks, but especially to the dual choice feeding system (phase I). Subsequently (phase II), in four tanks, one feeder was filled up with control diet supplemented with quinine (1.5%, negative diet) whereas the second feeder contained the control diet. In other three tanks, the tester-feeder distributed a positive diet (isoproteic and isolipidic but containing 46% fishmeal, 6% squid meal and 6% krill meal) and the second feeder delivered control diet. Finally, both positive and negative diets were confronted in the remaining four tanks. After eight consecutive days, the position of feeders was switched in the tanks and animals were allowed to feed for further 21 days (phase III). At the end of the experiment, the total amount of feed distributed was calculated by weighing the feed remaining in the feed hoppers. This quantity was used to calculate the delivery rate for each electronic feeder. The amount of feed delivered daily was calculated using the feeder delivery rate and number of daily demands. The experimental tanks were inspected daily to ensure the absence of feed on the bottom. Consecutively, and utilizing the same animals from the previous experiment, an additional trial was set up to corroborate the feeding deterrent effect of quinine using a single feeder per tank for 14 days. Three tanks were fed with quinine-supplemented diet and four tanks with control diet. Feed intake levels were calculated as before.

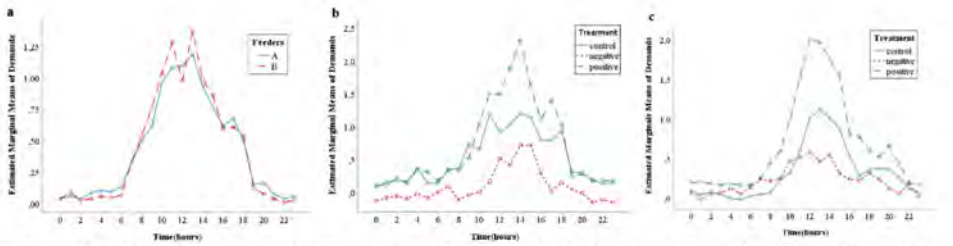

Fig. 1. Feeding demand profiles estimated during 24 h for a) phase I, b) phase II, and c) phase III (estimated marginal means are forecasted means in function of the independent variable).

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Results and discussion

During phase I of the dual-choice experiment, using a univariate general linear model that analyzes all sensors jointly, independently of the tank, no significant differences in feeder activation were found, thus demonstrating that fish demanded feed in a similar way independently of feeder. Sensor activation differed according to time (hour of the day), day and tank. It was demonstrated that fish exhibited a similar daily pattern of demands (figure 1a) in all sensors/feeders. Number of demands was different between tanks but also between the different experimental days, thus indicating the instability of feed intake (data not shown). During phase II, when experimental diets were introduced, we found significant differences in sensor activation, with the lowest values being measured in feeders provided with quinine-supplemented diet and highest demand levels being found in those containing positive diet (figure 1b). This demonstrated that fish exhibited preferences towards sensors coupled to feeders delivering positive diets but avoided feeders supplying a deterrent feed (provided by quinine inclusion), when compared to the control diet. Similarly to the accommodation phase (I), feed intake levels differed according to day and tank, but this time we also recorded significant differences in the 24h-feeding pattern. In this respect, the activation period of sensors delivering quinine-rich diet was narrower (figure 1b). Finally, in phase III when the position of feeders was inverted, fish continued to demand less from feeders delivering the quinine diet, and had higher demands from feeders supplying the positive diet. Results suggest that fish are able to discriminate the position of the feeder according to the type of diet delivered or, what is the same, they can discriminate the diet independently of the feeder position. Similar to phase II, feeder sensor activation differed according to day, tank and time (figure 1c).

Finally, using a different experimental set up in which animals were fed exclusively with one type of diet, either control or quinine-supplemented, we confirmed that, when presented with a diet containing quinine, gilthead seabream reduce their voluntary feed intake (data not shown), thus corroborating that the lower preference towards this feed, when an alternative feed is presented, is associated with a feeding deterrent effect.

Conclusions and future directions

Seabream exhibited variable feed intake levels along different days and fish groups/tanks but were able to discriminate the diet's organoleptic properties using a dual-choice self-feeding system. This opens the possibility to use the system to evaluate new raw materials in terms of feed acceptance/preference, as well as the potential of flavorings to overcome the negative effects of antinutritional compounds or medication on feed intake.

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IDENTIFICATION OF FUNCTIONAL GENES RELATED TO HOST RESISTANCE TO *Piscirickettsia salmonis* IN ATLANTIC SALMON (*Salmo salar*) USING RNA-SEQ

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Introduction

Salmon Rickettsial Syndrome (SRS), caused by the gram-negative bacterium *Piscirickettsia salmonis*, is one of the most serious infectious diseases affecting Atlantic salmon production, causing significant losses due to its great associated mortalities (30-90% per outbreak) (Almendras et al., 2003). These outbreaks occur in the seawater stage of production where economic losses in relation to biomass constitute a greater impact. In addition to direct losses through mortality, there are indirect losses through reduced growth rates and anticipated harvests (Rozas & Enriquez, 2014).

One potential avenue to tackle SRS is improvement of host resistance using selective breeding, and potentially genome editing in the future. To achieve this, knowledge about the genetic basis of host response and identification of specific genes and pathways involved in that response is valuable. Therefore, the purpose of this research is to discover functional genes impacting on host resistance to SRS using RNA-Sequencing of head kidney and liver samples from a large-scale SRS challenge. Following on from this, CRISPR-Cas9 genome editing of high priority candidate genes will be assessed in cell culture using an in vitro disease challenge with comparison to unedited control lines.

Materials and methods

2,377 Atlantic salmon (*Salmo salar*) smolts from 104 families were challenged against SRS infection by intraperitoneal injection with mortalities recorded for 47 days post infection (dpi). Mortality, days to death, weight and length data was collected daily and fin-clips samples were obtained for genotyping. Head kidney and liver samples for RNA-seq were obtained from a subset of individuals at 3 and 9 dpi. RNA-seq libraries were obtained using the Illumina Truseq stranded mRNA kit. Differential expression (DE) analyses were performed in R v.3.3.1 using the Bioconductor package DESeq2 v.3.4 (Love et al. 2014).

Results

RNA sequencing revealed a clear response to the pathogen in both head kidney and liver (Figure 1), with control, 3 dpi and 9 dpi samples clearly clustered separately (especially in liver) by the first principal component, which explains a large percentage of the variation in gene expression. Differential expression analyses revealed extensive regulation in head kidney in response to SRS, with 4,835 and 4,562 differentially expressed genes at 3 and 9 dpi respectively, while the values observed on the liver transcriptome were lower, with 1,192 and 2,431 differentially expressed genes at 3 and 9 dpi respectively. KEGG enrichment analyses revealed up-regulation of several immune pathway in both tissues, such as "TNF signalling pathway", "Toll-like receptor signalling pathway" or "Complement and coagulation cascades". Particular pathways identified as crucial for the host immune response against SRS were further evaluated, and key genes were selected for further functional studies to analyse their potential use for genome editing (Table 1).

Discussion and Conclusions

Current results have allowed the identification of genes and pathways that are important in host response to *Piscirickettsia salmonis*. A list of candidate genes putatively related to disease resistance was obtained by the analysis of pathways of differentially up-regulated genes during infection. Literature review of the biological function of these candidate genes highlighted the most suitable candidates for functional studies.

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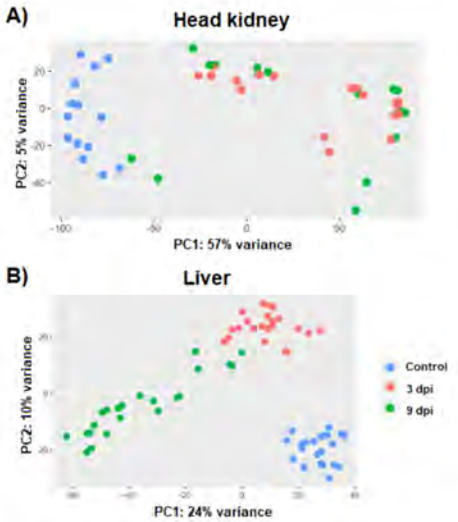

Figure 1: Principal component analyses of A) head kidney and B) liver RNA-seq samples from unchallenged controls, and infected fish 3 days and 9 days post infection.

Head kidney			
3dpi		9dpi	
Pathway	Candidate genes	Pathway	Candidate genes
Cytokine-cytokine receptor interaction	CCL19, CCL4, CXCR2, TNFRSF14	Phagosome	CORO1A, TFRC, TUBA, TUBB, FCGR1A, ITGAM
Salmonella infection	IPAF, ASC, CASP1	Cytokine-cytokine receptor interaction	IL1β, CCR4, CSF2RB, TNFRSF9
Legionellosis	NLRC3, NLRP3, IPAF, ASC, CASP1	TNF signaling pathway	TNF, TRAF2, NFKBIA, RIPK3, PIK3CA_B_D, P38

Liver			
3dpi		9dpi	
Pathway	Candidate genes	Pathway	Candidate genes
Complement and coagulation cascades	A2M, C3, C5, CFH, HF1	Apoptosis	EIF2S1, MAP3K5, CAPN1
Staphylococcus aureus infection	MBL, CFB, C2, C4	RIG-I-like receptor signaling pathway	CYLD, TBK1, IFIH1
Phagosome	TUBA, CALR, C3	TNF signaling pathway	RIPK3, ITCH, PIK3CA_B_D, P38

Table 1: List of differentially expressed pathways and candidate genes up-regulated during SRS infection at 3 and 9 dpi in head kidney and liver samples.

NEW HEX-X LINE DRUMFILTERS FOR WARM SALTWATER APPLICATIONS AND END OF PIPE SOLUTIONS

MSc. Biology, Henrik Mortensen

CMAQUA Technologies

Marine aquaculture is expanding as new and valuable species now can be domesticated. Farming of marine species are not easy and it is also a challenge to build RAS with equipment which can last under this very corrosive environment.

HEX filters are build with a view of high corrosion resistance, using high density PE, PVC and POM as main components. The new platform for construction of drumfilters is based on the **HEX-X** versions, where even large drums are constructed of a durable polymer formed as an **X** giving strength to the drum, both for the dynamic and static loading.

Resulting sludge from the backwash process from **HEX-X filters,** in various applications, needs to be concentrated to ease handling and the use of concentrated sludge. Sludge from saltwater installations are more difficult to treat than corresponding freshwater farms, a separation beltfilter system with a three chamber system is a possibility to treat the waste in an effective and safe process.

REPLACING FISH MEAL WITH DEFATTED INSECT MEAL (YELLOW MEALWORM *Tenebrio molitor*) IMRPOVES THE GROWTH AND IMMUNITY OF EUROPEAN SEABASS (*Dicentrarchus labrax, L.)*

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Introduction

This study was undertaken on behalf of Ÿnsect (France) by the Hellenic Centre of Marine Research (HCMR, Greece). The aim of this study was to assess the effect of 2 doses of dietary insect meal on the immune system of sea bass, *Dicentrarchus labrax, L.* This was achieved by feeding the fish for 2 months with a diet close to commercial diets and based on fishmeal and 2 diets where 30 or 100% of the fishmeal (TM30 & TM100 respectively) was replaced by a defatted meal of *Tenebrio molitor* larvae produced by Ÿnsect. The growth was assessed after 2 months. The digestibility of the 3 diets was assessed by collecting fish faeces for 2 weeks at the end of the growth period. The immune system was assessed using the fresh blood and serum collected after 2 months feeding. Also, fish resistance to disease was compared between fish fed the different diets.

Material and methods

European sea bass were obtained from Selonda (Psachna, Evia, Greece) and were kept in cylindrical 250 l-tanks for 1 month and fed 4 times daily with a commercial diet. Then, groups of 110 fish of initial weight 3.98 ± 0.04 g were anaesthetized using clove oil (diluted 1:10 in ethanol), weighed and randomly distributed in 9 cylindrical 250 l-tanks in an open-circulation sea water system following a photoperiod of 12L:12N (Fish density at 1,8 kg/m3). After 20 days, fish were weighed and 40 fish were removed from each tank to remain with 70 fish/tank (fish density at 2.1 kg/m3 at 21 days and up to 9.4 kg/m3 at the end of the experiment, after 65 days). Water temperature ranged from 25-27ºC.

After 9 weeks of feeding, 8 fish from each tank were anaesthetised and blood samples were collected by a caudal puncture. A histopathology procedure was carried out to assess gut health. Sixty fish per group were kept for an additional challenge test with an important pathogen in marine fish: *Vibrio harveyi.* The cumulative mortality and syndrome score were measured over 10 days.

Figure 1 Feed Conversion Ratio and the Weight Gain after 9 weeks of feeding trial (n=70)

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Results

Both the TM30 and TM100 diets improved feed efficiency and weight gain when compared to the control group. The best results were obtained at TM30, with a weight gain of +13.7% higher than the control (29.2g vs 25.7g) and the Feed Conversion Rate (FCR) was significantly lower (-7%). After 9 weeks, the lowest FCR was 0.77 for TM100 and 0.87 for CRTL, a reduction of 11.5%. The Total feed intake was similar between all the treatment groups.

TM30 and TM100 reduced the appearance of abnormal intestinal villi in the gut from 5 in the control group to 2 (n=15). Gut health was improved. During the challenge test, TM30 showed a reduction of cumulative mortality by 45% (TM30: 18.3% vs 33.3% in the control). The symptom scores also showed a similar pattern.

Conclusion and discussion

The dietary meal obtained from *Tenebrio molitor* used to replace partially or totally the fishmeal (69% crude protein content) in diets for European sea bass resembling the commercial type feed with 30% FM and plant proteins with supplementation of some amino acids was very promising. Fish feeding on diets where FM was totally replaced by the insect meal (TM100) showed higher respiratory burst activity and lower trypsin inhibition, while their resistance to *Vibrio harveyi* infection was not affected. Fish fed on diets where FM was partially replaced by insect meal (TM30) tended to resist better to *Vibrio harveyi*. Similar results have been assessed on red seabream (*Pargus major*) when high proportions of yellow mealworm defatted meal were included (Idol et al. 2019).

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MICROSATELLITE ANALYSIS OF *Dicentrarchus labrax* AND *Sparus aurata* POPULATIONS OF MEDITERRANEAN FISH FARMS

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Introduction

Genetic technologies play a crucial role in modern aquaculture. Genetics can determine a range of production characteristics linked to product marketability (desired color, shape, etc.), cultivability, disease resistance to monitoring and preserving the wild populations. In genetic analysis, it is important to distinguish if genetic differentiation of a given trait of interest for production is a consequence of individual or populational genetic variation. In this study we investigate the genetic structure of different population of *Sparus aurata* and *Dicentrarchus labrax* reared in Mediterranean hatcheries.

Materials and methods

97 samples of *D. labrax* and 218 samples of *S. aurata* were collected during 2018 from Mediterranean hatcheries. Specificall , *D. labrax* samples were collected from 10 Greek and 3 Italian hatcheries and *S. aurata samples* were collected from 8 Greek, 6 French and 8 Italian hatcheries. DNA extraction was carried out based on Aljanabi's protocol (Aljanabi and Martinez, 1997). For the microsatellite analysis, we used 4 validated primer sets to amplify 4 microsatellites for each of the species. Sequencing reads were performed in an ABI 3730XL and the output collected for genotype using GeneScan. The Structure program was used to infer the number of sea bream and sea bass populations (K) in the collected samples by plotting the second order of change of L(K) (Δ(K)) as described by Evanno and colleagues (2005). Phylogenetic analysis was carried out using the R phylogenetic package APE, and was based on the pairwise Fst values for every locus, which was extracted using the MSA 4.05 program.

Results

No clustering according to geographic location was observed in the phylogenetic tree of the *D. labrax* samples (Figure 1). The samples were grouped into two clades, which contained a mix of sea bass samples from Greek and Italian hatcheries. In contrast, two main groups were identified in the phylogenetic tree for the *S. aurata* samples (Figure 2). Group 1 consists exclusively of *S. aurata* from Greek hatcheries, while in group 2 a mix of *S. aurata* from both French and Italian hatcheries were identified. Analysis of the structure of group 2 revealed that the *S. aurata* from French hatcheries clustered in one clade, while *S. aurata* from Italian hatcheries clustered in a second independent clade. Finally, samples from one Greek hatcheries (GR3) was highly divergent from the other clades.

Best K values, calculated by plotting the second order of change of L(K) (Δ(K)), indicated K=2 for *D. labrax* and K=5 for *S. aurata*. Plotting the q-values for each of the individual *D. labrax* samples analysed indicated that the fish are most likely descended from two populations and in most cases reflect the genetic structure of that population (Figure1). In contrast, the *S. aurata* q-value plots were characterized by an admixture patterns. However, the q-value pattern differed between the different groups identified by the phylogenetic tree (geographical) populations (Figure 2)

Discussion

The diversity of fish farms and production tactics means that knowing the population structure is a difficult task, since frequently in fish farms the broodstock may be of unknown origin, there is frequently exchange of eggs and juveniles across the Mediterranean, crossbreeding with wild individuals occurs and breeding programs may be company specific. The results of the present study revealed that two main groups of *D. labrax* were under production in the hatcheries that participated in the study and these results probably indicated that two distinct populations of different origin are used. Moreover, the population structure analysis suggests that there is minimum genetic flow between these two groups. The case of *S. aurata* is more complex and the phylogenetic analysis revealed two main groups (one with the Greek samples and one with French and Italian). The population structure of *S. aurata* in both groups was characterized using the q-value to have an admixture pattern similar to what is observed in hybridization zone patterns. The population pattern of the *S. aurata* could be the result of a combination of an extensive exchange between breeders and selective breeding programs.

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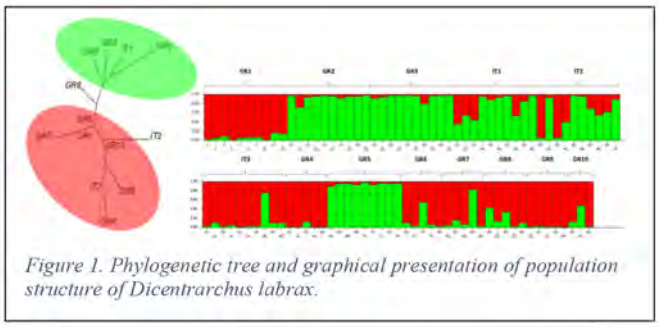

Figure 1. Phylogenetic tree and graphical presentation of population structure of Dicentrarchus labrax.

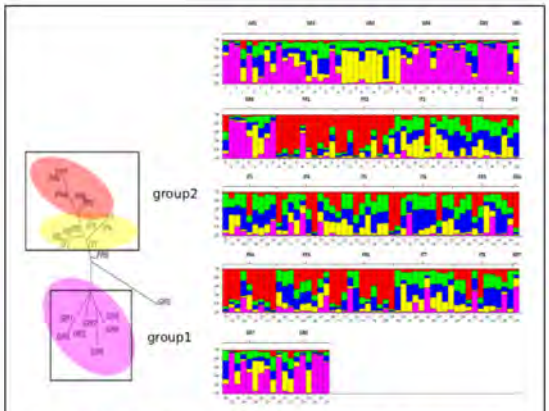

Figure 2. Phylogenetic tree and graphical presentation of population of Sparus aurata.

Acknowledgements

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APPLICATION OF PROBIOTIC AND PAPAIN ENZYME IN THE DIET OF TROPICAL SHORTFIN EEL *Anguilla bicolor*

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Introduction

Eels are the commercial fish in Indonesia, and at least seven species of eels have been recorded in Indonesian waters where three species of these were found in Aceh Province waters (Sugeha et al. 2006; Muchlisin et al. 2017). The tropical shortfin eel *Anguilla bicolor* is one of the promising species to be cultured intensively Muchlisin, 2013), and the local farmers have been initially cultured this species. But the local farmer claims that in the aquaculture system the growth performance and feed utilization were higher and therefore the profit margin was low. Probably, this is caused by the feed quality of the fish does not meet the needs of the fish. The probiotic and papain enzyme are the promising materials required to enhance digestibility of the feed and trigger the growth rate of the fish Therefore, the objective of the study was to evaluate the application of the probiotic and enzyme in the diet for eel fingerling.

Materials and methods

A completely randomized design with three levels of treatments were used in this study. The tested treatment is the application of probiotic and papain enzyme and its combinations with three replications as follows: (A)= Commercial feed without probiotic and papain enzyme, (B) = Experimental diet without probiotic and papain enzyme, (C)= Experimental diet with probiotic 10 ml kg^{-1} of feed, (D)= Experimental diet with papain enzyme 10 g kg^{-1} of feed, (E)= Experimental diet with probiotic 10 ml kg^{-1} of feed + papain enzyme 10 g kg^{-1} of feed, (F)= Experimental diet with probiotic 5 ml kg^{-1} of feed + papain enzyme 5 g kg^{-1} of feed.

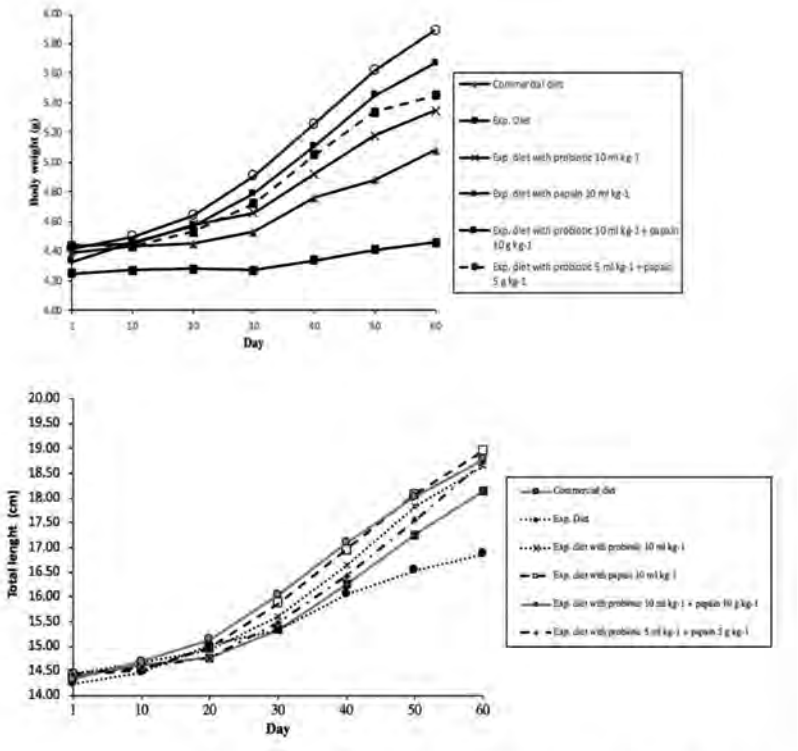

Figure 1. The growth trend of the eel during experiment (a) body weight growth, (b) total length growth

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The experimental diet was formulated from raw materials purchased from the local market in Banda Aceh City, Indonesia. It had 30% crude protein and 15% lipids. The raw materials are both animal and plant-based protein source, i.e. fishmeal, ebi shrimp meal, golden snail meal, blood meal, soybean meal, cornmeal, tapioca flou , and fine bran. The commercial probiotic of Raja Lele® containing Lactobacillus bacteria and papain enzyme flour was used. These materials were purchased from the local market in Banda Aceh City. The probiotic or papain enzyme was mixed with 5% egg yolk in the 100 ml distilled water, then sprayed onto experimental diet homogenously, then left for 30 min at room temperature before used for feeding. The fish were fed on a commercial diet two times a day at 08.00 AM and 06.00 PM for satiation during acclimatization process. It was distributed randomly into 18 plastic containers (vol. 25 L) which have been equipped with aeration at the stocking density of 15 fish per container. Individually, The fish initial body weight and total length were measured before been fed on an experimental diet of 10% of their body weight two times a day at 08.00 AM and 06.00 PM for 60 days. The data of every replication was calculated for average value then subjected to one-way Analysis of Variant (ANOVA) then followed by Duncans multiple range test. The percentage of data are arcsin transformation to normalize the data prior to analysis. The analysis was performed using the SPSS software ver. 22.0

Results

The results showed that the weight gain ranges between 4.6 g to 5.9 g with a daily growth rate of 0.06 g day^{-1} to 0.09 g day^{-1}, specific growth rate ranges between 0.72 % day^{-1} to 1.35 % day^{-1}, survival rate ranges between 50% to 73.3%, feed conversion ratio was 4.17 - 2.35, and its efficiency was 41.82 - 58.98%. The ANOVA test revealed that application of probiotic and papain enzyme gave a significant effect on the growth performance, survival rate and feed utilization of the tropical shortfin eel *A. bicolor* ($P<0.05$).

The Duncan multiple range tests showed higher weight gain, daily growth rate, and specific growth rate were found at fish fed on an experimental diet with 10 g kg^{-1}papain enzymes, these values are significantly different with other treatments. In addition, the higher feed conversion ratio and efficiency were also recorded in fish feed on the experimental diet with 10 g kg^{-1} papain enzyme. Moreover, the higher survival rate was also found at 10 gkg^{-1}papain enzyme, but this value was not significantly different from other treatment except to control B (experimental diet with probiotic or papain enzyme). Based on the body weight by 10 days interval, regular monitoring showed that the experimental fish are slow growing during the first 10 days with the body weight of the fish decreased and it consumed less of the experimental diet without probiotic and papain enzyme. However, the fish starts to grow after 10 days of the experiment. The fish that fed on an experimental diet with papain enzyme 10 g kg^{-1} feed was growing faster than other treatment (Figure 1).

Discussion and conclusion

The study showed that application of probiotic and papain enzyme results in the positive effect on the growth performance, survival and feed utilization of the eel *A. bicolor*, this is indicated by the higher value of these parameters compared to control treatments (without probiotic or papain enzyme). In comparison between probiotic and papain enzyme, the study revealed that papain enzyme produced better values of all measured parameters. However, when combined with probiotic at two different dosages, the results were decreased gradually. Therefore, this study shows that the application of the papain enzyme singly produces better results in eel *A. bicolor*. Papain is a proteolytic enzyme that functions to catalyze or breaks the protein into smaller fragments such as peptides and amino acids that can be absorbed by the intestine (Amri and Mamboya, 2012), while Lactobacillus is one of the probiotic bacteria that increase the digestibility rate and immune system of the fish (Feliatra et al., 2018). Lactobacillus hydrolyze the carbohydrates to lactic acid; then lactic acid will create an acidic condition in the digested tracts. In acidic conditions, Lactobacillus has the ability to inhibit pathogenic bacteria (Delgado et al. 2001). As already described previously that probiotic and papain enzyme produces a positive effect on the fish health and growth. However, the performance of probiotics is lower than papain in eel fingerling as reported in this research data. This is probably due to the fact that probiotic bacteria is a living organism that requires the energy sources for growing and produces byproduct materials such as carbon dioxide that is toxic for the cells [24], and in higher density (overgrow) results in the negative effect on the host (Dukowicz et al. 2007). Enzyme is a biological molecule (protein) that functions as catalysator and helps a complex reaction in the body (Cooper et al. 2000), therefore there is no side effect on living organism. In conclusion that probiotic and papain enzyme gave a significant effect on the growth performance, survival rate and feed utilization of the tropical shortfin eel *Anguilla bicolor*. The best results were discovered in fish fed on an experimental diet with papain enzyme 10 g kg^{-1} of feed.

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Blooms2Feeds^{+2}: PROCESSING SEAWEED TO CREATE FUNCTIONAL AQUAFEED INGREDIENTS

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The future of sustainability relies heavily on effective resource management. This includes utilising biomass in means and ways that have not been conceived of previously. Every year, green seaweed (*Ulva* spp.) blooms around our coasts, creating biomass that could be used as a supplement in fish feeds, potentially promoting gut health and integrity, stimulating appetite and feed utilisation, or illicit an immune response. The extraction of this biomass reduces the environmental burden on our coasts and reduces the carbon footprint associated with intensive fish farming. However, this raw product requires processing to unlock oligosaccharides bound in large complex carbohydrate units. These fractions can be used as a bioactive instrument to enact changes in the fish s digestive system, where some fractions provide nutrients and others challenge adaptive responses. The presence of beneficial bacteria within feed can also alter the gut microbiome and aid digestion. This study consolidates recent advancements in saccharification and fermentation while developing the most prudent processing measures that can efficiently scale. Key innovations, which include; novel bacterial lyase to break down structural polysaccharides that restrict access for further degradation; thermal treatment to both extract structural polysaccharides and kill naturally occurring decomposing agents; enzymatic hydrolysis to release oligosaccharides from large carbohydrates; and lactic acid fermentation to fully utilise the carbohydrates of the biomass thereby increasing the bioavailability of nutrients and provide a probiotic culture. These preliminary processes are intended to stabilise and confer enhanced seaweed properties in fish feeds.

THE REASONS BEHIND THE ESCAPES OF *Sparus aurata* FROM MADEIRA ISLAND (PORTUGAL) FISH FARMS - RISK ASSESSMENT THROUGH THE ANALYSIS OF ARCHIVE DATA

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Introduction
Offshore fish farming is a well-established industry in southern Europe and the gilthead seabream *Sparus aurata* the most commonly farmed fish species. Around Madeira, an archipelago situated in the North Eastern Atlantic, the species was recorded in the wild shortly after offshore farming started in 1997. Escape-events from fish farms are most likely the reason for the introduction of *S. aurata* to the waters of Madeira. Many factors are involved in escape events. Large scale escape incidents are related to the formation of holes in the net cage, which can be caused by rough sea conditions (e.g. storms), but also through predator attack and the net-biting activity of the farmed fish. Fish farms monitor regularly the condition of the farming facilities, particularly the nets and observed damages are recorded in archive files

Materials and Methods
Historical archive data from 2018 and 2019 collected from fish farms on Madeira Island in Calheta, Caniçal and Ribeira Brava. The data was obtained from regular inspection dives around farming facilities and related with associated operational data (age of nets and time in water, transfers of fish, information on stocks, other), the weather and sea conditions recorded at the time.

Results
Preliminary analysis of data revealed trends of high number of net holes associated with rough sea conditions. Detailed data analysis of present material has yet to be performed and results to be presented at conference may provide information on the most relevant causes of escape events from Madeiran fish farms.

Acknowledgements
This study was performed with the help of farm managers Pedro Diniz, Marismar Lda. and Élvio Pontes, from Ilhapeixe Lda.

NEW DATA FOR ARTIFICIAL PROPAGATION OF FISH BY USING OVARIAN LAVAGE WITH SPERM

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Introduction

Artificial spawning (AS) (or tank spawning) is a simple way of fish propagation. It can be induced by hormonal administration or the manipulation of the ambient water parameters (i.e. temperature, light regime, water conductivity). Many economical important fish species are produced by AS due to several reasons. First of all in case of the hard predictability of the ovulation time (european eel, pikeperch) the small size or sensitiveness (zebrafish), or its simplicity (spawning in pond or cage). In contrast *in vitro* fertilization (IVF) is a better tool for breeding programs, egg and sperm can be manipulated (cryopreservation, producing triploid fish) cross breeding of different lines and species (intra and inter specific hybrids) can be made in several combination. In our former studies (Müller et al., 2018a,b, 2019), we proved that sperm injected into the ovary by catheter through the oviduct 10-12 hours before hormonally induced ovulation, retains its fertilizing capacity for several hours. Using this method, we produced viable larvae via in vitro fertilisation. The aim of this study was to investigate the time-dependent fertilizing capacity of sperm which were introduced into ovary in African catfish as model fish

Materials and methods

Experiment I. Sperm samples (2 mL sperm / bodyweight kg) were incubated in gonad lobes 5, 10, 15, 20, 25, 36 and 48 hours before the gamete stripping. Ovulation was induced by extracted carp pituitary (CPE) hormonal administration (5 mg CPE / BW kg). Stripped and mixed gamete batches were fertilised through activation with aerated water. Five minutes after the water activation, three samples of eggs were collected from each batch and incubated in a Petri dish at 25°C.

Experiment II. Three different inseminated sperm dosages were tested on fertilisation from the same pooled sperm batch; 2 mL, 1 mL and 0.5 mL sperm volume / BW kg. Ovulation was induced by 5 mg CPE / BW kg. Stripped and mixed gamete batches were fertilised through activation with aerated water. Five minutes after the water activation, three samples of eggs were collected from each batch and incubated in a Petri dish at 25°C.

Results

Experiment I. There were no statistical differences ($p<0.05$) among the hatching rates in the 5-25h treatment groups, but we observed large individual fluctuations in fertilisation and hatching rate within the groups. However, at 48 hours the treated group showed low fertilisation and hatching rate indicating loss of fertilizing capacity.

Experiment II. The 0.5 mL sperm dosage showed statistically ($p<0.05$) higher fertilisation and hatching rate indicating that small milt volume was enough to apply for propagation.

Discussion and conclusion

Our improved method can combine the simplicity of induced spawning, with a less time-dependent delivery of the sperm compared to conventional in vitro fertilisation. An advantage of ovarian lavage with sperm and hormone preparations could also be in the fields of aquaculture management where it is important to maintain or increase genetic diversit .

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MARKETING OPTIONS ASIDE FOOD FISH PRODUCTION - APPLICABILITY OF RAS DERIVED PIKEPERCH (*SANDER LUCIOPERCA*) AS STOCKING MATERIAL FOR OPEN WATER BODIES

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In several Northern and Central European countries the pikeperch (*Sander lucioperca*) is well known and a highly estimated native food and game fish. It is not surprising, that research and industry intend to establish the pikeperch as an aquaculture candidate (Zienert and Heidrich 2005, Müller-Belecke and Zienert 2008, Brämick et al. 2014, Mylonas and Robles 2014, Steinberg et al. 2018, Zarki et al. 2019). Weaning to artificial dry feeds and production of 5–10g fingerlings within a period of less than three months is accomplished in first commercial hatcheries. Fattening to approx. 1 kg can then been achieved in recirculation aquaculture systems (RAS) at constant temperatures of 22-24°C within about one year. Meanwhile in several European countries pikeperches are produced in RAS at a commercial scale. Nevertheless, when targeting on the food fish market, most of the RAS-farmers suffer from comparably high production costs and concurrence from capture fishery derived pikeperch, imported frozen for low prices, predominantly from the Russian Federation and Kazakhstan (FAO 2019). Marketing individuals alive for stocking issues can be a promising, highly-paid alternative of an often underestimated volume (Pagel and Arlinghaus 2016). The aim of the present study was to evaluate whether pikeperches, intensively produced in RAS on basis of artificial dry feeds, are capable to readapt to their natural feeding basis and survive and grow after been stocked into adequate open water bodies.

In a first approach, five groups of RAS derived pikeperches of different initial mean weighs were stocked in predator protected, static 20m³ outdoor tanks at approx. 20°C together with adequately sized prey fish (*Rutilus rutilus*). After three weeks, the growth performance of the pikeperches and the decline of the offered prey fish biomass was analysed (Tab. I).

Tab. I: Growth performance of five pikeperch groups of different initial mean weights, stocked together with prey fish for three weeks

Stocked pikeperches				**Stocked food fish**		
No. (n)	Initial weight (g)	Output weight (g)	Increment (%)	Initial weight (g)	Output weight (g)	Decline (%)
20	149	170	14	2300	0	100
10	329	396	20	2100	390	81
10	739	715	-3	3640	440	88
8	1103	1129	2	2850	1540	46
5	1946	1811	-7	1700	1020	40

In all five groups, the declining prey fish biomass indicated a fast readaptation potential of RAS derived pikeperches back to life prey fish. Nevertheless, as their body weights stagnated or even decreased, the pikeperch groups of higher initial mean weights seemed to need more effort to readapt.

In a second approach, two summer-turbid flatland lakes (Bauersee, 20ha; Herrensee, 16ha), inhabited by self-recruiting pikeperch populations, where stocked with RAS derived, individually PIT-tagged pikeperch of 280g (n=12 per ha) and 560g (n=5 per ha) in late spring. Approx. 490 days later, after two summers of growth, recapture traits where conducted by trawl nets. Recapture rates, mean weight gains and specific growth rates (SGR) of the recaptured individuals are shown in Tab. II.

Tab. II: Recapture rates, specific growth rates (SGR) and mean weight gains for RAS derived pikeperches after two summers of growth in two natural lakes

	Stocked with 280 g	**Stocked with 560 g**
Bauersee (20ha)		
Recaptured after 2 summers	1.50%	2.00%
Mean weight at recapture	1081g	1587g
SGR d0 - d487	0.25%	0.24%
Weight gain d0 - d487	759g	897g
Herrensee (16ha)		
Recaptured after 2 summers	3.80%	5.00%
Mean weight at recapture	1127g	1397g
SGR d0 - d492	0.26%	0.26%
Weight gain d0 - d492	815g	880g

The recaptured pikeperches in both lakes and both size classes showed weight gains and SGR as expected among natural conditions in Northern Europe (Ložys 2004, Pérez-Bote and Roso 2012).

The obtained results indicate that RAS producers can market their pikeperches as stocking material for open water bodies with good conscience. Especially fish in size classes up to approx. 500g easily readapt to their natural food source and show potential to survive, grow and establish among natural conditions.

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EVALUATION AND COMBINATION OF MEANINGFUL PARAMETERS TO ACCESS FISH WELFARE IN RAS CULTURED PIKEPERCH (*SANDER LUCIOPERCA*)

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In aquaculture the consideration and documentation of animal welfare increasingly gains importance. Compliance with animal welfare in husbandry practices is a socially demanded principle in Europe and for many consumers a non-material criterion for purchase decisions. The aim of the research project was the development and verification of an index based model for the documentation of animal welfare in pikeperch, kept in recirculation aquaculture systems (RAS). The index model, combining several meaningful welfare parameters, can provide a basis for future certification standards. Otherwise it can represent a tool which allows pikeperch producers to present their approaches and results of animal welfare consideration transparently, without certification costs, to consumers, fishery and veterinary authorities.

In a first step ca. 50 potential indicators for animal welfare were grouped for the sections „environment“, „stock“ and „individual“ and evaluated with regard to their explanatory power as well as practicable and confident assessment. For the 18 indicators finally included in the model, practice-orientated levels were defined. Indicator scores, weighting scores, weighting factors and relative weighing factors were derived, following the procedures described by Stien et al. (2013) and Pettersen et al. (2014) to generate their Salmon Welfare Index Models SWIM 1.0 and 2.0.

The developed index model was tested in example surveys in RAS for pikeperch production in Germany. During on-site visits of eight RAS, the operators were informed about the background of the project and the intended procedure within about half an hour. Information needed to evaluate the section „environment“, using environmental parameter mean values from the last month of production, could be obtained within about one hour. Evaluation for the section „stock“ by observation of three separate rearing units and for the section “individual“, examining 10 randomly selected individuals could be conducted within about one hour. Thus, within a reasonable period of time it was possible to collect in-depth knowledge on the reared pikeperches welfare status among on-farm conditions. Tab. I shows the calculated welfare indexes for the different sections and the resulting weighted totals obtained from example surveys.

Tab. I: Pikeperch welfare indexes obtained from example surveys in eight RAS production sites

Section	Production site							
	1	2	3	4	5	6	7	8
„Environment“	1.00	1.00	1.00	1.00	0.96	0.77	0.96	0.91
„Stock“	0.89	0.97	1.00	0.89	0.92	0.88	0.97	0.76
„Individual“	0.97	1.00	1.00	0.96	1.00	0.98	0.97	0.91
Total	**0.95**	**0.99**	**1.00**	**0.95**	**0.96**	**0.89**	**0.97**	**0.87**

The practitioners included in the example surveys evidently placed great value on providing close to optimal rearing conditions for their fish. The examined stocks and individuals showed a correspondingly good condition. At site 6 with a suboptimal pH, slightly increased ammonium and quite high nitrate concentrations several environmental parameters were outside the defined optimum ranges. Despite suboptimal

environmental conditions, the condition of the individuals was close to the optimum. The example surveys finally proved the applicability of the index model for welfare assessment, basing on a methodologically uniform, objectively recorded data basis.

An effect of high stocking densities on the condition of the examined individuals could not be determined during the example surveys. Thus, at site 6, individuals stocked with 110kg per m^3 showed a comparable condition to individuals kept under identical environmental conditions at 60kg per m^3.

Future surveys in further intensively reared pikeperch stocks should be conducted to examine whether a correlation between individual size and eye condition can be manifested. In the example surveys, individuals with lens opacities were more likely to be found in stocks with large individuals than in stocks with smaller fish. Physically plausible, the scales, becoming rougher with increasing size, as well as stronger frictional forces in body contacts between large individuals could justify this observation.

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FISHMEAL FROM ENSILAGED FRESHWATER FISH PROCESSING BY-PRODUCTS IN A RAINBOW TROUT DIET: GROWTH PERFORMANCE, PRODUCT QUALITY AND ENVIRONMENTAL IMPACT

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Processing freshwater fish results in around 50% of by-products with potential for reutilization as fish feed ingredients. Especially in small-scale operations, corresponding by-products are accumulated in low amounts throughout the whole year and have to be protected from spoilage. As deep freezing demands a high input of energy and equipment, approaches to ensilage fish processing by-products by acidification were tested and successfully established. Ensilaged fish processing by-products can now be stored long-term in a sustainable way, until sufficient amounts for centralized transports to fishmeal-plants were collected. The present project stage aimed on the evaluation of fishmeal, derived from ensilaged freshwater fish processing by-products (fish-silage meal) as ingredient for rainbow trout (*Oncorhynchus mykiss*) diets.

Six 1.3m³-tanks of a laboratory scale recirculation aquaculture system were stocked with each 60 rainbow trout with initial body weights of 236±33g (MW±SD) and fed with two diets in 3 replicates per diet close to saturation at rearing temperatures between 13°C and 15°C for 112 days. The control diet (control) as well as the experimental diet including 50% of fish-silage meal (FSM) contained 48% of crude protein and 17% of crude fat. As indicated in Tab. I the fish receiving FSM showed a comparable or even slightly better performance than the individuals fed with the control diet.

Tab. I: Performance of rainbow trout receiving diet with fish-silage meal (FSM) or control diet (mean values from 3 replicated groups per diet)

	Control	**FSM**
Experimental period (d)	112	112
Initial body weight (g)	239.2	232.1
Final body weight (g)	767.1	820.8
Weight gain (g)	527.9	588.7
Specific growth rate (%)	1.04	1.13
Feed conversion ratio (kg/kg)	1.06	0.97
Survival rate (%)	97.6	98.8

The diet containing fish-silage meal had no considerable effects on the carcass composition, but resulted in significantly increased red and yellow levels in the coloration of filets and livers (Tab. II). Furthermore the FSM diet caused a slight but noticeable influence on sensory parameters (in adaptation to DIN EN ISO 5495:2007), with a more pronounced color, odor and taste in cooked filets. The sensory product quality was not promoted by the use of fish-silage meal. However, possible losses in sensory quality parameters could be reduced or eliminated by purging before slaughtering.

With regard to potential environmental impact the FSM diet showed disadvantages. With 7.8% (control) and 7.7% (FSM) both diets contained similar percentages of total nitrogen (N). However, due to the higher proportion of solid structures from bones and carcasses in fish-silage meal derived from fish processing by-products, the total phosphorus (P) content of the FSM diet was clearly higher (3.28%) than in the control diet (1.45%). Assuming contents of 2.72% N and 0.43% P in rainbow trout wet mass (Schreckenbach et al. 2001) and the obtained feed conversion ratios, nutrient emissions resulting from both diets can be balanced as shown in Tab. III.

Tab. II: Differences in brightness and coloration of filet and liver in rainbow trout receiving diet with fish-silage meal (FSM) or control diet (Konica Minolta CIE L a b coordinates analyzed in 29 fish per diet)

	Control		FSM		
Parameter	**MW**	**SD**	**MW**	**SD**	***p***
Filet brightness L	42.05	3.06	41.46	2.54	0.357
Filet color a	4.70	1.54	5.85*	1.03	0.000
Filet color b	1.52	0.88	3.32*	1.62	0.000
Liver brightness L	25.21	4.32	27.71*	5.17	0.016
Liver color a	17.59	2.02	19.59*	2.32	0.000
Liver color b	-0.83	3.13	0.62*	3.39	0.041

MW: mean value; SD: standard deviation. *:significantly different to control ($p \leq 0.05$)

Tab. III: Balance of nutrient releases resulting from administration of the tested diets

	Control		FSM	
	N	**P**	**N**	**P**
Input per kg of administered diet (g)	77.9	14.5	77.1	32.8
Extracted by fish biomass (g)	25.7	4.1	25.7	4.1
Emission per kg administered diet (g)	**52.2**	**10.4**	**51.4**	**28.7**

Compared to the control diet, phosphorus emissions from the FSM diet can be expected to be approx. 2.8 times higher. Especially when applying diets which include fish-silage meal derived from fish processing by-products, approaches to reduce phosphorus emissions, as faeces stabilization by guar gum supplementation (Brinker et al. 2005), effective mechanical purification systems (Sindilario et al. 2009a/b) and/or P-elimination modules (Müller-Belecke et al. 2018) in the farm outlets should be implemented.

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APPLICATION OF SUNFLOWER PROTEIN CONCENTRATE TO SUBSTITUTE FISHMEAL IN A RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) DIET

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In line with the production of sunflower oil, press cakes rich in protein were generated as by-products. These press cakes can be processed into sunflower protein concentrates with protein contents of up to 85%. So far, there are only few information published with regard to the implementation and utilization of such sunflower protein concentrates (SPC) in diets of commercially important aquaculture species (Stickney et al. 1996; Lovatto et al. 2017; 2018). The present study aimed on the evaluation of a sunflower protein concentrate, obtained from the ESPACE Prozess Technologien GmbH Berlin, as substitute for fishmeal in a rainbow trout (*Oncorhynchus mykiss*) diet.

The pelleted control diet (control) and the experimental diet (SPC) were nearly isoenergetic and isonitrogenous (Tab. I). Instead of 35% fishmeal as the control diet, the SPC diet contained 17.5% fishmeal and 17.5% sunflower protein concentrate.

Tab. I: Properties of the formulated control and experimental (SPC) diets

	Control	SPC
Dry matter (%)	83.4	87.2
Ash (%)	5.5	4.8
Crude protein (%)	47.7	47.3
Crude fat (%)	17.5	16.8
Gross energy (MJ kg^{-1})	23.1	23.3

Six 1.3m^3-tanks of a laboratory scale recirculation aquaculture system were stocked with each 55 rainbow trout with initial body weights of 158±39g (mean value ± standard deviation) and fed with the two diets in 3 replicates close to saturation at rearing temperatures between 5.9°C and 15.7°C for 84 days. During the first 28 days the rearing temperature was slowly adapted from suboptimal 5.7°C to the optimal range for this species of between 14 and 16°C. In the following 56 days of the performance test the temperature was kept within the optimal range. The performance comparison at the end of the trial was based on (i) feed intake, survival rates, specific growth rates (Busacker et al. 1990) and feed conversion ratios as well as (ii) slaughtering of 24 individuals per diet in order to record the percentages of intestinal fat, the percentages, brightness and coloration (Konica Minolta CIE L*a*b* color space) of livers and fillets.

All control and experimental groups completed the performance test with survival rates of 100%. The substitution of 50 % of the fishmeal by the tested sunflower protein concentrate had no apparent effect on feed intake of the supplied rainbow trout. As indicated in Tab. II at low, slowly increasing water temperatures during the first phase of the performance test and thus suboptimal conditions for the fish, the SPC diet led to noticeably lower specific growth rates and decreased feed conversion ratios. Kept among optimal temperatures in the second phase of the performance test, specific growth rates and feed conversion ratios of the groups fed with the SPC diet declined, when compared to the control groups, but only in a magnitude of 4 %.

The SPC diet, containing sunflower protein concentrate had no considerable effects on intestinal fat percentages, fillet yields and brightness as well as coloration of fillet and liver. Nevertheless, the liver weights (4.00g versus 4.97g) and liver percentages (1.36%

versus 1.63%) of the fish fed with the SPC diet were significantly smaller ($p \leq 0.05$) than from the fish receiving the control diet.

Tab. II: Performance of rainbow trout receiving diet with sunflower protein concentrate (SPC) or control diet at suboptimal and later at optimal rearing temperatures (mean values from 3 replicated groups per diet)

	Temperature range			
	Suboptimal (d0-d28)		Optimal (d28-d84)	
	Control	**SPC**	**Control**	**SPC**
Experimental period (d):	28	28	56	56
Initial body weight (g):	159.9	155.9	180.7	169.1
Final body weight (g):	180.7	169.1	336.1	303.6
Weight gain (g):	20.8	13.2	155.4	137.0
Specific growth rate (%):	0.44	0.29	1.11	1.07
Feed conversion ratio (kg/kg):	1.79	2.71	0.98	1.02

Depending on price and availability, the tested sunflower protein concentrate thus represents an option for the substitution of appreciable amounts of fishmeal in rainbow trout diets, when the fish can be kept among optimal rearing conditions.

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ONGROWING OF SOLE (*Solea senegalensis*) SUBJECTED TO THREE FEEDING PATTERS IN A RECIRCULATION SYSTEM

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Introduction

The Senegalese sole (*Solea senegalensis*) is one of the most studied species in the last decades due to its economic value and high market demand (Morais *et al*., 2014; Rodriguez and Peleteiro, 2014). In recent years, important progress has been made in larval culture and ongrowing; however, courtship in F1 is still a key bottleneck for Senegalese sole aquaculture (Morais *et al*., 2014). Selecting and using suitable feed during the growth cycle is crucial to maximize profitabilit . The Senegalese sole exhibits a particular feeding behaviour, as it feeds from the bottom of the tank and it has night feeding patterns. Here we present the results of ongrowing of three batches of Senegalese sole, in which the daily food intake was distributed in three doses (3, 6, 9).

Materials and methods

The experience was carried out in the facilities of the Instituto Galego de Formación en Acuicultura (IGAFA). A batch of 180 individuals was randomly split into 9 tanks, of 1 m^2 and operating with a recirculation system. The fishes were fed with specific dry feed using automatic feeders, in a period of 21h. The feeding rate was initially 1%, and then it was progressively reduced to 0.77% and 0.67%. In batch A, in which individuals were 17.83±1.0 cm and weighed 76.72±17g, the fed was distributed in 3 doses per day. In batch B, in which individuals were 18.10±1.0cm cm and weighed 77.68±19g, the fed was distributed in 6 doses per day. In batch C, in which individuals were 18.10±1.0cm and weighed 77.13±17g, the fed was distributed in 9 doses per day. Culture parameters were kept constant within the optimal range for this species: salinity 38‰, temperature 19 ±1ºC, pH 7.45±0.11. Levels of nitrogen compounds (ammonium, nitrites and nitrates) were monitored twice a week. Water samples for microbial cultures were also regularly taken. Weight and size were regularly measured, and these data were used to calculate the following growth indexes: condition factor (CF), feed conversion rate (FCR), specific growth rate (SGR) and thermal growth coeffic ent (TGC). Both partial (for each sampling interval) and global indexes were calculated. Statistical analysis was performed using Wilcoxon signed-rank test at a significance level of 0.05 (Table I)

Results

Growth during the 165 days of this experience was similar in all 3 batches. The fish in batch A reached a final weight of 303.68±65g and a total weight gain of 226.96g. In batch B final weight was 305.77±68g and weight gain was 228,09g. In batch C final weight was 308.68±65g and weight was 231.55g. CF was similar in batches B and C and slightly higher in batch A. SGR was nearly identical in all 3 batches (0.84 for A and C and 0.83 for B). FCR was 0.92 in all batches; and TGC was 0.77 for batches A and B, and 0.78 for batch C. CF was significantly different between batches A and B, as well as A and C. Differences between all other parameters were not statistically significant. In this experience no pathological problems or deaths were observed.

Day	N	BATCH A						BATCH B						BATCH C					
		L (cm)	W (g)	CF	SGR	FCR	TGC	L (cm)	W (g)	CF	SGR	FCR	TGC	L (cm)	W (g)	CF	SGR	FCR	TGC
0	20	17,83	76,72	1,35				18,10	77,68	1,31				18,10	77,13	1,30			
21	20	18,72	98,12	1,50	1,24	0,68	0,98	19,07	100,23	1,44	1,27	0,65	1,02	19,10	101,60	1,46	1,37	0,60	1,11
41	20	19,70	119,82	1,57	1,00	0,86	0,82	20,07	123,03	1,52	1,02	0,84	0,85	20,18	123,90	1,50	0,99	0,87	0,83
63	20	20,98	147,50	1,59	0,95	0,91	0,83	21,28	149,62	1,55	0,89	0,98	0,78	21,53	154,23	1,54	0,99	0,87	0,88
83	20	22,22	174,08	1,58	0,83	1,05	0,77	22,57	175,57	1,54	0,80	1,10	0,74	22,70	179,98	1,54	0,77	1,14	0,80
103	20	23,37	202,62	1,59	0,76	0,89	0,74	23,55	198,55	1,52	0,61	1,91	0,59	23,92	209,50	1,53	0,76	0,89	0,75
124	20	24,52	235,37	1,60	0,71	0,96	0,74	24,73	236,62	1,56	0,83	0,81	0,86	24,93	243,60	1,57	0,72	0,95	0,75
144	20	25,60	270,55	1,61	0,69	0,85	0,75	25,82	270,93	1,57	0,67	0,90	0,72	26,00	278,14	1,58	0,66	0,91	0,72
165	20	26,52	303,68	1,63	0,55	1,09	0,61	26,77	305,77	1,59	0,58	1,00	0,64	26,98	308,68	1,57	0,50	1,22	0,56
		8,69	226,96	1,56	0,84	0,92	0,77	8,67	228,09	1,51	0,83	0,92	0,77	8,88	231,55	1,51	0,84	0,92	0,78

Table I. Results obtained from batches A, B and C: L: average length, W: average weight, CF: condition factor, FCR: feed conversion rate, SGR: specific growth rate, and TGC: thermal growth coefficient

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Discussion and Conclusion

According to these results, there are not significant differences for most growth indexes in the three populations analyse. However, CF was significantly difference in population A compared to B and C, which could be due to the fact that individuals from population A reached a slightly smaller size. SGR and FCR exhibit good values and similar to those achieved by (Rodríguez and Souto, 2003). The results from this experience in a recirculation system are better than those in an open system (Peleteiro *et al*., 2010; Rodriguez and Peleteiro, 2014). Overall, these results show that the ongrowing phase in Senegalese sole does not depend on the number of doses in which the feed intake is distributed.

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A NOVEL METHOD FOR THE DEVELOPMENT OF SILVER NANOWIRES-BASED HIGHLY ELECTRO-CONDUCTIVE MEMBRANE WITH ANTIFOULING PROPERTY FOR EFFICIENT MICROALGAE HARVESTING

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1. Introduction

Membrane filtration is one of the most extensively used technique for material separation in various fields such as water and wastewater treatment, biotechnology, pharmaceutics, and food industries [1–3]. However, the major issue that is associated with membrane-based technology is fouling, which causes reduction of membrane performance and operation stability [4]. Typical ways of overcoming fouling includes; optimizing operation conditions, surface modifications and feed pre-treatments. One of the newest anti-fouling approaches, and also arguably the most effective and promising one, is so-called electro-bubble-filtrati n, in which foulants are physically detached from membrane surface by means of micro-bubbles generated directly at membrane surface via water electrolysis. For this method to work, electrically conductive materials must be either used as a membrane, in a pure form or made of composite materials. However, the major issue with electro-conductive membranes is their weak stability and lower electrical conductivity. Therefore, in this study, we propose a novel method for the fabrication of a highly electro-conductive yet stable membrane via relatively simple but yet not reported technology i.e. electroplating.

2. Methods

A Poly(ethersulfon) support membrane was used for the fabrication of the composite conductive thin film membrane. AgNWs were uniformly coated on a polymeric support by means of dead-end vacuum filtration. The coated membranes were dried overnight at 50°C in a controlled heating system. The AgNWs-coating layer was subjected to Ag-based electroplating, in a custom-built electroplating cell, to ascertain durability and filtration performance, properties fit for commercial applications. The Ag electroplated membrane (C-AgNWs) was then washed with an excess of DIW, followed by overnight drying at 50°C. This electro-membrane was characterized, in terms of physical properties and microalgae harvesting.

3. Results and discussion

Preliminary results showed that the fabricated membrane exhibited excellent electrical conductivity (figure 1) along with workable stability (figure 2) of conductive membrane. Moreover, the fabricated membrane performed better performance in both continuous (figure 3) and intermittent mode (figure 4) of microalgae electro-filtrati

4. Conclusions

Findings from this study confirm that the electroplating not only elevates the electrical conductivity but also strengthens the stability of the membrane. This suggests that the electroplating method offers a promising new way of fabricating electro-membranes with finally-workable-qualit , for the purpose of water and wastewater treatment including microalgae harvesting. The electro-filtratio performance of the membrane, when evaluated for microalgae harvesting, was found to be promising both in continuous and intermittent modes of electro-filtration

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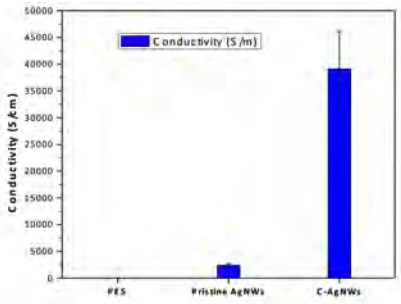

Figure 1. The electrical conductivity of the PES support membrane, pristine AgNWs membrane, and C-AgNWs membrane

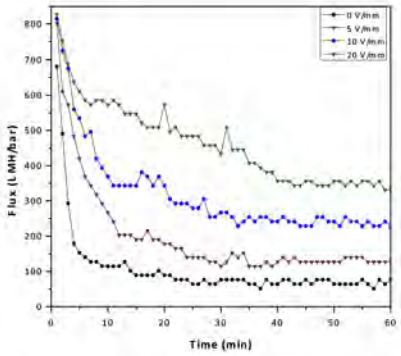

Figure 3. Microalgae filtration under continuous electric field application

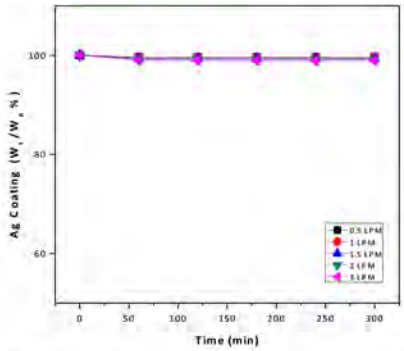

Figure 2. Effect of shear rate on the stability of Ag coating

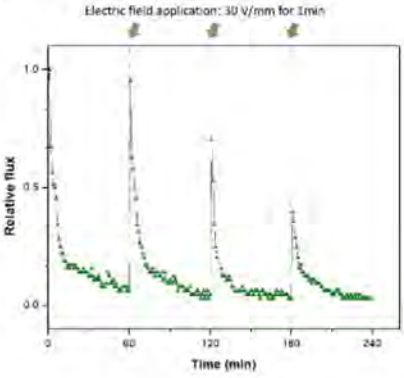

Figure 4. Microalgae filtration under intermittent electric field application

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INDIAN ALMOND TREE (*Terminalia catappa* Linn.) AS HERBAL BIOMEDICINE IN AQUACULTURE

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Aquaculture is growing drastically over the years with intensification. On the other hand intensification increased the stress, occurrence of diseases, introduction of new pathogens and application of the synthetic chemical substances to overcome these problems. Recently adoption of herbal medicine in aquaculture practices is becoming the trend due its advantages over the chemical substances. Plants are storehouses and sources of safer and cheaper chemicals. Numerous herbal plants have been identifi d for its anti-microbial, antifungal, anti-parasitic, growth promotion, appetite stimulation, immunostimulation and stress reducer properties. Indian almond tree (*Terminalia catappa* Linn.) is one among them. Tannin is the major chemical component of *T. catappa* which exhibits the antimicrobial property. The reports on use of Indian almond leaves as herbal biomedicine have been reviewed in the present article. The extensive work need to be done to optimize the dose and duration of treatment against the most common pathogens.

Chemical Composition of Indian Almond leaves	Tannins (punicalagin, punicalin, terflavin A and B, tergallagin, tercatain, chebulagic acid, geranin, granatin B, corilagin), Flavanoids, Isovitexin, Vitexin, Isoorientin, Rutin and Triterpenoiods (ursolic acid, 2α, 3β, 23-trihydroxyurs-12-en-28 oic acid)

Indian almond leaves as Herbal medicine

SL. No	Activity	Principle	Effective against
1.	Antimicrobial	Lyse the cell wall, block the protein synthesis and DNA synthesis, inhibit the enzyme secretions and interfere with the signalling mechanism of quorum sensing pathway	*Staphylococcus aureus, A. Hydrophila,*
2.	Anti-parasitic	Crude extracts of Indian almond at 800 mg/L significantly eliminates the Parasites.	*Trichodina sp, Zoothamnium spp., Gyrodactylus and Dactylogyrus,*
3.	Anti-fungal	Herbal extracts involve the fungal cell wall lysis, altering the permeability, affecting the metabolism and RNA and protein synthesis which leads to death	*Pythium ultimum, Rhizoctonia solani, Sclerotium rolfsii,* and *Aspergillus fumigatus*

MICRO WORMS (*Panagrellus sp)* - NUTRITIOUS LARVAL FEED!

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Larvi-culture is the most crucial stage of the aquaculture practices. The survival rate during the nursery period completely depends on availability of nutritious appropriate size feed. Larvae generally get attracted towards the live food and that must be of appropriate size for the mouth of the fry and nutritive. Micro worms are tiny in size than the newly hatched brine shrimps and have less ability to move in the water which enables the fries to easily catch and eat them. Micro-worms are live bearing, releasing 10 to 40 young every 1 to 1.5 days for a 20 to 25 day life span. The young reach sexual maturity in three days. The live nematodes contain 76% water and 24% dry matter. The dry matter contains 48% protein, 21% lipids, 7% glycogen, 1% organic acids, and 1% nucleic acids. The high fat content makes a particularly attractive food source for larval fish

Enrichment of Micro-worms:
Enrichment can be simply carried out by adding a fortification additive (vitamin premix for example) to the culture medium. Micro-worms enriched with fish oils have higher levels of highly unsaturated fatty acids (HUFAs). Micro-worms have also been enriched with the carotenoid astaxanthin, which can enhance pigmentation in some fish species.

Advantages:

1. Nutritionally rich
2. Easily digestible by the juvenile fish which promotes the excellent growth
3. Culture technique is easily adaptable
4. Cost of production is negligible

Conclusion:
Micro-worms are nutritionally rich live feed source with appropriate size. Micro-worms can be easily cultivable with low cost of production. Hence the technology can be applicable in nursery rearing of fish and shrimp

META-ANALYSIS OF GROWTH PERFORMANCE OF FRESHWATER FISHES UNDER SALINE ENVIRONMENTS IN AQUACULTURE

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Osmoregulation is discussed to be responsible for consuming a major portion of the fish s available energy. Therefore, a common aquaculture practice is the addition of salt to the rearing environment, assuming that besides e.g. reducing stress after handling, it has further beneficial effects on the fish. In theory, freshwater fish kept under slight saline conditions require less energy to maintain their body homeostasis and are able to utilize the remaining energy for somatic growth. To investigate this theory, a meta-analysis on growth performance of freshwater fish in saline environments under aquaculture conditions was conducted.

First, a literature search on fixed word terms was conducted. From all suitable references available, general data for a descriptive review were collected and the specific growth rate (SGR) of the fishes in relation to the salt concentration was extracted. After calculating a percentage-SGR-deviation between growth in the control and in the different salt concentrations, somatic growth of fishes was also compared by taking into account their taxonomic allocation

In total, 56 publications including 59 growth studies were used for this meta-analysis, out of which 32 studies were used to calculate the percentage-SGR-deviation. Growth was studied in salinities ranging from 0 to 36g NaCl l^{-1}. On average, 4.7 salt concentrations were investigated per experiment, fish had a start weight of 92.1g, the experimental duration was 51 days, the rearing volume per tank was 0.44m^3 and 518 fish were used in each trial. Based on all authors' statements, an optimal salinity for freshwater fishes is supposed to be 2.6g NaCl l^{-1} whereas the suboptimal salinity was postulated with 10.6g NaCl l^{-1}.

The percent-SGR-deviation of the 32 studies showed a significantly negative correlation with the salt concentration. The higher the salt concentration in the environment, the lower the growth performance of the fishes. Also, the differentiation of the calculated percentage-SGR deviations based on taxonomic orders resulted in significant negative correlations with the exception of Perciformes.

With this meta-analysis, we revealed that the somatic growth of freshwater fishes under saline regimes is by far not as increased as it is expected according to common theory. In the majority of the studies, growth decreased with increasing environmental salt concentrations. We propose not to overestimate salt as a growth enhancer for freshwater fishes in aquaculture.

INDIVIDUAL GROWTH PERFORMANCE OF JUVENILE PIKE PERCH (*Sander lucioperca*) UNDER SALINE CONDITIONS IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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Scientific studies on fish call for individual recognition in order to detect individual performance parameters. For this purpose, either parts or all fish of a group have to be tagged for experiments. In particular, in cases where only a part of the experimental fish has been tagged for economic reasons, it has to be excluded that tagging effects occur. Literature recommends allowing fish to recover for 14 days prior to the start of an experiment in order to avoid tagging induced biases.

RAS-reared juvenile pike perch (22±3g, n=150) were marked using *passive integrated transponder* (PIT, 11x2.2mm, HDX). PIT were implanted between the pelvic fins into the abdominal cavity using a syringe implanter. Fish were allowed to recover post-tagging for 14 days under identical rearing conditions in the same RAS, together with their untagged conspecifics. Subsequently, the growth performance of tagged and untagged pike perch was observed in a 78 days trial under different salinity regimes (0, 3, 6, 9, 12g NaCl l^{-1}) in five RAS.

We did not observe tagging induced mortality. The tag retention from the time of tagging until termination of the experiment was 100%. Even though, tagged pike perch had a significant lower body weight at the start of the experiment compared to their untagged conspecifics, no significant differences were detected at the end of each trial under salinities from 0 to 9g NaCl l^{-1}. In four out of five treatments, tagged pike perch showed signs of compensatory growth. In all tested salinity treatments, both groups of fish responded identically to saline regimes. Based on group comparison, pike perch in 6 and 9g NaCl l^{-1} showed growth depression. However, tagging data revealed that individual growth still occurred. Therefore, tagging allows for individual conclusions that might deviate from generalized group observations. Still, tagging did not affect the overall results of this growth study but additionally allowed for individual based conclusions. A post-tagging recovery period of 14 days is sufficient for juvenile pike perch that are to be utilized in growth studies.

EARTHWORM VERMICOMPOST TO ENHANCE SHRIMP WHITE SHRIMP *Litopenaeus vannamei* GROWTH AND INHIBITE AHPND DISEASE IN A EXPERIMENTAL CULTURE

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Introduction

In Mexico the annual shrimp aquaculture rate production is growing, and thus demands more feeding inputs and diseases inhibitors. Among organic fertilizers, *Eisenia foetida* solid humus is considered as a great promoter for plankton growth, helping to develop these aquatic organisms. In other hand, the excessive use of antibiotics in aquaculture has affected negatively those species who are farmed (Marshall y Levy, 2011), and also has decreased shrimp growth (Bray et al., 2006) because bacteria are more resistant to antibiotics (Karunasagar et al., 1994). That's why it is important to find environmental alternatives to reduce shrimp diseases. The aim of this study was to evaluate different solid earthworm vermicompost doses as growth promoter and AHPND inhibitor.

Methodology

Five treatments with three replicates each one were seeded with 120 shrimp larvae in 120L tanks with zero water exchange. The tanks were fertilized with initial doses of: control, 0.00mg·L^{-1} solid Vermicompost [VC], 275, 550, 825 and 1100 mg/L^{-1}. The doses were, thereafter, reduced by half at day fifteen. The experiment lasted 45 days. Growth, Food Conversion Ratio and survival were measured. After the growth experiment, mortality accumulation was evaluated for each treatment when shrimp were challenged against *Vibrio parahaemolyticus* in three liters bowls with three replicates with 10 shrimp of 1.5g each bowl.

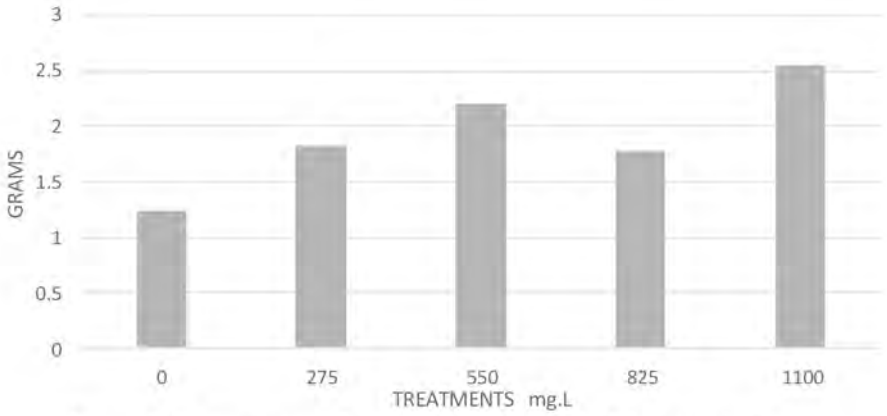
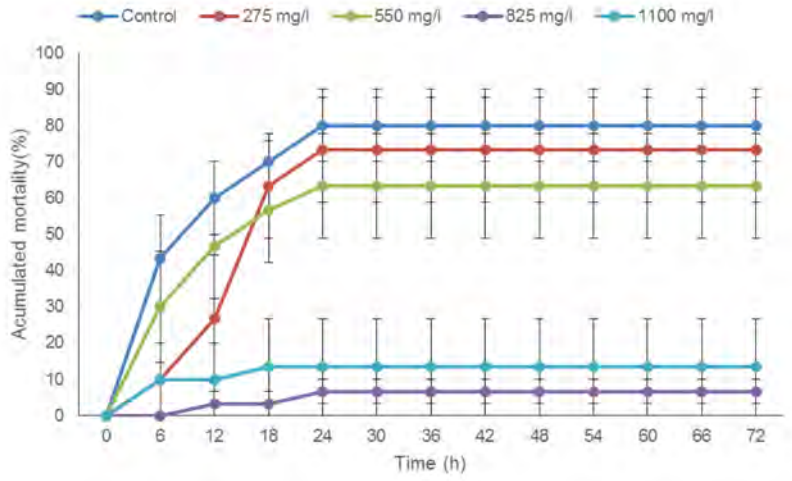


Figure 1. Shrimp growth by treatment at the end of the experiment.



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Results

Water quality parameters were not constant during the experiment and had significant difference (P>0.05) between oxygen and pH treatments. We found that shrimp reared with the organic fertilizer had the best growth (Figure 1), and the lowest food conversion average.

On the other hand, we have that treatment over 550mg/l of vermicompost were effective against *V. parahaemolyticus*. Less than three shrimps died in this treatments in a period of 72h, contrary to treatments less than 550mg/l who had over 50% of the mortality in the first 18h of the challenge. Control had the highest mortality with 80% of the shrimp dead before the 24h trail.

Some studies suggest that vermicompost is an important food resource for juvenile shrimp (Chakrabarty, 2008). This organic fertilizers improves water quality and enhanced phytoplankton production (Bwala and Omoregie, 2009; Chakrabarty et al. 2009). We find that vermicompost is a real option to shrimp growth and can lower productions costs. It also can enhance shrimp survival and increase shrimp production when is used in the first stages of shrimp when it is more susceptible to diseases, but further investigation must be done to find what bacterium conglomerate in the vermicompost acts as inhibitor to AHPND .

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EFFECTS OF DIFFERENT DIETS AND INCORPORATION OF PROBIOTICS ON THE SKIN MORPHOLOGY OF ATLANTIC SALMON (*Salmo salar*)

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Introducti
on

Commercial salmon feeds used in Norwegian aquaculture industry are based on a mixture of plant and marine ingredients. Over the last decades the proportion of the plant ingredients in the feeds have increased while marine ingredients decreased. The current ratio of plant : marine ingredient composition is 70:30 (Ytrestøyl et al., 2015). Plant proteins used in the feeds are derived from protein concentrates, usually with reduced content of antinutritional factors (ANFs). Nevertheless, amino acid imbalance and presence of ANFs are still a concern of the aquafeed industry. Furthermore, plant lipids have an unfavorable ratio of n-3:n-6 and does not provide the recommended EPA and DHA to the fish. Hence, ingredient composition of feed may have an effect on the health of the fish (Torrecillas et al., 2015). In addition, dietary administration of probiotics can influence the mucosal immune system of the fish (Hoseinifar et al., 2015). Skin, which is the largest first line defense organ of the fish, plays a key role in protecting the fish from the surrounding environment which is rife with opportunistic pathogens. Therefore, skin mucous morphology (Vatsos et al., 2010) which is considered as an indicator of fish s skin health, may be affected by several environmental factors including those linked to feeds. This study investigated the histo-morphological alterations of the skin of Atlantic salmon (*Salmo salar*) fed different diets with or without probiotics.

Material and methods

A feeding experiment was conducted with Atlantic salmon (mean weight of 146.97 ± 4.9g SD). The fish were fed three types of feeds with different basal diets with or without probiotics. The ingredient composition of the diets were, Diet 1: fish meal/ fish oil based, Diet 2: a commercial like diet dominated by plant ingredients (plant : marine is 70:30), and Diet 3: a fish meal/ fish oil based diet in which soybean meal replaced 20% of the fish meal. Dietary probiotic was cultured in the laboratory and vacuum coated on the diets. Dorsal skin samples were collected from 12 fish (mean weight 201.4 ± 37.8g SD) per treatment and fixed in 4% formalin. Samples were decalcified with 10% formic acid for 5 hours, prior to processing. After paraffin embedding, tissue sections of 4µm were prepared and stained with Hematoxylin and Eosin (H&E) and Alcian Blue – Periodic Acid Schiff (AB-PAS). Images (n= 9/ fish, N=108/ diet) from different locations of skin were acquired using light microscope. Quantitative analysis of skin morphology was performed; average area of mucous cells (AAM), ratio between total area of mucous cells and total area of epithelium (TAM/ TAE), and number of mucous cells per epithelium (M/E) were determined. The analysis was done using Image J (1.52a).

Results and discussion

The results did not reveal any significant differences in the average area of the mucous cells (AAM), and ratio between total area of mucous cells and total area of epithelium (TAM/TAE). However, the number of mucous cells per epithelium (M/E) was significantly influenced by diet and probiotics. Fish fed Diet 2 and 3 had significantly more M/E compared to those fed Diet 1. Addition of probiotics to Diet 1 increased the M/E, an indication of a strengthened skin barrier.

Conclusion

The results of this study revealed that the ingredient composition of diets directly influence the number of mucous cells per epithelium. Dietary administration of probiotics increased the number of mucous producing cells per epithelium, and this increase may have improved the barrier function of the skin of Atlantic salmon.

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ESTABLISHING A NON-INVASIVE METHOD FOR SEXUAL MATURATION MONITORING IN ATLANTIC SALMON (*Salmo salar*) MALES AND FEMALES USING ULTRASOUND TECHNOLOGY

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Introduction

Atlantic salmon broodstock maturation monitoring relies on invasive gonado-somatic index (GSI; gonad weight as percent of total body weight) measurements requiring sacrifice of fish or measurements in deceased individuals that might not be representative of the population. Other invasive and semi-invasive methods available (direct gonad inspection through incision, endoscopy, blood sampling) come with increased risk of handling stress, infection and mortality. The main objective of this study was to establish a non-invasive method for maturation monitoring to reduce stress and improve animal welfare in broodstock management of wild and farmed Atlantic salmon populations.

Materials and methods

One-year old Atlantic salmon smolts of the AquaGen strain were transferred to seawater in May 2013 and kept under regular production conditions until freshwater transfer in May 2015, after to seawater winters. Fish were given additional 24h light during first and second seawater winters to prevent grilsing and advance maturation, respectively. From one year before expected stripping date, in September 2014, 20 males and 20 females were examined monthly until a maturation inducing temperature drop was given in freshwater in August 2015, after which five males and five females were examined weekly until running milt and ovulation was detected. Samplings included body weight and length, ultrasound-based gonad length measurements, blood sample, gonad tissue sample and gonad weight and length.

Results and discussion

Ultrasound measurements were related to measured gonad length and weight and used to establish models for ultrasound-based GSI (US-GSI) estimation in both sexes. US-GSI models had higher correlations and were more usable in females ($R > 0.9$, $p < 0.05$) than in males ($R = 0.8$, $p < 0.05$). Differences between sexes lie mainly in measurement challenges, as ovaries were easier to measure accurately than testes. Observations from ultrasound images were related to plasma sex hormone levels from blood samples and gonad tissue histology and added nuance to US-GSI measurements. This somewhat compensated for less accurate US-GSI measurements in males.

Conclusion

Ultrasound based GSI measurements are very well suited for maturation monitoring in Atlantic salmon females. Adding other information from ultrasound image to US-GSI registrations, ultrasound is also a useful tool for maturation monitoring in Atlantic salmon males.

THE EFFICACY OF VARIOUS FILTER MEDIA IN REMOVING COPPER FROM TREATED SEAWATER

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Antiparasitic copper treatment is an integral part of routine marine aquaria quarantine, and, at times is even used to treat fish on exhibits. It is very effective at treating marine aquarium fish infected with protozoan ectoparasites.Free copper will react with calcareous materials (e.g. coral, rock or limestone), to form insoluble copper carbonate. This will leach out into fresh, untreated saltwater once a bath treatment is completed. As copper is extremely toxic to invertebrates, any residual copper can cause unexpected mortality.

This project investigated and compared the efficacy of various filter systems in removing residual copper in saltwater. The four filter media examined were activated carbon, zeolite, poly-filters and powdered banana peel

The experiment was undertaken using five glass tanks each having seawater, airlines, copper lines and canister filters. The first tank was used as a control. There were three phases to the experiment. The first phase took place from the 26 February 2016- 01 March 2016.The first phase involved copper treatment to all five glass tanks at the same flow rate. Copper tests using copper reagents and a colorimeter was performed daily until therapeutic levels of 0.25mg/l were reached. Temperature (using the LDO) and pH (using wtw 330i pH meter) readings were also taken as these factors influence the absorption of the free copper.

The second phases started on the 1 April 2016 were gravel and rockwork of the same size and quantity were introduced to all five glass tanks. The copper lines were then closed off. This allowed for the free copper to be absorbed by the rockwork and gravel. Copper tests were done once again using the colorimeter together with pH and temperature readings. The results of the copper testing showed that free copper also leached out some instances. The leaching resulted in therapeutic levels been reached. This phase was monitored from 1 April 2016- 31 May 2016.

The third phases started on the 1 June 2016 were various filter media (activated carbon, zeolite, polyfilter and powdered banana peel) was placed in polyester stocking and put into the canister filte . Activated carbon of porous size was used, zeolite (cationic Aquaculture filter medium), Poly-Filter was cut into squares and powdered banana peel were observed. The banana peels were first dried using a food dehydrator and then ground down to a powder form using a mill with a blade of 8 um diameter. During this phase the copper was tested daily using the colorimeter and the pH and temperature readings were recorded. This phase took place from 1 June 2016- 31 September 2016.

My results showered that zeolite together with powdered banana were the most successful in absorbing the most amount of free copper. The pH within this time frame was 7.8-8 and temperatures were relatively stable. After two months the zeolite became deactivated and activated carbon achieved good absorption of free copper. The reason that activated carbon could have took long in the absorption process was due to the pore size were if the pore size was larger the surface area would be larger and be able to absorb more free copper. Powdered banana showed immense absorption however the pH levels would drop on certain days. The Poly-filter showed very slow absorption during the entire experiment through all three phases. In September 2016 I did a few microbiological tests each filter medium was swabbed and plated onto nutrient agar and incubated for 24 hours. Total counts were done on these plates.

In conclusion activated carbon was the best filter media although taking a long period of time to absorb the free copper. Some results were zero or extremely low levels of free copper detected. Powdered banana was the second filter media that absorbed the most amount of free copper however the pH readings took time to come up. The zeolite was also very good absorbent however will have to be reactivated after two to three months or new zeolite to be added to canister filte . The microbiology that was done showed that the filter medium with the most absorption i.e activated carbon had the least amount of microbial load compared to Poly-filter which had the least amount of absorption but had the most amount of microbial load.

Further studies will be conducted with other filter media (with varying pore sizes) to determine which absorbs the most amount of free copper. Nutrient tests (ammonia, nitrites and nitrates) will be performed to investigate the effects of free copper absorption.

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The findings of this project will assist quarantine in their copper treatment and will ensure a safe living environment for the marine animals in our care.

Environmentally these filters can be used in waste water treatment plants to remove heavy metals such as copper either by having canister were the media is added in or in biofilters were the various media can be added. The reason to have these filter media is to prevent excessive heavy metal leaching into the ocean especially when treatment water meets the ocean. Many waste water treatment plants around the world are using biological media such as banana peels, orange peels and ginseng beside the typical filter media that can be obtained

Copper accumulation in marine fish can cause toxicity of the precious sea life over time. The public that consume fish which were exposed to high copper levels will be at risk when consuming theses fish over a period of time. During this experiment it was noted that fish also consume the algae that grow on the rocks and gravel which have absorbed the copper over a period of time. By consuming the algae the fish are at high risks of copper toxicit .

EVOLUTION OF THE COMPLEMENT SYSTEM IN FISH

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Introduction

The complement system is an important component of innate immunity and causes the lysis and elimination of microorganisms. In some teleosts, multiple copies of the complement 3 (*c3*) gene have been described (the functionally active form of complement component C3 exists as the product of a single gene. We have now identified and characterized three functional C3 proteins (C3-1, C3-3, and C3-4Sunyer et al., 1997; the functionally active third component of complement (C3Forn-Cuní et al., 2014) suggesting that the complement system and its regulation is more complex in fish compared to mammals (Sun et al., 2010). Taking into consideration the microorganism rich environment, which fish inhabit we studied complement evolution with a focus on aquaculture species.

Material and methods

Orthologues of the human complement system were procured in fish using BlastX and the ENSEMBL (http://ensembl.org/, July 2018) database. Phylogenetic trees were constructed for C3 and the related C4 and C5 proteins. The deduced protein sequences of the fish and tetrapod genes were aligned and for the complement phylogenetic tree orthologues of two invertebrate deuterostomes genes were included (Vase tunicate and cephalochordate). Sequences were aligned using the MUSCLE algorithm in the AliView platform (Larsson, 2014)i.e. FASTA, Phylip, Nexus, Clustal and MSF. The intuitive graphical interface makes it easy to inspect, sort, delete, merge and realign sequences as part of the manual filtering process of large datasets. AliView also works as an easy-to-use alignment editor for small as well as large datasets. AVAILABILITY AND IMPLEMENTATION AliView is released as open-source software under the GNU General Public License, version 3.0 (GPLv3 and phylogenetic trees were constructed using Maximum-Likelihood (ML) analysis. The ML analysis was performed in PhyML 3.0 software (http://www.atgc-montpellier.fr/phyml/)(Guindon et al., 2010).

Results

The number of predicted *c3*, *c4*, *c5* transcripts was established by sequence similarity and clustering in the phylogenetic tree (Table 1). The C3 clade possessed the largest number of members with two main clusters C3.1 and C3.2. For C4, the tree topology suggests that two distinct clusters exist, and they were named C4.1 and C.4.2. Within the C5 cluster two main branches existed one contained C5 from ray-finned fishes and the other cluster contained C5 from the cartilaginous fish, the coelacanth and tetrapods (Figure 1).

Discussion and conclusion

The expansion of the *c3* gene in teleosts suggests that the alternative complement pathway was favoured during evolution. The acquisition of a strong innate immune response against pathogens may explain the success of teleosts (>25,000 exist). Overall, the results suggest that the humoral component of the innate response may be more complex in the fishes compared to terrestrial vertebrates that are not exposed to such a microbial rich media. We propose that the evolution of the *c3* and *c4* genes was under different pressures and that *c3* gained (and potentially *c4*) a diversity of functions in teleosts suggesting that aquatic environments may have uniquely shaped this system (Meng et al., 2012). A notable expansion of C3 genes occurred in the aquaculture species sea bass, tilapia and Atlantic salmon and most are species-specific. Understanding how the complement system evolved and functions in fish may help to better understand fish immunity and potentially predict species susceptibility to pathogenic agents in aquaculture environment.

(Continued on next page)

Table 1. Number of predicted C3, C4, C5 genes and transcripts identified in fish.

	Human	Chicken	Coelacanth	Spotted gar	Seabass	Tilapia	Atlantic salmon	zebrafish
C3	1	1	2	3	4 (3)	4 (1)	5 (2)	8
C4	2	2	1	1	1 (1)	2	3	2
C5	1	1	1	1	1	1	1	1

Partial sequences found are within brackets. * Transcript data. ni not identified

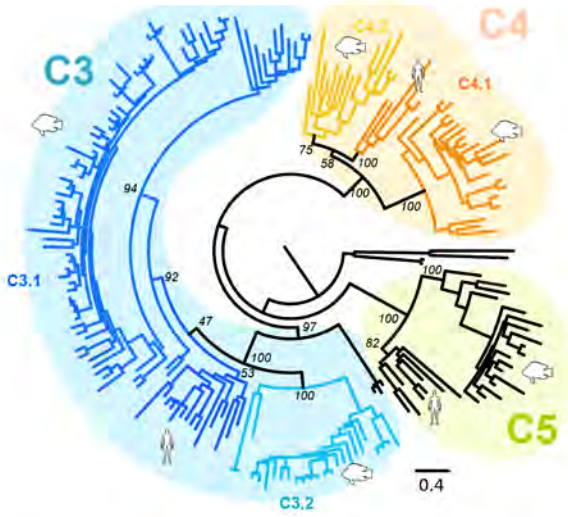

Figure 1. ML phylogenetic analysis of the fish and other vertebrate C3 (blue), C4 (orange) and C5 (green). Tree was rooted with human alpha-macroglobulin (AAT02228) and CD109 (AF410459_1) and bootstrap values for major branches are shown.

Acknowledgements

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NATIVE FLAT OYSTER RESISTANCE TOWARDS *Bonamia ostreae*: A HOLISTIC APPROACH COMBINING OMICS AND PHYSIOLOGY

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Introduction

Over-exploitation has dramatically depleted European natural *Ostrea edulis* stocks in the 19th century (Laing, 2006). During the past six decades, wild European flat oyster beds have experienced a further decline in extent and abundance. Consequently, the flat oyster fishery has suffered a ~90% decrease in production, from 30 000 tonnes in 1960 to 3 000 tonnes in 2014 (FAO, 2019). Habitat degradation, competition and primary diseases (e.g. Bonamiosis and Marteliosis) have contributed to the recent decline in production (Haelters and Kerckhof, 2009). *Bonamia ostreae* infection was first diagnosed in Europe in 1979 (Pichot et al., 1979) and since then has been linked to mass mortalities of commercial and wild *O. edulis* stocks, significantly contributing to a loss in production. No treatment is currently available for Bonamiosis. Strict regulation of oyster movement between disease-free and *Bonamia* positive areas is the only preventive measure available to avoid the further spread of this parasite. Selective-breeding has been highlighted as key to the sustainable intensificatio of the aquaculture industry, especially for the development of disease and climate change resilient stocks (FAO, 2016). Despite early experimental indication of heritability of disease tolerance to Bonamiosis, as well as the presence of heavy selection pressure in this oyster disease model, tolerance has not evolved to a meaningful level in either farmed or wild *O.edulis* populations. This project investigated this counterintuitive lack of adaptation in *O. edulis*, and the context (e.g. host response) in which this disease was able to manifest. By challenging naïve oysters with *B. ostreae*, we characterise animals using an integrated approach, combining -omics (genomic, transcriptomic and metabolomic profiling) with the physiological assessment performance and fitness (metabolic rate, condition index and clearance rate) during different disease stages.

Material and Methods

Oysters were collected from a managed *Bonamia* positive UK population and brought to Cefas facilities (Weymouth, UK). *Bonamia ostreae* positive individuals were identified by microscopy and subsequently used to infect naïve animals (West coast of Scotland) by injection, with infected oysters subsequently acting as trojans. Following this, a further 1 500 naïve oysters (West coast of Scotland) were placed into tanks with the trojan animals, initiating a disease challenge. Prior to challenging, oysters were individually tagged and a biopsy from the dorsal posterior end of the gill arch was sampled for genotyping. Disease progression and mortality rates were followed throughout the challenge. During this period, proxies for fitness (condition index; CI) and physiological performance (O_2 consumption (OC) and clearance (CR) rates) were assessed monthly to infer physiological phenotype, highlighting the profile of susceptible and resistant animals.

Results and future outlook

Monitoring physiological and health status of naïve individuals exposed to *B. ostreae* enabled us to follow the gradual effects of this disease on the European flat oyster. We hypothesise that shifts in metabolic energy requirements are related to disease progression and implicate physiological differences between susceptible and resistant animals. A clear understanding of the context and condition of animals in which disease is emerging is vital to clarify disease mechanisms. Results from this study are being combined with transcriptomic, genomic and metabolomic data to provide an extended profile of susceptible and resistant individuals. Moreover, this fully integrated approach will provide important insights on the nature (if genetic or plastic) of physiological traits. Future results will be applied to aid in the development of *O. edulis* breeding programs contributing to the aquaculture industry as well as conservation efforts of this valuable keystone species.

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IMPROVING SALINITY ADAPTATION IN NITRIFYING BIOREACTORS BY SEAWATER PRIMING

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Introduction

Recirculating aquaculture systems (RAS) for Atlantic salmon (*Salmo salar*) are often operated on varying salinities in different production phases of the fish. The nitrification process in RAS may be adversely impacted by salinity changes, leading to the accumulation of ammonia or nitrite, which are toxic to fish (Chen et al., 2006). Thus, salinity changes in RAS must be performed with caution and with the least possible impact on the nitrification process

An increase in salinity can drastically reduce the nitrification rate in freshwater bioreactors, mainly because osmotic stress can reduce cell activity by inhibition or plasmolysis of microbes (Moussa et al., 2006; Uygur and Kargi, 2004). Stress priming is a strategy where bacterial communities exposed to a mild stress become more capable of dealing with a more severe stress in the future (Andrade-Linares et al., 2016). However, studies on osmotic stress priming in microbial communities are limited (Moussa et al., 2006). This study was undertaken to verify if the stress priming strategy was applicable to salinity changes in RAS bioreactors in the production of Atlantic salmon post-smolt.

We investigated the impact of seawater priming on freshwater and brackish water moving bed biofilm reactors (MBBR). It was hypothesized that biofilms that had previously been exposed to seawater (primed biofilms) would be more robust than un-primed biofilms

Methodology

The experimental setup consisted of eight continuously run 37L MBBRs operated on synthetic medium. The biofilm carriers were obtained from two RAS MBBRs that had been started up in freshwater (0‰ salinity) and brackish water (12‰ salinity), respectively. F1 and B1 were primed in seawater (32‰) for two weeks while F0 and B0 were maintained at their native salinities. Thereafter, all treatments were first operated on freshwater (24 days), and subsequently on seawater (31 days). Salinity changes were conducted by changing the salinity of the buffer tank providing makeup flow to the MBBRs, ensuring a gradual salinity change in the reactors overnight. The nitrification performance was evaluated by performing capacity tests to determine the maximum specific oxidation rates of ammonia (AO_{max}) and nitrite (NOR_{max}).

Results & Discussion

After the second transfer to seawater, the primed freshwater treatment F1 showed little reduction in AOR_{max} or NOR_{max}, whereas F0 had a 50% reduction in AOR_{max} and up to 95% reduction in NOR_{max} (Fig. 1). The results contradict those from a study on nitrifying sludge, where prior adaptation did not improve salinity resistance (Moussa et al., 2006). This suggests that biofilms may show different salinity responses than nitrifying sludge. In the brackish water treatments, AOR_{max} was relatively unaffected by the salinity increase. This is in alignment with other studies reporting that brackish water MBBRs (22‰ salinity) may be more robust to salinity changes than freshwater MBBRs (Gonzalez-Silva et al., 2016; Sudarno, 2011). However, in the freshwater phase, AOR_{max} in the brackish water treatments dropped by ~50%, but recovered to the original rate after about 15 days. Further, although NOR_{max} in B0 and B1 was reduced by 50% in seawater, there was no nitrite accumulation, verifying complete nitrification under the prevailing ammonia loading rate.

Conclusions

The results indicate that stress priming by prior exposure to seawater can significantly improve salinity adaptation in nitrifying freshwater MBBRs. In comparison, brackish water MBBRs are not greatly impacted by seawater priming as they are relatively robust to salinity increase. The results highlight how microbial management strategies may be applied to nitrifying bioreactors for the selection of bacterial communities with the desired functionalities. In conclusion, seawater priming of young freshwater biofilms is a feasible strategy for RAS bioreactors operating on varying salinities.

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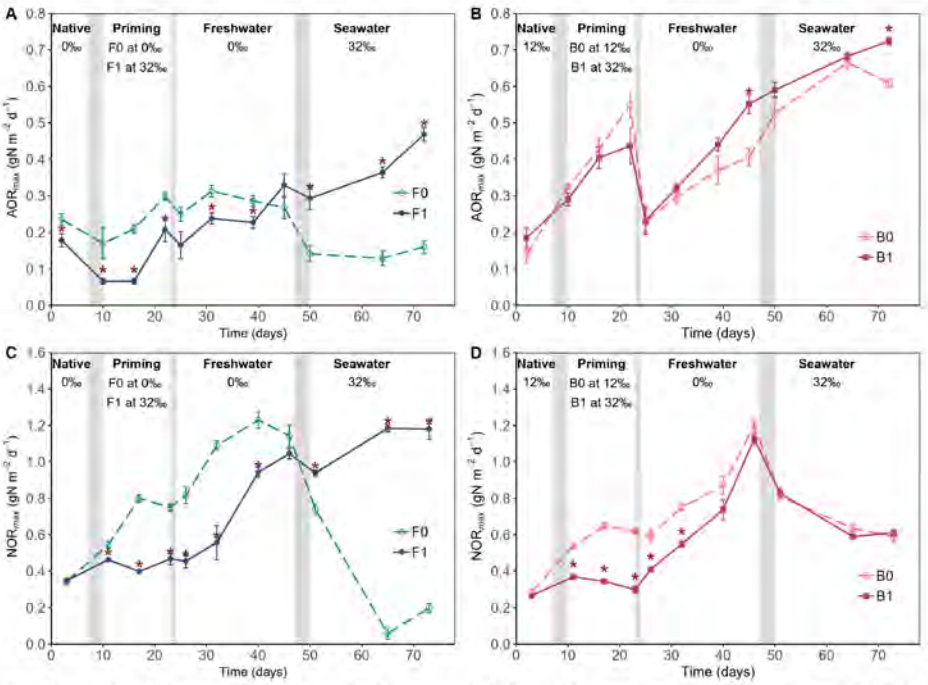

Figure 1.1 Maximum ammonia oxidation rates (AOR_{max}) for treatments A) F0, F1 and B) B0, B1 and maximum nitrite oxidation rates (NOR_{max}) for treatments C) F0, F1 and D) B0, B1, during phases with different salinities (‰, parts per thousand). Grey shaded regions indicate periods of salinity change. In each graph, asterisks above the data points indicate that the primed treatment (F1 or B1) was significantly different from the un-primed treatment (F0 or B0) ($p < 0.05$).

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ENHANCING FISH DIGESTIVE EFFICIENCY THROUGH NUTRITIONAL MODULATION AT EMBRYONIC STAGE

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Introduction

New and more efficient methods to "sustainably intensify" Aquaculture production are essential to attain the seafood demand for direct human consumption in the near future. Nutrition has been identified as one strategy of early exposure that might affect animal early development and later phenotype. This strategy may have positive consequences in the modulation of fish digestive physiology, which will correlate with higher performance outputs. Thus, improving fish digestive efficiency will lead to higher productivity and lower biogenic emission from aquaculture facilities. This will minimise the impact on the environment while increasing the biological efficienc . The present work will present results on early metabolic programming in fish, regarding enhancing fish performance through the modulation of digestive capacity during fish embryogenesis

Material and methods

An innovative *in ovo* nutritional modulation technique based on low-frequency ultrasounds was used to enhance the transport of compounds across the embryo membranes. An early stimulus with bioactive compounds (D, E, F) involved in gut maturation was applied in zebrafish embryos at 3.5 hours post-fertilization (hpf). At 22 days post-fertilization (dpf), growth performance, digestive enzyme activities and gut microbiota composition were analysed to evaluate the larval nutrition-induced metabolic plasticity and the effects on fish digestive efficien .

Results and Discussion

The preliminary results showed that fish survival was not affected either by the sonophoresis technique or bioactive compounds supplementation ($p>0.05$). Final dry weight (DW) at 22 dpf was statistically higher in larvae from F treatment when compared to the control ($p=0.027$). In fish supplemented with bioactive compound D, it was observed a progressive DW increase with increasing D concentrations.

The results of the present work indicate that some bioactive compounds may act as promoters of early intestinal maturation, improved digestive capacity and growth performance in fish larvae. We also identified a "developmental window" in which nutritional programming has short-term effects on digestive functionality. Overall, the present study supports sustainable self-sufficiency in fish aquaculture by integrating dietary strategies and relevant data obtained from new tools. The perspective of applying this novel concept to aquaculture industry provides numerous advantages, since digestive capacity is considered key to fish resilience and qualit .

Acknowledgments

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ASSESSMENT OF FEEDING BEHAVIOUR IN JUVENILE SEABREAM Sparus aurata UNDER RECIRCULATION

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Introduction
Studies with self-feeder devices have been carried out previously with seabass (Covès et al., 2006, Di-Poï et al., 2008) but further research is needed with gilthead seabream (*Sparus aurata*). Efforts are focused on establishing links between the social structure and different behavioral patterns derived from divergent stress coping styles (SCS), checking the possible effect of boldness, curiosity or dominance hierarchies in the effectiveness of self-feeding devices and investigating whether they can be used as an operational welfare indicator in farmed fish (Attia et al., 2012; Ferrari et al., 2014; Millot et al., 2008; Millot and Bégout, 2009).

Present experiment consisted in investigating (Sparus aurata) the social structure that builds around a self-feeding system. We tested the functionality of two self-feeding methods also compared with hand-feeding. We determined social structure based on the individual contribution to total food demand, characterizing individuals according to high triggering (HT, >15% actuations), low triggering (LT, 3-15% actuations) and zero triggering (ZT, 0-3% actuations), (Covès et al., 2006). We carried out two group based personality tests to identify individuals of divergent stress coping styles (SCS) and we established links between the social structure around the self-feeding device and behavioural differences between proactive, intermediate and reactive individuals.

Materials and methods
To carry out this experiment, 360 gilthead seabream juveniles were distributed in four tanks (90 fish/tank). Two of them were fed by hand and the other two were under self-feeding conditions throughout the experiment. We characterized the personality of gilthead seabream through the group-based Hypoxia and Risk-tacking tests that allowed us to differentiate between proactive, intermediate and reactive stress coping styles (SCS) and establish the relationship between personality traits and triggering activity. Finally, an acute stress event by hypoxia was conducted to study variations in social structure and food demand when adverse conditions and basal plasma cortisol and glucose were analysed.

Results
Social structure by triggering activity was as it follows: Most of the fish were Zero triggering (ZT) from 89.84% to 93.1%. Low triggering (LT) fish represented 9.30% and 5.75%. Only one High triggering (HT) fish was responsible for about 30.5% and 32.1% of the total number of actuation, representing 1.16% and 1.15% of the population respectively. High triggering (HT) fish weighed 67.1 g (above the average, > 60.26 g) and 47.9 g (below the average, < 58.68 g) respectively. Most of the individuals involved in food demand responded to reactive and intermediate coping styles and showed higher SGR. There were no differences in body weight between coping styles.

Results obtained from the personality tests, showed that the smallest part of fish within tanks belonged to proactive coping style, representing from 4.76% to 26.19%. Reactive ones ranged from 9.52% to 44.05% and the intermediate behaviour included the most part of individuals (51.19% to 64.29%).

None of the two acute stress events caused appreciable alterations in the food demand during the hours or days following the stress event. Nevertheless, the acute stress produced changes in triggering roles. High triggering (HT) fish reduced its contribution to the feed demand, being replaced by LT and ZT fish.

Plasma glucose analysis did not show signifiant differences between tanks nor SCS. Cortisol levels indicated a lower stress in fish under self-feeding conditions, if compared with those fed by hand.

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Discussion and conclusion

We verified that in a small population of 90 seabream, the same pattern is always observed, where only one individual is responsible of more than 30% of total food demand, characterized as high triggering (HT) fish.

Consistency through time and situations in responses to group based tests, allowed the characterization of fish as proactive, reactive and intermediate SCS, as demonstrated in Castanheira et al. (2013). Fish belonging to the intermediate SCS represented more than 50% and showed the importance of the intermediate group in reared populations of gilthead seabream.

Results suggest that bold fish (proactive ones) could be highly competitive individuals and have a priority access to the food resources, since they know where the food falls and they frequent the area under the food-dispenser by establishing as routines. Shyer individuals need to find other strategies and actuate the trigger (which feed the entire group) since they do not have that priority to feed themselves, also observed in other studies conducted with seabass (*Dicentrarchus labrax*) (Di-Poï et al., 2008; Ferrari et al., 2014). This link between SCS and the triggering status could explain the social structure built under the self-feeder apparatus.

A regular food demand by fish can be indicative of lower stress and fish welfare in reared fish. Demand-feeding systems could optimise production allowing less stressful culture conditions if provided with regularity and in a sufficient quantity to allow fish expressing their normal feeding behaviour, but further research is needed to better assess growth performances under self-feeding conditions.

Aknowlegements

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EFFECT OF EARLY LIFE HYPOXIA AND DIET COMPOSITION ON GROWTH, FEED INTAKE AND OXYGEN CONSUMPTION OF RAINBOW TROUT (*Oncorhynchus mykiss*) IN LATER LIFE

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Introduction

Factors and mechanisms controlling voluntary feed intake (FI) are less studied in fish compared to mammals. However, a good understanding of these factors and mechanisms is required to optimize feeding management in aquaculture practices, but also to achieve maximal FI in control groups during experiments (Saravanan, 2013). It is well known that under hypoxia conditions, FI is regulated by dissolved oxygen concentrations in the water (Tran-Duy et al., 2012). Under normoxia conditions other factors play a role maximizing FI. One of these factors is a low oxygen level in the early life of fish. Here it is hypothesized that exposure to a low oxygen level in early life adjust gene functions (i.e. epigenetics), resulting in an increased oxygen uptake capacity, and therefore potential for a higher maximal FI, of fish in later life. Another factor influencing voluntary feed intake under normoxia conditions is diet composition. Macronutrients differ in their degree of oxidation in aerobic metabolism, and therefore oxygen consumption per unit feed can be altered by dietary macronutrients, resulting in diets contrasting in dietary oxygen demand (DOD) (Saravanan, 2013). When fed to satiation it is expected that diets differing in DOD result in similar oxygen consumption (i.e. based on maximum aerobic capacity), but different FI. This is described by Saravanan (2013) as the oxystatic concept. It is unknown if the oxystatic concept holds true for fish exposed to hypoxia conditions in early life.

This study aims to determine for Rainbow trout (*Oncorhynchus Mykiss*) the impact of early life history (exposure to hypoxia or normoxia) on FI, growth and oxygen consumption in later life, either at a high (i.e. high in starch) or low (i.e. high in fat) oxygen demanding diet.

Materials and Methods

The effect of early life hypoxia on FI, growth and oxygen consumption in later life was studied in a 2 x 2 factorial experiment, with oxygen history and contrasting DOD as independent factors. The oxygen history was created by exposing fish two days post hatching to normoxic (100% oxygen saturation) or hypoxic (60% oxygen saturation) conditions for a period of 17 days. Thereafter all fish were maintained at similar, normoxic conditions until the start of the experiment. For contrasting DOD, two diets were formulated where either fat (low-DOD) or starch (high-DOD) was used as non-protein energy source.

During the experimental period Rainbow trout (average initial weight 201 ± 8 gram) differing in oxygen history were fed ad libitum for a period of 7 weeks with one of the two experimental diets. Growth rate, feed intake, apparent digestibility and oxygen consumption were measured.

Results

Results of this study show that fish exposed to hypoxia at early life, have an increased oxygen consumption and feed intake at later life. The impact of early life on oxygen consumption was dependent upon DOD, indicated by the interaction effect between oxygen history and diet for oxygen consumption.

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STRATEGIES TO IMPROVE THE EFFECTIVENESS OF COMMERCIAL PURIFICATION (DEPURATION) IN REMOVING NOROVIRUS FROM OYSTERS

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Abstract/Introduction

Norovirus (NoV) is the principal agent of bivalve shellfish–associated gastroenteric illness worldwide. One treatment option for controlling the risks associated with shellfish originating from moderately polluted areas (e.g. class B areas in Europe) is for them to undergo purification (depuration) in tanks of clean seawater to allow them to purge themselves of microbiological contaminants. Whilst bacteria may be rapidly and effectively removed during this process it is well documented that purified shellfish shown to be free of *E. coli* (the statutory faecal indicator in European legislation) have been associated with outbreaks of NoV. This study investigates potential improvements in commercial purification practices to assess their NoV removal potential from bivalves, as recommended by EFSA (2012).

Methods

Experiments were conducted using environmentally contaminated oysters in laboratory scale and commercial scale purification systems see figure 1) during February and March 2019 – a period falling within the usual winter NoV season in the UK. The study focussed on Pacific oysters (*C. gigas*), a species that dominates the oyster trade in Europe and is implicated in most bivalve-associated illness outbreaks. In these experiments, oysters were placed in purification tanks under the typical conditions encountered with commercial practices in the UK and NoV removal compared against potential enhanced strategies. These strategies included the following: elevated temperatures, feeding, salinity and light vs dark.

The depuration kinetics were assessed by testing the shellfish pre, mid and post-cycle for NoV by PCR, bacteriophage by both PCR and by viable culture. The bacteriophage testing by PCR and viability assay was intended to provide a surrogate measure of NoV infectivity. *E. coli* testing was also carried out to confirm clearance of this statutory faecal indicator. The enhanced purification strategies will be validated in experiments using commercial purification tanks and assessed in collaboration with an industrial partner.

Fig. 1. Commercial purification tanks in laboratory conditions during feeding trial

(Continued on next page)

Results

Analysis of samples is currently incomplete, however, preliminary data obtained from this study suggests that NoV is better removed at 18°C then at lower temps (8°C). NoV genogroup II was consistently better removed than GI. A possible beneficial effect was observed with darkness (vs light) and algal feeding (vs no feeding) using a commercially available algae mix, however, further data is awaited for confirmation of this

Conclusions

Whilst NoV GI removal levels were negligible, NoV GII was consistently better removed, indicating that commercial purification may well be effective for some genotypes/strains of NoV. This study underlines the importance of establishing different approaches for reducing consumer risks associated with the consumption of bivalve shellfish

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THE DRAFT GENOME OF PIKEPERCH *Sander Lucioperca*

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Introduction
The Percidae family contains about 266 fish species distributed in 11 genera. Several of these species are economically relevant in recreational and commercial fish farming. *Sander lucioperca* is one of the species that are considered a particular promise in aquaculture. The relative faster growth compared to other percids, the resilience and diversification potential make S. lucioperca an attractive species for intensive rearing, as these traits are crucial for the potential yields in commercial production. While the global capture production of pikeperch has halved since 2010, its aquaculture production has increased two fold in the same time and reached about 900 tons per year (Food and Agriculture Organization (FAO), 2018). This illustrates the increasing consideration of pikeperch for commercial aquafarming, but also suggests that pikeperch is still a niche-market species. Though, there is still a tremendous lack on genomic tools for pikeperch breeding. In the present work, we report the first draft genome of pikeperch including comprehensive gene annotations. The initial de novo assembly consisted of 1966 contigs with outstanding metrics in term of contiguity, completeness and structural accuracy.

Materials and methods
The genome assembly of pikeperch (*Sander lucioperca*) was constructed using erroneous PacBio long reads and by taking advantage of accurate Illumina short reads. We sequenced one paired-end (2 × 150 bp) short insert library (470 bp) and two mate-pair libraries (2-8kb and 2-10kb) . In addition, a 20kb library was prepared and sequenced with the PacBio Sequel platform. We assembled the raw PacBio reads into draft contigs using Flye vers.2.3.7 (Mikhail et.al, 2018). To annotate protein-coding genes in pikeperch genome, we combined ab initio and homology-based methods along with RNA-Seq evidences.

Results
In total, 530 GB and 62 GB of DNA sequencing raw data were generated respectively by Illumina HiSeq X ten platforms and PacBio Sequel System. These data were assembled into a final assembly size of 900 Mb covering 88% of the 1014 Mb estimated genome size (Fig. 1). The initial draft genome consisted of 1966 contigs with a N50 length of 3.0 Mb. The final assembly yielded 1313 scaffold with N50 of 4.9 Mb. The identified repetitive structures accounted for 39% of the genome. By combining homologies to other ray-finned fishes, and de novo gene prediction methods, we annotated 21,249 protein-coding genes in the *S. lucioperca* genome, which is consistent with the gene content reported for other Perciformes fishes. The orthologous analysis between selected Perciformes fish species suggested that, 35 orthogroups with 317 genes were pikeperch-specific. Finally, the quality assessment of our assembly highlighted that 96% of short reads have been properly aligned to the draft genome with the correct distance to their mates. The assembled genome spans 98.24% and 96.3% of Vertebrate respectively Actinopterygii single-copy genes spaces. This suggests not only that most gene-rich regions have properly been assembled, but demonstrates also the high structural accuracy of the assembly.

Discussion and conclusion
We successfully sequenced and assembled the genome of the pikeperch using long reads from the third generation and taking advantage of the Illumina short reads accuracy. The generated 900 Mb assembly demonstrates outstanding metric in contiguity and structure, compared to other high quality published fish genomes, in particular, species in the Perciformes order. We have annotated 21,249 protein-coding genes in the pikeperch genome, of which 350 are pikeperch-specific. This novel genome assembly is a valuable genomic resource for further genome-wide studies. In particular, it will facilitate the construction of genetic linkage maps and the identification of molecular markers associated with commercial traits. Finally, this draft genome will further enhance investigations of the adaptability of pikeperch in captive conditions.

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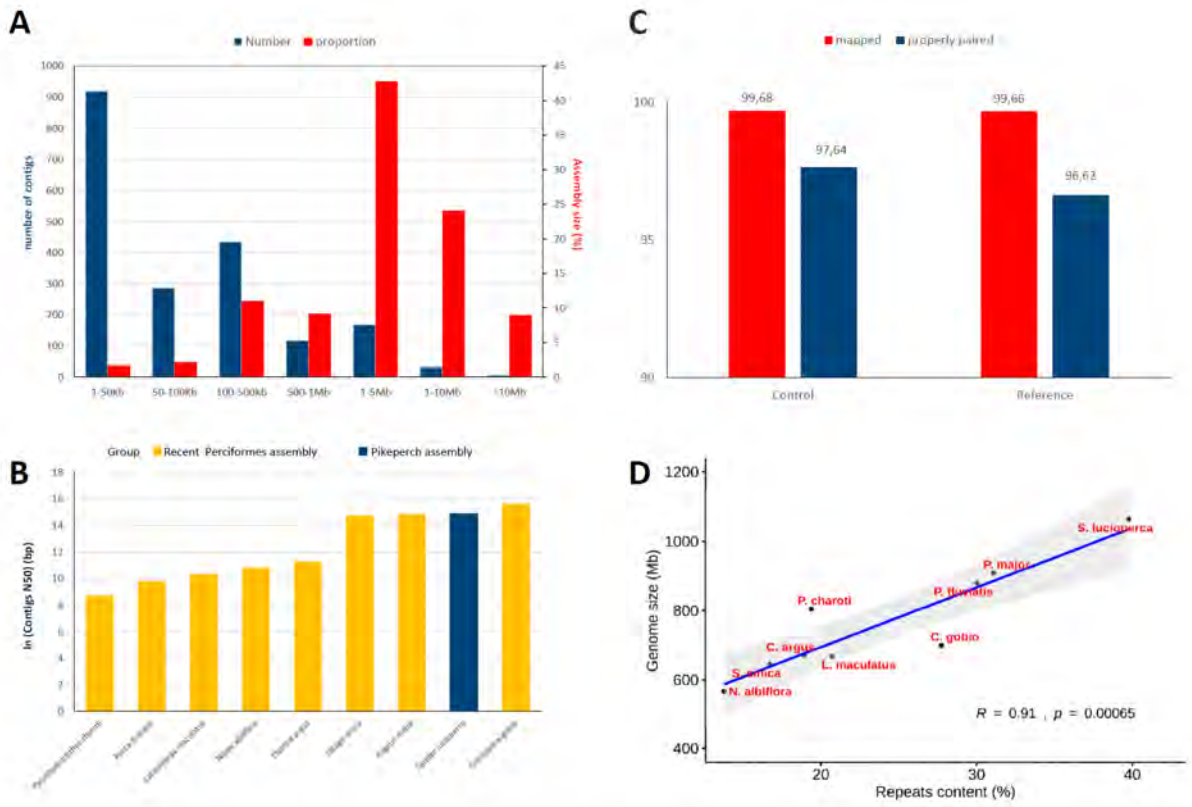

Fig. 1: Assembly assessment. **A**: Number of contigs and their proportion to the overall size of pikeperch's – draft assembly. **B**: Contigs N50 comparison with recently published assemblies of Perciformes species. **C:** Mapping rate of PE-reads from the reference and a control individual, to the PacBio-based assembly of Pikeperch. **D**: Comparison of repeats content and genome size in recently published Perciformes species.

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BIOCHEMICAL AND PROTEOMIC CHARACTERISATION OF EXTRACELLULAR PROTEINS FROM THE PROTOZOAN PARASITE *Paramoeba perurans* REVEALED BY AN *IN VITRO* MODEL

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Introduction

The parasite *Paramoeba perurans* is suspected to have a set of extracellular virulent proteins (Butler and Nowak, 2004; Bridle et al, 2015) that result in the clinical manifestation of AGD in susceptible farmed fish. Bridle et al (2015) demonstrated that extracellular secretions from a virulent 'wild type' *P. perurans* isolate produced a greater cytotoxic response in a Chinook salmon embryo (CHSE -214) cell line compared with secretions from a long-term cultured *P. perurans* isolate. However, the causative proteins responsible for the host cytotoxic response were not determined. This study aims to characterise the extracellular proteome of *P. perurans*.

Method

Media was collected and pooled from *P. perurans* cultures and filtered through a 0.22µm polyethersulfone filter to obtain cell-free supernatants. The supernatants were concentrated 10-fold using centrifugal concentrators and protein quantification measured with the BCA assay. One dimensional (1D) gels coupled with liquid chromatography tandem mass spectrometry (LC- MS/MS) was used in separating out the soluble extracellular fraction of a virulent and non-virulent isolate of *P. perurans*. The extracellular proteins were also subjected to enzymatic activity using a protease assay and extracellular degradation capabilities of the parasite was also determined using gel zymography. An epithelial cell line was used to assess the host cytotoxic effects of the extracellular proteins from *P. perurans*. The epithelial gill cell line viability was assessed using a trio of assays.

Results

Cytotoxicity was detected when the epithelial cell line was incubated with *P. perurans* extracellular proteins. Confirmation of protease activity via the protease assay and gel zymography was also noted for both the virulent and avirulent strain. The proteins responsible for the cytotoxicity are currently being identified via LC MS/MS

Discussion and conclusions

A trio of assays was used to validate cytotoxictiy of the virulent and avirulent *P. perurans* strain on an epithelial cell line. The causative cytotoxic proteins are currently being analysed and identified using LC MS/MS. The proteins are suspected to play a role in Amoebic Gill Disease progression.

Determining the causative proteins will enhance our current knowledge of *Paramoeba perurans* and additionally facilitate future research efforts in developing therapeutics.

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IS THERE A NEED FOR FLAT OYSTER (*Ostrea edulis*) RESTORATION IN DENMARK?

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Introduction

Historically, the European flat oyster (*Ostrea edulis*) population in Denmark has been mainly located in sheltered areas on the West coast, i.e. Wadden Sea, Ringkøbing Fjord and in the Limfjorden, and less so in the Kattegat. The current flat oyster population is located in the Limfjorden, where it has survived for more than 165 years after it re-colonized the Limfjorden again, as the resumed salt water inflow increased the salinity in the Limfjorden when the North Sea broke through at Agger Tange in 1825. Although the population has been fluctuating, the feral flat oyster population is currently thriving and is supporting a sustainable regulated dredge fishery with landings up to 320t in 2018/19. For the last three years, the population has increased substantially from 1,500t to 6,000t and a similar trend is observed in 2019. Although, *Bonamia* disease was registered in 2014 in the Limfjorden and it lost its status as being declared *Bonamia* free. However, no mass mortality has been observed neither in aquaculture facilities nor in the feral population. The reasons for such increase are mainly due to successive successful natural recruitments and the establishment of flat oysters in new areas. Thus, unlike most other European flat oyster populations, which have reduced and sometimes disappeared from overfishing and disease, the flat oyster population in the Limfjorden is flourishing despite external pressure. A natural question could then be if there is a need for a flat oyster restoration program in the Limfjorden

The recent discovery of *Bonamia*, the irregularity in successful recruitment of flat oysters and the recent massive expansion of the invasive Pacific oyster (*Crassostrea gigas*) in the Limfjorden have raised some concerns regarding the future of the feral flat oyster population in the Limfjorden and aquaculture development. Spat collectors for flat oysters are colonized by Pacific oysters and mixed bottom oyster populations are now emerging in some areas of the Limfjorden. Despite the positive development in the flat oyster population in the Limfjorden, research efforts at DTU Aqua are focusing on: i) developing and securing a hatchery production from a potentially genetically diverse wild population of flat oyster to supply both restoration and aquaculture activities, ii) monitoring the possible development of *Bonamia* in the Limfjorden, iii) mapping the development, dynamic and interactions of both native and invasive oyster beds in the Limfjorden and iv) investigating potential areas for flat oyster restoration in the Limfjorden but primarily elsewhere in Denmark

Results and discussion

The presentation will give a short status of the development of both hatchery production and the feral population of flat oysters in the Limfjorden. It will also describe the various challenges facing the industry regarding: i) recent observations of the parasite, *Bonamia ostrea*, ii) competition from non-native species, iii) natural fluctuations in population size and recruitment but also new possibilities for marked development regarding i) breeding programs, ii) restoration and aquaculture production. Furthermore, an outline of perspectives for initiating flat oyster restoration programs in Denmark will be presented.

IS THERE A NEED FOR FLAT OYSTER (*Ostrea edulis*) RESTORATION IN DENMARK?

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MOLECULAR IDENTIFICATION OF *Photobacterium damselae* subsp. *piscicida* ASSOCIATED WITH PASTEURELLOSIS IN MEAGRE

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Introduction

Photobacterium damselae subsp. *piscicida* is a gram-negative bacterium that causes a serious health condition of both farmed and wild fish populations known as pasteurellosis (Pečur Kazazić al., 2019). However, both subspecies of *Photobacterium damselae* have been described as disease causative agent in marine fishes, and due to their genetic proximity are extremely hard to distinguish. This study characterizes genetically the isolate 42019IPMA, obtained from a possible pasteurellosis outbreak in meagre (*Argyrosomus regius*) produced in an open intensive system at Aquaculture Research Station, Portuguese Institute for the Sea and Atmosphere (EPPO-IPMA).

Material and methods

DNA from the isolate 42019IPMA was used to amplify and sequence the *csp*, 16S rRNA, *atp*A, *rpo*A and *rpo*D genes. The amplified PCR fragments were purified and sequenced (SECUGEN, Madrid). BLAST algorism was applied in partial nucleotide sequences obtained and ClustalW software was used to align the sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the model recommended by MEGA6 software to each partial sequence of 16S rRNA, *atp*A, *rpo*A and *rpo*D genes (Tamura et al. 2013).

The molecular fingerprinting of *Photobacterium* strains was performed with enterobacteria repetitive intergenic consensus (ERIC) PCR and repetitive extragenic palindromic (REP) PCR methods (Rodríguez et al. 2006). The strains used were reference strains *Photobacterium angustum* CECT5690T, *P. damselae* subsp. *damselae* CECT 626T, *P. damselae* subsp. *piscicida* CECT 5895, *P. angustum* CECT 5690T *P. iliopiscarium* DSM 9896T, *P. kishitanii* DSM 19954T, *P. leiognathi* CECT 4191T and *P. phosphoreum* CECT 4192T; the isolate 42019IPMA; and *P. damselae* subsp *piscicida* isolates 316, 319, a321, 354 and 356 recovered from Senegalese sole adults (*Solea senegalensis*), isolates 576 and 577, recovered from gilthead sea bream adults (*Sparus aurata*) and isolates 580, 581 and 582 from wild adult striped mullet (*Mugil cephalus*). Similarity between isolates and the reference strains was estimated using the Dice similarity coefficient with the software FAMD (Schlüter & Harris, 2006).

Results

The isolate 42191IPMA partial sequences of 16S, *atp*A, *rpo*A and *rpo*D genes showed similar percentage of similarity with *P. damselae* subsp. *damselae* and *P. damselae* subsp. *piscicida* strains available in Genebank dataset. The partial sequence of *csp* gene presented, however, 99.5 % of similarity with *P. damselae* subsp. *piscicida* and only 95.3 % with *P. damselae* subsp. *damselae*.

The phylogenetic trees derived from 16S, *atp*A, *rpo*A and *rpo*D partial sequences illustrates the position of the isolate 42019IPMA, clearly associated with *P. damselae*. The housekeeping genes, *rpo*A and *rpo*D, confirmed the clustering of the isolated 42019IPMA with *P. damselae* subsp. *piscicida* strain 91-197 (Teru *et al*. 2017) with a bootstrap value of 98 and 93 %. Therefore, these results support the diagnosis of isolated 42019IPMA as *P. damselae* subsp. *piscicida* strain.

Finally, molecular fingerprintin of *Photobacterium damselae* strains show that the isolated 42019IPMA is clearly grouped with both subsp. of *P. damselae* (subsp *piscicida* and *damselae*) and more closely related with the strains of *P. damselae* subsp *piscicida* CECT 5895 and isolated 316, 319, a321, 354 and 356 recovered from Senegalese sole adults (*Solea senegalensis*).

Conclusion

In conclusion, the genetic results show clearly that the strain 42019IPMA isolated from meagre (*Argyrosomus regius*) at EPPO-IPMA facilities is a strain of *P. damselae* subsp. *piscicida*.

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Acknowledgements

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GROWTH INHIBITION EFFECT OF *Edwardsiella tarda* by LYTIC PHAGE ETP-1 ALONE AND ITS COMBINED EFFECT WITH AMPICILLIN

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Introduction

The frequent use of antibiotics to prevent the spread of diseases or to control the infections in aquaculture has shown development of antibiotic resistance strains. Hence, overuse and inappropriate usage of antibiotics needs to be minimized to reduce the risk of spreading resistance strains. Phages are gaining high importance as an alternative therapeutic agent to control the rise of multi-drug resistant (MDR) bacteria in aquaculture. In this study, we isolated and characterized a lytic phage (ETP-1), as an alternative bio-control measure of MDR pathogenic *E. tarda,* which causes edwardsiellosis to both freshwater and marine animals.

Methods

ETP-1 was isolated and characterized for its morphology and growth performance and growth inhibition effect of *E. tarda*. *E. tarda* growth inhibition was evaluated using different multiplicity of infections (MOIs) (10-0.01) of ETP-1 phage treatments. We further exemplified the study to identify the traits of growth inhibition by ETP-1 when combined use of ETP-1 plus ampicillin 2.5-25 μg/mL for 24 h.

Results and discussion

Morphological analysis revealed that ETP-1 belongs to *Podoviridae* family. Phage treatment alone showed MOI dependent bacteria growth inhibition, and ampicillin treatment showed higher *E. tarda* inhibition compared to control, but inhibition effect was lower than the phage treatment. Combined treatment was more effective in killing *E. tarda* showing the highest growth inhibition at ampicillin (5 μg/mL) plus ETP-1 (10 MOI). Our results showed that ETP-1 could inhibit growth of *E. tarda* more effectively than ampicillin, suggesting that ETP-1 has potential to be an important tool for controlling bacteria propagation, and combine with low dose of antibiotics (ampicillin) have greater effect.

CUSTOMIZED FEEDING TABLES FOR PRECISION FARMING: DEMONSTRATION FOR THE GILTHEAD SEABREAM

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Introduction

Optimization of feeding in aquaculture is crucial to ensure the sector sustainability from both environmental and economic perspectives. Herein, a tool that aims to guide farmers to estimate feeding rates for their own targeted growth and the feeds in use is presented and illustrated for the Gilthead Seabream: The customized feeding tables (CFT).

Methodology

This tool is based on the Energy and Protein fluxes (EP model), which is a generic modelling approach (Nobre et al., 2019) derived from the bioenergetic factorial approach (Lupatsch, 2003). The CFT calculates the feed requirements for the user targeted growth, whereby the resulting feeding rates ensure a balanced feed DE/DP ratio. The CFT can be generated per feed or combined for a given set of feeds such that the best feeds concerning energy and protein requirements are selected. The tables can be generated for custom user-defined temperature and weight classes.

Table 1. Comparison of the DP/DE requirements for *S. aurata*: CFT and published data

DP/DE ratios: Digestible protein (mg) / digestible energy (kJ)			
Fish size (g)	**FAO (2019)**	**Lupatsch (2003)**	**CFT ***
0.8–3.3	30.2–27.6	–	–
10	–	30.0	28.9
5.6–29.8	25.9–22.7	–	–
17.5 - 49.9	28.1–21.5	–	–
50	–	24.4	26.2
42.5–146.3	22.4–20.8	–	–
100	–	22.2	24.0
209.0–333.0	23.8–23.8	–	–
300	–	18.9	23.1

* Calculated for the same conditions as for Lupatsch (2003) trials.

$$\text{Feed intake (g fish}^{-1}\text{ day}^{-1}) = 1.38\ (\pm 0.39) * BW^{0.681\ (\pm 0.026)} * e^{0.073\ (\pm 0.012) \cdot T} \quad \text{(Eq.1)}$$

Where, BW is fish body weight expressed as kg, T is temperature expressed as ºC.

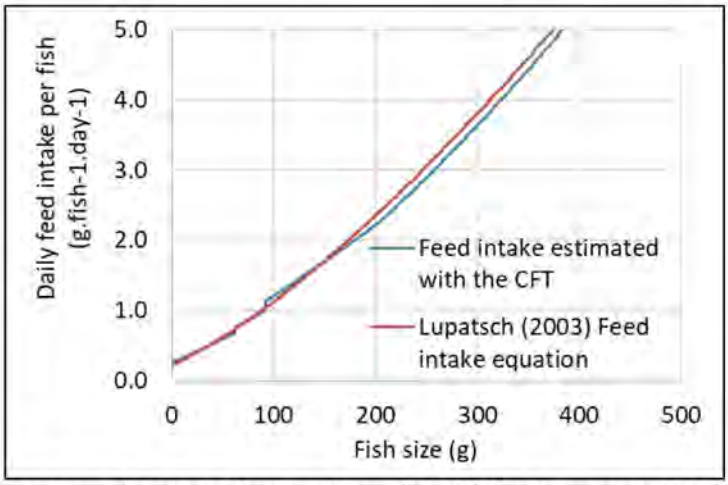

Figure 1. Comparison of the daily feed intake per fish: CFT and Lupatsch (2003) equation

(Continued on next page)

Results

The CFT is herein illustrated for the Gilthead Seabream (*Sparus aurata*). The evaluation of the CFT was carried out by: i) comparing the outputs of estimated ratios DP/DE requirements with those published by Lupatsch (2003) and FAO (2019), shown in Table 1; ii) comparing the generated feeding tables with the outputs of the feed intake equation (Eq.1) defined by Lupatsch (2003), shown in Figure 1.

Conclusions

Herein is shown a tool to calculate feeding rates for a given custom or targeted growth and considering the feeds in use. The CFT will be applied in the context of the MOONSHINE project in order to plan the seabream trials to be carried out for the testing of the control and novel feeds based on algae biomass. The outputs of the ad libitum trials will provide data for further evaluation of the CFT.

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Acknowledgements

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DATA ANALYSIS AND SIMULATION APPROACHES FOR AQUACULTURE PRODUCTION MANAGEMENT

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Introduction

Currently aquaculture operations generate a large number of variables and data. Data analysis and simulation methodologies can support informed decision making. However, given the level of expert knowledge required, few organizations are using these techniques to improve their decisions and knowledge about their production. Herein, is presented a suit of tools that can be used by fish farmers, to for instance: i) create feeding tables customized for the farm standard growth, ii) predict the impact of different feeding strategies on their farm performance, iii) to extract insights from the historical farm production data. The objective of this presentation is to illustrate the potential of the data analysis and simulation methodologies in a bream and bass farm and main take homes regarding the adoption of these technologies by other farmers.

Methodology

This presentation illustrates the application of several data analysis and simulation approaches to a commercial bream and bass farm located in located in southwest Europe (Ria de Alvor, Portugal): 1. Energy and protein flux model (EP model, Nobre et al. 2019) for generating customized feeding tables. 2. Nutrient based model (Conceição et al. 2018) to predict fish weight, time to harvest, estimate feed consumption, oxygen consumption and nutrient excretion among other variables. 3. Data analysis to extract insights from the farm production data. At an initial stage the datamining process was carried out at a fish farm based on a very limited dataset to illustrate how these techniques can be applied even in early stages of adoption of advanced data management systems.

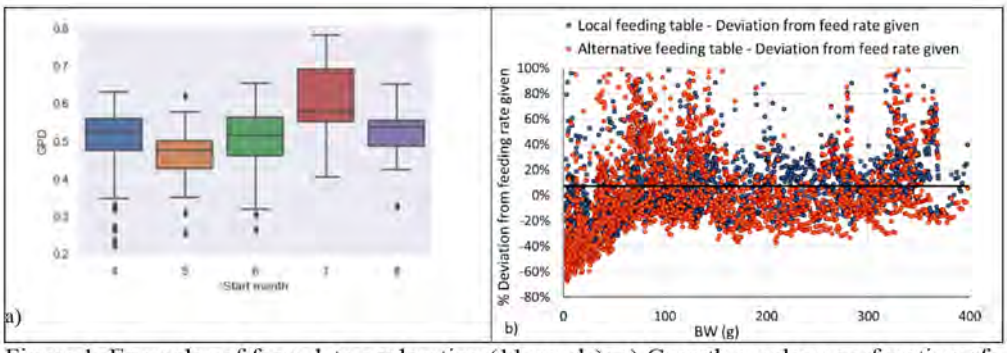

Figure 1. Examples of farm data exploration (11 ponds): a) Growth per day as a function of the start of production, b) comparison of feeding tables with feeding rate given.

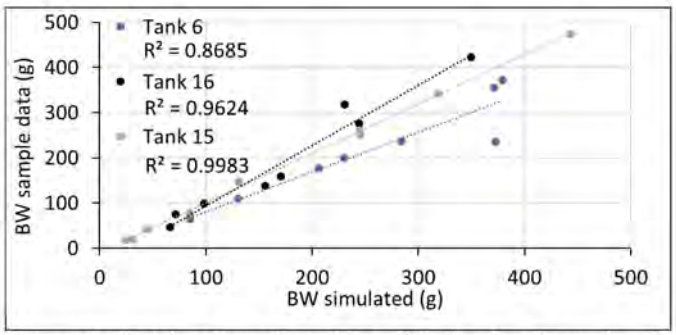

Figure 2. Nutrient based model validation for 3 ponds of seabream at the commercial farm.

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Results

As an example of the data exploration carried out at the fish farm, Figure 1 a) shows the influence of the start month of production in the fish growth per day. Figure 1 b) illustrates other application of datamining to provide insights to farm managers: in this case for the farmer to compare the feeding rate given two feeding tables: its own and an alternative based on the EP model. The advantage of the customized feeding table is that it is calculated based on the fish energy and protein requirements for a given feed and on the farm target growth curve.

Figure 2 shows the validation outputs of the nutrient based model for 3 tanks of the commercial farm.

After ensuring confidence on the model a dashboard was prepared to provide updated estimates about fish weight, time for reaching harvestable weight and feeding requirements up to the end of the production cycle.

Discussion and conclusions

Critical points a farmer should consider before carrying out system data analysis and simulations: i) define specific objectives and questions to be analysed given the wide range of possible analysis, and ii) establish a data flo , which requires a data management system for gathering and integration of the relevant data. Analysis and simulation of aquaculture systems is an evolving task, during which are identified new questions, variables to be collected and further analysed.

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HOW DO HYDROLYZED INSECT MEALS AFFECT SEA TROUT (*Salmo trutta trutta*) MICROBIOTA?

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Introduction

Insect meals are known mainly as an excellent source of protein which is between 9.5% to 70.1% (Sánchez-Muros et al., 2014), although there are a good source of antimicrobial peptides (AMP) and chitin, which can promote animal health (Gasco et al., 2018; Józefiak and Enberg, 2017). These compounds exhibit activity against bacteria, fungi, and viruses (Józefiak and Enberg, 2017) and they start playing a key role in the animal gut. Besides, Wang et al. (2018) commented that the microbiota of the gastrointestinal tract is modulated by environmental and nutritional factors. Considering these aspects the aim of this test was to evaluate the effect of two hydrolyzes insect meals inclusion in the gut microbiota of sea trout.

Materials and methods

After 60 days, sea trout (*Salmo trutta* m. *trutta*) fingerlings were fed with three experimental diets: control diet with only fishmeal (CON); TMD: 10% of hydrolyzed *Tenebrio molitor* meal; and ZMD 10% of hydrolyzed *Zophobas morio* meal. At the end of the trial, the fish were sacrificed and the samples of the gastrointestinal content were collected and immediately stored at –80ºC. for FISH analysis. The analysis was performed according to Józéfiak et al., (2011) method. The oligonucleotide probes used for this study were selected from the literature. To distinguish the total count (DAPI) of bacteria from other particles in the samples, oligonucleotide probes were labeled with DsRed and Alexa Fluor fluorochromes. The filters were left at -80°C for 24 h in the dark until visualization with the use of a Carl Zeiss Microscope Axio Imager M2. The numbers of detected bacteria are expressed in colony-forming units/g of digesta (CFU/ml) and were calculated according to the equation given below:

$$\log CFU / g = \log(N \times (\frac{WA}{PA}) \times (\frac{Sweight + Dweight}{Sweight}) \times (\frac{1000}{Svolume}))$$

where N = number of visible bacterial cells, WA = the work area of the filte , PA = picture area, S_{weight} = sample weight, D_{weight} = diluting factor weight, and S_{volume} = volume of the sample pipetted onto the filte

Results

The inclusion of hydrolyzed ZM meal decreased significantly the quantification of *Aeromonas* spp (AER 66), *Enterococcus* spp as well as in the *Carnobacterium* spp, which was increased with TM meal too ($P < 0.05$). In the case of the *Lactobacillus* group, the inclusion of TM meal reduced significantl . The total number of bacteria, and *Aeromonas* spp (AER 642), as well as *Bacillus* spp, did not exhibit any significant di ferences among treatments (Table I).

Table I. Microbiology results of the digesta of sea trout fed with different diets at the end of the experimental period

	Treatment		
	Control	*T. molitor*	*Z. morio*
Total number of bacteria	9.49	9.52	9.54
Aeromonas spp. (AER 66)	9.34[a]	9.36[a]	9.24[b]
Aeromonas spp. (AER 642)	9.28	9.18	9.13
Carnobacterium spp.	9.34[a]	9.17[b]	9.10[b]
Enterococcus spp.	9.29[a]	9.29[a]	9.15[b]
Lactobacillus group	9.38[a]	9.18[b]	9.23[ab]
Bacillus spp.	9.13	9.12	9.19

Values in the same row having different superscript letters are significantly different at $P < 0.05$.

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Discussion and conclusion

According to Wang et al. (2018), microbiota presence is conditioned by the type of diets and in the case of freshwater fish species, *Aeromonas* is one of the dominant intestinal microbiota. Besides, Egerton et al. (2018) remarked that they are especially found in carnivorous and omnivorous ones. Moreover, Skrodenytė-Arbačiauskienė et al. (2008) reported that 22% of the gut microbiota of sea trout was *Aeromonas* and 6% were *Carnobacterium*, and these amounts were conditioned by the type of insect consumed, which were different from *Salmo salar*. Although, these authors did not report *Enterococcus* genus in sea trout only in Salmon fed with another amount of insect and species. Similar to this genus, *Lactobacillus* and *Bacillus* were not found in sea trout only in salmon (Egerton et al., 2018). Considering that the genera *Enterococcus*, *Lactobacillus* and *Bacillus* are not part of the microbiota of sea trout; beside the "host species" shapes microbial communities in fish (Larsen et al., 2014). As the fishmeal was not totally replaced in this test, the gut microbiota of all the animals kept similarities and insect meals only reduced the number of certain genera of bacteria that in natural conditions would not be presented in the fish gut. To confirm these findings would necessary to do further research to establish these findings.

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TRIALS WITH JELLYFISH SPECIES: IMPROVEMENTS ON THE BIOLOGY OF THE SPECIES AND REARING TECHNOLOGY

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Introduction
Jellyfish is the major subject of study of "GoJelly - a gelatinous solution to plastic pollution" (2018-2021), a project financed by programme Horizon 2020 of the European Union. The project involves 15 European research institutions and private companies and has the participation of the Chinese Academy of Sciences.

Results
Under GoJelly the closing of the life cycle of several jellyfish species was made in captivity at Madeira Oceanic Observatory (OOM) laboratories namely, *Rhizostoma pulmo* (Rhizostomatidae), *Aurelia solida* and *Chrsyaora hysoscella* (Semaeostomeae) and several trials were performed to evaluate the influence of environmental factors on the reproduction of these species. Experiments using jellyfish in aquafeeds were also performed. This communication reports the preliminary results and comments on the major improvements on biological and ecological knowledge of the species and rearing technology.

Acknowledgements: The study was supported by project "GoJelly - a gelatinous solution to plastic pollution" (2018-2021), under European Union´s Horizon 2020 research and innovation programme, grant agreement Nº 774499.

PUBLIC PERCEPTIONS OF AQUACULTURE IN ATLANTIC ISLANDS: IS THERE ROOM FOR MSP?

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Introduction

Aquaculture is developing rapidly at a global scale[1] and sustainable practices are an essential part of meeting the protein requirements of the ballooning human population[2]. Although aquaculture as a whole is expanding, marine aquaculture accounts for a smaller percentage of current production (about one third) compared to more inland fish-farmin [1]. Within the UE, Macaronesia Archipelagos- Madeira and Canary Islands) have been developing aquaculture activities in the last 25 years, mostly devoted to coastal farming of seabream and seabass. Still, the competition for the use of space with other economic activities, namely tourism, has brought attention to public perception and is rising a number of questions that have to be addressed. In order to manage and facilitate economic growth, whilst safeguarding environmental objectives in the marine environment, legislation mandating the development of Maritime Spatial Planning (MSP) was introduced in the UE in 2014. MSP turns to be a place-based, multi-sectoral decision-making approach that is being widely promoted for reducing the conflicts and impacts commonly encountered in conventional sector-by-sector planning[3,4]. Current situation in the portuguese archipelago of Madeira is an example of how public perception and social media can condition the development of coastal aquaculture and MSP process.

Analysis of the aquaculture industry in Madeira Archipelago

Although Portugal has the highest *per* capita fish consumption rate in the EU (54 kg/year in live weight terms)[5], in 2016, national aquaculture production accounted for only 5.9% of fish unloaded in port, demonstrating not only yhat aquaculture was not considered by national consumers as an alternative, but also that efforts were needed to develop the sector. In the last three years, with the support of the European Maritime and Fisheries Fund, national efforts resulted in increase of aquaculture output. In Madeira archipelago, aquaculture as an industrial activity had small expression as until 2016, only one fish farm existed producing between 300-600 tons per year. The recent implementation of MSP and the lawful changes in order to reduce license time, conducted to a sudden increase in the number of companies applying for coastal zones defined within the MSP as Zones of Interest for Aquaculture (ZIA) that would turn Madeira the biggest national seabream "producer", accounting for more than 60% of the production. Consequently. this has risen questions and called social media attention. In less than two years, more than 20 news came out in social platforms, newspapers, local TV and radio. Underlying most of the news were visual impacts in a touristic island and environmental impacts. Experience from other areas of the globe, including the close archipelago of Canary Islands, has shown that accelerated growth of fish farms may lead to important socio-environmental conflicts that decrease or even in some cases stop the expected growth of the industry. Consumers are exposed to numerous, and often contradictory, messages with respect to issues such as food safety and environmental conflicts. The media coverage of an issue therefore may have an impact on the public's demand for (politically) solving an issue[6].

Material & Methods

Analyses of newspaper articles, national and regional was done through content analysis, with the common ground of extracting the meaning from the context of text to understand what that text meant to the audience[6].

Results & Discussion

Analysis of newspapers and Tv programmes showed that confusing messages were being diffused to the consumers. Although there was a broad representation of actors that contributed to the newspaper articles and debates in TV shows, where local scientists, NGO's, Public Administration and Political parties were represented, final messages regarding the characterization of the sector were not clear. It is clear that opposition political parties and NGO's make their statements underlying their position trough fear of environmental impacts and consequences to the tourism activity. Newspaper articles were either 100% positive, announcing Madeira´s ambition to increase the production or either reporting the controversy around the installation of new fish cages. Technology was only included in the news by means of explaining that collar fish

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cages are the most common equipment used that justify coastal aquaculture. Human food and food security were hardly mentioned and most time were referred by the NGO´s. Public Administration comments were regularly directed to the importance of MSP process, already implemented in the archipelago and the fact that the ZIA´s were established after a public process consultation. Industry actors were hardly present in the news and in all cases expressed positive opinions towards coastal aquaculture. Scientific experts and public administrators not directly involved in the licensing process, represented more neutral positions or conservative opinions presenting both positive and negative contributions written by these groups. Major explicit negative opinion was on the environmental impacts, using the salmon industry as an example. Expressions such as "Degradation of the water quality", "water pollution" and "aquafeeds" were the words used to contextualize those negative impacts. Implicitly there was an association with tourism negative reaction to the visual observation of the collar cages, but no inquiries were made.

These findings can be seen easily understood by contextualizing today´s society. We live on an era in which the media desire to attract consumers by preferring conflict over success (e.g., by sensationalizing problems)[6]. General public many times is not aware of the amount of aquaculture products consumed on daily basis in their houses. Often there is also poor knowledge and understanding of aquaculture biological and ecological principals as most of the available information is of scientific character and not available.

The current situation on social media leveraged by public unawareness has delayed the authorization of several projects in the archipelago questioning the entire MSP implementation.

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FROM LARVAE TO PLATE – AN EXPERIMENTAL LAND-BASED RAS CULTURE OF *Litopeanaeus vannamei* IN POLAND

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Introduction

The Pacific whiteleg shrimp *Litopenaeus vannamei* is a key shrimp species in global aquaculture. The European market is dominated mainly by frozen or cooked shrimps imported from Asia or South America, where they are grown mainly in ponds. Such breeding is however possible only in warm climatic zones. In order to become independent from imports as well as to introduce a fresh (unfrozen) product to the market, a number of farms producing *L. vannamei* in the recirculating aquaculture system (RAS) have recently been created in Europe. This way of breeding not only allows the production of exotic species beyond their native ranges, but also the control and optimization of growth conditions. So far, two farms producing *L. vannamei* have been established in the Baltic Sea region. However, cultivation of this exotic species using innovative techniques raises an interest of many potential producers, including those from Poland. To support small and medium-sized enterprises representing the aquaculture sector in Poland, which are potentially interested in production of *L. vannamei*, an experimental culture of this species was initiated at the University of Gdańsk (Poland) under the InnoAquaTech project. In addition to the demonstrative character, the culture also served as a study site to examine growth rate, health status and nutritional value of the studied shrimps.

Materials and methods

Two laboratory RASs, each consisting of three units: (1) culture tank (500 l of volume), (2) main unit with filters (solid, ammonia and protein removal) and set of sensors for controlling water parameters (temperature, salinity, pH, redox) and (3) water preparation tank (250 l), were installed at the University of Gdańsk (Poland) in 2017. Specific pathogen free larvae and post-larvae of *L. vannamei* (approx. 2000) were imported from a hatchery in Florida, US. They were gradually acclimatized to a higher temperature (25°C) and lower salinity (28ppt). Two breeding cycles, lasting 18 and 32 weeks respectively, were carried out and in each of them different feed was used (Gemma DIAMOND, Scretting, Norway and CreveTec, AQUABIO, Belgium). During the experiment, shrimp behavior and mortality were observed. In addition, every two weeks, the length (± 1mm) and weight (± 0.001g) of 20 randomly collected shrimps were determined. After the end of experiment the shrimps were killed by chilling in an ice slurry and then preserved for further analyses which included health status (examination of any bacterial, fungal and viral diseases as well as abnormalities in digestive, circulatory and respiratory systems), elemental composition (contents of carbon, hydrogen, nitrogen) and calorific value (Grodzinski et al., 1975).

Results

During the breeding, cannibalistic behavior was observed, resulting in the increased mortality of individuals, as well as mechanical damage to appendages such as antennas, rostrums, paraeopods, pleopods, telson and uropods. In majority of cases, such an exoskeleton lesions were accompanied by melanization, i.e. the accumulation of blood cells at the site of infection and the deposition of pigment (melanin). The morphological and anatomical changes indicating the poor health condition were observed only in single individuals. The exceptions were fungal infections and inflammation. More than half of the analyzed shrimps had fungal infections in the form of white spots, mainly on the inner side of the carapace, on paraeopods and uropods, less frequently seen on antennas and rostrum. Regardless, the shrimp mass increased at a rate of 0.41-0.54g per week. In both breeding cycles, the final shrimp mass was about 10 g. The mean content of carbon, hydrogen and nitrogen in the dry weight (DW) of abdominal muscle was respectively 45.5 ± 1.6%, 6.8 ± 0.4% and 13.5 ± 0.5%, whereas energy value amounted to 19.9 ± 0.9J mg^{-1} DW.

Discussion and conclusion

Despite the relatively high mortality rate of shrimps caused mainly by cannibalism and RAS system failure, the experimental seems to be successful. Shrimps were characterized by good condition and health, and their weight gain was satisfactory. Thanks to the culture, it was possible to learn the biology and ecology of *L. vannamei*, and the acquired skills can be transferred in the future to stakeholders developing production of this species in Poland.

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VALINE REQUIREMENT OF AFRICAN CATFISH (*Clarias gariepinus*) JUVENILES

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Introduction

Valine is an essential branched amino acid (BCAA) that has great impact on lipid and glucose metabolism. It could prevent high–fat diet induced obesity in animals. Valine can be used as an energy source in the muscles, and preservation of glucose usage. It has been associated with maintenance of the lean body mass during prolonged exercise in both man and mammalian models; and assists in the synthesis of glutamine and alanine and in balancing of other BCAAs such as leucine and isoleucine. However, over dosage causes skin crawling and hallucinations. As there is dearth of information on valine metabolism in African catfish, the study investigated and determined the optimum level of valine required for the production of African catfish

Materials and Methods

African catfish were fed to apparent satiation daily for 56 days, with five diets containing different levels of valine at, 0.00, 0.40, 0.80, 1.20 and 1.80% making treatments 1-5. The feeding trial was conducted in triplicates in glass tanks. Standard methods were used in the generation of data and analyses, while fourth polynomial degree regression analysis was used to estimate the valine requirement based on the specific growth rate of the fish. ANOVA was applied while Duncan Multiple range test was used to separate the means.

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Results and Discussion

Although there was no definite pattern of increment in the dietary amino acids as a result of the supplemental valine, addition of valine in the diets slightly increased other amino acids in the diets particularly the other BCAAs, Leucine and Isoleucine. Supplementation of valine in the diets improved the growth performance until in the fish fed diet containing 0.8% of valine and then declined consistently, suggesting that level as the optimum. Nevertheless, a fourth degree polynomial regression analysis indicated 0.72g/100g diet as optimum (Fig. 1). Similarly, supplemental valine significantly improved the carcass minerals (Table 1) but slightly increased some amino acids. Interestingly, the carcass cholesterol decreased with increasing values of the supplemental valine (Table 2). Manipulation of the dietary valine will reduce the minerals required for the production of aqua-feeds, thereby reducing the unit cost of aquaculture production. Also valine can be used to produce fish with low level of cholesterol which will reduce cardiovascular related diseases in people that consume the fish. This supports the work of Huang et al. (2018).

Conclusion: Addition of valine in the diets improved fish growth performance and nutrient utilization. And the valine requirement of African catfish was estimated as 0.72g/100g diet

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Table. 1. Carcass minerals of African catfish fed valine diets (mg/g)

Diets/Parameters	Na	K	Ca	Mg	P
Diet 1 (control)	8.90±0.06^{a}	47.8±0.06^{a}	7.36±0.08^{a}	2.92±0.05^{a}	51.9±0.12^{a}
Diet 2 (0.40 %)	9.40±0.01^{b}	51.6±0.05^{b}	9.80±0.04^{b}	4.70±0.03^{b}	52.3±0.50^{b}
Diet 3 (0.08%)	9.60±0.25^{c}	55.4±0.10^{c}	10.5±0.19^{c}	4.96±0.35^{c}	53.2±0.26^{c}
Diet 4 (1.20%)	10.4±0.32^{d}	60.2±0.08^{d}	12.6±0.09^{d}	5.57±0.62^{d}	54.3±0.48^{bc}
Diet 5 (1.60)	15.8±0.25^{e}	61.7±0.67^{e}	13.5±0.36^{e}	6.68±0.61^{e}	56.6±0.12^{d}

Values on the same column with similar superscripts are not significant (P>0.05)

Table.2. Carcass cholesterol levels of African catfish fed valine diets

Diets/Parameters	Cholesterol (mg/dl)
Diet 1 (control)	148.6 ±0.07^{e}
Diet 2 (0.40 %)	133.0 ±0.01^{d}
Diet 3 (0.08%)	130.0 ±0.06^{c}
Diet 4 (1.20%)	123.9 ±0.04^{b}
Diet 5 (1.60%)	121.3 ±0.05^{a}

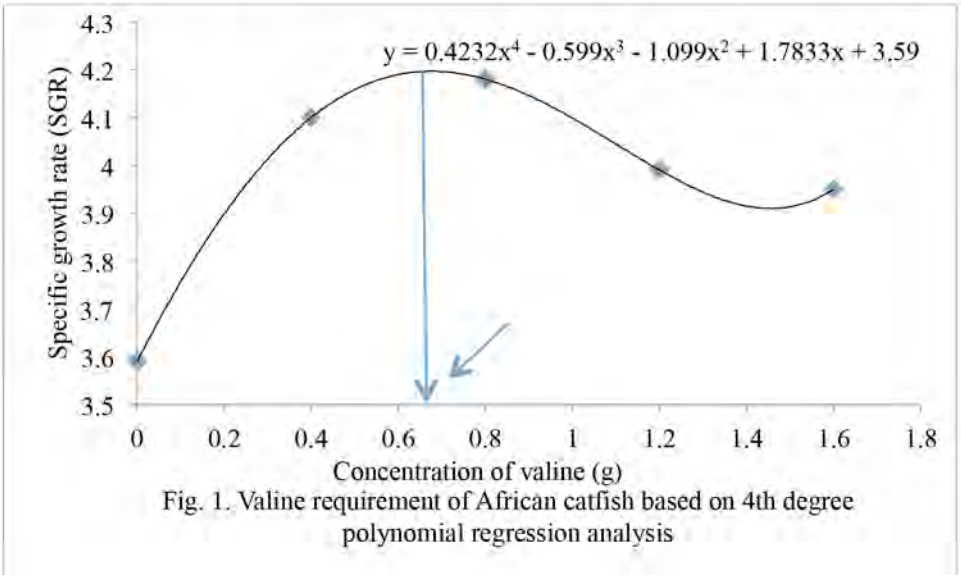

Fig. 1. Valine requirement of African catfish based on 4th degree polynomial regression analysis

INFESTATION PRESSURE OF SALMON LICE (*Lepeophtheirus salmonis*) ON RETURNING ATLANTIC SALMON (*Salmo salar*) IN CENTRAL NORWAY

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Introduction

The salmon louse (*Lepeophtheirus salmonis*) is a directly transmitted marine ectoparasite found on salmonids in the North Atlantic and North Pacific oceans. The parasite grazes on skin, mucus and underlying tissue of the fish, causing osmoregulatory dysfunction and physiological stress responses, making the fish more susceptible to secondary infections and mortality (Finstad, et al., 2000).

The parasite has 8 developmental stages, where the infective copepodite stage attaches to the young post-smolt salmon as the fish migrate out to open sea to graze. During this sea phase the lice molt to become gravid females on the host, producing egg-strings that hatch to produce new generations of infective copepodites. Louse have been recorded to survive for up to 15 months under laboratory conditions (Hamre, et al., 2009), but information regarding the survival and life cycle of salmon lice in the open ocean is scarce (Costello, 2006). Jacobsen and Gaard (1997) observed densities of up to 30 adult female lice per fish returning from the sea, and early developmental stages of lice have previously been observed on trout returning from the ocean in Caster, England, indicating that lice infections can occur in areas where host densities are low and salmon farming is non-existent (Tingley, et al., 1997). The possibility of open sea re-infection of copepodites from gravid females, originating from chalimus from near-shore waters is therefore hypothesized (Jacobsen, et al., 1997), and may contribute to explain the observed stability of sea lice densities in areas with low impacts from farmed salmon.

Materials and methods

This work will address the potential impact of sea lice infections from lice on the annual return of salmon, analyzing historical data from the returning salmon to Agdenes years 2012-2018, accounting for lice and biomass data for <1000 individuals annually in order to contribute to existing knowledge about the association between lice infestations on Atlantic salmon, timing for return and molting stage of *L. Salmonis*.
Further, such information is important as very little is known about survival of salmon lice during the sea migratory stage of salmon. The effect of sea lice on salmon population size, age- and size distribution will be evaluated.

Results and discussion

The main outcomes will be presented.

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ANTIVIRAL EFFECTS IN *L. vannamei* OF FEED ADDITIVE OF MICROENCAPSULATED PHENOLIC EXTRACTS OF *M. Umbellata*

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Introduction

The aim of the current study was to determine the stimulation of a microencapsulated *Malpighia umbellata* phenolic extract of the immune response of *Litopenaeus vannamei*, in laboratory conditions, against the white spot syndrome virus (WSSV). The phenolic composition and carotenoid contents of leaves, bark, and fruits of *M. umbellata*; the changes in the phenolic composition and contents of carotenoids and vitamin C of its fruits in different stages of maturity; and the antioxidant properties of its leaves, bark, and fruits were assesed. Leaves accumulated the highest levels of flavonoids (10.55 mg/g dry extract), tannins (21.16 mg/g dry extract), and carotenoids (424 63 µg/g dry tissue); whereas, the highest level of total phenolics was found in bark (47.12 mg/g dry extract). Twenty-two phenolics were characterized by high performance liquid chromatography with diode array detection (HPLC-DAD). Apigenin-7-O-glycosides, phenolic acids, and flavonols were predominant in leaves, bark, and fruits, respectively. Important chemical variations were found during fruit ripening. A three-days bioassay was carried out to evaluate the imunoestimulating effect of microencapsulated extract added to food, evaluating the genic expression of six antioxidant enzymes, forming part of the immune system of *L. vannamei*. The bioassay treatments were: I) control diet (Camaronina®), II) 0.1 mg of microcapsuled per gram of food, III) 0.5 mg of microcapsuled per gram of food, and IV) 1.0 mg of microcapsuled per gram of food. The semiquantitative expression of six immune system genes were carried out by RT-PCR. The addition of microencapsules containing the leaf extract of *M. umbellata* stimulated the expression of antioxidant enzyme genes, mainly at 48 h and especially the proPO gene (Fig. 1).

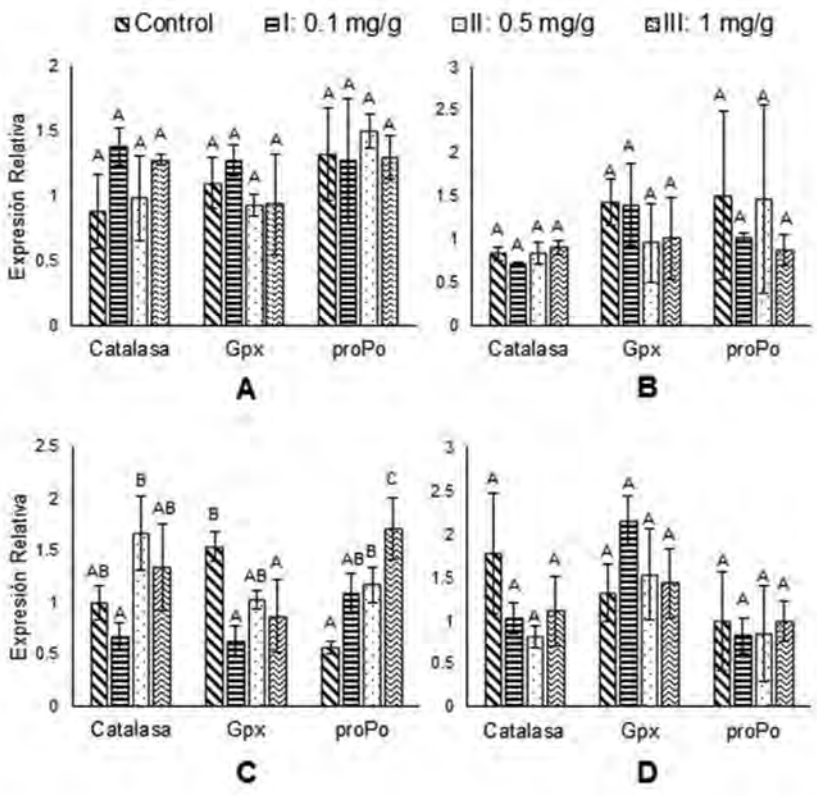

Fig. 1. Relative mRNA expression of SOD and lysozyme in hemocytes and, HSP70, HSP90, chymotrypsin, and trypsin in hepatopáncreas of *L. vannamei*. Values are mean ± SE (n = 9). Different superscript indicates significant differences (P<0.05).

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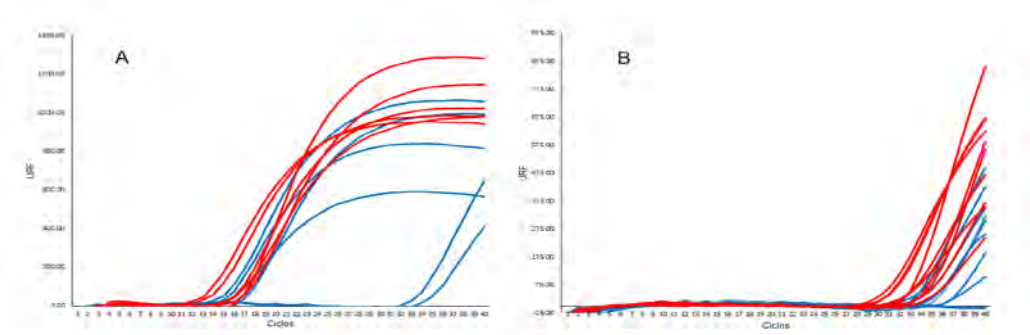

Fig. 2. Análisis de qPCR en la detección de SVMB en Litopenaeus vannamei a los 15 (A) y 30 (B) días de la alimentación con 1 mg g-1 de extracto microencapsulado foliar de Malpighia umbellata. Camarones control: rojo; tratamiento (1 mg de microencapsulado/g de alimento): azul

A second bioassay for 30 days revealed that the addition of microcapsules to food had no effect in the growth of shrimp. survival was significantly different between treatment and control. The shrimp fed the microcapsule diet remained alive during the 30 days of the bioassay, while the survival of the control shrimp decreased to 93.8 ± 5.57%. The addition of the microencapsulated phenolic extract in the feed revealed a positive effect on the immune response of *L. vannamei*, since in the control shrimp, higher expression of viral protein was found than in those fed microcapsules mixed in the feed. The addition of microcapsules to food significantly increased shrimp survival and significantly decreased the prevalence of white spot syndrome virus on *L. vannamei* (Fig 2.).

The 15-day infection of the bioassay revealed a survival of 80% in the shrimp fed microcapsules in the formulation of their diet, while the control showed a survival of 66%. Survival and prevalence of SVMB in shrimp fed microcapsules (1 mg g^{-1}) at 15 and 30 days of the bioassay (Table 1).

The addition of microencapsules containing leaf extract of *M. umbellata* stimulated the expression of antioxidant enzyme genes, mainly at 48 h and especially proPO. The addition of microencapsulates containing leaf extract of M. umbellata had no effect on the Shrimp Specific Growth Rate. On the contrary, it had a positive effect on the survival and prevalence of SVMB, mainly at 30 days of bioassay. *M. umbellata* represents an alternative for the prevention and treatment of SVMB of shrimp *L. vannamei* that can contribute to improve the culture and production of this crustacean.

LIVER TRANSCRIPTOME OF JUVENILE DIPLOID AND TRIPLOID ATLANTIC SALMON (*Salmo salar* L.)

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Introduction

Triploid fish are of interest to solve key issues for the sustainable development of Atlantic salmon (*Salmo salar* L.) farming in Norway and other producing countries, namely the impact of farmed escapees on wild stocks and pre-harvest sexual maturation. Despite this, uncertainties regarding the performance of cultured triploid stocks have hindered the widespread adoption of triploid salmon by the industry, excluding in Tasmania (Benfey, 2015). The liver is the main organ involved in fish metabolism and plays roles in detoxification and immunity with effects on fish health, welfare and growth. Previous work has focused on the molecular mechanisms occurring in the liver of diploid and triploid salmon in fish fed plant-based diets up to 24 weeks after start-feeding (Vera et al., 2017). In this study, we compared the liver transcriptome of diploid and triploid salmon fed a standard fishmeal commercial diet from fry stage to the completion of parr-smolt transformation.

Materials and methods

The detailed description of the experimental set up, fish and feed used in this experiment is reported in Peruzzi et al. (2018). Fish were reared following standard husbandry procedures and sampled at 21, 30 and 38 weeks post start-feeding (2370, 3010 and 3665 degree days, dd) corresponding to fry, parr and smolt stage, respectively. Transcriptome techniques involved the extraction (QIAGEN) and use of total RNA from fish liver for RNA-seq library preparation (NEBNext), next-generation sequencing on the NextSeq500 (Illumina), and bioinformatic analysis using TopHat2 for mapping and DESeq2 (Love et al., 2014) for differential expression (Odei et al., in preparation).

Results

The PCA (Principal Component Analysis) plot for sequenced data did not indicate clustering by ploidy; instead, the data clustered in fry, parr and smolt groups, with the exception of one smolt fish (Fig. 1). There were 34 DEGs (Differentially Expressed Genes) with an absolute fold-change > 2 and an adjusted *p*-value <0.05 for comparisons which define the ploidy effect. In contrast, comparisons between ontogeny stages from fry to smolt within each ploidy resulted in 5642 DEGs. GO (Gene Ontology) enrichment analysis for all DEGs returned no significantly enriched GO terms associated with ploidy while comparisons for ontogeny in each ploidy group showed significantly enriched GO terms within down-regulated DEGs only (Benjamini correction < 0.05).

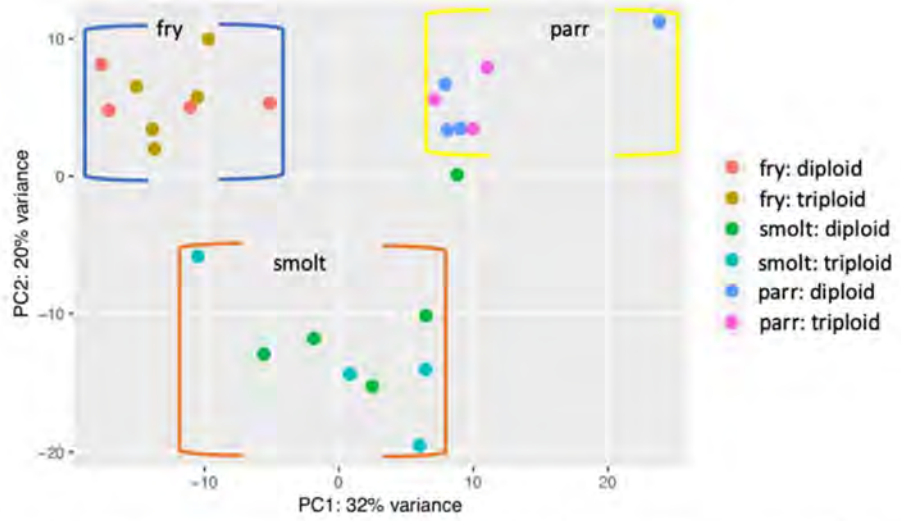

Figure 1. Principal Component Analysis (PCA) plot for all sample points. Individual samples (n=4-5 fish/ploidy/stage) of diploid and triploid individuals cluster by ontogeny stage (fry, parr and smolts) rather than ploidy.

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Discussion and conclusion

Findings from this study revealed a relatively small number of DEGs between ploidy groups, which suggests a close similarity in the liver transcriptome between diploid and triploid Atlantic salmon. In contrast, the effect of ontogeny assessed by comparing fry, parr and smolt showed very high liver transcriptome responses for both diploid and triploid fish. Similar functional categories identified for top DEGs for both ploidies contribute to parr-smolt transformation, while few top DEGs were ploidy-specific. The majority of significantly enriched biological processes for GO terms down regulated in fry compared to parr were related to nucleotide and energy metabolism, while immune system processes were significantly down regulated in parr when compared to smolts in both ploidies. The striking resemblance in GO terms for both ploidies is likely due to a phenomenon of genome dosage compensation, which may be involved in polyploidy regulation (Shrimpton et al., 2007).

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A MACHINE-LEARNING-BASED APPROACH TO FORECAST HARMFUL ALGAL BLOOMS

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Introduction

Harmful algal blooms (HABs) and the environmentally persistent toxins they produce affect virtually every coastal region. HABs are a natural phenomenon resulting from mass proliferation of phytoplankton in waterbodies and are generally caused by diatoms and dinoflagellates in seawater or cyanobacteria in freshwater (Sanseverino et al. 2016). These blooms can have devastating effects on aquaculture leading to fish kills in cage systems (Anderson and Rensel 2016) or the closure of shellfish growing sites (Hoagland and Scatasta 2006) resulting in economic losses in the millions of euros and hampering industry development. The causes and consequences of these blooms have spurred interest in developing a capacity for predicting HAB events facilitate public and private decision making.

The challenges of predicting HABs relate to the broad spatial and temporal scales involved in conjunction with the complex relationships between environmental drivers and varied algal species. These complexities have led to concern that forecasting HABs in dynamic ocean environments is not possible and is an area of ongoing research (Anderson and Rensel 2016). The most common approach defines algal cells as passive particles or tracers within a hydrodynamic model and simulates their fate and transport within a waterbody (e.g., Velo-Suárez *et al.*, 2010). More sophisticated approaches also consider the biological activity of the algae by coupling hydrodynamic models with biogeochemical submodules that consider advection, diffusion, and reaction of cells (e.g., McGillicuddy *et al.*, 2011). Current challenges in predicting HABs relate to developing regional models, the transferability of these models to other geographies, and the integration of real-time data with predictive models.

An alternative to physics-based modelling, is to leverage the large volumes of data collected by satellite and in-situ sensors to develop data-driven approaches. In this work, we developed a computationally lightweight machine-learning-based approach to make seven-day forecast of algal blooms (using Chlorophyll-*a* (Chl-*a*) as a proxy) and applied the model at 718 locations globally. The predictions demonstrated accuracies comparable to state-of-the-art models at a fraction of the computational cost, while the data-driven nature means the model can be easily applied at arbitrary locations (because the model predicts based on data not any parametrisation related to species or geography).

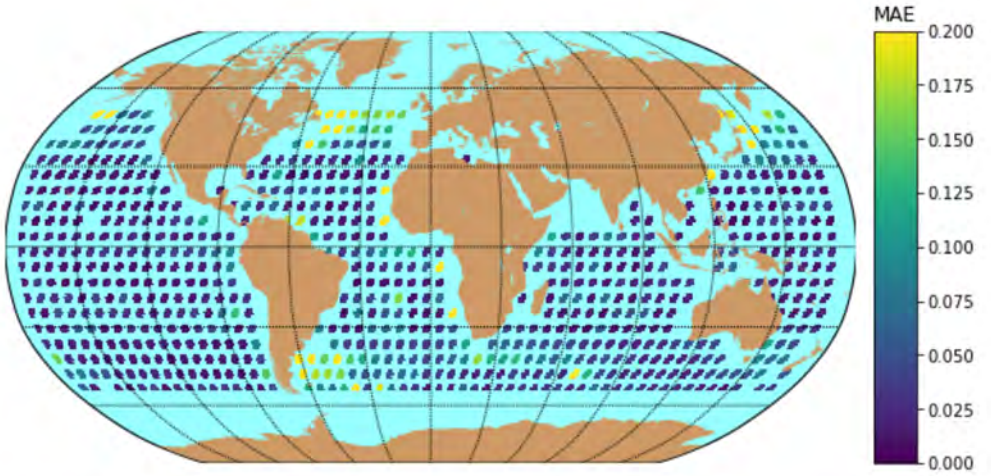

Figure 1: MAE between seven-day model forecasts of Chl-a and MODIS measurements at all global locations (mg/m^3).

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Methodology and Results

Training data for the machine learning models was 17 years' worth of Chl-*a* data from the MODIS instrument aboard the NASA Aqua satellite. Although global coverage can nominally be achieved every 1–2 days, the actual temporal resolution is reduced to 5–10 days because of cloud cover. A multilayer perceptron model was developed to learn Chl*a* forecasts based on: (a) MODIS-measured Chl-*a* concentrations, (b) sea surface temperatures (SST), and (c) light intensities (LI). An automated hyperparameter tuning exercise was undertaken at an initial mid-Atlantic location using data from 2002 through 2018. The optimised MLP network consisted of eight hidden layers and 32 nodes, which closely captured measured dynamics of Chl-*a*.

Model performance was evaluated at 718 points equally distributed around the globe. For each location, the model was trained (using the same set of hyperparameters) from data at that coordinate and validated against the 10% of data held back (approximately 19 months). For each point, mean-average-error (MAE) was computed by comparing the seven-day forecast against measurements. Figure 1 presents the MAE for each location. The machine-learning approach adapted readily to the notably different dynamics across the geographically distinct locations. Average MAE was less than 0.12 mg/m^3 and, notably, the data-driven approach produced consistently accurate results for all locations. The applicability of the approach can be of significant value toward managing and responding to HABs in coastal and freshwater sites

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TOOLS FOR NEW AND FLEXIBLE APPROACHES FOR AQUACULTURE LICENSING AND REGULATION

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Introduction

While the annual consumption of fish is increasing, overall EU production is considerably lower than global volumes. Aquaculture is one of the pillars of the EU's Blue Growth strategy and its development forms part of the Europe 2020 strategy. The stagnated growth of the EU aquaculture industry is well-documented and the consenting process has been highlighted as a key bottleneck to growth. There is a need for improvements to the consenting system to facilitate growth within the sector. Key to growth and development is a reduction of administrative burdens, costs and uncertainties related to the approval of production licences. Europe plans to help increase the sector's production and competitiveness and key priorities to facilitate the sustainable development of aquaculture include simplifying administrative procedures; ensuring access to space; enhancing competitiveness; and promoting a level playing field for operators

The H2020 project TAPAS, Tools for Assessment of Planning and Aquaculture Sustainability (http://tapas-h2020.eu/), aims to support EU member states to establish a coherent and efficient regulatory framework to support sustainable growth of the aquaculture sector in line with the EU's Blue Growth Strategy. The project, aims to establish new strategies and models for sustainable growth in the aquaculture industry. With the intention of creating new, cost-efficient, management tools and flexible approaches for the European aquaculture secto .

Methods

As part of this process a critical review of existing legislation and practises across European countries and sectors and analysis to identify the perceived impacts and bottlenecks of the current regulatory frameworks, was carried out. Extensive consultation with stakeholders and regulators was conducted to identify the key bottlenecks and issues in the licensing regulation across jurisdictions. These consultations and responses were categorised to identify areas of commonality and key areas. Recommendations were collated that could lead to efficiencies and improvements in licensing and regulation.

Results and Discussion

The results of the consultation process identified key issues and bottlenecks in the licensing process. Based on the collected data, research was conducted to resolve issues through the suggestion of novel approaches and the creation of tools to improve efficiency in the regulatory and licensing process. The flexible approaches and tools were ground-truthed during continuous consultation with stakeholders and industry to ensure acceptability and utility of the approaches. This presentation outlines the results from the final stakeholder input and makes concluding recommendations on the establishment of new, flexible approaches and tools for aquaculture licensing and regulatory policy.

TOXICITY STUDY ON *Allium cepa* LINN. BULB IN HEALTHY *clarias gariepinus* (BURCHELL, 1822) SUB-ADULT

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Introduction

Allium cepa Linn. (Onion) is widely researched for its biological activities in animal health management and had been well documented to have a wide range of therapeutic potentials (Griffith et al., 2002; Augusti, 1996) but little has been reported on its toxicity potential on experimental animals because of the belief that it is natural and therefore safe (King et al., 2009) . Hence, this study assessed the toxic potentials of *A. cepa* by exposing healthy *clarias gariepinus* sub-adults to varying concentrations (200, 150, 100, 50 and 25 g/Kg) of *A. cepa* via diet and bath (5, 3, 1.5, 0.7 and 0.4 g/L) for two weeks.

Materials and methods

The fish were sacrificed and the liver and kidneys collected, processed and examined for histopathological changes. Proximate analysis of the bulb, qualitative and quantitative phytochemistry was determined using standard methods. Brine Shrimp lethality assay (BSLA) and mean lethal dose of the onion on experimental fish was investigated using Finney linear regression.

Results

The protein content of the onion was 8.48% while that of carbohydrate was 5.94%. Saponins, tannins, phenols, flavonoids and alkaloids were found in trace amount. The LC_{50} of the onion extract was between 0. 51mg and 0.64mg in the BSLA while mean LC_{50} for experimental fish exposed via diet and bath were 447.1g/kg and 12.2g/l fish. Histopathological changes observed in the liver were vacuolar degeneration of the hepatocytes and congestion of the central vein (Plate 1), while necrosis of the epithelial cells and haemosiderosis (Plate 2) were the most consistent findings in the kidney, especially in fish exposed via bath.

Discussion and conclusion

Toxic effect of the fresh onion bulb was observed in the livers and kidneys of fish treated with different concentrations of *A. cepa* bulb in this study. The BSLA categorized the onion bulb as a plant with mild toxicity which was confirmed by the various degeneration stages observed in the livers and kidneys of the experimental fish. This finding is in agreement with previous reports by various authors on the ability of the *Allium* species to cause a clinical condition referred to as 'toxicosis'. This could be due to the presence of n-propyl disulphide, S-methyl and S-propenyl cysteine sulphoxides in the onion bulb (Parton, 2000). These compounds may be broken down into various sulphides with hemolytic capability (Parton, 2000). Moreso, secondary renal failure may result due to hemolytic crisis (Al-Salahy, 2002). *Allium cepa* bulb though have high therapeutic ability could be toxic to fish when administered via feed and/or by bathing at higher concentrations

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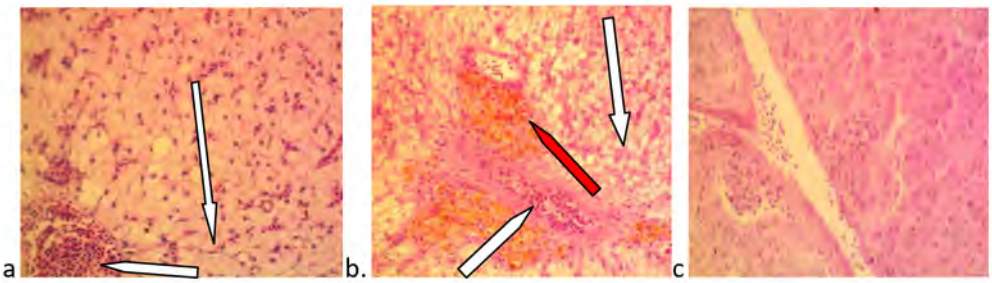

Plate 1: Liver sections in both routes of administration and control

a. Section of the liver of fish treated orally with *A. cepa* showing severe vacuolar degeneration of hepatocytes (arrow) and congestion of sinusoid and central vein (arrow head) b. T8 Section of the liver of fish treated through bath with *A. cepa* showing vacuolar degeneration (arrow) and pigmentation of the hepatocytes (red arrow head) and congestion of the central vein (arrow head) c Section of the liver of fish not treated with *A. cepa* appearing apparently normal (x400; H 7 E).

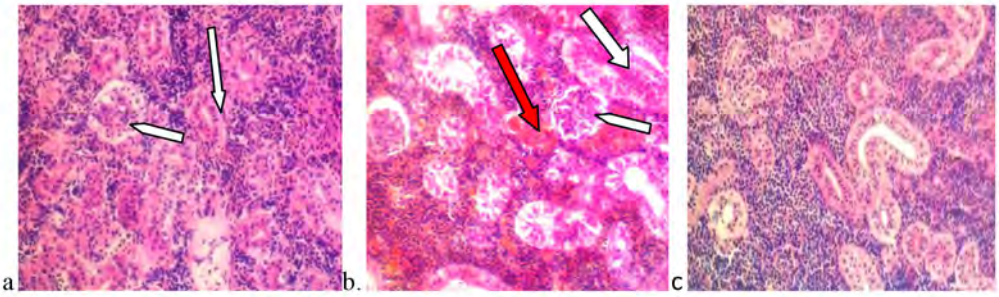

Plate 2: Comparative kidney sections in both routes of administration and control

a. Treatment 3(100g/Kg) Section of the kidney of fish treated with *A. cepa* via diet showing degenerations and necrosis of tubular (arrow) and glomerular epithelial cells (arrow head) b. Treatment 6 (5g/L) Section of the kidney of fish treated with *A. cepa* via bath showed degeneration and necrosis of tubular (white arrow) and glomerular (red arrow) epithelial cells (arrow) with haemochromatosis (white arrow head) c. Section of the kidney of fish not treated with *A. cepa* appearing apparently normal (x400; H & E)

IDENTIFICATION OF EGG SPECIFIC TRANSCRIPTS IN FERTILIZED EGGS OF JAPANESE EEL (*Anguilla japonica*)

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Introduction

Survival rates of *Anguilla japonica* embryos and larvae are extremely low and still far from industrial scale, although artificial production of glass eel was succeeded in some laboratories (Tanaka et al., 2003; Kim et al., 2013). This is largely due to the poor quality of eggs. However, assessment of egg quality in this species is not properly established. Egg specific transcripts, particularly maternal origin, could be placed on the checklist for the quality assessment. In this study, we identified transcripts specifically expressed in fertilized eel eggs by RNA-se

Materials and Methods

Total RNA was extracted from fertilized eel eggs produced by artificial maturation and fertilization, and also from tissues (brain, testis, ovary, liver, kidney, muscle) of immature eels (TL 64±3 cm, BW 445.3±55.1 g) using TRIzol® Reagent (ambion, USA). RIN values for all samples were higher than 8.1 (Bioanalyzer, Agilent). RNA-seq was carried out according to the manufacturer's instructions (Illumina Truseq stranded mRNA library prep kit + Illumina cluster generation system + Illumina Novaseq 6000 sequencing system). To obtain quantification scores for eel transcripts in all tissues, TPM (transcripts per kilobase million) values were calculated. This value was used to create a heatmap and identify egg specific transcripts. Identified egg specific genes was confirmed by the conventiona T-PCR.

Results

Transcripts obtained from RNA-seq were mapped to previously constructed reference transcriptome for this species (30,847 transcripts; 16,720 genes). TPM values for these genes were used to calculate a correlation matrix and produce a heatmap (Fig. 1). This hierarchical clustering helped us identify 10 most highly expressed genes in fertilized eggs of eels (Fig. 2).

Discussion

Identified top 10 genes include genes for *nadh dehydrogenase, hnrpa01, cyclin-b1, myristoylated alanine-rich c-kinase, upf0054 protein c2orf43-like protein, sal-like protein 4, nynrin-like protein, pcna-interacting partner, cytochrome c* and *lamin-b1*. Fertilized eggs and tissue of eel was investigated by real-time PCR (RT-qPCR). Tissue specificity of these genes were also confirmed by RT-PCR. All of these genes are highly likely to be maternal origin because the eggs were sampled far earlier than MBT (mid-blastula transition).

Many of these genes are known to have an important role during oocyte growth and early development (*Sal-like protein 4, cyclin-b1 and lamin-b1* / Wang et al., 2011; Deborah et al., 1993; Izumi et al., 2016). This implies that the identified egg specific genes might be associated with egg quality of this species. Further study is required to relate these transcripts to hatching and survival of fertilized eggs.

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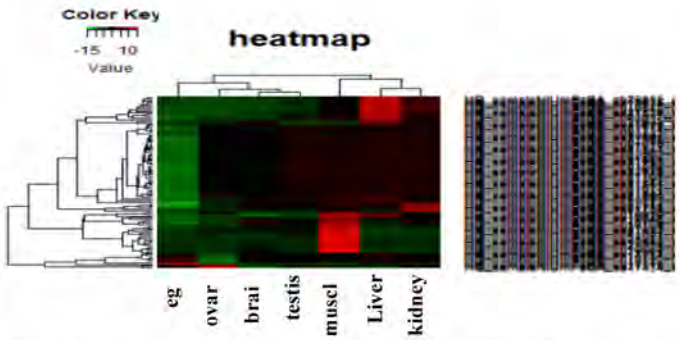

Fig. 1. Hierachical clustering of the top 100 genes expressed in different tissues of the eel *Anguilla japonica*.

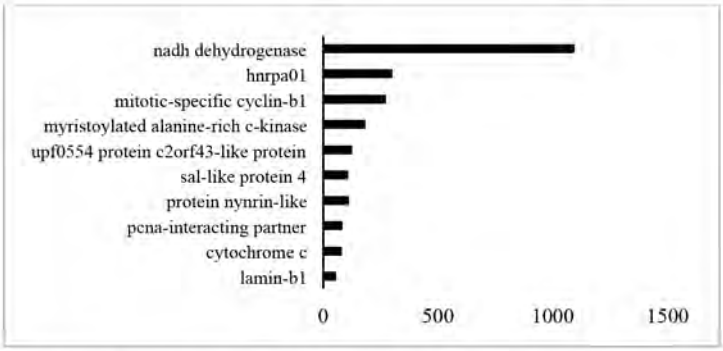

Fig. 2. Comparison of expression levels (TPM) of top 10 genes by RNA-seq in fertilized eggs of eel *Anguilla japonica*.

EFFICACY OF *Ocimum gratissimum* (BASAL SCENT LEAF) POWDER AS ANAESTHETIC AND ITS EFFECT ON THE HAEMATOLOGY OF *Clarias gariepinus* JUVENILES

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Introduction

Clarias gariepinus is the most cultured fish in nigeria due to it wide acceptability, commanding market value, high tolerance to diseases and ability to withstand wide changes in water quality. the practice of aquaculture has increased tremendiously in recent years, marking it the the fastest food producing venture in the world (FAO, 2018). Intensive nature of aquaculture have subjected farmed fish to a number of stressors due to handling proceedures and transportation from hatcheries to final stages (Akinrotimi *et al* 2015). These stressors have most often been responsible for high mortalities recorded, hence hindering the growth of fish farming. Anaesthetics are used in aquaculture, fisheries and biological researches, as a way to minimize hypermotility during handling and transportation to reduce stress and mortality (King *et al* 2005; Ross Ross 2008; Okey *et al* 2013). Previous studies have reported the use of some plant materials to anaesthetized various fish species including *C. gariepinus* (Olufayo and Ojo 2018; Ademale *et al* 2017; Adebayo and Olufayo 2017; Okey *et al* 2018). This studies aimed to investigates the efficacy of *Ocimum gratissimum* as anaethetic and determine itn some haematological parameters of *C. gariepinus* juveniles.

Materials and methods

Clarias gariepinus juveniles of mean weight and length (24.50±4.25g and 27.60±6.75cm) were procured from University of Calabar Fish Farm and transported to the Department of Fisheries and Aquatic Science Cross River University of Technology (CRUTECH). Fresh leafs of *O. gratissimum* were obtained from within the university campus, identified in Forestry Department, air dry for 5 days and blended to powder. The experimental treatment were prepared from a stock solution of 2g in 10 litres of water (200mg/l) into five concentrations (0, 50, 100, 150 and 200mg/l) in 20 litres of water. Stages of induction and recovery were monitored and recorded using a stop watch as descibed by Coyle *et al* (2004). Blood was ollected by severing the caudal peduncle into Ethylene Diamine Tetra acetic Acids (EDTA) for the analysis of various hemaetological parameters using standard methods.

Results

The result revealed that *O. gratissimum* caused anaesthesia which was concentration dependent. Induction time (min) reduced with increase in concentration while recovery increases as induction time reduces (Table 1 and Figure 1).

Discussion and Conclusion.

The observed behavioural changes including initial hypermotility, hyperventilation, loss of equilibrium and no reaction to handling suggest the fish were immobilised (anaesthetized) by the plant material. This was in line with the reports of several researchers who used anaesthetics on fish during handling and transportation (Agokei and Adebisi 2010; Velisek *et al*. 2011; Simeos *et al*. 2011). Slight changes in the haematological parameters reported in this study corroborates with the studies of many other researchers who have use plant materials as anaesthetic (King *et al* 2005; Sudagara *et al* 2009; Adebayo and Olufayo 2017). Minimal changes in RBC, Hb and PCV recorded on fish exposed to 150mg/l which were not different ($P > 0.05$) from the control but induces anaesthesia in 6.22 mins shows it is an ideal concentration. However, Sudagara *et al* (2009) reported a 48 hours reversal to the haematological paramters of fish exposed to clove powder. Further research will be required to investigate the effects of *Ocimum* of the biochemical paramters of *C. gariepinus*.

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Table 1: Induction and recovery time (min) of of *Clarias gariepinus* juveniles' exposure to *Ocimum gratissimum* powder anaesthetic for 30min.

Concentration (mg/l)	Induction		Recovery	
	Induction 1	Induction 2	Recovery 1	Recovery 2
0	-	-	-	-
50	26.61 ± 2.34	-	-	2.82 ± 2.44
100	11.12 ± 1.54	15.26 ± 0.81	1.26 ± 1.38	4.43 ± 0.36
150	4.16 ± 2.36	6.08 ± 0.34	2.35 ± 2.14	6.76 ± 2.18
200	1.24 ± 4.56	3.22 ± 1.26	4.81 ± 0.19	11.67 ± 1.44

Mean with the same superscript are not significantly different at p< 0.05, Induction1 (loss of equilibrium), induction2 (deep anaethesia), recovery 1 (regain equilibrium), recovery2 (normal swimming).

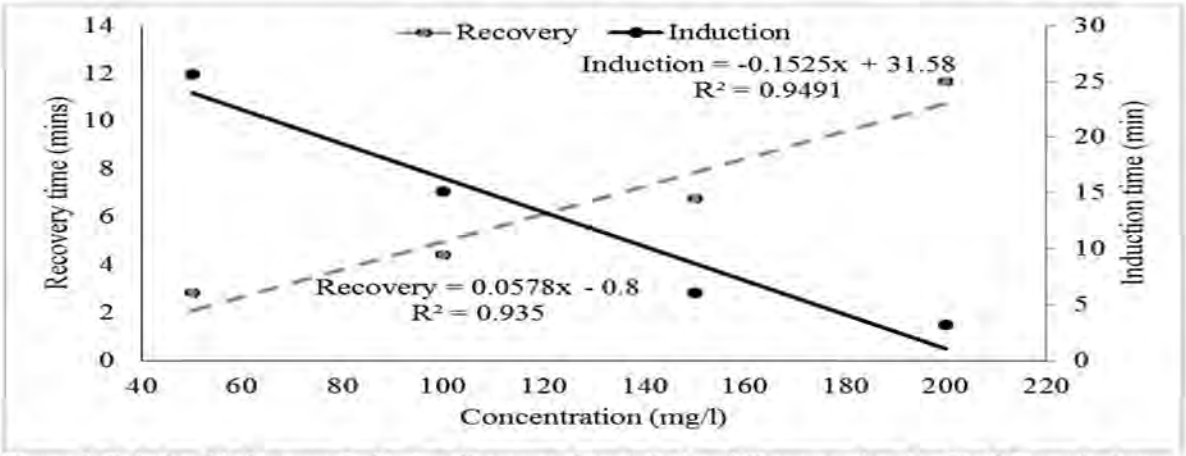

Figure 1: Relationship between Induction, Recovery time (mins) and Concentration (mg/l) of *O. gratissimum*

Haematological parameters showed some slight changes exspecially at higher concentration however some were not significant (p> 0.05). the mean values of red blood cells, haemoglobin, pack cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, basophile, eosinophile and neutrphile were decreasing with increased concentration of clove powder. Others such as white blood cells, platelets and lymphocytes increased with concentration (Tables 2 and 3). Fish exposed to 150mg/l cause induction shows that RBC, Hb and PCV were not significantly (P > 0.05) from those exposed to 50mg/l which were however not different from the control (0.0mg/).

Table 2: The mean values of selected haematological indices of *Clarias gariepinus* juveniles' exposure to *Ocimum gratissimum* powder anaesthetic for 30min.

Conc. (mg/l)	Haematological parameter							
	RBC (10^{12} cells/L)	WBC (10^{9}cells/L)	Hb (g/l)	Plt	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/l)
0.00	8.34 ± 1.05^{a}	30.95 ± 1.79^{e}	10.34 ± 0.70^{a}	58.77 ± 1.07^{c}	38.05 ± 3.20^{a}	$66.30\pm 1.92c$	21.17 ± 1.92^{c}	30.84 ± 1.28^{a}
50.00	7.06 ± 0.08^{ab}	33.12 ± 4.30^{c}	9.26 ± 0.34^{ab}	61.82 ± 1.71^{bc}	33.97 ± 1.58^{ab}	$67.88\pm2.49bc$	23.38 ± 0.58^{bc}	28.38 ± 1.98^{ab}
100.00	6.82 ± 0.70^{ab}	42.47 ± 0.87^{b}	8.86 ± 0.19^{bc}	70.63 ± 1.46^{b}	30.32 ± 1.27^{bc}	78.69 ± 5.73^{c}	24.60 ± 0.65^{b}	27.87 ± 2.22^{a}
150.00	5.93 ± 0.90^{bc}	50.07 ± 1.41^{a}	7.99 ± 0.54^{bc}	77.00 ± 3.05^{ab}	28.64 ± 0.34^{bc}	93.76 ± 4.80^{a}	26.44 ± 0.82^{ab}	26.59 ± 0.78^{a}
200.00	4.80 ± 0.33^{c}	52.87 ± 2.36^{a}	7.31 ± 0.80^{c}	98.14 ± 4.93^{a}	26.17 ± 1.34^{c}	102.52 ± 4.53^{a}	28.70 ± 1.28^{a}	25.31 ± 1.08^{c}

Mean with the same superscript are not significantly different at p< 0.05, Conc.= concentration, PCV = packed cell volume , RBC= red blood cell Hb = haemoglobin, MCV =mean cell volume MCH= mean cell haemoglobin MCHC= mean haemoglobin concentration, WBC =white blood cell, Plt = platelet..

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Table 3: The mean values of selected Differential white blood cell counts of *Clarias gariepinus* juveniles' exposure to *Ocimum gratissimum* powder anaesthetic for 30min.

Conc. (mg/l)	Differential white blood cell count (%)				
	Neut	Lymp	Baso	Mono	Eosin
0	14.71 ± 1.92^{a}	56.25 ± 1.40^{b}	4.88 ± 0.20^{a}	5.99 ± 0.32^{b}	8.07 ± 0.17^{a}
50	13.74 ± 1.48^{a}	58.02 ± 1.49^{ab}	4.74 ± 0.36^{a}	7.01 ± 0.14^{b}	7.66 ± 0.44^{ab}
100	12.18 ± 0.60^{bc}	59.40 ± 1.78^{ab}	4.31 ± 0.31^{a}	7.92 ± 0.53^{b}	6.61 ± 0.36^{ab}
150	12.11 ± 0.83^{bc}	59.82 ± 1.42^{ab}	3.95 ± 0.10^{ab}	9.08 ± 0.05^{ab}	5.98 ± 0.48^{ab}
200	10.79 ± 0.31^{c}	65.44 ± 1.91^{a}	3.07 ± 0.77^{b}	10.27 ± 0.85^{a}	4.14 ± 0.42^{b}

Mean with the same superscript are not significantly different at $p< 0.05$, Conc.= concentration, Neut = neutrophil Lymp= lymphocytes Baso = basophil, Mono = monophil, Eosin = eosinophil,

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GOVERNANCE AND PERCEIVED POWER IN THE SALMON VALUE CHAIN

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Introduction

The functioning and performance of food value chains are affected by the way stakeholders are embedded in the chains, by the coordination modes, and by the type of governance (Carbone, 2017). An industry can have various types of governance structures in a given location or across varying segments of the chain and these can develop with time. The global value chains (GVC) approach (Gereffi *et al*., 2005) provides a theoretical framework for understanding the governance of commodity production. The governance types can range from market, relational, modular, captive to hierarchy governance forms, which are characterized based on e.g. the degree of complexity of inter-firm transactions and the decision making process of the lead firm.

This research is part of the VALUMICS project with the overall aim to develop tools for decision makers to assess the impacts of policy in food value chains. The main goal of this study was to provide a better understanding of the functioning of the salmon value chain and identify practices and structural elements where fairness is or could be an issue. The focus is on assessing the perception of actors and stakeholders in the salmon value chain on power asymmetries and how power is exercised, the fair value distribution in the chain, the role of government and industry oversight groups in the power dynamics and the transparency and trust in the chain that would support fairness.

Methods

A governance framework for global value chains according to Gereffi *et al.* (2005), was applied to identify the characteristics of the governance forms along a buyer-supplier power continuum from farming to final product in retail. Six interviews were conducted with business experts from Norway, UK and Iceland in the salmon chain according to a semi structured interview guideline. The interviews lasted for approx. one hour, they were recorded, transcribed and anonymised and further input has been gained from discussions with stakeholders in workshops conferences and via teleconferences.

Results

The salmon value chain is producer driven which is contrary to agriculture farms who have a weak position in the value chain and are susceptible to be captive suppliers. The large integrated aquaculture companies are competing on the global market and have a strong bargaining position against the concentrated supermarket chains who are the lead firms in the value chain. The inter-firm relations of producers and their buyers is characterized by free market exchanges where products are sold on the spot market, however, there is a trend of long term contracts in particular between large integrated companies and retail or large secondary processors. "Market" governance is thus applicable for salmon producers and primary processors who are selling commodity products on the spot market, where transactions are easily codified and suppliers are capable of making products based on technical standards and there is no input from buyers. The specifications are based on industry standards and the complexity of information exchanged is low, and therefore transactions need no explicit coordination. The standards facilitate transactions since information about the products and their specifications according to best aquaculture practices can be codified. The essential point is that the costs of switching to new partners are low for both parties. This is typical for free market exchanges where buyers respond to specifications and prices set by sellers (Gereffi *et al.,* 2005). The linkages between secondary processors and retail can be characterized as modular or relational, for example, when producing a differentiated branded product. "Relational" linkages emerge if a product specification is hard to codify, transactions are complex, and supplier capabilities are high, where trust and reputation are built up over time. "Modular" linkages are distinctive in that they are based on codified knowledge rather than on prices, and suppliers take full responsibility of the process technology. Secondary processors are stuck in the middle of the chain, and are reliant on farmers / primary processors or wholesalers for raw materials where they buy on the spot market. They are vulnerable when prices on the spot market are high and have little influence to negotiate the price with retailers, who normally operate at a fixed ma gin.

A “Hierarchy” governance is characterized by high incentives to centralize control of strategic investments and this is typical for the vertically integrated large companies of the salmon value chain who mainly target high end retailers. The increasing number of requirements from large retail chains has been a main driver for vertical and horizontal integration of aquaculture companies as well as the food industry globally. The objective has been to increase the negotiation power towards the retailers with respect to price, product, and volume.

The salmon value chain governance is influenced by network governance, contracting and informal relationships and the governance structure is perhaps best described as a “Hybrid” form. The large integrated firms and their subsidiaries constitute a network of firms that organize their transactions through a combination of different arrangements. This is characteristic of plural hybrid forms whereby a firm (or a network of firms) could partially produce in-house (or distribute through its own outlets), outsource other parts of its activity through contracts with specific firms, and possibly use spot markets, all at the same time (Menard, 2017).

Discussion and Conclusion

Price is considered to be one of the most important factors that will increase a supplier’s perceptions of fairness. As far as suppliers are concerned, the impact of price on a long-lasting supply chain is complicated due to the complexity of the relationship, the cooperative nature of relationships, and various market circumstances. In agricultural supply chains, suppliers lack pricing power because of their inferior position. Therefore, suppliers will pay more attention to retailers’ procurement pricing criteria, which involves examining product quality, purchase quantity, geographic position, and relationship. It can be argued that the impact of price satisfaction on fairness perception is not only related to whether suppliers can fairly gain profits but is also connected to the endurance and stability of the cooperative relationship in question. Therefore, suppliers are sensitive to prices and high levels of price satisfaction will effectively enhance the cooperative stability of the supply chain in question. The interviewees with agents across the salmon value chain suggest that producers are satisfied with the value distribution in particular with respect to the current high prices of salmon, while secondary producers and feed producers are less content.

Acknowledgements

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EFFECT OF PROTEIN SOURCE AND PROTEIN/CARBOHYDRATE RATIO ON APPETITE REGULATION IN GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Optimization of feed efficiency and growth are main goals in fish production. Thus, for sustainable growth of aquaculture it is of outmost importance to understand the processes that regulate feed intake (FI) in fish. It is well known that diet composition affects FI; hence, it is important to increase knowledge on the physiological effects of dietary replacement of fishmeal (FM) by plant-feedstuffs (PF), or protein (P) by carbohydrates (CH). Several studies linked FI with endocrine control and metabolic pathways in different fish species (Ronnestad et al. 2017). Nonetheless, little is known about appetite regulation in gilthead seabream (*Sparus aurata*) and the respective link with diet composition. Thus, the present study aimed to obtain an insight on how nutritional parameters such as P/CH ratio and protein sources (FM vs. PF) affect appetite regulation mechanisms in seabream.

Materials and Methods

Gilthead seabream (140g) were fed four isolipidic (18% lipids) diets with two P/CH ratios (P50/CH10 or P40/CH20) and protein sources (FM or a mixture of 20% FM/80% PF), respectively named FMP50/CH10, FMP40/CH20 and PFP50/CH10, PFP40/CH20. Each diet was fed to triplicate groups of fish twice daily until visual satiation, 6 days a week, during 6 weeks. At the end of the trial, fish were bulk-weighed after 1 day of feed deprivation. Three fish per tank were also sampled 5 hours after the morning meal for collection of brain, intestine, liver, and stomach for gene expression analysis. Quantitative real-time polymerase chain reaction (RT-qPCR) was performed according to Salmerón et al. (2015) to assess the expression of genes involved in growth and appetite regulation. Statistical analysis was carried out by two-way ANOVA, using a probability level of $p<0.05$ for rejection of the null hypothesis. In case of interaction, one-way ANOVA was performed for protein source within P/CH ratio and for P/CH ratio within protein source.

Results

Fish fed diet FMP40/CH20 presented lower final body weight (FBW) than fish fed diet FMP50/CH10. Feed efficiency (FE) was higher in fish fed diet P50/CH10, independently of protein source. Gene expression of leptin receptor (LepR) in brain, cholecystokinin (CCK) in intestine, and growth hormone receptor (GHR II) in liver were influenced by P/CH ratio. LepR expression was higher in fish fed P40/CH20 diets, while for CCK, and GHR II the opposite was observed. Insulin-like growth factor I (IGF-I) expression was higher in fish fed FMP50/CH10 than PFP50/CH10, and within the FM diets it was higher in diet P50/CH10. The remaining analyzed parameters were not affected by dietary composition. These included FI; cocaine-amphetamine-related transcript (CART), corticotropin-releasing hormone (qCRH), ghrelin receptor A (GhrR A), leptin, and neuropeptide Y (NPY) in brain; ghrelin receptor B (GhrR B), and leptin in liver; and ghrelin (Ghr) in stomach. Undetectable levels of expression of the following genes was detected under the present experimental conditions: Ghr and GhrR A in intestine and liver; GhrR B in brain; and leptin in intestine and stomach.

Conclusions

P/CH ratio had a stronger effect than PS on the analyzed parameters. Despite of the observed modulation in the expression of appetite regulatory genes, these effects were not reflected in differences in FI. From a commercial point of view, it is important to observe that growth, FE, FI, and appetite regulation related genes expression were not affected by the PF dietary incorporation.

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Table I. Growth, feed intake and efficiency, and growth and appetite regulation related gene expression of gilthead seabream fed the experimental diets.

P source (PS)	FM			PF			Two-way ANOVA		
P/CH ratio	P50/CH10	P40/CH20	SEM	P50/CH10	P40/CH20	SEM	PS	P/CH	I
Growth, feed intake and efficiency									
FBW	217.4^{b}	195.9^{a}	4.6	205.0	206.9	3.5	ns	ns	*
FI	13.68	12.19	0.4	12.97	14.13	0.6	ns	ns	ns
FE	0.77	0.66	0.02	0.71	0.66	0.02	ns	**	ns
Normalized expression (ΔΔCq) from growth and appetite regulation genes									
Brain									
CART	0.00009	0.00163	0.0004	0.00085	0.00037	0.0002	ns	ns	ns
qCRH	0.0067	0.0108	0.002	0.0061	0.0108	0.003	ns	ns	ns
GhrR A	0.00005	0.00007	0.00001	0.00006	0.00007	0.00001	ns	ns	ns
Leptin	0.00003	0.00002	0.00001	0.00002	0.00002	0.00000	ns	ns	ns
LepR	0.00008	0.00015	0.00002	0.00008	0.00015	0.00002	ns	*	ns
NPY	0.0368	0.0629	0.0143	0.0710	0.1286	0.0274	ns	ns	ns
Intestine									
CCK	0.3794	0.2205	0.0346	0.3416	0.2957	0.0373	ns	*	ns
Liver									
GhrR B	7.8E-08	6.1E-08	1.3E-08	3.8E-08	5.3E-08	7.6E-09	ns	ns	ns
GHR II	0.00084	0.00058	0.0001	0.00058	0.00051	0.0000	**	**	ns
IGF-I	0.0389^{Bb}	0.0223^{a}	0.002	0.0304^{A}	0.0306	0.002	ns	**	**
Leptin	0.00031	0.00017	0.00004	0.00018	0.00028	0.00004	ns	ns	ns
Stomach									
Ghr	0.5972	0.5793	0.06	0.7356	0.8077	0.06	ns	ns	ns

Values presented as means (n=3) and pooled standard error of the mean (SEM). I: interaction. Two-way ANOVA: ns: not significant; *P ≤ 0.05; **P ≤ 0.01. Different lower-case letters denote for significant differences between P/CH ratio, upper case letters denote for significant differences between protein sources (P<0.05).

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FISH-GUT *Bacillus* SPP. AS POWERFUL ANTAGONISTS OF BACTERIAL AQUACULTURE FISH DISEASES

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The aquaculture industry is the world's fastest-growing food protein producer. It is indispensable to satisfy the world's fish demand, being responsible for 50% of global seafood consumption. However, aquaculture development and expansion is constantly challenged by bacterial disease outbreaks, a major constraint to the economic profitability of the industry according to the United Nations. *Aeromonas* spp., *Vibrio* spp., *Photobacterium* spp., *Tenacibaculum* spp., and E*dwardsiella* spp. are among the most common bacterial agents affecting marine aquaculture fish. Although antibiotics have been extensively used as prophylactic and therapeutic agents to treat bacterial infections, the selective pressure created by their misuse has contributed to the development and spread of antibiotic-resistant bacteria, posing a serious threat to public health. In a post-antibiotic era, where decreasing efficacy of antibiotics might turn minor infections into global problems, it is urgent to find new and natural alternatives that assure an advanced and integrated health care for humans, animals, and the environment. One promising strategy is the use of probiotics, "live organisms which when administrated in the adequate amounts can confer a health benefit on the host". *Bacillus* spp. have been recognized as attractive probiotics for aquaculture due to their endospore-forming nature, which represents an advantage for industrial applications. Moreover, *Bacillus* spp. are known for the production of Natural Antimicrobial Compounds (NACs) antagonistic of the growth, biofilm formation, and communication (quorum-sensing) of several Gram+ and Gram- bacteria. Harnessing the fish-gut microbial potential, we aimed to isolate and characterize different *Bacillus* spp. from the gut of aquaculture fish, capable of producing NACs active against fish bacterial diseases

For that purpose, *Sparus aurata*, *Diplodus sargus*, and *Dicentrarchus labrax* were fed with the same commercial diet for 6 weeks and their heat-treated intestinal contents were used to obtain the gut spore-forming community. All isolates were screened for NACs production against major bacterial pathogens, including *Aeromonas hydrophila*, *A. salmonicida*, *A. veronii*, *A. bivalvium*, *Vibrio anguillarum*, *V. harveyi*, *V. parahaemolyticus*, *V. vulnificus*, *Photobacterium damselae* subsp. *damselae*, *Ph. damselae* subsp. *piscicida*, *Tenacibaculum maritimum*, *Edwardsiella tarda*, and *Shigella sonnei*. The NACs production by *Bacillus* spp. was accessed through different antagonistic assays: (i) colony-overlay assay, to screen the entire collection for NACs production; (ii) well-diffusion and 96 microplate assays, to evaluate NACs extracellular nature; (iii) anti-biofilm assay, to evaluate the potential of extracellular NACs in reducing biofilm formation, and (iv) anti-quorum-sensing (QS) assay, to access NACs ability to interfere with acyl-homoserine-lactone signals used in QS. Significance of inhibition was evaluated by repeated measures ANOVA or by one-way ANOVA. Sporeformers with the most promising characteristics were identified by 16S rRN gene sequencing.

A total of 176 isolates representing different colony morphologies and samples were selected. Spore production was confirmed by phase-contrast microscopy revealing 172 isolates as producers of endospores with different sizes and shapes. Screening for NACs production revealed that 52% displayed antimicrobial activity against at least one pathogen tested. Using the colony-overlay assay, we selected the 8 most promising isolates based on the size of the inhibitory halos produced. These 8 isolates were capable of inhibiting the bacterial growth of *Aeromonas* spp., *Vibrio* spp., *Photobacterium* spp., *Tenacibaculum* sp., or *Edwardsiella* sp. By characterizing the localization of the inhibitory molecules, we further observed that cell-free supernatants of 3 isolates (identified as *B. subtilis* by 16S rRNA sequencing) inhibited the growth of all pathogens tested, except for *A. salmonicida*. Additionally, the extracellular NACs were capable of interfering with

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biofilm formation of *A. salmonicida*, *A. hydrophila*, *V. anguillarum*, *V. parahaemolyticus*, *Ph. damselae* subsp. *damselae*, *T. maritimum*, and *S. sonnei.* Although the extracellular NACs were not capable of interfering with the growth of *A. salmonicida* they significantly decreased its biofilm formation. Moreover, the 3 isolates produced extracellular NACs capable of interfering with acyl-homoserine-lactone signals, used in Gram- bacterial communication. Further, the 3 isolates were sensitive to all antibiotic classes demanded by the European Food Safety Authority (EFSA) as mandatory to comply with the minimum safety requirements to be considered as probiotics. Based on these *in vitro* tests, the 3 isolates were considered the most promising to be used as probiotics or as a source of bioactive molecules able to inhibit important bacterial fish pathogens

The extracellular NACs responsible for the anti-growth, anti-biofilm and anti-QS activities are being identified and characterized, to be used as future disease-preventive molecules in aquaculture.

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IN VITRO EVALUATION OF THE INTERACTION BETWEEN EXOGENOUS CARBOHYDRASES PRODUCED BY SOLID-STATE FERMENTATION OF BREWERS' SPENT GRAIN AND DIGESTIVE ENZYMES

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Introduction

The presence of non-starch polysaccharides (NSP) in most plant feedstuffs (PF) has been associated with adverse effects on carnivorous fish nutrient digestibility and, ultimately, on growth and health. The lack of enzymatic machinery and well-developed microbiota in the digestive tract of carnivorous fish for processing NSP are possible causes for such effects. A promising nutritional strategy to improve nutrient digestibility of plant-based diets is the use of exogenous enzymes (e.g. carbohydrases, proteases) as feed additives. Solid-state fermentation (SSF) is an eco-friendly and cost-effective biotechnology process that allows converting inexpensive agro-industrial by-products into added-value products such as carbohydrases. As a low-cost and lignocellulosic by-product of the brewing industry, brewery spent grain (BSG) is an attractive substrate for microbial enzyme production by SSF. The present study was designed to assess the potential of a carbohydrase enzyme extract obtained by SSF to release total amino acids (AA) and monosaccharides (pentoses) from plant-based diets in an *in vitro* gastrointestinal model with enzyme extracts of European sea bass (*Dicentrarchus labrax).*

Material and methods

Three isoproteic (48% crude protein) and isolipidic (16% crude lipids) diets were formulated with 15% fishery products (fish meal and fish protein concentrate), 5% hemoglobin, and 61% plant feedstuffs (wheat gluten, soybean, wheat, rice bran, sunflowe , rapeseed). In all diets fish oil was the main lipid source. Diets were similar in starch (10%), hemicellulose (2.4%), cellulose (1.6-%) and lignin (2.3%) contents. The diets were unsupplemented (control) or supplemented with an enzyme extract obtained by SSF at 0.1 or 0.4%. The enzyme extract was obtained from SSF of BSG using *Aspergillus ibericus* MUM 03.49 as the inducer for enzyme production. The enzyme extract was a combination of cellulase (1343U g^{-1} crude extract) and xylanase (15885U g^{-1} crude extract).

Table I. Total amino acids (AA, mg g^{-1}) and pentoses ($\mu g\ g^{-1}$) released by gastric (G) and intestinal (I) digestion of the experimental diets with active (A) or inactive (W) fish enzyme extracts[1]

Diet	Control				0.1% BSG				0.4% BSG			
Digestion phase	G		I		G		I		G		I	
Incubation mixture	A	W	A	W	A	W	A	W	A	W	A	W
AA	18.7 ±0.1	14.9 ±0.1	27.3 ±0.5	24.3 ±0.2	19.2 ±0.3	18.3 ±0.4	27.7 ±0.5	11.4 ±0.0	18.9±0.0	17.3 ±0.3	29.4 ±0.1	23.9 ±0.6
Pentoses	179.1 ±26.8^{a}	197.2 ±10.9^{a}	232.1 ±32.0^{a}	267.0 ±27.5^{a}	198.3 ±40.5^{b}	217.1 ±7.5^{b}	282.3 ±65.3^{b}	355.2 ±10.1^{b}	261.5 ±48.4^{c}	291.2 ±11.3^{c}	328.3 ±26.2^{c}	394.3 ±13.2^{c}

3-way ANOVA (P-value)

Factor	diet	digestion phase	incubation mixture	diet x digestion phase	diet x reaction mixture	digestion phase x reaction mixture	diet x digestion phase x reaction mixture
AA	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Pentose	≤0.001	≤0.001	≤0.001	0.179	0.663	0.103	0.774

[1]Value are mean ± SD (n = 3). When significant interaction between factors was found, one-way ANOVA was performed for each factor. Different superscript letters stand for statistical differences across experimental diets as determined by the Tukey test ($P < 0.05$).

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The *in vitro* methodology involved two-step hydrolysis designed to simulate stomach and intestinal digestion of European sea bass, as described in Morales and Moyano (2010). Briefl , the digestion was simulated using the following conditions: samples of each diet (between 20 and 80 mg mL^{-1}) were incubated in triplicate in a 10 mL closed reactor at 25ºC simulating gastric and intestinal digestions. The gastric digestion was done at pH of 5.0 with 9846 U mg^{-1} acid proteases and the intestinal digestion was done at pH of 8.5 with 507 U mg^{-1} alkaline proteases from sea bass stomach and intestine, respectively. A negative control assay including inactivated fish enzyme extracts was also carried out. The release of hydrolysis products, AA and pentoses, were monitored along the course of each digestion phase (at 0, 0.5, 1, 2h for gastric digestion; at 3, 4, 5, and 6h for intestinal digestion). Results are presented as total amount of product released during the different digestion phases.

Results and Discussion

After the pelleting process, 0.1 and 0.4 % BSG supplemented diets presented 1.76 and 5.76 U g^{-1} cellulase activities and 5.11 and 8.80 U g^{-1} xylanase activities, respectively. The simulated gastric and intestinal digestion demonstrated that AA release remained unaffected by dietary treatments (Table I). In contrast, a significant increment of pentoses release in the presence of increased incorporation of extracts in the diets was observed throughout gastric and intestinal incubations. Independently of dietary treatment, AA and pentoses release were higher at intestinal than at gastric digestion. In all dietary treatments, inactivation of fish enzyme extracts (W), promoted higher pentoses release than active fish enzyme extracts(A). Contrarily, inactivation of fish enzyme extracts did not affect AA release from experimental diets, except in the 0.1% BSG diet.

In conclusion, dietary supplementation of plant-based diets with 0.1 or 0.4 % enzyme extracts obtained by SSF of BSG seems to contribute to enhancing monosaccharide availability. Results also suggest that fish enzyme extracts interact with carbohydrases extract reducing their ability to hydrolyze NSP. Thus, coating the enzyme extract should be considered to maximize their potential. Studies *in vivo* still need to be performed to confirm the results of the present *in vitro* results.

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PRODUCTION OF ENZYMATIC EXTRACTS FOR AQUAFEEDS BY SOLID-STATE FERMENTATION WITH *Aspergillus ibericus* OF WINERY AND OLIVE MILL WASTES

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Introduction
The replacement of fishmeal by plant ingredients in aquafeeds imposed new formulation strategies to overcome some nutritional restrictions associated with these alternative ingredients. Thus, supplementation of plant-based diets with feed additives, as exogenous enzymes and antioxidants compounds, has recently attracted increasing interest to improve feed utilization and to promote animal health. Solid state fermentation (SSF) of agro-industrial wastes has a high potential for the production of these additives, being a practical, economical, and environmentally-friendly process. Olive mill and winery wastes have valuable compounds that may be valorized thought SSF and that may be used as additives for aquafeeds. This study was conducted to optimize the production of non-starch carbohydrases through the SSF of the olive mill and winery wastes and to test its efficacy to improve the release of pentoses during digestion of a plant-based diet in European seabass.

Materials & Methods
Olive mill wastes (crude and exhausted olive pomace; COP and EOP, respectively) and winery wastes (exhausted grape marc and vine trimming shoots; EGM and VTS, respectively) were fermented by *Aspergillus ibericus* MUM 03.49 (10g solid; 75% moisture; $2*10^6$ spores). A simplex-centroid design was performed to optimize the production of cellulases, xylanases, and β-glucosidases with the four solid wastes in 15 different mixtures (4 runs with single wastes; 6 runs with binary mixtures; 4 runs with ternary mixtures; and three central points with quaternary mixture of wastes). After SSF, the enzymes produced were extracted with distilled water. The recovered extract was lyophilized and added (0.4% diet) to a plant-based diet (15% fish meal + 60% plant feedstuffs), and an *in vitro* assay simulating European seabass sequential acidic and basic digestion was performed according to Morales & Moyano (2010).

Results & Discussion
Compared to the use of single wastes as substrate, mixtures olive mills and wineries wastes increased the production of lignocellulolytic enzymes by SSF. The mixture of solid waste that maximized xylanases, cellulases, and β-glucosidases production was 30% EGM + 36% VTS + 34% EOP (Fig.1a-c). The SSF of this mixture leads to an enzymatic activity of 78.2 U xylanase; 39 U cellulose; 21 U β-glucosidase per g of lyophilized extract.

In vitro digestibility trials confirmed that supplementation of a plant-based diet with 0.4% of this extract increased the release of pentoses during alkaline digestion (Table 1). During acid digestion, this effect was significant only when the fish enzymes were inactivated.

Conclusions
Solid-state fermentation by *A. ibericus* of olive mill and winery wastes mixtures allowed higher production of lignocellulolytic enzymes compared SSF of the wastes separately. Overall, the mixture of 30% EGM + 36% VTS + 34% EOP was the one that provided better results. Further, the enzymatic activity of the extract was more active during alkaline than acidic digestion.

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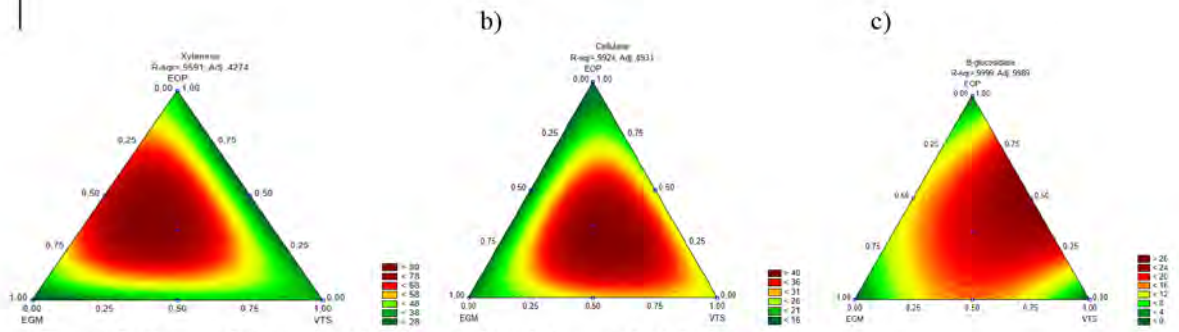

Figure I: Contour diagram for (a) xylanase, (b) cellulase, and (c) β-glucosidase.

Table 1. Pentoses release (µg g^{-1}) during acid and alkaline *in vitro* digestion of the experimental diets with active (A) and inactive (I) European seabass digestive enzymes.

Digestion	**Acid**				**Alkaline**			
Diet	Control		0.4% SSF-extract		Control		0.4% SSF-extract	
Fish enz.	A	I	A	I	A	I	A	I
	98.2± 2.8	43.1± 6.3	102.3± 10.5	80.2± 4.3	96.4± 5.6	38.7± 1.7	129± 4.1	57.8± 2.4
Two-way ANOVA					**Two-way ANOVA**			
Factor	diet	Fish extract	Interaction		diet	Fish extract	Interaction	
	≤0.001	≤0.001	≤0.001		≤0.001	≤0.001	n.s	

SHOULD THE AQUACULTURE INDUSTRY USE BLOCKCHAIN TECHNOLOGY FOR DATA RECORDING?

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Introduction

Blockchain technology has existed since 2008 and it is expected that this technology will disrupt many traditional business sectors and models, in particular those that are virtual in nature; online banking is one such example. Blockchain technology will definitely have relevant applications also in the aquaculture industry, but there is no doubt that the blockchain suppliers are currently overselling their products and they are promising more than they can deliver. This presentation aims to disentangle hype from truth when it comes to the capability of blockchain technology to achieve traceability in aquaculture supply chains by attempting to answer two fundamental questions:

1. How does blockchain compare and contrast with alternative technologies and methodologies to achieve a similar outcome and what are the key selection criteria for deciding which technology to adopt?
2. What are the costs, benefits and practical considerations of blockchain as applied in the aquaculture industry

Blockchain-based traceability in the aquaculture industry

This presentation outlines applications, limitations, costs, and benefits related to the use of blockchain technology in the aquaculture industry, and in particular evaluates the pros and cons of having a blockchain-based traceability system compared to a traditional electronic traceability system. The core principles of blockchain technology are outlined, as well as the fundamental requirements and drivers relating to an electronic traceability system. The presentation compares the functionality of traditional vs. blockchain-based food traceability systems, evaluates costs and benefits, and provides some practical advice on implementation issues.

Discussion and conclusion

The overall conclusion is that unless speed of operation or confidentiality are considered to be the most important characteristics of the traceability system, a blockchain-based implementation may be very suitable. The main benefit related to a blockchain-based traceability system is that, at least for now, the blockchain-based systems are more homogenous than traditional electronic traceability systems, so interoperability between different blockchain-based systems is likely to be easier to implement than interoperability between different traditional electronic traceability systems. Lack of interoperability is one of —, or probably the biggest current obstacle preventing system-wide, farm-to-fork aquaculture product traceability, so this advantage associated with blockchain-based implementations is significant

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MOLECULAR, TRANSCRIPTIONAL AND FUNCTIONAL PROFILING OF TWO GLUTAREDOXINS FROM BIG-BELLY SEAHORSE (*Hippocampus abdominalis*)

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Introduction

Glutaredoxins (Grxs) are known as glutathione-dependent thiol-disulfide oxidoreductases that are ubiquitously distributed in all living organisms (Bräutigam et al., 2013)we present a biochemical and biophysical characterization of zebrafish glutaredoxin 2, focusing on iron-sulfur-cluster coordination. The coordination of [2Fe2S]2+-clusters in monomers of this protein was revealed by both absorption and Mössbauer spectroscopy as well as size exclusion chromatography. All other holo-glutaredoxins represent [FeS]-cluster bridged dimers using two molecules of non-covalently bound glutathione and the N-terminal active site cysteines as ligands. These cysteine residues were not required for [FeS]-cluster coordination in zebrafish glutaredoxin 2. A crystal structure of the teleost protein revealed high structural similarity to its human homologue. The two vertebrate-specific cysteines as well as two of the teleost-specific cysteines are positioned within a radius of 7Å near the C-terminus suggesting a potential role in [FeS]-cluster coordination. Indeed, mutated proteins lacking these teleost-specific cysteines lost the ability to bind the cofactor. Hence, the apparent mode of [FeS]-cluster coordination in zebrafish glutaredoxin 2 could be different from all yet described [FeS]-glutaredoxins. © 2013 Elsevier Inc.","author":[{"dropping-particle":"","family":"Bräutigam","given":"Lars","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Johansson ","given":"Catrine","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Kubsc ","given":"Bastian","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"McDonough","given":"Michael A.","non-dropping-particle":"","parse-names":false,"suffi ":""},{"dropping-particle":"","family":"Bill","given":"Eckhard","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Holmgren","given":"Arne","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Berndt","given":"Carsten","non-dropping-particle":"","parse-names":false,"suffix":""}],"containe -title":"Biochemical and Biophysical Research Communications","id":"ITEM-1","issue":"3","issued":{"date-parts":[["2013"]]},"page":"491-496","title":"An unusual mode of iron-sulfur-cluster coordination in a teleost glutaredoxin","type":"article-journal","volume":"436"},"uris":["http://www.mendeley.com/documents/?uuid=ba31254f-1f59-4c23-af7b-d922d4aae154"]}],"mendeley":{"formattedCitation":"(Bräutigam et al., 2013. They are small antioxidant enzymes that belong to the thioredoxin superfamily but exhibit more versatile substrate activity than thioredoxins (Trxs) (Vlamis-Gardikas and Holmgren, 2002). Grx family proteins have several isoforms with quite different structures and catalytic activities. They regulate essential and distinct biological functions in organisms. Seahorses are a group of aquatic animals that have exploited for years for their medicinal and ornamental properties. Big-belly seahorse (*Hippocampus abdominalis*) is the largest seahorse species and is naturally distributed in the Korean peninsula and around the ocean. Being a large, smooth-skinned species provides a high market value for big-belly seahorses. However, fulfilling the demand for seahorses is still a problem as they are extremely difficult to culture. One of the major threats for seahorse cultivation is their poor adaptability to higher densities and stressful environments, thus increasing their vulnerability to pathogenic infections (Woods, 2007). Therefore, understanding the immune responses of big-belly seahorse against bacterial and pathogen-associated molecular pattern (PAMP) stimuli as well as assessing the functional aspects will facilitate the development and maintenance in seahorse cultivation. Accordingly, the present study investigated the molecular, transcriptional, and functional properties of Grx1 and Grx2 from big-belly seahorse (HaGrx1 and HaGrx2) to understand its role in immunity and redox homeostasis.

Materials and methods

In order to assess sequence characteristics, the *in-silico* analysis was performed by, ExPASy Protparam, SWISS model workbench, Clustal Omega, EMBOSS needle tools and MEGA7 software. Tissue specific expression of *HaGrx1* and *HaGrx2* was analyzed using 14 tissues of healthy seahorses. Temporal expression analysis of *HaGrx1* and *HaGrx2 in* blood was carried out by using lipopolysaccharides (LPS), polyinosinic:polycytidylic acid (poly I:C), *Edwardsiella tarda* and *Streptococcus iniae*. Further, Insulin reduction and dehydroascorbate reduction assays were proceeded to find out the redox activity.

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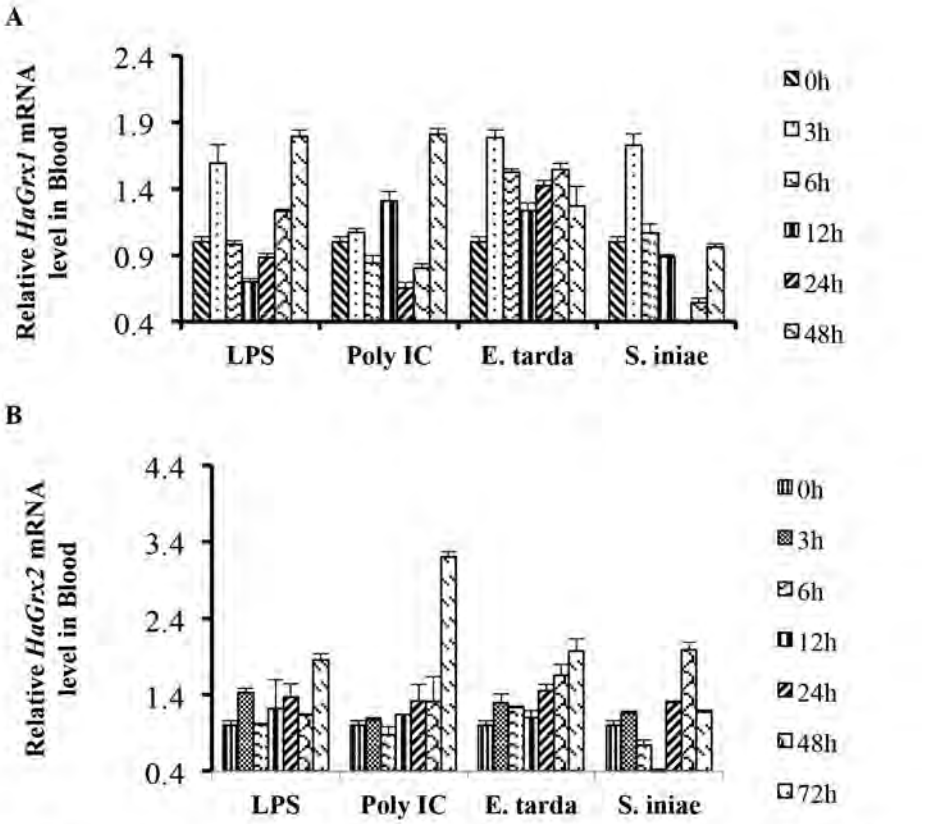

Fig. 1. Temporal expression profiles of (A) *HaGrx1* and (B) *HaGrx2* in the blood after immune stimulation. For the immune challenge, PAMP (LPS and poly I:C) and bacterial (*Edwardsiella tarda* and *Streptococcus iniae*) were used. The Livak method was used to calculate the fold changes in mRNA expression, and seahorse 40S ribosomal protein S7 gene was used as an internal control gene in the qPCR experiment. The relative fold changes in expression were compared with those of the PBS-injected control at different time points. The data are represented as mean values (n = 3) ± S.D.

Results

In-silico analysis showed that HaGrx1 contained the classical glutaredoxin 1 structure with a CSYC thioredoxin active site motif. HaGrx2 possess Glutaredoxin 2 structure with CPYC active site. According to multiple sequence alignment and phylogenetic reconstruction, HaGrx1 and HaGrx2 presented the highest homology to the Grx1, and Grx2 ortholog from *Hippocampus comes* respectively. Transcriptional studies demonstrated the ubiquitous distribution of *HaGrx1* and *HaGrx2* transcripts in all the seahorse tissues tested. HaGrx1 represented the highest expression in muscles whereas HaGrx2 highly expressed in brain and skin. Significant modulation ($p < 0.05$) of *HaGrx1* and *HaGrx2* transcripts were observed in blood upon stimulation with pathogen-associated molecular patterns and live pathogens (Figure 1). Further, dehydroascorbate reduction and insulin disulfide reduction assays revealed the oxidoreductase activity of HaGrx1 and HaGrx2

Discussion and conclusion

According to the study, HaGrx1 and HaGrx2 consist of typical glutardoxin structure and showed highest identity and similarity with *Hippocampus comes*. HaGrx1 and HaGrx2 can be expressed by immune stimulant and its redox activity might be act as a barrier for invading pathogens and PAMP stimuli. Altogether, results in this study suggests that HaGrx1 and HaGrx2 actively involved in host redox homeostasis as well as immune responses.

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MODELLING SUITABILITY OF ERO RESERVOIR AND ITS ENVIRONS FOR AQUACULTURE IN EKITI STATE, NIGERIA

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Introduction

Reservoirs in Nigeria are primarily constructed for public water supply and characterized by artisanal fishery (Daramola *et al.*, 2007). Most of the reservoirs are surrounded by large expanse of land that could be assessed for fish culture. However, utilising these areas for successful and sustainable aquaculture operations requires assessing different environmental factors (soil, hydrology, topography, drainage and climate) that would guide in site selection (Andi *et al.*, 2013). Such approach as defined by Andi *et al.* (2013) is a strategic planning tool that could predict the expected benefits and constraints of productive land use and environmental degradation that might occur due to the use of land. The study was purposively conducted in Ekiti State, Nigeria due to the need to increase fish production; attributed to the landlocked nature (neither enclosed by land with neither sea nor ocean surrounding for fishing purposes) of the State (Omobepade *et al.*, 2014).

Materials and Methods

Landsat 8 OLI satellite datasets and Shuttle Radar Topography Mission were acquired from the Global Land Cover Facility to determine the land use/land cover and the digital elevation model respectively within a 500m buffer. Soil samples were collected from a 1.5m (0 – 37.5cm, 38 – 75.5cm, 76 – 112.5cm and 113 – 150cm) profile dug at three locations and analysed for physiochemical properties. Hydrological, soil, land use/land cover, vegetation, and elevation parameters were subjected to an analytical hierarchical process to develop a suitability map for aquaculture development in the study area according to standard methods.

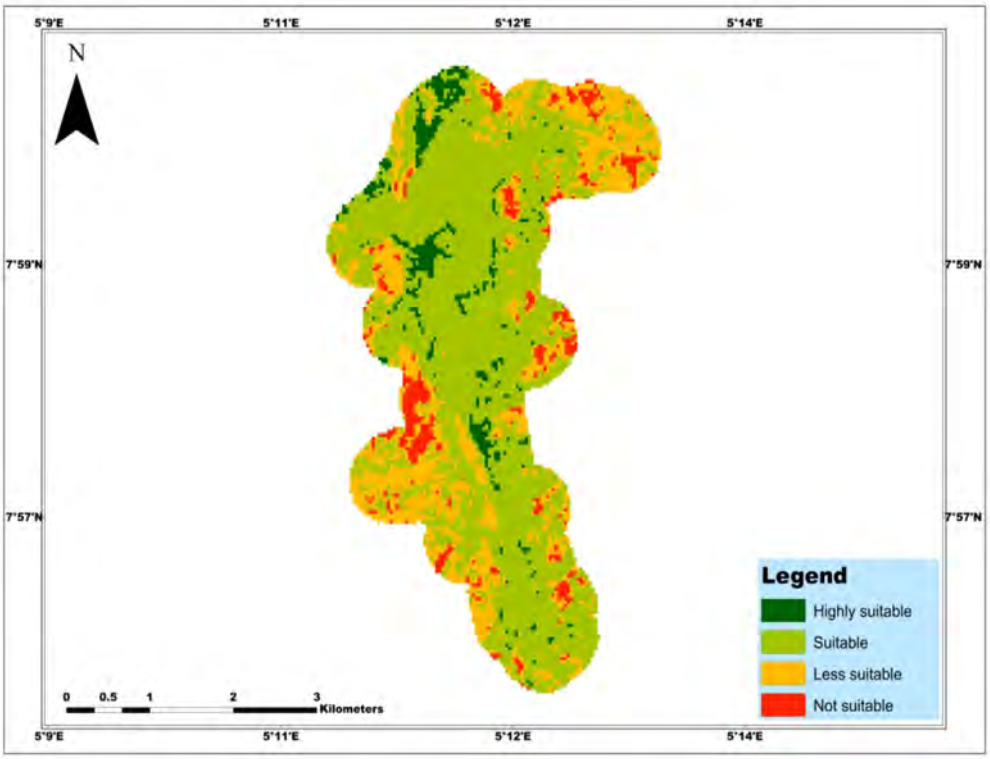

Figure 1: Land suitability model for Aquaculture Development in and around Ero Reservoir, Nigeria

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Results

Results indicated that 20.99%, 47.74%, 12.96, 6.03% of the buffered area were water body, light vegetation, dense vegetation and built up areas respectively. Soil classifications in the study area were Lixisols (Sandy-clay-loam) which covered lesser land mass (11.63%) while Nitisols (Clay-loam) covered the higher land mass (88.37%). Suitability model for the study area ((Figure 1) showed that land mass unsuitable for aquaculture development represents 6.07% (89.73 hectares) of the study area, 359.88 hectares (24.35%) were less suitable, and 950.76 hectares (64.33%) were suitable while 77.63 hectares (5.25%) were highly suitable. Despite the large sparse of the reservoir and its environ belonging to the suitable classes, land unsuitable for aquaculture could be attributed to the presence of hills, rocks, undesirable soil parameters, high slopes and dense vegetation.

Conclusion

Hence, individuals and corporate bodies could invest in the suitable areas for aquaculture development, which at the long run will increase the fish availabilit , create employment, improve livelihood and enhance economic profile of the State

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BIOACTIVITY OF GARLIC *Allium sativum* AGAINST *Dermestes maculatus* ON SMOKE-DRIED AFRICAN CATFISH *Clarias gariepinus*

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Introduction

The geometric increase in world population and need to boost daily dietary protein intake, fish remains the major source of protein in most developing countries. However, due to poor handling, crude processing methods, inadequate storage facilities and lack of proper preservation techniques, about 30 – 50% of fish harvested are wasted in Nigeria, Bate and Bendall (2010). Infestation of smoked / dried fish by *Dermestes maculatus* results in quality and quantity deterioration, hence, the need for preservation. In the past, chemicals have been used to preserve fish products, thus, leaving an after effect detrimental to human – the end consumer. Furthermore, the high cost of these chemicals has led to the use of natural plants to reduce the cost. *Allium sativum* a naturally and readily available plant contains one of the active ingredients (allicin) known for insect repellant.

Materials and Methods

The study was carried out at the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria. Fresh *A. sativum* bulbs harvested form the crop section of the Uiniversity's farm was oven-dried at 50^{o}C for 72 hours. Dried bulbs was grinded into powder using a kitchen blender. Adults and larva of *D. maculatus* were isolated from heavily infested dried fish and cultured under laboratory conditions for 21 days. Fifteen *Clarias gariepinus* (150±5)g from the fish farm were smoked at 60˚C in a coal-powered kiln for 12 hours. Smoke-dried fish samples were placed in separate plastic containers while *A. sativum* powder was sprinkled on fish at varying proportions (0.0, 0.1, 0.3, 0.5 and 0.7)g. Each treatment (in triplicate) was infested with 20 adults of *D. maculatus*, containers were covered air tight and kept for 7days.

Results

Mortality of *D. maculatus* was higher in treatments with higher quantity of *A. sativum* after 7 days (figure I)

Biochemical composition analyzed in fish samples following AOAC (1995) methods also showed significant variations ($p<0.05$) in parameters with the least infested fish treatments having values closer to the initials (able 1).

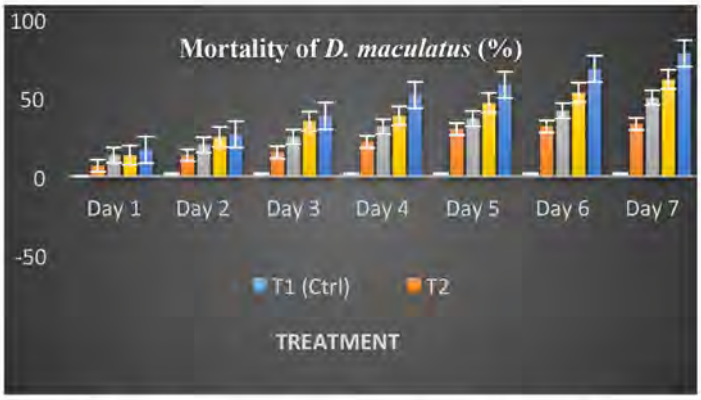

Fig. I: Toxicity of *A. sativum* on *D. maculatus* in smoke-dried *C. gariepinus*

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Discussion and conclusion

High mortalities recorded in the adult stage of *D. maculatus* observed on smoke-dried catfish treated with *A. sativum* powder could be attributed to the active bio-components (allicin, ajoene, flavonoids and alkaloids and some phenolic compounds) found in the plant. This agrees with the assertions of Ayeloja et al. (2016) that garlic is an effective insecticide against *D. maculatus*. Andargae et al. (2013) reported that some ethnobotanicals such as garlic have been found to be effective against short-lived insects or pests in the field as well as in food (during storage). Lawson (1998) reported that the most biologically active compound in garlic is allicin, and its present both in fresh and dry garlic bulbs. The insecticidal property of any plant material is dependent on the active constituents of the plant material. Similarly, Saad et al. (2006) reported that this component (allicin) contributes to antibacterial, fungicidal, insecticidal and anti-parasitic properties of garlic. *A. sativum* has been reported to possess repellant properties which deter insects from infesting smoked fish Ayeloja et al. (2016). The mortalities caused by *A. sativum* which in linked to the secretion of allicin by the plant that blocks the spiracles of insects when it comes in contact with their bodies. Egwunyenga et al. (1998) attributed this repelling function to the olfactory sensation in *D. maculatus*.

Analysis of fish samples showed significant variations in biochemical compositions of smoke-dried fish samples upon exposure to *D. maculatus*. Uneke (2015) confirmed variations in biochemical components of dried catfish infested with insects. Douiri et al, (2013) reported that *D. maculatus* caused losses in the amount of nutrient available to consumers resulting in loss of quality and quantity of smoked *C. gariepinus*. Atijegbe (2004) opined that protein, fat and nitrogen free extract contents in smoked/dried fishes reduce significantly as the days of infestation increases, suggesting that *D. maculatus* impairs the fish quality. Similarly, observations from this result is connected to the assertions of Fasakin and Aberejo (2002) that *D. maculatus* needs a good supply of protein, vitamin and minerals for optimal growth according to their nutritional requirements adequately supplied by *C. gariepinus*.

Preservation of smoked dried fish with *A. sativum* powder against the infestation of *D. maculatus* has been proven highly effective to curb the menace of post-harvest losses, maintain quality and promote sustainable aquaculture. Therefore, *A. sativum* is a potential replacement for chemical preservatives against *D. maculatus* in smoke-dried *C. gariepinus*.

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EFFECTS OF HEAT-KILLED *Lactobacillus plantarum* (HK L-137) ON THE HEALTH STATUS AND GROWTH-RELATED GENE EXPRESSION OF GENETICALLY IMPROVED FARMED TILAPIA

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Introduction
Genetically improved farmed tilapia (GIFT), an enhanced strain of Nile tilapia (*Oreochromis niloticus*), exhibit better growth and survival rates than routinely available strains of tilapia. Currently, intensive culture systems are common for tilapia culture and often cause stressful conditions that reduce fish growth and wellbeing. The use of functional feed additives as bio-friendly agents is a sustainable way to improve cultured fish performance, and the use of functional ingredients with immune modulatory properties has become more prominent across the animal feed industry.

Heat-killed *Lactobacillus plantarum* (HK L-137) can positively impact the performance and wellbeing of aquatic animals, as reported in several studies.

Thus, the present study investigated the potential value of HK L-137 as a dietary probiotic for GIFT.

Materials and methods
For 12 weeks, fish were fed a control diet (HKL0) or a diet supplemented with HK L-137 at a concentration of 50 (HKL50), 100 (HKL100) or 1000 (HKL1000) mg kg^{-1} feed.

Results
At the final sampling, the HKL100 group had significantly ($P < 0.05$) increased performance parameters (FBW, WG, SGR and FER) compared to the control group, while the HKL50 and HKL1000 groups showed weaker improvements. Mucosal thickness and villus length were significantly ($P < 0.05$) increased in the HKL50 and HKL100 groups in the anterior, middle and posterior intestine, but muscle thickness was significantly ($P < 0.05$) improved only in the anterior and middle intestine. Amylase, lipase and protease activity was significantly ($P < 0.05$) increased in fish fed 50 or 100 mg HK L-137 per kg diet compared to control fish. Significant modulation of blood haematocrit, haemoglobin levels, and RBC and WBC counts ($P < 0.05$) occurred in fish fed HK L-137, while total cholesterol and GPT were decreased by HK L-137. Furthermore, antioxidative enzyme (SOD and CAT) activity were significantly ($P < 0.05$) higher in the HKL100 group than in the control group, while MDA levels were lower. Furthermore, fish fed HK L-137 showed enhanced total serum protein and IgM levels. Interestingly, qRT-PCR revealed significant ($P < 0.05$) upregulation of the growth-related gene IGF-I and the glucose regulation gene G6PD but downregulation of the fatty acid synthase (FAS) gene in all HKL groups compared to the control group.

Conclusion
Thus, we conclude that the use of HK L-137 is an efficient strategy to achieve economically feasible and sustainable tilapia production.

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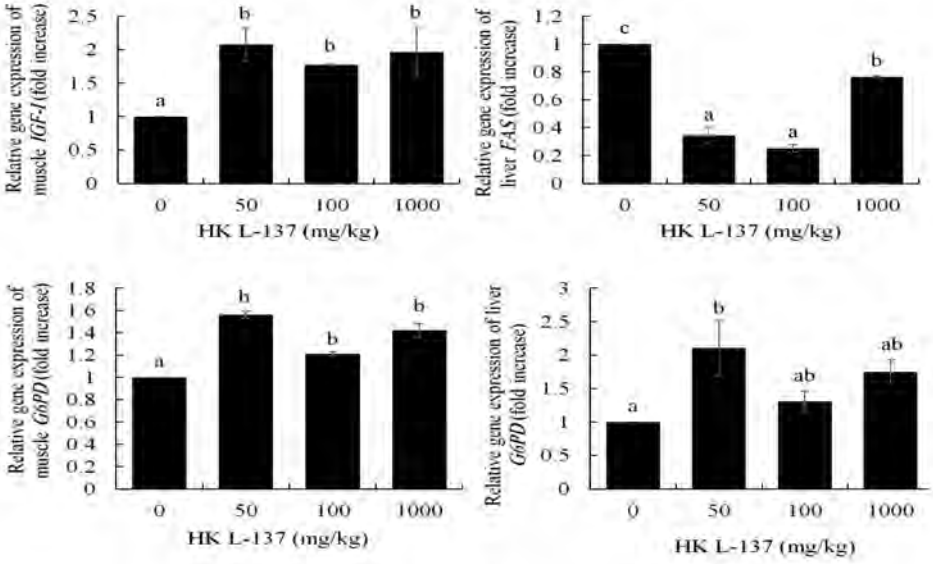
Table 1. Growth performance and nutrient utilization in GIFT tilapia*

Item	Test diets			
	HKL0	HKL50	HKL100	HKL1000
Initial body weight (g)	16±0.04	16±0.03	16±0.03	15.97±0.04
Final body weight (g)	36.16±0.9^{a}	41.2±1.3^{b}	45.2±0.7^{c}	42.2±0.93^{b}
Weight gain (%)	126.3±5.4^{a}	158.3±8.3^{b}	183.4±4.3^{c}	167.4±5.83^{b}
Specific growth rate (% BW/ day)	1.82±0.1^{a}	2.1±0.1^{b}	2.3±0.04^{c}	2.04±0.05^{b}
Feed efficiency ratio	0.54±0.02^{a}	0.61±0.03^{ab}	0.73±0.02^{c}	0.66±0.02^{b}
Survival	91.1±2.2^{a}	97.8±2.2^{b}	100±0.00^{b}	98.9±1.1^{b}
Condition factor (%)	1.82±0.1	1.83±0.03	1.84±0.06	1.84±0.04
Hepatosomatic index (%)	2.24±0.2	2.39±0.13	2.09±0.34	2.24±0.17
Viscera somatic index (%)	2.9±0.1	2.87±0.13	2.6±0.03	2.74±0.1

*Values expressed as means ± SE (n = 3). Different superscript letters indicate significant differences for each pairwise comparison between treatments.



BIOREMEDIATION OF ORGANIC SLUDGE FROM A MARINE RECIRCULATING AQUACULTURE SYSTEM USING THE POLYCHAETE *Abarenicola pusilla*

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Introduction

Organic sludge, the principal residual product from recirculating aquaculture systems (RAS), is one of the main environmental and logistical challenges associated with aquaculture production (Martins et al., 2010; van Rijn, 2013). Polychaetes have been described as potential remediating organisms of aquaculture sludge due to its ability to survive in organic enrichment conditions and its capability to assimilate particulate organic waste from intensive aquaculture (Brown et al., 2011; Robinson et al., 2015). The aim of this study was to evaluate the removal performance of *Abarenicola pusilla* when fed with organic sludge from a marine RAS.

Materials and methods

A. pusilla (mean weight 2.00 ± 0.07g) were implemented in a prototype system with biofiltration and water recirculation. The polychaetes were fed with aquaculture sludge from a marine RAS with *Seriola lalandi* (average weight=3000.2 ± 110.4g, culture density = 10.85kg.m-3). The experiment consisted of the addition of 10% of fish sludge respect to the total sediment in the experimental units, at different densities of *A. pusilla* (60, 75, 150 and 200 organisms.m-2), with three replicates each, and during 45 days.

Results

The total organic matter (TOM) removal rate achieved by *A. pusilla* is shown in Table I. The results showed higher TOM removal at 150 and 200 organisms.m-2. In addition, total nitrogen (33.63 ± 0.003mg.d-1) and total carbon (236.78 ± 0.003mg.d-1) removal rates were obtained with the highest density of polychaetes.

The results also showed the assimilation of organic components presented in the sludge, with a significant increase ($p<0.05$) in total carbon (24.75%), organic carbon (24.68%) and lipid content (0.65%) in the biomass of the organisms. The highest specific growth rate (SGR) of *A. pusilla* was 3.06% per day. The survival rate was 91.67% over the 45 days trial period.

Discussion and conclusions

The results of this study indicated that *A. pusilla* can be used as a bioremediating species for organic compounds contained in marine RAS aquaculture sludge. Other research in bioremediation with polychaetes reported lower TOM removal rates (Palmer, 2010; Fang et al., 2016; Marques et al., 2017). These results agreed with Palmer (2010), where the highest TOM removal rate was obtained at the highest density of organisms per area. Total nitrogen and carbon removal rate from the sludge by *A. pusilla* was also higher than reported in other species (Honda and Kikuchi, 2002; Fang et al., 2016).

The biomass production of *A. pusilla* (approx.. 400g.m-2 in 45 days) demonstrated that this species is able to consume aquaculture sludge as the only source of feed. The significant assimilation of lipid content, total and organic carbon obtained, suggested that this species is a potential candidate for nutrient recycling from marine RAS. Further studies should determine the possible changes in the fatty acid profile of *A. pusilla* in order to use it in aquaculture feeds.

Table I. Total organic matter (TOM) removal rate by *A. pusilla* during the trials (mean ± S.D.). Significant differences ($p<0.05$) respect to units with 60 y 75 org.m-2 are expressed with (†).

Density (org.m-2)	60	75	150	200
Biomass (g.m-2)	58.48 ± 9.77	71.11 ± 9.10	155.21 ± 16.49	195.86 ± 11.07
TOM removal rate (g.m-2.d)	13.65 ± 0.04	18.53 ± 0.05	33.86 ± 0.05†	35.77 ± 0.05†

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EFFECTS OF REPLACING FISH MEAL WITH COCKROACH *Periplenata americana* MEAL IN THE DIET OF *Clarias gariepinus* FINGERLINGS

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Introduction

High cost of fish meal necessitates need for non-conventional protein source like feather meal, poultry droppings, Insects, worms, garden snails and tadpoles among others (Devendra, 1988). Housefly maggots have been reported to be a dietary supplement in tilapia and *Clarias gariepinus* and *Heterobranchus longifilis* by (Madu and Ufodike, 2003). American cockroach *(Periplaneta americana)* can be cultured to supply protein in fish feed and thus, this research investigated the effect of cockroach *(Periplaneta americana***)** meal on the performance of *Clarias gariepinus* fingerlings

Materials and Methods

Materials used included cockroach meal, fish meal, groundnut cake (GNC), soymeal meal, maize meal, vitamin mineral premix, palm oil, electronic sensitive weighing balance (SF-400;Capacity 3000gx1g/106ozx0.1oz) etc. one hundred and eighty (180) catfish fingerlings were randomly distributed in triplicate of 20 fish per tank for three treatment

Three 45% cp experimental diets (Table 1) were fed to the fish for 56 days.

Statistical analysis

Statistical package minitab release 14 was used to analysis the data generated.

Results

The highest mean weight gain, lowest FCR and high SGR, PER, ANPU and survival were recorded for diet 3 (cockroach meal) than other diets (P0.05) (Table 2).

Discussion

The high protein content in the test diets could be responsible for high growth performance of catfish fingerlings, which is in agreement with the report of (Tran *et al*., 2015) and could be attributed to high performance of catfish fingerlings fed with cockroach meal in this experiment. The lipid content of fish meal based diet was higher than that of cockroach meal diet which could be responsible for high diet consumption than cockroach meal diet though with no significant differences. Kiriratnikom and Kiriratnikom, (2012). The optimum dietary protein levels however depend on the fish growth rate, feed intake, amount of non-protein energy in the diet, protein quality, presence of natural food and management practices (Davis *et al*., 2009). This could be responsible for high performance of catfish fingerlings fed with cockroach meal in this experiment. Fats are highly digestible and an important source of concentrated energy and play several key roles in the growth and development of fish (Robinson and Li, 2009

Table 2: Growth Parameters of *Clarias gariepinus* fingerlings fed experimental diets for 56 days.

Growth Parameters	Diet 1	Diet 2	Diet3	SD±
Initial weight gain (g)	1.13 ± 0.03^{a}	1.10 ± 0.00^{b}	1.10 ± 0.00^{b}	0.02
Final weight gain(g)	1.87 ± 0.27^{b}	2.08 ± 0.23^{b}	2.49 ± 0.39^{b}	0.30
Mean weight gain (g)	1.15 ± 0.48^{a}	0.98 ± 0.23^{a}	1.39 ± 0.39^{b}	0.38
Feed fed (g)	3.63 ± 0.90^{a}	3.81 ± 0.29^{a}	3.53 ± 0.29^{a}	0.57
FCR	2.01 ± 0.79^{a}	1.86 ± 0.32^{a}	1.43 ± 0.15^{b}	0.50
SGR (%/day)	0.89 ± 0.30^{c}	1.13 ± 0.19^{b}	1.44 ± 0.29^{a}	0.27
PER	0.69 ± 0.15^{b}	0.58 ± 0.17^{b}	0.87 ± 0.20^{a}	0.17
ANPU (%)	1.78 ± 1.26^{b}	4.19 ± 4.87^{b}	16.23 ± 7.19^{a}	5.07
Survival (%)	37	42	62	2.31

Means on the same column with different superscripts are significantly different (P< 0.5)

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Table 1: Formulated diets containing cockroach meal.

Feed ingredients (%)	Diet 1	Diet 2	Diet 3
Fish meal	0.00	100.00	0.00
Groundnut cake	416.50	284.90	294.50
Soybean	416.50	284.90	294.50
Cockroach meal	0.00	0.00	100.00
Maize meal	67.000	230.30	211.10
Lipid	50.00	50.00	50.00
Vitamin mineral premix	50.00	50.00	50.00
Total	1000.00	1000.00	1000.00

Table 2: Proximate Compositions of formulated diets

Diets %	Crude Protein	Lipid	Ash	Moisture	Fibre	Carbohydrate
Diet 1	45.05	9.08	14.88	7.81	1.80	21.38
Diet 2	45.09	13.85	14.89	7.18	0.80	18.19
Diet 3	45.04	8.88	12.04	8.20	4.50	21.34

Conclusion

Cockroach meal in the diet of *Clarias gariepinus* fingerlings as a protein sources was evaluated, and from the study it can substitute fish meal without compromising the nutrient utilization, growth and survival

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GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF *Clarias gariepinus* FINGERLINGS FED WITH ROSELLE (*Hibiscus sabdariffa*) CALYX AS DIETARY ADDITIVE

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Introduction

Antibiotics are chemical compounds used in treating and preventing diseased in aquaculture. Farmers also use antibiotics in fish feed to enhance growth. Antibiotics growth promoters (AGP) were supposed to increase growth rate as a result of improved gut health, resulting in better nutrients utilization and improved feed conversion. However, these drugs are expensive, promotes microbial resistance, breakage of the animal intestinal micro-ecological balance and the presence of antibiotics residues in resultant fish produce. Natural materials such as medicinal plants could be widely accepted as feed additives to enhance fed utilization and sustainability and aquaculture productive performance. World Health Organization encourages using medicinal herbs and plants to minimize the use of chemicals. Hence a call for research into accessible, potent, cheaper, eco-friendly, alternative natural antibiotic. A particular medicinal plant of interest is the Roselle, *Hibiscus sabdariffa*.

Materials and Methods

The study was conducted at the research farm of the Department of Fisheries and Aquaculture, Federal University Dutsinma, Katsina State, Nigeria. Dried flower (Calyx) of *Hibiscus sabdariffa* was purchased, milled with a blender into powder form, sieved and stored in an air tight container until when ready use. The dosage values of the Roselle used in the experimental diet were 4g, 6g and 8g / Kg, while the control, 0g had no Roselle Calyx. Four diets (40%) crude protein) were formulated and coded Diet 0 control, Diet 1 (4.0g), Diet 2 (6.0g) and Diet 3 (8.0g). Feed ingredients were bought, ground and measured using a sensitive weighing scale. The ingredients were mixed thoroughly and pelleted, sundried, labeled accordingly and stored at room temperature until it was ready for use. Ingredients used were fishmeal, Soybean meal, Yellow maize, palm oil, Vitamin/mineral, Premix, Cassava starch (acted as a Binder),common salt, bone meal and *Hibiscus sabdariffa* (Roselle). Two hundred *Clarias gariepinus* fingerlings with initial mean weight of 4.25 ± 0.04g were obtained from a reputable fish farm and transported in oxygen bags to the experimental site. The fish were acclimatized for 14 days in holding tanks and fed commercial diet (Copens feed). After which they were randomly stocked in triplicates per treatments. The fish were fed twice daily at 5% body weight at 9:00 am and 5:00 pm for 12 weeks. Fish in each aquarium were sampled biweekly and the amount of feed adjusted accordingly. Dead fish were daily recorded and removed. At the end of the study, fish were individually weighed

Results

Table 1: Growth performance and nutrient utilization of *Clarias gariepinus* fingerlings fed experimental diets.

Parameters	0	1	2	3	Mean	SD	S.E±
Initial mean weight (g)	4.3^{a}	4.27^{a}	4.23^{a}	4.20^{a}	4.25	0.04	0.022
Final mean weight (g)	21.31^{a}	20.8^{a}	21.67^{a}	27.97^{b}	22.94	3.37	1.69
Mean weight gained (g)	17.01^{b}	16.53^{b}	17.43^{b}	23.77^{a}	18.69	3.41	1.70
Mean weight gained per day (g)	0.21^{b}	0.19^{b}	0.21^{b}	0.28^{a}	0.22	0.04	0.02
Specific Growth rate (%)	1.46^{b}	1.45^{b}	1.47^{b}	1.64^{a}	1.51	0.09	0.05
Total Feed fed (g)	374.80^{b}	343.70^{b}	390.80^{b}	491.50^{a}	400.20	63.93	31.97
Feed conversion ratio	2.13^{b}	2.09^{b}	2.06^{b}	0.93^{a}	1.80	0.59	0.29
Protein Intake	44.20^{a}	38.40^{a}	24.10^{b}	22.70^{b}	32.35	10.62	5.31
Protein Efficiency ratio	38.48^{c}	43.08^{c}	72.43^{b}	104.5^{a}	64.62	30.54	15.27
Average Survival (%)	55	55	46	24	45	14.63	7.31

*Values with the same super script within the rows are not significantly different from each other.

(Continued on next page)

Discussion and Conclusion

The mean weight gain (MWG) of the fish fed diet 0, 1, 2, and 3, were 17.01g, 16.53g, 17.43g and 23.77g respectively. The group of fish fed diet 3 had the highest mean weight gain, and this is significantly different ($P<0.05$) from the weight gain of the group of fish fed on diet 0, 1 and 2. An interesting characteristic of Roselle calyx is the presence of polyphenols, ascorbic acid (Escribano-Bailón et al, 2006) http://www.epj.eg.net/article.asp?issn=1687-4315;year=2016;volume=15;issue=2;spage=78;epage=87;aulast=El - ref38, and red pigments (anthocyanin), which show antioxidant activity (Sáyago-Ayerdi, et al, 2007). These components could participate in fish metabolism, helping to improve health and growth. These results are also in agreement with the results of Pérez *et al.* (2012), who showed that increasing anthocynain in Roselle calyx/kg diet increased the growth rate, weight gain and specific growth rate of goldfish (*Carassius auratus*). In the present study, feed intake was higher for fish fed diets containing *H. sabdariffa* calyx at all levels, except fish fed with the Diet. 1 This gradation, which was in the favor of fish on *H. sabdariffa* calyx, could be attributed to a high content of vitamins and minerals in *H. sabdariffa* calyx, which enhanced appetite (Mahadevan 2009) and it is evidenced by higher weight gain. The fingerlings fed diet 3 had the highest specific growth rate (SGR) of 1.64. Those fed with Diet 1 and Diet 0 had the least value of 1.45 and 1.46 respectively. The present data also showed that feed efficiency ratio was significantly higher ($P<0.05$) for 8% *H. sabdariffa* calyx diet, whereas there was no significant difference in protein efficiency ratio between the control and 4% of *H. sabdariffa* calyx. This is in agreement with El Mesallamy AM (2016).These results suggested that *H. sabdariffa* calyx supplementation did play a role in enhancing feed intake with a subsequent enhancement of the fish body composition. Moreover, Pérez et all 2012 showed that increasing anthocynain in *H. sabdariffa* calyx/kg diet, increased feed conversion rate of goldfish (*C. auratus*). The feed conversion ratio (FCR) observed in fingerlings fed diet 0 (2.13) was higher than those fed other diets, with the lowest value in fingerlings fed diet 3 (0.93). The lower FCR value in treatment Diet 3 showed that it was highly digested by the fish than other treatments which may be due to the high content of vitamins and minerals in *H.sabdariffa calyx*.

The Protein efficiency ratio (PER) results indicated that there was no significant difference ($P<0.05$) between the control Diet 0 and Diet 1 but there was a significant difference ($P> 0.05$) between the control Diet 0, Diet 2 and Diet 3.The PER was highest in fish fed Diet 3 and lowest in fish fed diet Diet 0. This indicates that the protein content of diet 3 was efficiently utilized by the fish. Olaniyi (2009a &b) asserted that the higher SGR and smaller FCR values, the better the feed quality. Also Adikwu (2003) reported that a lower FCR value implies efficient feed utilization by fish. Based on these result, fish fed with 8% roselle calyx inclusion (Diet 3) performed better without compromising growth rate or causing any deleterious effect on the fish. It is hence concluded that 8% inclusion of roselle calyx as an additive in the diet of *Clarias gariepinus* fingerlings supported growth and nutrient utilization of the fis

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ASSESSMENT OF ABUNDANCE AND DISTRIBUTION OF FISH SPECIES IN SOME DAMS IN EKITI STATE, NIGERIA

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An assessment of abundance and distribution of fish species in Ureje, Itapaji and Egbe dams in Ekiti State, Nigeria was conducted where samples were collected fortnightly with the assistance of artisanal fishermen operating in the study areas. The population structure and distribution patterns were determined using the number of occurrence index (NOI) and histogram. A total number of 148 individuals comprising 5 species occurred throughout the sampling period. *Oreochromis niloticus, Tilapia zillii* and *Sarotherodon melanotheron* occurred in the three dams, *Mormyrus hasselquistii* occurred in two of the three dams i.e. Itapaji and Egbe while *Hemichromis fasciatus* was found in Itapaji dam only. *Oreochromis niloticus* was abundant in Ureje and Egbe dams with 52.38% & 52% respectively, but occurred in a low quantity in Itapaji dam with 15.66%, *Tilapia zillii* had 28.30%, 22%, 6.67% at Ureje, Itapaji and Egbe dams respectively; *Sarotherodon melanotheron* had 51.1% in Itapaji dam where it was dominant and recorded 18.87% and 8% in Ureje and Egbe dam respectively. *Mormyrus hasselquistii* had 18% and 8.89% in Egbe and Itapaji dams respectively and *Hemichromis fasciatus* recorded 17.7% at Itapaji dam where it only occurred. *O. niloticus* dominated the entire catches in all the dams with 41.2% followed by *S. melanotheron* 25%, *T. zillii had 19.6%, M. hasselquistii* had 8.9% and *H. fasciatus* had 5.5%. The study revealed that there was a generally high similarity among the freshwater fish communities in the selected dams. It is worthy of note that these dams have capacity for fish production that could serve as a sustainable means of livelihood for the populace around the areas if properly managed.

BACTERIAL OUTER MEMBRANE VESICLES OF *Aeromonas salmonicida* INDUCE A PRO INFLAMMATORY IMMUNE RESPONSE IN VITRO AND IN VIVO

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High mortality rates after bacterial and bacterial-viral superinfections cause high losses for the aquaculture industry. As treatment with antibiotics is not an alternative, bacterial vaccines for intramuscular or intraperitoneal injection were developed resulting in inflammatory granulomas and stress. Within the project "Modular oral applicable Multi-Vaccine – principle solutions (MoMV)" we propose therefore the design of a modular vaccine based on outer membrane vesicles (OMV´s) of the bacterial fish pathogen *Aeromonas salmonicida*. The simple preparation, the safety due to their non-replicative nature as well as the composition of natural surface exposed membrane antigens in their native confirmatio are the advantages of such a vaccine design. In the present study we are focusing on characterization of the immunogenic potential of the OMV´s.

Methods: Fish were treated with a bacterial vaccine formulation either by intraperitoneal or oral vaccination. At different time points after vaccination peritoneal, blood, spleen and head kidney leukocytes were isolated and comparatively characterized by flow cytometry in target immune organs (gut, peritoneum) and effector immune organs (spleen and head kidney) labelled with a cocktail of lineage marker specific antibodies. Moreover, using the lineage marker specific antibodies the recognized cell populations were sorted at function related time points after stimulation. Finally, the mRNA was prepared form these isolated cell populations to characterize their mRNA profile

Results: A comparable kinetics of distinct leukocyte populations was seen after both intraperitoneal administration of inactivated bacterin as well a preparaed OMV from *A.salmonicida* especially in the targets immune organs. Moreover after 14 and 28 day post vaccination a high titer of *A.salmonicida* specific antibodies was measured in both treated fish groups indication an antigen specific immune response

DO DOWN-STREAM PROCESSING OF BAKERS YEAST (*Saccharomyces cerevisiae*) AFFECT DIGESTIBILITY AND IMMUNE RESPONSE IN ATLANTIC SALMON (*Salmo salar*)?

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Introduction

The increased demand for high-quality feed ingredients for the aquaculture industry have given increased focus on microbial ingredients as sources of both protein and lipids. However, limited information exist on the effect of different down-stream processing of yeast on nutrient digestibility and immune response in fish. In the present study, we used three different down-stream processing methods for yeast: spray drying, autolysis or cell crushing and evaluated their effects on nutritional and health for Atlantic salmon (*Salmo salar*).

Materials and methods

In detail, we first re-suspended fresh live bakers yeast (*Saccharomyces cerevisiae*) in deionized water and then processed using one of the following six methods: spray dried at 1) 180 or 2) 250°C, treated with a high shear processor LM20 Microfluidizer with a force of 3) 10 000PSI or 4) 20 000PSI, and autolyzed using a 30l Bioreactor system at 50°**C** for 5) 8 or 6) 16h. Nitrogen leakage and fixation of yeast cells for electron microscope were performed before final spray drying. Solubility of β-glucan and mannan were tested in pre-digested (*in vitro*) spray dried yeast. The experimental diets consisted of 700g kg^{-1} of a fishmeal based reference diet (including yttrium as a digestible marker) and 300g kg^{-1} of one of the processed yeast candidates. Fish were fed with one of the seven resulting diets for 30 days, and faeces, spleen, head kidney, distal intestine and plasma were collected from fish at the end of the experiment

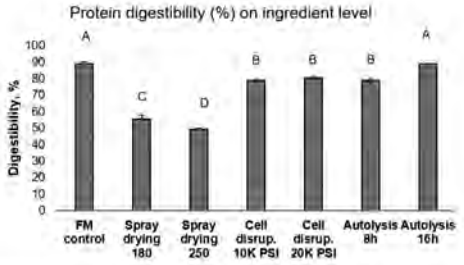

Fig. 2. Protein digestibility of different processed *S. cerevisiae* in Atlantic salmon.

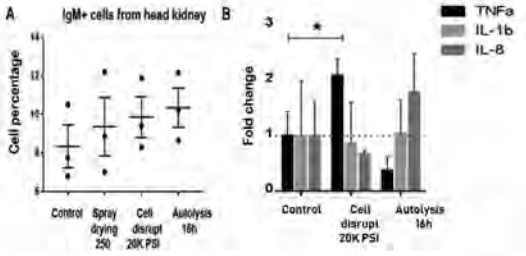

Figure 5. A. Percentage of leukocytes IgM+ from head kidney of Atlantic salmon fed different processed *S. cerevisiae*. B. Detection of immunological markers by indirect ELISA in distal intestine from *Salmo salar* fed processed yeasts by cell disruption (20k PSI) and autolysis (16 hours). *: $p<0.05$ (Dunnett's multiple comparisons test).

(Continued on next page)

Results and discussion

Processing of yeast resulted in increased level of soluble protein in the yeast cream post-treatment, with the highest level for yeast disrupted at 20K PSI. This was confirmed with scanning electron microscopy, were disrupted and cracked cells could be observed (Figure 1). Fish fed yeast that was only spray dried resulted in a lower growth and higher FCR compared to the FM control. Significant lower protein digestibility of spray dried yeast support the growth performance data (Figure 2). Autolysis for 16h resulted in the highest protein digestibility, which was similar to the FM control.

There was normal histology observed in DI of all fish. Interestingly, 16h autolyzed yeast induced the secretion of IL-8, while cell crushed yeast induced the secretion of TNFα in the distal intestine, analyzed by ELISA. The expression of IgM in leukocytes was analyzed by flow cytometry. Thus, processing of yeast could have an effect on immune stimulation on fish.

Conclusion

Different down-stream processing methods of *S. cerevisiae* led to:

- Increased protein and β-glucan solubility of yeast
- Increased protein digestibility in Atlantic salmon
- Enhanced immune stimulatory effect in Atlantic salmon

DECIPHERING THE GENOME OF THE RIVER MONSTER - THE EUROPEAN CATFISH (*Silurus glanis*)

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The European catfish (*Silurus glanis*) is one of the largest freshwater fish species, top predator and an iconic trophy-fish for recreational fishermen. *S. glanis* is a highly valued species for its high quality boneless flesh and has a long tradition of aquaculture in Eastern and Central Europe. The interest of rearing European catfish continues to grow and the aquaculture production of this species has almost doubled during the last decade. However, despite its high ecological, cultural and economical importance the available genomic resources for *S. glanis* are very limited.

To fulfill this gap we generated *de novo* assembly of the whole genome sequence of a female European catfish. The linked-read based technology with 10X Genomics Chromium chemistry and Supernova assembler produced a highly continuous draft genome of *S. glanis*: ~0.8Gb assembly (scaffold N_{50} = 3.1Mb; the longest individual scaffold 13.7Mb; BUSCO completeness of 85.5%), which included XXX Mb of putative repeated sequences.

By using *S. glanis* transcriptome assembly based on RNA-seq data from eleven tissues we subsequently annotated catfish genome. A total of YYY protein-coding genes were predicted, ZZZ (NN%) of which were annotated functionally from either sequence homology or protein signature searches. Finally, we demonstrate the power and usefulness of the annotated catfish genome by sequencing of 62 individuals in three DNA pools (Pool-Seq) comprising one albino and two normal strains of *S. glanis* from the Czech Republic and by identifying the molecular basis of albinism.

The highly continuous genome assembly will be an invaluable resource for aquaculture genomics, genetics, conservation, and breeding research of the European catfish

DO TROPICAL CORALS GET STRESSED DUE TO SHIPPING?

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Introduction

Live corals are being increasingly shipped worldwide, either from the wild or from *in situ* coral farms located in tropical regions, to supply wholesaler aquarium companies, hobbyists, researchers and general public (Leal et al., 2015). Corals are usually shipped, over long distances, in plastic bags filled with seawater and oxygen. During this period corals can be affected by temperature fluctuat ons, mechanical damage or hypoxia. These stressors can compromise organisms' wellbeing and induce several stress responses, which ultimately can lead to death. It is important to understand the physiological effect of transport on different coral species. Stress biomarkers can be a valuable tool to access the sub lethal impacts on organisms' metabolism, oxidative stress and oxidative damage (Rodrigues et al., 2017). Thus, it is possible to evaluate the resistance and resilience of different coral species submitted to a shipping practice, and even identify biochemical parameters to predict whether the coral is in good shape to be transported, or otherwise, if harvesting and shipping may lead to coral death. In this way, two soft coral species *Sinularia polydactyla* and *S. asterolobata* were submitted to a usual 48 h shipping, and their oxidative stress response, oxidative damage and energy budget changes were evaluated.

Materials and methods

Corals were purchase to wholesaler company, which transported them in plastic bags, containing seawater and oxygen. Shipping lasted approximately 48 h, since harvest in Batam, Indonesia, until arrival at our laboratory facilities in Ecomare, Aveiro, Portugal. Coral colonies were sampled at arriving (Ti – initial time) and after 3 months (Tf – final time), to analyze biochemical biomarkers of antioxidant defenses (catalase – CAT, glutathione S-transferase – GST, total glutathione – tGSH), oxidative damage (lipid peroxidation – LPO) and energy consumption (Ec – estimated by the electron transport system activity).

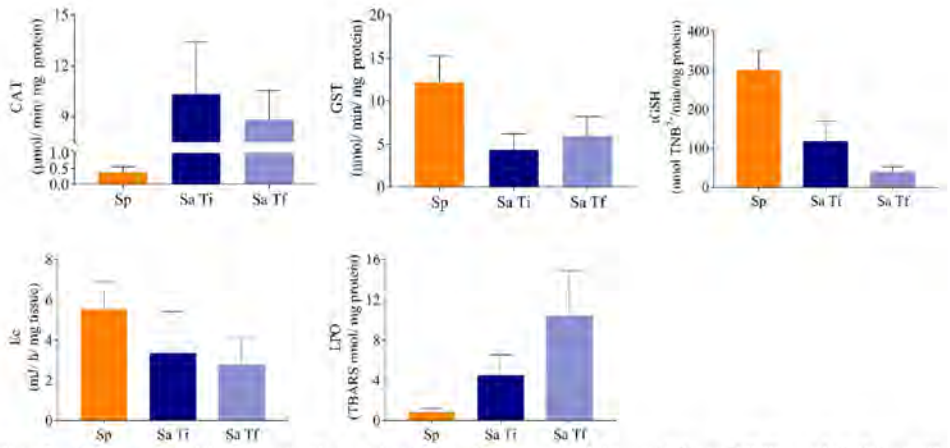

Fig. 1 - Effects of shipping on the antioxidant defenses: CAT – catalase, GST – glutathione S-transferase, tGSH – total glutathione; on metabolic respiration: Ec – energy consumption; and on oxidative damage: LPO – lipid peroxidation, of *Sinularia polydactyla* (Sp - orange) and *Sinularia asterolobata* (Sa - blue), in two different sampling points (Ti – initial time, and Tf – final time). All values are presented as mean ± SD.

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Results

S. polydactyla colonies did not recovered and died one week after arrival. Results of oxidative stress response of *S. polydactyla* for Ti show high values of antioxidant defenses activities and levels (GST and tGSH), combined with a high metabolic activity (Ec values). Regarding *S. asterolobata* results, it had high values of CAT activity at Ti sampling, but low GST activity and tGSH levels. Surprisingly, LPO values were slighter higher at Tf than in Ti samplings.

Discussion

The *Sinularia* species had different reactions to handling and shipping. The transport induced more stress in *S. polydactyla* than in *S. asterolobata*, ultimately leading to *S. polydactyla* colonies dead. In Ti, CAT activity was partially suppressed in *S. polydactyla,* and antioxidant activity was mainly performed by GST and tGSH. As expected, stress caused a higher energy consumption for *S. polydactyla*. The low lipid peroxidation observed may be explained by cell apoptosis, leading to a stage that LPO cannot be measured. In terms of antioxidant defenses and metabolic respiration for *S. asterolobata* we do not verify any damage and see a visible recovery after shipping. In this way, the LPO values observed for the Tf sampling may be close to normal, as these organisms live in association with photosynthetic dinoflagellates, and have a daily cycle of ROS production, but further investigation on this matter needs to be done. In sum, shipping can cause cellular damage, depending on corals species resistance, even in organisms belonging to the same genera.

Note that this work does not encourage the harvest of wild animals from their natural habitat and it mainly focuses on a procedure commonly performed. Besides, it can add value to tropical corals sustainably produced *in* or *ex situ*.

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TESTING DIFFERENT PROTOTYPE DIETS FOR CORAL AQUACULTURE

Ana P.L. Costa*, Igor S. Bernardo, Davide A.M. Silva, Andreia C.M. Rodrigues, Catarina R. Marques, Amadeu M.V.M. Soares and Rui J.M. Rocha

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Introduction

The increasing demand of corals led to the need to create a sustainable solution for coral delivery without compromising natural stocks, already affected by climate change and human impact. Coral aquaculture *ex situ* can be a viable option to meet the needs (Leal et al., 2013)growing evidence suggest that their symbiotic bacteria produce most of these bioactive metabolites. The ex hospite culture of coral symbiotic microbiota is extremely challenging and only limited examples of successful culture exist today. By contrast, in toto aquaculture of corals is a commonly applied technology to produce corals for aquaria. Here, we suggest that coral aquaculture could as well be a viable and economically feasible option to produce the biomass required to execute the first steps of the NP-based drug discovery pipeline.",”author”:[{“dropping-particle”:””,” family”:”Leal”,”given”:”Miguel C.”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:” ,”family”:”Calado”,”given”:”Ricardo”,”non-dropping-particle”:””,”parse-names”:false,”suffi ”:””},{“dropping-particle” :””,”family”:”Sheridan”,”given”:”Christopher”,”non-dropping-particle”:””,”parse-names”:false,”suffi ”:””},{“dropping-particle”:””,”family”:”Alimonti”,”given”:”Andrea”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dro pping-particle”:””,”family”:”Osinga”,”given”:”Ronald”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””} ,”container-title”:”Trends in biotechnology”,”id”:”ITEM-1”,”issue”:”10”,”issued”:{“date-parts”:[[“2013”,”10”]]},”pa ge”:”555-561”,”publisher”:”Elsevier Ltd”,”title”:”Coral aquaculture to support drug discovery”,”type”:”article-journa l”,”volume”:”31”},”uris”:[“http://www.mendeley.com/documents/?uuid=1f15707b-e78a-4214-931f-7cbece2580d5”]}],”mendeley”:{“formattedCitation”:”(Leal et al., 2013, but is important to take into account species requirements and economic questions for an optimal culture (Rocha et al., 2013). Symbiotic corals, that live in association with unicellular photosynthetic dinoflagellates, require an appropriate supply of light intensity and spectra, but still needs the input of heterotrophy for supplementary essential nutrient acquisition (Houlbrèque and Ferrier-Pagès, 2009)that they are both auto- and heterotrophs, was recognized early in the twentieth Century. It is generally accepted that the symbiotic association between corals and their endosymbiotic algae (called zooxanthellae. Thus, understanding coral development-related questions, as mixotrophy, can be crucial for the appropriate control of culture parameters and maximization of coral growth (Costa et al., 2016). Four coral species commonly reared and with potential for bioactive compounds production were selected. Three microencapsulated diets' prototypes formulated by SPAROS were provided and their effect on growth, photosynthetic efficiency and cellular ene gy allocation evaluated.

Materials and methods

Colonies of the four selected species (*Sinularia brassica*, *Sarcophyton glaucum*, *Zoanthus* sp. and *Palythoa* sp.) were purchase from wholesaler company, which collected them in Indonesia. After an acclimatization period in the culture systems, all coral colonies were fragmented in mini colonies using a sterilized scalpel and sewed in aragonite and cement bases. Recovered coral fragments were placed in four independent recirculated systems (for system detail see Rocha et al. (2015) to test 3 prototype diets. the corals of the fourth system were not fed during the whole experiment. Each experimental system was stocked with 9 replicas (mini colonies) of each species. Experimental diets (squid and fishmeal-based diet, artemia-based diet, and microalgae-based diet) were provided two times per week. During feeding time experimental culture tanks were disconnected from the filtration tank for a period of 30 minutes, to allow corals to feed before the experimental diets begin removed from the system by the protein skimmer. This experiment will have a duration of 5 months. At the beginning (Ti) we performed measurements of: (1) coral size to assess growth - photographic registration of octocorals and polyp count of hexacorals, (2) *in vivo* chlorophyll fluorescence, using a pulse amplitude modulation (PAM) fluoromete , (3) biochemical biomarkers of cellular energy allocation. At the end, the same methodologies will be performed for all treatments. Additionally, the polyp number count and *in vivo* chlorophyll fluorescence measurements were made in the middle of experimental period, as non-invasive methods, to obtain some early results.

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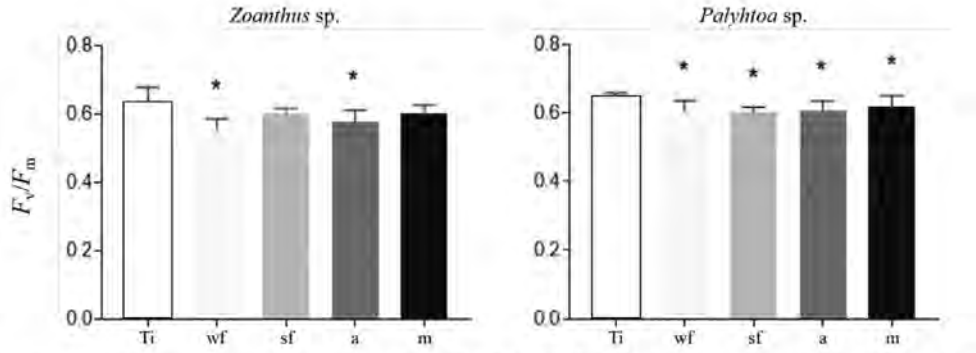

Fig. 1 –Maximum quantum yield of PS II (F_v/F_m) of coral fragments (mean ± SD, n = 9) at the beginning (Ti) and at the middle (wf – without feed, sf - squid and fishmeal-based diet, a - artemia-based diet, m - microalgae-based diet) of the experiment. Statistically significant differences between treatments are distinguishable by symbol (*) ($p < 0.05$ for all comparisons).

Results

Results of coral growth show that Zoanthus sp. had a higher percentage of polyp growth with squid and fishmeal-based diet, whereas Palythoa sp. had higher polyp development without feed. Comparisons between intial F_v/F_m of coral fragments from same species show that they had the same photosynthetic performance. The mid-stage results show statistical differences in Zoanthus sp. F_v/F_m, between squid and fishmeal-based diet and artemia-based and initial sampling. For Palythoa sp., all treatments had differences in F_v/F_m values comparing with initial sampling.

Discussion

It is visible a different response by Zoanthus sp. and Palythoa sp. towards feed after 2,5 months of supply. Zoanthus sp. colonies seem to have a greater growth with squid and fishmeal-based diet, presenting an approximate photosynthetic capacity towards initial values. In contrast, Palythoa sp. fragments had a greater development without feed, although all treatments showed a reduction on the photosynthetic capacity through the experiment. This contrasts to what it was expect for hexacorals, as the availability of external feed induces a photosynthesis enhancement (Anthony and Fabricius, 2000; Houlbrèque and Ferrier-Pagès, 2009)phototrophic energy gains can be diminished due to light absorption by suspended particles, and stress from particle abrasion or deposition on tissues. However, energy gains are enhanced if organisms are able to use SPM as a food source. For photosymbiotic benthic suspension feeders, increases in SPM concentrations may require both phototrophic and heterotrophic acclimation to sustain a positive energy balance. This study provides an experimental analysis of the effects of contrasting light and SPM regimes on the energy budget (scope for growth. Photobiological and biochemical changes will be presented and discussed into further detail, but overall our data highlights the differences in nutritional requirements among coral species.

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MICROBIOLOGICAL LOAD OF BIVALVE MOLLUSCS, CULTURED IN CLASS C AREAS, AFTER A DEPURATION PROCESS TESTING WATER TEMPERATURES

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Introduction

Consumption of bivalve molluscs may represent a risk to human health (Campos and Cachola, 2007) and so, depending on the microbiological load, bivalves require depuration before commercialization, to ensure food safety. European legislation requires the classification of production areas in class A, B or C, based on *Escherichia coli* load, which must not exceed 230, 4600 and 46000 *E. coli*/100 g of flesh and intravalvular liquid, respectively. Class C areas are the most problematic, due to the requirement of transposition of bivalves to relying areas for more than two months prior to depuration, leading to organism's mortality and economical costs, that may be unbearable. Marine bivalve depuration can be performed in flow through or recirculating systems using filtered seawater during a period of 24 h or more. Usually, the depuration systems used in Europe are equipped with mechanical filters, UV sterilizers, temperature control systems (water chillers), and, in some cases, protein skimmers, using the same water parameters for all purified species. However, due to physiological specificities, it might be relevant to adapt water parameters, namely temperature, to the physiological requirements of each species, in order to optimize the depuration process. This study aimed to assess whether it is possible to depurate marine bivalves reared in class C areas, reducing *E. coli* to legally accepted levels in 24 h, using different water temperatures.

Materials and methods

For this study were selected three species of bivalves with a broad distribution and high economic value in Europe: clam (*Ruditapes decussatus*), razor clam (*Solen marginatus*), and cockle (*Cerastoderma edule*). Bivalves were harvested in class C zones in Algarve, South Portugal, transported to laboratory facilities and depurated in different temperature scenarios: 10, 15, 20, and 25 °C. Experiments were performed during 24 h in 250 L modular depuration systems, individually equipped with filtration (UV-C unit and protein skimmer) and temperature control systems. Before and after the depuration process, edible tissue and intravalvular liquid from bivalves were sampled to analyse microbiological content. The most probable number (MPN) of *E. coli* present in the bivalves was calculated according to the method described by ISO 16649-3:2015 (ISO, 2015).

Results

The better temperature for depuration, regarding *E. coli* values, differ between species. While *R. decussatus* depurated better at 20 ºC, *S. marginatus* presented lower values of *E. coli* at 10 and 15 °C. For *C. edule*, depuration process was more efficient at 20 and 25 ºC.

Discussion

According to the European legislation, bivalves produced or harvested in class C areas cannot be commercialized for direct human consumption, which can represent significant economic losses, hindering the aquaculture of bivalves in many areas with conditions for the development of this activity. However, the results obtained for studied species suggest that it is possible to depurate bivalves harvested in class C areas in 24 h, using adequate methodologies, guarantying the quality and safety of the product for human consumption. It is clear that different species may have specific physiological requirements that must be considered during the depuration process. In this study the results shown that temperature can have a preponderant effect in the depuration process, which might be related with physiological or morphological aspects, or even, with the ecological niche occupied by the different species, since studied bivalves live buried at different depths in the substrate. In the future, other tests should be carried out to study the interaction between temperature and other water parameters (e.g. salinity), in order to optimize the purification process, considering the physiological requirements of each species.

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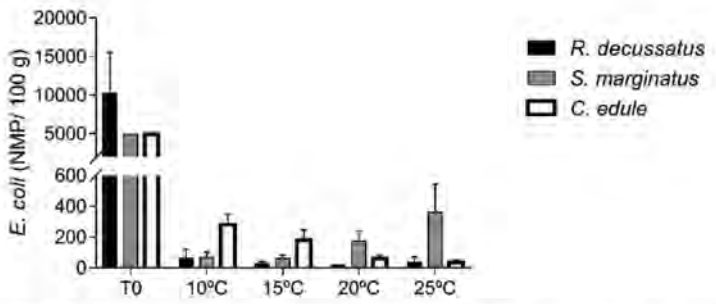

Fig. 1 – The most probable number (MPN) of E. *coli* per 100 g of flesh and intravalvular liquid before (T0) and after a 24 h depuration process, at each tested temperature (10, 15, 20, and 25°C), for *Ruditapes decussatus, Solen marginatus* and *Cerastoderma edule*. All values are presented as mean ± SD.

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IMPLEMENTATION OF LOW COVERAGE GENOTYPE BY SEQUENCING IN THE SELECTIVE BREEDING PROGRAM OF ARCTIC CHARR (*Salvelinus alpinus*) IN SWEDEN

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Introduction

The Arctic charr breeding program has been a main driving force for developing the aquaculture industry in Sweden. Selection has been performed for over 30 years using only animals from a closed breeding nucleus. Substantial growth improvement compared to wild stocks has been realized over the years (Eriksson *et al.* 2010)a breeding programme and the study of the biological and behavioural characteristics of the species. Traits of three different Arctic charr populations differing in ecology and appearance were compared during initial 2-year trials under farming conditions. The best-performing population with respect to the growth rate and the lowest frequency of early sexual maturation was a piscivore form and this became the foundation for a breeding programme intended to select for an Arctic charr strain suitable for farming. After 23 years and 7 generations, our selective breeding has resulted in a fast-growing, late-maturing strain much appreciated by farmers. The biological and behavioural characteristics studied included annual and diel locomotor activity, feeding, social and thermal behaviour. Applying our findings in these areas has greatly improved both profits and conditions for the fish. Other investigations have focused on the application and further evaluation of the results from research in practical farming trials, such as evaluation of growth at different farms with different temperature conditions, optimal time and stocking density for start-feeding and evaluation of different feeding schedules. In Sweden, Arctic charr is mainly farmed in net pens situated in nutritionally depleted and extremely unproductive water reservoirs formed by damming rivers to create electric power. Judicious farming of Arctic charr in such reservoirs can restore their nutritional and productivity state to that which existed before regulation. Site selection criteria for Arctic charr farming in such waters have been developed. The development of intensive farming of Arctic charr in Sweden is discussed together with current limitations and future possibilities. © 2010 Springer Science+Business Media B.V.”,”author”:[{“dropping-particle”:””,”family”:”Eriksson”,”given”:”Lars Ove”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Alanärä”,”given”:”Anders”,”non-dropping-particle”:””,”parse-names”:false,”suffi ”:””},{“dropping-particle”:””,”family”:”Nilsson”,”given”:”Jan”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping particle”:””,”family”:”Brännäs”,”given”:”Eva”,”non-dropping-particle”:””,”parse-names”:false,”suffix” ””}],”container-title”:”Hydrobiologia”,”id”:”ITEM-1”,”issue”:”1”,”issued”:{“date-parts”:[[“2010”]]},”page”:”265-274”,”title”:”The Arctic charr story: Development of subarctic freshwater fish farming in Sweden”,”type”:”article-journal”,”volume”:”650”},”uris”:[“http://www.mendeley.com/documents/?uuid=c18f1f29-9421-4532-abf5-c013d8977afd”]}],”mendeley”:{“formattedCitation”:”(Eriksson et al. 2010. Nevertheless, up to date selective breeding has been practiced solely relying on estimating breeding values through traditional pedigree-based approaches. A main advantage of genomic tools over pedigree lies on the ability of providing higher resolution relationships amongst the breeding candidates. Genotype by sequencing (GBS) technologies offer a cost-effective approach for utilizing modern genomic-based selective breeding practices. Low sequencing coverage GBS has the potential of further lowering genotyping costs, while maintaining the ability of accurately estimating relationships amongst the tested population (Dodds et al 2015).

Materials and methods

Arctic charr of year class 2016 from the national Swedish breeding program was used for constructing GBS sequencing libraries. In particular, 372 animals from 12 full-sib families were chosen for genotyping purposes. The aforementioned animals were reared in three different farms in Sweden. GBS libraries were constructed according to Elshire et al (2011) with modifications described in Dodds et al (2015). Sequencing was performed in two sequencing lanes of an Illumina HiSeq2500. Read demultiplex was performed using GBSX (Herten et al 2015) and SNP discovery using Stacks (Catchen et al 2011). Genomic relationship matrices were constructed following the methodology of Dodds et al (2015). Finally, single-step GBLUP approaches (Aguilar *et al.* 2011; Legarra *et al.* 2014) using the genotyped animals and additional pedigree records of 8,903 animals were tested to investigate the potential of low coverage GBS on improving the accuracy of breeding values regarding growth traits in Arctic charr.

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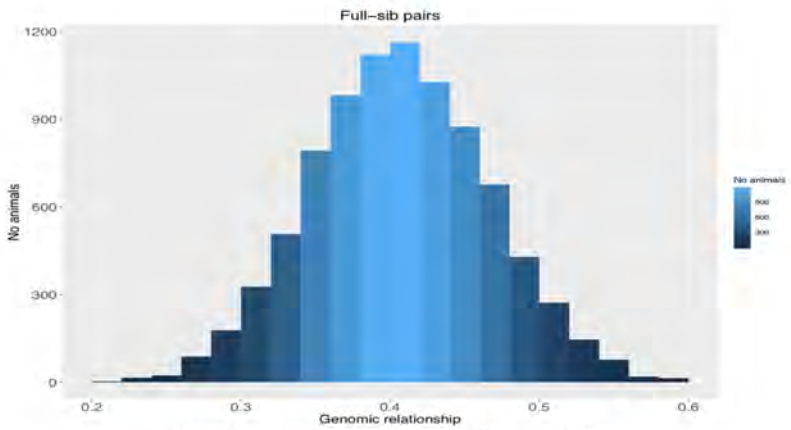

Figure 1. Relationships amongst full-sibs.

Results

Approximately 14,500 SNPs with minor allele frequency and call rate above 0.05 and 50% respectively were detected. The mean obtained coverage was 4X across the genotyped samples. A mean relationship of 0.44 was estimated across all tested full-sibs (Figure 1).

Using a cross-validation scheme (replicated 5 times) with 1/3 of genotyped animals being used as a validation set ssGBLUP resulted in 10 – 14 % increase of breeding value accuracy compared to pedigree BLUP (best linear unbiased predictor).

Discussion and conclusion

The current study provides the first identification of SNP markers in the selective breeding stock of Arctic charr in Sweden. Application of low-coverage GBS was efficient in identifying relationships amongst the tested animals. A higher resolution of realized relationships was obtained as opposed to traditional pedigree-based approaches. Despite the small number of genotyped animals (372) ssGBLUP resulted in 10 – 14 % increase in the accuracy of estimated breeding values. Increasing the number of genotyped animals is expected to provide more accurate measures regarding the advantages of genomic technologies. Overall, the incorporation of GBS is expected to boost the Arctic charr breeding program allowing more efficient management of inbreeding accumulation through estimating realized relationships and increase the accuracy of selection amongst the breeding candidates.

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NUTRITIONALLY CHALLENGED EURASIAN PERCH LARVAE (*Perca fluviatilis*) SHEDS LIGHT ON ADAPTABILITY OF FISH TO COMPOUND DIET

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Introduction

Domestication is an indispensable element of expansion of aquaculture sector. By phenotypic modifications domestication makes the organisms more adapted to the culture conditions (Diamond, 2002). There is very few fish species with the highest level of domestication in aquaculture (Teletchea and Fontaine, 2014). And these fully domesticated ones are among the best performing species (exhibiting high survival, feed conversion ratio, spawning performance etc.) in aquaculture. Therefore, any new species in aquaculture, having huge importance in the expansion of the sector as a whole, is exposed this complicated process. However, there is very little known about the mechanisms conditioning adaptability of the fish to the intensive culture environment. Consequently, reaching higher levels of domestication is a very difficult and slow process.

Larval rearing in intensive culture conditions is the first challenge test for the fish being, in fact, first step of selection. Only the most adaptable individuals will survive and grow during this period. During this period quality of the commercial feed is one of the biggest challenging factor during as it highly deviates from the food available in the nature. Understanding of this adaptation process is crucial in development of effective rearing protocols as well as selection strategies.

The aim of this study was to investigate the effect of two different types of compound diets with different protein sources – easily digestible fish-meal-based (control; C) diet and hardly digestible soy-bean-meal-based diet (experimental; E) – on growth and survival of Eurasian perch (*Perca fluviatilis*) larvae. In this study two separate rearing trials were performed where larvae coming from domesticated and wild fish were used

Materials and methods

Eurasian perch larvae were originated from "Żurawia" pond system (wild fish) or from commercial intensive farm (SARL Asialor, France) (domesticated fish). Both populations were reproduced with the same method (as described by Żarski et al. 2017). Larvae, following incubation, were reared in recirculating aquaculture system, for 18 days at temperature 23°C. After 18 days fish were weaned sharply (without co-feeding) to compound diet. Photoperiod was constant (24L:0D) throughout the study.

As a control feed (Perla larva, Skretting, Norway) was used. Experimental feed was prepared in extruder and the composition was as follows: 50% protein, 12.7% fat, 6.03% and 1.7% fibe . Fish were fed with compound diet 6 times a day, each time until apparent satiation for 12 days.

During the study larvae were monitored for growth (total length [TL] and wet body weight [WBW]) and survival. Data between the two populations (domesticated vs wild) were compared using t-test ($P<0.05$).

Tab. 1. Results of 12-day-long rearing trial with control (C) and experimental (E) diets offered to Eurasian perch larvae. Data in rows, among the respective parameter and between wild and domesticated population, marked with different letter index were statistically different ($P<0.05$).

Type of feed	Wet body weight [mg]		Total length [mm]		Mortality [%]	
	Wild	Domesticated	Wild	Domesticated	Wild	Domesticated
C	48.87 ±14.86^{b}	60.79 ±18.71^{a}	15.17 ±2.28^{b}	17.14 ±2.06^{a}	19.25 ±0.89^{a}	12.04 ±1.26^{b}
E	31.1 ±9.87^{a}	20.69 ±6.12^{b}	13.54 ±1.29	13.46 ±0.94	72.25 ±2.12	69.23 ±1.98

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Results

At the time of weaning domesticated fish were found to have significantly higher ($P<0.05$) TL (12.15 ±0.81 mm) and WBW (13.02 ±1.26 mg) than the wild-origin larvae (TL=10.84 ±1.23 mm; WBW=9.53 ±2.08 mg). After 12 days of rearing with the use of C feed domesticated fish were found to perform much more better as all the zootechnical parameters were significantly higher than recorded in wild-origin fish (Tab. 1). However, following application of E diet wild fish were found to grow better reaching significantly higher WBW at the end of the experiment ($P<0.05$). Even though, the TL were similar between the groups at the end of feeding period with E diet, it need to be emphasized that wild fish compensated growth differences recorded at the beginning of the challenge. The domesticated perch larvae performed lover mortality rate in diet C ($P<0.05$), but in diet E, no significant changes were found ($P>0.05$) (ab. 1).

Discussion

The results of our study indicates that domesticated fish were performing much more better – whenever typical rearing procedure is applied along with the high quality commercial feed. Interestingly, however, the same fish fed with feed having lowered expected digestibility, were found to utilize offered food less efficiently when compared to the wild-origin larvae. Digestion capabilities were reported to undergo diet-caused modifications which were then further inherited in higher vertebrates (Axelsson et al. 2013). This allows to suggest, that domesticated fish are very well adapted to particular composition of the feed (in this study protein source being crucial during larval period), but significant changes in composition had negative effect on their growth rate. High mortality rate (c.a. 70%) in both groups suggest that only some fish are able to adapt to such an ‘inadequate’ food, though wild-origin larvae were found to cope with this unfavorable diet much more better. This can be associated with the fact that wild fish over generations had to preserve adaptability to various food sources and their plasticity remained at higher level than the fish ‘selected’ on the base of their adaptation to specific compound diet.

Findings of the present study indicates that domesticated fish has narrowed adaptation capabilities when compared to wild fish. Even though, domestication improves adaptability of the fish to culture environment, it may be speculated that this applies only to the particular conditions they used to be exposed to. Therefore, any changes in production strategy (such as compound feed) may require longer re-adaptation to the new conditions, than it would take place when the domestication would start from the very beginning (with the use of wild-origin fish). In addition to that, it remains unclear whether such a re-adaptation would not lead to deprivation of some commercially valuable traits, what should be reconsidered in further studies.

Acknowledgements

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THE NEW DEFINITION OF AQUAPONICS: FUTURE PERSPECTIVES AND CONSTRAINTS

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Introduction

Traditional aquaponics is known to combine aquaculture and soilless plant production without details on production scales or species preferences. So far, many different aquaponic systems have been developed that show a wide range of production scales for private and commercial purposes. The European network "The EU Aquaponics Hub COST Action FA1305" has presented a new classification, nomenclature and definition of modern aquaponic systems in order to support their future development (Palm et al. 2018).

Aquaponics can be divided into I) open aquaponics, II) domestic aquaponics, III) demonstration aquaponics and IV) commercial aquaponics (Palm et al., 2018). Common systems range from outdoor facilities that involve pond fish farming (open aquaponics) to large industrial facilities that operate entirely indoors. The different production sizes enable private use with mini/hobby or backyard systems (domestic) for home-grown production, aquaponics for education and exhibition (demonstration units) or small-scale/large scale commercial ventures with access to the wholesale or retail markets. The main difference of each scale is found in the new nomenclature of aquaponics in the narrower sense (sensu stricto - *s.s.*) with traditional hydroponics compared to aquaponics in the wider sense (sensu lato - *s.l.*) with the use of aquaculture wastewater in horticulture or in agriculture (Palm et al., 2018).

The common feature of all aquaponic facilities is the combined production of aquatic organisms with different plants that are cultivated with the help of feed-derived nutrients converted through bacteria. Most aquaponics have been constructed as closed systems (*coupled aquaponics*) for freshwater fish and plants such as basil, mint, salad, greens, and tomatoes. They are often small or medium sized and operate economically only under both an optimal choice of location and an adequate market outlet. It is also known that salinity is a major ecological barrier in commercial crop production, and saltwater or marine aquaponics designers are still searching for suitable fish and plant combinations for inland production, in deserts, and along the coasts (Gunning et al., 2016, Appelbaum & Kotzen, 2016). New systems separate the fish and plant units at the larger commercial scale (category IV, *decoupled systems*) with fertilizers to support plant growth. Such systems have been tested especially with tomatoes as the main target crop, and compete with technologically advanced hydroponics production systems that can reach 100 kg of tomatoes per plant. A mixture of aquaponics and freshwater with fertilizer might be counter-productive in achieving the best plant yields, and economically successful production in these systems, of tomatoes for example, still awaits verification

Future perspectives and constraints

Aquaponics is defined as "a production system of aquatic organisms and plants where the majority (> 50%) of nutrients sustaining the optimal plant growth derives from waste originating from feeding the aquatic organisms" (Palm et al., 2018). This definition opens new options for aquaponic products but on the other hand enables authorities to clearly distinguish these products from hydroponic plants or produce originating from regular agricultural production systems.

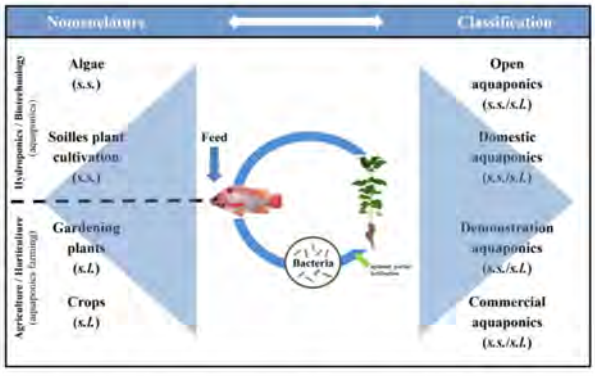

Figure 1 The correlation of new aquaponics nomenclature and classification (*s.s.* sensu stricto, *s.l.* sensu lato according to Palm et al., 2018) that allows new products.

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Future areas of development include the demarcation and expansion of the classical cultivation method. The soilless plant production principle is only one variant of integrated aquaponics production. In addition to hydroponic subsystems such as DWC, NFT, ebb and flo , drip or aeroponics, the use of algae is also possible at an industrial scale. The use of aquacultural process water in horticulture or on the open field by regular farmers, termed aquaponics (*s.l.*) farming, represents two new categories with soil or soil-like substrates (aquaponics *s.l.*- horticulture and agriculture). Native peat alternatives or humus substrates such as Biohumin® (Knaus et al., 2019) can replace fertilizers and upgrade the plant quality even under the direct use of the process waters. This allows the production of a wide range of other aquaponic products from ornamental plants to agricultural crops by the integration of nomenclature items to different scales of aquaponic systems (Figure 1).

One future challenge of modern aquaponics is the spread of these new aquaponics products in often saturated markets, requiring the proof of concept and economical business models. At the same time, the growth of aquaponics plants must have a relative agronomic effici ncy of over 50%. In addition to the use of aquacultural process water on cereal fields or for vegetable cultivation outdoors, other cultivation methods that do not rely on the process water but reuse the (processed) sediments or regained nutrients from aquaculture are also possible. This allows the processing, transportation and reuse of aquacultural waste based nutrients to other agricultural production sites and decouples the fish from the plant production facilities.

There is no doubt that we must move towards a circular economy. Aquaponics (*s.s.*/soilless; *s.l.*/with soil) allows a better reuse of aquacultural waste products. Because of the nearly unlimited possibilities for system designs, local adaptations and a wide range of produce, these systems still have high potential for further development.

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EFFECTS OF DIETARY GLYCEROL SUPPLEMENTATION ON LIVER AND MUSCLE METABOLOME: COMPARATIVE STUDY IN EUROPEAN SEABASS AND RAINBOW TROUT

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Introduction

The sustainable development of aquaculture still relies in the substitution of fishmeal for alternative ingredients, especially for carnivorous fish (Oliva-Teles et al., 2015; FAO, 2018). Glycerol has been already used as an alternative energy source in diets for farmed animals, sparing amino acids to other functions such as growth (Lall and Dumas, 2015; Rito et al., 2019). The aim of the work was to evaluate the effects of dietary glycerol supplementation in European seabass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) muscle and liver metabolome, as two of the most representative species for freshwater and saltwater aquaculture in Europe.

Material and Methods

Fish juveniles were fed diets with 0%, 2.5% and 5% glycerol, and groups were assigned respectively as S0, S2.5 and S5 in European seabass; and T0, T2.5 and T5 in rainbow trout. Muscle and liver aqueous fraction was extracted. ^{1}H 1D Nuclear Magnetic Resonance spectra were acquired and its metabolite profile was assessed. Multivariate (Principal Component Analysis – PCA) and univariate statistical analysis were applied. The energy charge was determined in muscle.

Results

In European seabass, PCA scores plots of both muscle and liver aqueous fraction shown no separation between groups. Univariate analysis revealed a decrease in L-leucine, isoleucine and valine between both experimental groups and the control group. Glycerol concentration in muscle had an increase of around 11 times between control group and S5.0 group. In liver, taurine, glycine and glycerol increased around four, three and five times respectively, from control to S5.0 group (Table I). Regarding the energy charge, no differences were identified between groups. The mean values of the three groups were around 0.8.

Table I. Summary of the metabolites identified in the muscle and liver aqueous fraction and its fold-change variation, between groups fed with the control diet with no glycerol (D0); and two experimental diets, supplemented with 2.5% (D2.5) or 5.0% (D5.0) glycerol. Key: (-) fold change with non-significant variation; (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$.

	Muscle	D2.5/D0	D5.0/D0	Liver	D2.5/D0	D5.0/D0
European seabass	L-leucine	0.78 *	0.78 *	Taurine	-	4.11 *
	Isoleucine	0.72 ***	0.73 ***	Glycine	-	3.18 *
	Valine	0.75 ***	0.75 ***	Glycerol	-	5.57 **
	Glycerol	-	11.79 **			
Rainbow trout	Choline	-	0.62 ***	Sarcosine	0.16 *	-
	Betaine	-	0.68 ***			
	Lactate	-	-			
	Niacinamide/Nicotinurate	1.56 *	-			

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Concerning rainbow trout, PCA scores plots of both muscle and liver aqueous fraction showed no separation between the metabolite composition of samples from the three groups. Regarding univariate analysis, in muscle, choline and betaine decreased around 0.6 times from T0 group to T5.0 group. On the other hand, niacinamide/nicotinurate increased 1.5 fold between T2.5 and T0 groups. In liver only sarcosine revealed a slight decrease from control group to T2.5 group (Table I). The mean values of energy charge of all groups were around 0.9 and no differences were identified between them

Discussion and Conclusion

Generally, dietary glycerol inclusion seems to promote different responses in rainbow trout and European seabass. Muscle seems to be more affected than liver in both species. In rainbow trout, muscle is more affected by the higher glycerol content diet, while in seabass variations were observed in both glycerol concentrations. Regarding liver, trout had only one metabolite with significant differences at 2.5% glycerol group, while in seabass metabolites varied only at 5% glycerol group.

In trout, regardless of the tissue and the dietary glycerol concentration, results were indicative of variations in choline-related metabolism. Differences in seabass were in general related with protein biosynthesis pathways. Only seabass showed increased glycerol concentrations in both tissues, which suggests its higher incorporation into tissues or its slower and/or incomplete metabolic utilization. Noteworthy, the triacylglycerol turnover rate in rainbow trout is indeed higher than the one in other fish species (Magnoni et al., 2008)

Regarding the effects on the general metabolism, at the tested concentrations, rainbow trout seems to be more prompt to this dietary glycerol inclusion.

NMR-metabolomics approach generates an optimized data output, requiring simple extraction procedures and short NMR time acquisitions. Concerning aquaculture production, this approach also allows an easy proceeding to assess muscle/fillet composition that could be applied in several experimental designs.

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PAN-EUROPEAN MODELLING OF PACIFIC OYSTER (*Crassostrea gigas*) GROWTH IN THE OFFSHORE ENVIRONMENT: INDICATORS, REGIONAL COMPARISON & CLIMATE CHANGE

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Introduction

Mariculture continues to rise in response to increased demand for seafood and stagnating wild fisheries. However, expanding shellfish aquaculture in intertidal areas, where cultivation traditionally takes place, is spatially limited. The possibility of expanding production offshore offers a solution to this problem, and its potential has been demonstrated *in situ* for several sites (e.g., Pogoda et al., 2011). Through modelling and mapping, this work examines the biological suitability of the offshore environment for Pacific oyster (*Crassostrea gigas*) cultivation across Europe and northwest Africa, and investigates the potential impact of climate change on future growth.

Materials and methods

Daily maps (0.1° spatial resolution) of various physical and biogeochemical parameters that influence the spatial range suitable for *C. gigas* cultivation and that underlie its growth rate were generated using the POLCOMS-ERSEM model (Butenschön et al., 2016; Ciavatta et al., 2016, 2018) for the North East Atlantic Ocean, North Sea, and Mediterranean Sea (see model domain in Fig. 1). Chlorophyll (Chl), as a proxy for phytoplankton (picoplankton size class excluded), and water temperature were used to force Dynamic Energy Budget (DEB) modelling, which quantitatively describes energy uptake and flow through an organism, including storage, growth, and reproduction (Kooijman, 2010). Growth of both *C. gigas* adults and spat was modelled and mapped.

Parameterization of *C. gigas* DEB modelling was as per Thomas et al. (2016), except for the half-saturation coefficient (Xk), which was calibrated using *in situ* data collected during offshore experiments by the *Syndicat Mixte pour le Développement de l'Aquaculture et de la Pêche en Pays de la Loire* (SMIDAP). Modelled results were then independently corroborated, using *in situ* data from the same site from a different year, as well as different years from another site from the literature (German Bight; Pogoda et al., 2011). In addition to an early-century reference period, a late century period (2090-2099) was also modelled and mapped, under varying climate change scenarios (RCP 4.5, 8.5).

Results and discussion

Several industry-relevant indicators were elaborated from the mapped growth data, including time to minimum market size, size in time for key markets (Fig. 1), and quality index, underlying market classification as "special", "fine", or "normal". Areas where salinity, current speed, temperature, and Chl ranges preclude *C. gigas* cultivation were masked, and two bathymetric ranges were considered: < 25 m depth, suitable for smaller-scale farmers, and < 200 m depth, with larger-scale operations with greater investment capacity in mind. Following the transformation of daily growth maps for the early- and late-century period scenarios into indicator maps (e.g., Fig. 1), several growth "hot spots" were revealed at the regional level, to be looked at in greater detail. Some were found to have high growth, but over a relatively small spatial area, and some were found to have high growth in the early-century reference period, but to be negatively affected by climate change. Areas where growth is consistently high across an area, and into the future, as well as under different climate scenarios (i.e., climate robust) are suggested to be better choices to investigate, as they offer a sort of buffer against the uncertainties of future climate change. Likewise, from a policy and industry-development standpoint, areas where growth is currently not exceptional, or where farms are currently not situated, but for which modelling suggests high and climate-robust growth in the future may be of interest.

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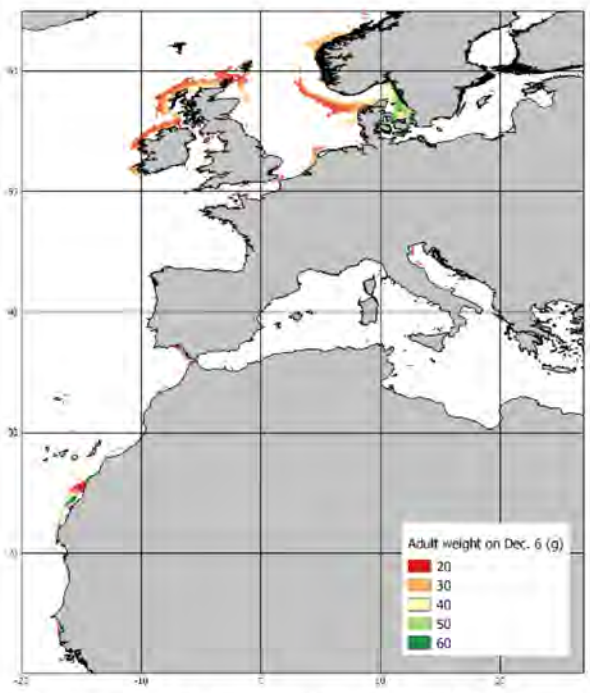

Figure 1. The model domain extent and an example of a mapped Pacific oyster growth indicator, adult weight in time for the December market, for the early-century (2000-04) reference period. Indicator maps and raw modelled growth data are also available for the two future scenarios (2090-99; RCP 4.5 and 8.5).

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TESTING RECOMBINANT GONADOTROPINS FOR THE PROPAGATION OF EUROPEAN EEL (*Anguilla anguilla*), PRETREATED BY FEMINIZATION, SIMULATED MIGRATION AND STEROID IMPLANTS

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Introduction

The current protocol for the induced maturation of female European eel consists of long-term weekly injections with carp or salmon pituitary extract to stimulate sexual maturation and with 17α,20β-dihydroxy-4-pregnen-3-one (DHP) to induce ovulation (Palstra et al., 2005). However, egg quality is often poor and when larvae are produced they die before functional exogenous feeding.

As these problems may well originate from the used protocol and broodstock background, we commence the conditioning of female broodstock from the glass eels stage by feminization, simulated migration and steroid implants. This study is the second in a series of ongoing experiments in our lab aiming to replace treatment with pituitary extract by highly stable eel-specific recombinant gonadotropins in order to improve gamete quality and reproductive success. In a preliminary trial with wild eels we managed to mature one female with recombinant FSH up to a gonadosomatic index (GSI) of 38.

Materials and methods

Single chain recombinant gonadotropins (recFSH and recLH) were obtained by fusing the respective β and α European eel specific subunits with a linker peptide. These sequences were expressed in a mammalian cell line (CHO) and subsequently the gonadotropins were semi-purified by ion exchange chromatograph .

Experimental eels had been subjected to a simulated lifecycle approach: A batch of wild glass eels was feminised by feeding them with 17β-estradiol (E2) coated pellets over a 6 month-period. After an additional 6 months of feeding them with a custom-made broodstock diet, eels of ~400 g were selected, transferred to seawater and fed no longer. For 2 months, eels were then subjected to simulated migration: constant swimming in the dark at daily alternating temperatures between 10 and 15 °C to make them silver (Mes et al., 2016).

Sixteen of these eels were then randomly selected, PIT-tagged, weighed and measured for body girth, body length and eye diameters to calculate the Pankhurst eye index. For an additional 2 months, eels were treated with a steroid implant (Thomson-Laing et al., 2019) containing 17-methyltestosterone (5 mg) and E2 (0.5 mg). Again, eels were weighed and measured, and GSI was non-invasively determined by applying ultrasound. Eels were then divided between two groups for hormonal stimulation of sexual maturation by weekly intramuscular injection of either carp pituitary extract (CPE at a dose of 20 mg kg^{-1}; CPE group; N=8) or recFSH (12 µg; REC group; N=8).

Starting in week 7, two days after each injection, eels were weighed to determine whether oocyte hydration had commenced as indicated by an increased body weight index (BWI). At BWI increase and at recFSH injection 16, final oocyte maturation (FOM) was induced by injecting CPE in the CPE group, or by injecting recFSH in combination with recLH (8 µg) in the REC group. Ovulation for eels in both groups was induced by injecting DHP (2 mg kg^{-1}). Eels were stripped for eggs which were fertilised and reared.

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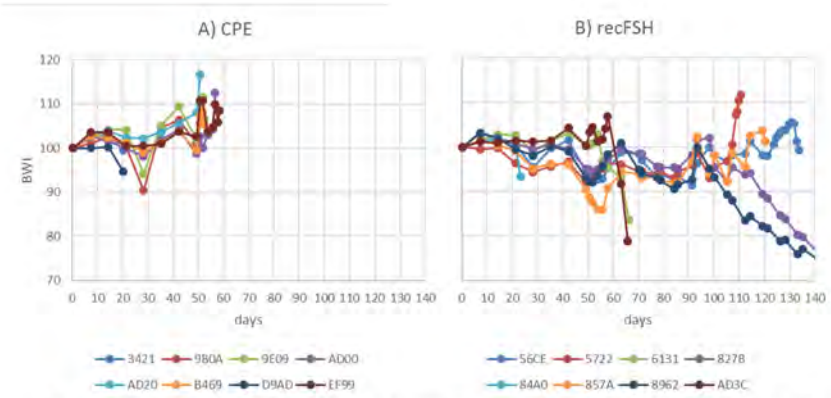

Fig. 1. Body weight index (BWI) during the period of weekly hormone injections with (A) CPE and (B) recFSH.

Results and discussion

At the end of migration and start of injecting implants, eels were 59 ± 3 cm long, weighed 402 ± 33 g, body girth was 11.5 ± 0.4 cm and eye index was 7.19 ± 1.46. At the end of this period when dividing both treatment groups, eels increased significantly in weight up to 418 ± 33 g, in body girth up to 12.7 ± 0.5 cm and in eye index up to 10.64 ± 1.09, indicating initiation and progression of vitellogenesis. Indeed, the GSI values were between 3.1 and 7.3, well above values of 2-2.5 which mark the initiation of vitellogenesis (Jéhannet et al., 2019).

Eels from the CPE group matured much faster than eels from the recFSH group: 8 ± 1 vs. 19 ± 3 injections (i.e., after combining recFSH with recLH). Six eels (75%) in the CPE group matured of which two yielded batches of larvae surviving for 4 and 9 days post hatching. Three eels (38%) in the REC group matured and from one eel (AD3C) eggs could be stripped (80% floated). However, no larvae were obtained from eels from this group. Four other eels in the REC group increased in BWI just before dropping weight and dying. Recombinant FSH appeared to work very well but the FOM protocol using recLH needs further improvement and is under current investigation.

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ACCELEROMETRY OF SEABREAM IN A SEACAGE: EFFECTS OF FLOW CONDITIONING ON ACTIVITY PATTERNS, GROWTH AND ROBUSTNESS

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Introduction
The flow regime can have great physiological impact resulting in enhanced growth and increased robustness (Palstra and Planas, 2013). By holding juvenile Gilthead seabream (*Sparus aurata*) on-land to sizes of ~20 g, and with a future perspective of holding them on-land even longer, farmers can benefit from applying an optimal flow regime. In a first study in RAS tanks (IRTA facilities, Sant Carles de la Ràpita), experimental fish were conditioned to flow regimes inducing swimming exercise at 0, 1 and 2 Body Length (BL) s^{-1} for a period of 8 months (Feb.-Oct.). Subsamples of these fish (N=100 per treatment) were then transferred to an experimental seacage in Mallorca (LIMIA facilities, Port d'Andratx) of which ten PIT tagged fish per treatment were implanted with accelerometer tags to investigate activity patterns. Activity will then be related to growth and robustness parameters of seabream in a cage in general, and to the effects of flow conditioning in particular. Accelerometry has been performed on Atlantic salmon in floating sea-cages (Føre et al., 2011, 2018), this is the first study investigating the activity of seabream in an experimental seacage

Materials and methods
Sizes of the experimental fish that were used for accelerometry were BL 174 ± 10 mm, BW 157 ± 28 g for fish conditioned at a flow of 0 BL s^{-1}; BL 185 ± 12 mm, BW 173 ± 36 g for fish conditioned at a flow of 1 BL s^{-1}, and BL 188 ± 24 mm, BW 189 ± 47 g for fish conditioned at a flow of 2 BL s^{-1}, indicating the exercise-enhanced growth that had occurred during flow conditioning in the RAS tanks. These fish were anaesthetized and then accelerometer tags (AccelTag AT-LP7; 21 x 7.3 mm, 1.9 g in air, 1 g in water; Thelmabiotel, Trondheim, Norway; Fig. 1) were implanted by a surgical incision on the ventral side in the body cavity and then sutured (Arechavala-Lopez et al. 2012). The accelerometers record every 60 s the gravity forces and movement along the three axes which can be converted to acceleration in m s^{-2} which is then used as a proxy for activity. Recordings were monitored by one receiver (TBR700; Thelmabiotel) placed at the bottom of the cage. Activity patterns were monitored for a period of 6 weeks (Nov.-Dec., with decreasing temperatures from max. 17.9 to min. 15.5 ºC) in a 2 x 2 x 2 m experimental sea-cage (fish density ~10 kg m^{-3}). Fish were fed manually once per day *ad libitum* in the morning at 11 h. After the 6 weeks period, fish were collected measured, weighed, and heart, liver, intestine, spleen, intestinal fat and fillet were dissected and weighed.

Results and discussion
Day/night rhythms in swimming activity under the experimental conditions were characterised by more active periods from 6 to 14 h and 18 to 0 h and less active periods from 0 to 6 h and 14 to 18 h. The peak in activity was not during but just before feeding indicating that experimental fish may have good ability to predict and time a re-occurring event such as feeding.

Growth performance was similar between fish of all three treatments. The initial differences that were due to exercise-enhanced growth therefore remained similar. Significant differences were found in overall activity patterns between flow conditioned groups. Exercised fish conditioned at 1 BL s^{-1} were more active than the 0 BL s^{-1} controls. This group of exercised fish also showed the largest relative heart size. Exercised fish conditioned at 2 BL s^{-1} were less active than the exercised fish at 1 BL s^{-1} and the 0 BL s^{-1} controls. These fish had the lower relative spleen size. These results indicate that the flow conditioning at 1 BL s^{-1} was optimal for robust juvenile seabream, a conclusion that was also supported by the results of the first study in RAS tanks

Activity patterning can be useful for timing feeding events. Under these conditions it may be advisable to feed seabream at the start of active periods; for example from 6 to 11 h and from 18 to 22 h, to favour muscle building at the cost of intestinal fat deposition. During the on-land phase in tanks, fish and farmer can benefit from applying flow enhancing swimming exercise at 1 BL s^{-1}.

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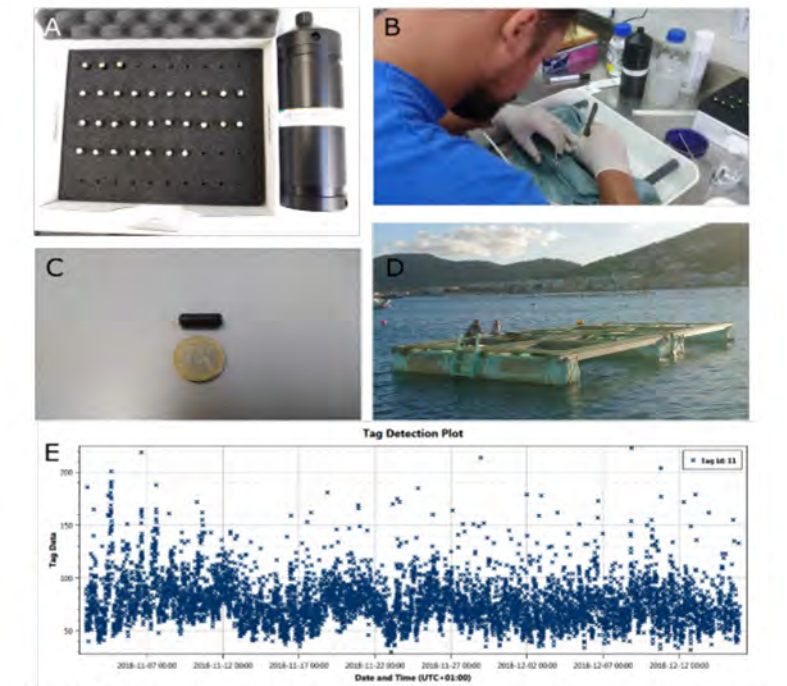

Fig. 1. (A) 30 accelerometer tags and 1 receiver, (B) seabream surgery to implant the tag, (C) tag size, (D) the experimental seacage, (E) recordings for one fish over the monitoring period of 6 weeks.

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ORGANIC REDUCTION AND NUTRIENT RECOVERY PERFORMANCES OF PIKEPERCH (*Sander lucioperca*) SLUDGE AEROBIC DIGESTION AND LETTUCE GROWTH PERFORMANCE IN ITS EFFLUENTS

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Introduction

Recycling of nutrients from fish sludge is needed to close the nutrient loop and make aquaculture and aquaponic systems more resources efficient and sustainable. Until now, only a few authors have examined the recovery of nutrients from fish sludge. While discussed to some extent in theory (Goddek et al. 2016; Yogev et al. 2017), practical investigation is further needed to better understand organic reduction and nutrient mineralisation performance of aerobic and anaerobic treatments of fish sludge, and its effect on the water composition and plant growth. Therefore, we investigated the mineralisation and sludge reduction performance of pikeperch RAS sludge aerobically digested in a long-term unique experiment. Hence, the first objective was to obtain the nutrient mineralisation performance by using specific mass balances equations developed in that specific purpose (Delaide et al. 2018). The second objective was to calculate the sludge reduction performance. The last objective was to test the suitability of aerobic effluent as a nutrient solution for direct use in a hydroponic system.

Materials & Methods

The experimental setup was divided in two parts running in parallel. One part consisted into a lab scale experiment to focus on nutrient mineralisation and sludge reduction performances using a setup of 3 blocks of 4 reactors each to assure repeatability and accuracy. The reactors were run in a fed-batch mode. Once every 7 days, supernatant was removed and of fresh sludge was added. This led to a hydraulic retention time (HRT) of 30 days. No sludge was discharged during the experiment thereby the sludge retention time (SRT) corresponded to the duration of the experiment. In order to determine the performances of the reactor, a mass balance approach was used based on Delaide et al.(2018) The experiment was run for 121 consecutive days. The second part focused on the yield and quality of lettuce grown in aerobic reactor effluents. The experimental setup consisted of growing lettuce in deep flow technique (DFT) filled with 3 different nutrient solutions (i.e. 3 different treatments): a standard hydroponic solution for lettuce (Std treatment) used as a control, the aerobic supernatant (AE treatment) and the hydroponic aerobic solution (HPAE treatment). The HPAE solution was made with rain water and chemical fertiliser for mimicking the AE solution. Each DFT contained 6 lettuces and 4 replicates were used. *Lactuca sativa* cv. Presteria were sown on turf blocs on the 26th of October 2018. On the 13th of March 2019, after 57 days of growth in the DFT the lettuces were harvested. The head and roots fresh mass were recorded, and their head colour was scored.

Results and discussion

Part1-Aerobic digestion: pH and EC were monitored weekly during the experiment. During the first six weeks, the pH decreased from 8 to 6 while the EC increased continuously. From the 10th week until the end of the experiment pH and EC stayed relatively stable, oscillating around 6,75 and 3,20mS/cm, respectively. An organic reduction performance of 40 ± 3% was observed during this experiment. The mineralisation performances were in a range of 10 to 40% for the macronutrients, except for P. Interestingly, P had a negative value indicating that instead of being mineralised this element stayed in its solid insoluble forms or precipitated or was assimilated by the microorganisms developing into the reactor. Except for B, the microelements had a very low mineralisation performance, i.e. < 1.6%. The aerobic treatment seems not efficient to mineralise the microelements. However, in the point of view of plant fertilisation suitability of the treatment effluent, the examination of the nutrient concentration profiles in pikeperch rearing water and then in the AE supernatant showed that the macro- and micronutrients were substantially increased by the aerobic treatment.

Part 2-Lettuce growth in aerobic supernatant: The HP standard treatment presented the highest head mass (675 ± 70g) while significant differences were not found ($P \uparrow$ 0.05) between aerobic supernatant and HP aerobic treatment, with a final head fresh mass of 528 ± 62g and 562 ± 68g respectively. During the experiment until the harvest day, no shape disorder was noticed for the lettuce heads in all treatments. However, a yellowing of the leaves was noticed in the AE and HPAE treatments. Nutrient monitoring revealed that P, K and Mn were relatively low in AE and HPAE solutions compared to the standard and were totally uptake by lettuce until depletion after 36 days (or even before) of culture. It is therefore supposed, that lettuce grown in these treatments suffered from nutrient deficiencies which influenced the final growth. Curiously, the AE treatment had no more yellowing leaves at the harvest time. It is presumable that other factors were present in the AE supernatant that allowed the lettuce to overcome the yellowing. This seems to be indicated by the significant differences in head to root ratio and a higher root mass that was observed in this treatment.

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Conclusion

This experiment investigated the feasibility of treating RAS sludge directly onsite by an AE treatment and the reuse of its effluents as nutrient solution for growing hydroponic lettuces. The lack of P and Mn observed in the DFT experiment could easily be linked to the poor performance of their mineralisation observed during the aerobic treatment. While they were present in substantial amount in the solid fraction of the sludge, they stayed in their insoluble forms. Regarding this low mineralisation performance, especially for P and the microelements, improvements of the treatment should be explored as adding an acidic step that can promote P mineralisation (Conroy and Couturier, 2010)"ISSN":"00448486","abstract":"Bio logical degradation of fish waste solids in the anaerobic zones of settling basins can reduce water quality and increase the environmental impact of aquaculture farms. The objectives of this study were to measure the rate of hydrolysis of waste solids from a salmon smolt hatchery and to investigate the concomitant dissolution of phosphorus and nitrogen. Hydrolysis followed first order kinetics with a reaction time constant of about 2 days. The rate of production of volatile fatty acids (VFA.

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EFFECT OF PHOTOPERIOD ON PIKEPERCH (*Sander lucioperca*) LARVAE PERFORMANCE IN RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

Pikeperch is considered to have the highest potential for inland aquaculture diversification in Europe. Throughout the last decades the culture of pikeperch in recirculating aquaculture systems (RAS) has increased. Nevertheless, larvae culture phase still represents a main bottleneck during commercial production. Several studies have described the influence of density, feeding strategy and tank designs in the performance of larvae reared in RAS (Kestemot et al. 2015). However, few studies about the influence of light conditions during this phase can be found (Tielmann et al. 2017). The aim of the present study was to investigate the influence of photoperiod on the performance of pikeperch larvae reared in intensive conditions until 30 days post hatch (DHP).

Materials and Methods

Pikeperch larvae, coming from the off-season reproduction in 2018 at Inagro Practical Research Aquaculture Center, were reared under 3 different photoperiod regimes Light: Dark (12L:12D, 16L:8D, 24L:0D) until 30 (DPH). Twelve 220 l RAS tanks (4 replicates per treatment) were used with and initial density of 110 larvae per liter. Light intensity at water surface was kept at 100 lx and water temperature at 16^{0}C. Feeding regime was similar amongst treatments using a constant feeding protocol (mixed diet: enriched artemia + dry feed). Performance parameters like length, body weight, survival rate, specific growth rate (SGR), swimming bladder inflation and % of deformities were measured at different DPH during the trial (n = 30 per tank). Data recorded was statistically analyzed to find significant d ferences ($\alpha = 0.05$) amongst treatments.

Results

Differences in larvae performance were observed amongst treatments during the experiment. In the first 15 days of rearing, an equal growth ($P > 0.05$) of larvae in weight and length was recorded (Figure 1). However, at the end of the trial, significant di ferences were found ($P < 0.05$).

Larvae reared in 16L:8D photoperiod presented the highest growth in wet weight (55.03±7.1mg) and standard length (19.23±2.1mm) compared to larvae reared in 12L:12D (41.35±3.2mg, 17.56±1.8mm) and 24L:00D (48.98±7.04, 18.1±2.59mm) respectively.

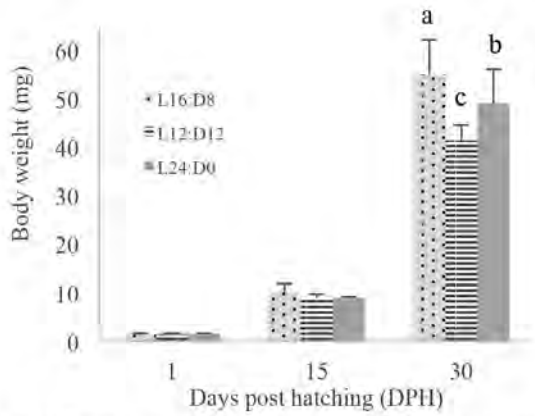

Figure 1. Body weight (wet weight) in pikeperch larvae reared until 30 days post hatching (DPH) under 3 different photoperiods Light: Dark (16L:8D, 12L:12D, 24L:D0). Data (mean ± SD), different letters mean significant difference amongst the groups ($P < 0.05$).

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Discussion and conclusion

The results obtained during this experiment indicate that pikeperch larvae reared under the photoperiod 16L:8D have a better performance compared to larvae reared under photoperiods of 12L:L12 and 24L:00D. Between 11- 15 DPH, important morphological changes on pikeperch larvae occurred (swim bladder inflation). After this period, larvae with noninflated swim bladders have higher metabolic demands due to erratic swimming and constant movement, which compromise their feeding capacity and therefore affecting their growth (Demska-Zakęś et al. 2003). More results and conclusion of this experiment will be presented.

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INDUCTION OF TRIPLOID DEVELOPMENT IN THE EUROPEAN GRAYLING *Thymallus thymallus*

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Introduction

European grayling (*Thymallus thymallus*) is a freshwater salmonid fish species. . Unfortunately populations of grayling have declined mostly due to the water pollution, overfishing and construction of dams. In several European countries stocking programs have been established to compensate for this loss. Unfortunately, this process included domesticated and non-native stocks and affected genetic pools of the grayling non-indigenous populations. To prevent local populations, sterile triploid graylings might have been used for stocking. However to date no protocol for induction of triploid development in grayling is available. Thus, the main goal of the present research is to elaborate conditions of successful triploidization of grayling.

Material and methods

Gametes for the experiment were provided from the grayling broodtsock kept in the Dept. of Salmonid Research in Rutki (Poland). Grayling eggs were inseminated and subjected to the High Hydrostatic Pressure (HHP) shock (8500psi for 5min) 10, 12.5, 15, 17.7, 20, 22.5 and 25 minutes after fertilization (*maf*). Part of the fertilized eggs were not subjected to HHP to develop as normal diploid (2n) specimens (controls). Fertilized eggs were placed in a hatching apparatus and incubated at 9°C under routine conditions. The survival of embryos was calculated at the eyed stage. Cytological examination of the hatched larvae including chromosome counting, measurement of the cell size and staining of the Nucleolar Organizer Regions (NORs) was performed on the

Results

Diploid graylings (2n= 100) from the control group exhibited high survival during the embryonic development (c. 80%). The highest survival rates in the experimental variants of the experiment were observed among embryos developing eggs that were HHP treated 17.5 *maf* and 20 *maf* and equaled 61.7% ± 0,6 and 63.2% ± 1.2, respectively. Larvae hatched from eggs exposure to HHP 20 maf had also significantly bigger cells when compared to cells from the diploid specimens and individuals from other experimental groups. Modal number of NORs in the interphase cells varied from 1 to 3 however three NORs were found only in larvae that hatched from the ggs shocked 20min after fertilization. Triploid number of chromosomes (3n= 150) was found in graylings that were developing in eggs exposure to HHP 17 to 22.5 maf.

Discussion and conclusions

Provided results confirmed that High Hydrostatic Pressure shock (8500psi) applied from 17 to 22.5min after fertilization for 5 minutes enabled successful triploidization of the European grayling. However, further studies including histology of the gonadal development is indispensable to confirm functional sterility of the triploid graylings and their application for the stocking programs.

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PATHOGEN AND BIOMASS GROWTH RISK ASSESSMENT MODEL FOR INLAND AQUACULTURE IN EASTERN EUROPE UNDER CLIMATE CHANGE

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Introduction

Carp farming has a long tradition in Eastern Europe and current yearly production is about 60 000 tonnes for the 5 top countries. Cultivation is typically extensive and takes place in earthen ponds, and Poland's production is 20% of the total volume. In multi-pond systems, fish reach a market size of 1.5 - 2 kg in a three-year period—however, the production (kg ha^{-1} y^{-1}) depends on water temperature, which can both influence growth rates and affect resistance to pathogens. Water temperature also directly influences fish immune responses and pathogen replication, leading to the development of specific host–pathogen interactions that can lead to manifestation of disease. The greatest threats faced by carp farmers are spring viremia of carp virus (SVCV) and koi herpesvirus (KHV), two economically important pathogens [2,3]. However, in recent years carp edema virus (CEV) has been described as a new pathogen which may induce substantial mortalities in common carp aquaculture in mixed infections together with KHV[4]. Tools which can aid carp farming stakeholders to plan for potential changes in water temperatures, under different climate change scenarios, can be valuable in mitigating the impacts of pathogens on the sector and supporting optimization of the farming process.

Material and methods

Our study aimed to develop a mapping tool capable of assisting the farming sector by estimating water surface temperatures using available air temperature data as a predictor (Figure 1), based on the methodology developed by Thrush and Peeler, 2013[4],.

GIS tools were employed to produce these maps, indicating the number of days per year where water temperature in ponds is above a specific threshold. These thresholds cover temperatures required for carp growth, and which condition disease expression caused by SVCV and KHV. Maps were developed using climate model data obtained from Plymouth Marine Laboratory on complete land surface temperature (LST) values for Poland for three time periods: 1. Present: 2000-2019; 2. Mid-term: 2040-2059 and 3. Long-term: 2080-2099, which were converted to water surface temperature (WST) values using an algorithm: and empirical data on water temperatures in ponds.

Results

The maps produced (Figure 2) show the estimated number of days per year with WST above the established threshold under two Radiative Concentration Pathways (RCPs), i.e. RCP 8.5 and RCP 4.5. The temperature time series for various climate change scenarios were also used to drive the Aquaculture, Biosecurity and Carrying Capacity (ABC) model, in order to examine direct effects of climate change on growth and environmental externalities. These tools are of use to carp farmers, and for the industry in general, as well as for government stakeholders, to understand the direct and indirect effects of climate change on the triple bottom line of people, planet, and profit.

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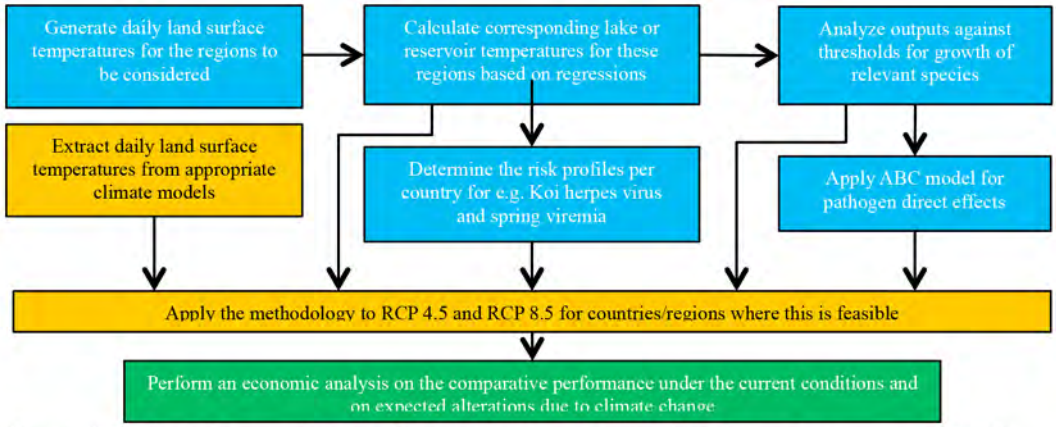

Figure 1. Methodology applied to obtain water temperatures based on air temperatures, and application to climate change scenarios.

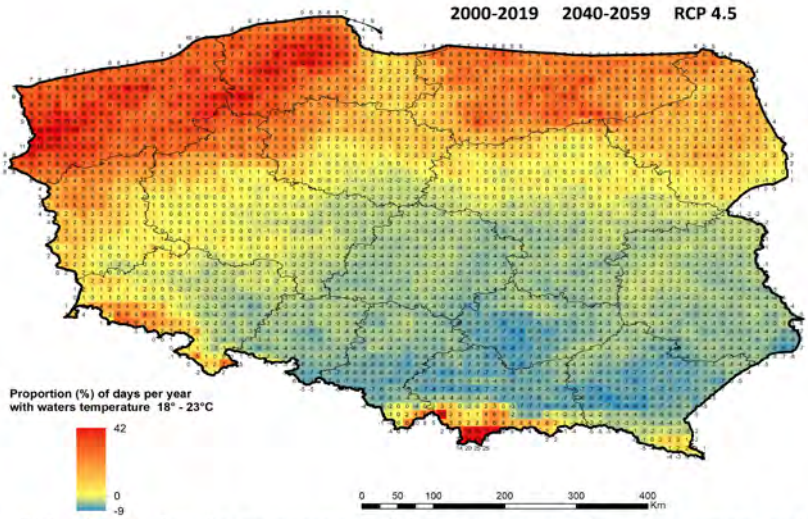

Figure 2. Risk change for the propagation of koi herpes virus between current and mid-term predictions in a conservative climate change scenario (RCP 4.5).

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NUMERICAL SIMULATION OF THE MACROALGAE MOVEMENT WITHIN IMTA-RAS SYSTEMS

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Integrated multitrophic aquaculture (IMTA) production systems, where the synergic effect of an aquaculture (usually in form of a Recirculation Aquaculture System -- RAS) and a macroalgae (seaweed) culture system is exploited, could reduce the pressure on both, the open sea fishing and the use of terrestrial and inland water resources [1]. IMTA-RAS systems represent a promising emerging research topic due to their biotechnological potential with an impact on human health and wellbeing [2].

However, there are some limiting factors or drawbacks in case of seaweed, e.g., *Ulva* sp. cultivation, integrated within RAS: (i) the large area required, (ii) the energy cost, (iii) lack of reliable mathematical models. The second point, energy issue, depending of the system designed to make move (to tumble) seaweeds either by the bottom aeration or by an impinging jet system, was studied experimentally in the work [3]. Here, we shall treat the third point, i.e., we aim to make one step towards modeling and *in silico* simulation of the multiphase flow in tanks for macroalgae cultivation. Modelling and eventually optimization of macroalgae photosynthetic growth within IMTA-RAS systems is left to the near future.

The cornerstone of modeling of the hydrodynamic conditions in seaweed tanks is the problem consisting in how to describe the tumbling pattern of seaweed within the tank in function of the operating conditions (air flow rate or the liquid velocity in the jet outlet in case of the impinging jet system) and tank geometry. Afterwards, having a mathematical model describing the relation between the hydrodynamics (flow pattern of algae) and the nutrients uptake and photosynthetic activity of seaweeds, e.g., in form of some constraints to be fulfilled, an optimization of IM A-RAS can be carried out.

Experiments at laboratory scale or at field are scarce [3], since they are laborious and time consuming. In this context, it becomes of utmost importance to develop a Computational Fluid Dynamics (CFD) based methodology for IMTA-RAS design since it can provide the full flow field description and a further coupling with the algal growth model. Finally, an ease of performing a large range of parametric studies for optimization is a crucial issue [4].

The present work gives guidelines to apply the CFD code STAR-CCM+, which offers an efficient and accurate set of fluid dynamics models and solvers with excellent parallel performance and scalability [5]. The circular tank (with diameter of 20 cm) with bottom air injection and a height of water equal to one diameter is used for our analysis. This set up ensures the formation of two rotating flow cells placed, in the vertical section of the tank, at both sides of the aeration inlet. Both 2D axi-symmetric and full 3D simulation of seaweds-like particles clumps movement are being performed. Based on the selected clumps trajectories, the probabilistic description of the random variable T_{cycle} describing one period of rotational movement, detected by passing through a horizontal plane, was assessed. Obviously, the numerical results have to be verified in subsequent laboratory or field studie

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CFD SIMULATION OF FLUID FLOW AND RTD ANALYSIS FOR AQUACULTURE SYSTEMS: WITH AND WITHOUT FISH

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The improved design of aquaculture systems is needed facing the demand for increased production as well as increased concern of fish wellbeing. Here, we make a step towards use of computational fluid dynamics (CFD) for design and operation of fish tanks. The proposed CFD based methodology allows the modeler (the designer) to have an overall picture about hydrodynamic conditions within the fish tank and to manipulate (*in silico*) all key phenomena involved, both physical (i.e., the tank geometry and operating conditions) and biological (fish density). Further optimization of the tank hydrodynamics, mainly to ensure the optimal rearing conditions (including the fish exercising) and fast biosolids removal, is being expected.

The cornerstone of modeling of the hydrodynamic conditions in fish tanks is the problem consisting in how to describe the flow pattern of water within the tank as a function of (i) tank geometry, (ii) the operating conditions (water recirculation rate or the liquid velocity in the inlet and all details concerning the boundary conditions), and (iii) fish biomass. Although there are some studies predicting the three-dimensional field of water velocity within aquaculture systems using computational fluid dynamics (CFD), see e.g. [1] for floating closed sea cages, and [2] for circular aquaculture tanks, the CFD simulation of a real system including the influence of fish swimming is still a big challenge, in point of view of both numerical and biological complexity. Therefore, some assumptions are further undertaken. First, we limit ourselves to perform numerical simulations of the stationary flow of incompressible viscous fluid in three-dimensional domains corresponding to the respective fish tank (all simulations were performed using Reynolds-averaged Navier-Stokes equation system and a suitable turbulence model). Second, the fish were simulated as a set of immobile objects (with the corresponding fish density

Obviously, this is not the first work where the influence of fish swimming was studied. Nevertheless, to the best of our knowledge, there are only two works presenting the field measurements, see [3] and [4]. These works are used for the validation of our CFD model. Let us remind that experiments at laboratory scale or at field are laborious and time consuming. In this context, it becomes of utmost importance to develop a reliable CFD based methodology being able to provide the full velocity flow field description and an ease of performing a l ge range of parametric studies for optimization.

Finally, based on the comparison of experimentally measured and simulated values of velocity profiles, we argue that CFD code ANSYS Fluent [5] represent a modern and reliable tool for the simulation of hydrodynamic flow field within a production unit, as well as for design and optimization of aquaculture systems.

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EFFECT OF DISINFECTION WITH NATURAL SUBSTANCES ON HATCHING OF GILTHEAD SEABREAM EGGS

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Introduction

The medicinal plant extracts can reduce the use of chemicals in aquaculture. Essential oils have antibacterial, antifungal, antioxidant, anti-inflammatory, anthelminthic, anesthetic and digestive properties (Asbahani et al., 2015). Disinfection involves the use of physical or chemical agents to remove microorganisms. In aquaculture disinfection of eggs is important for the treatment of diseases and increases the chance of hatching (Swaef, 2015). In addition, the disinfectants include compounds used to destroy microorganisms living on the surface of fish eggs (Assefa, 2018). Presently, the hatching parameters of gilthead seabream (*Sparus aurata*) eggs disinfected with natural substances, was assessed verifying the benefits found from the usage of non-chemical substances as disinfectants

Materials and methods

The seabream eggs arrived at the laboratory in tanks. Upon arrival, they were weighed and disinfected with the essential oils and NaCl for a specific period of 15 minutes. The disinfectant essential oils used were oregano oil (*Origanum vulgare*) 100, 250, 500 ppm, tea tree oil (*Melaleuca alternifolia*) 100, 250, 500 ppm and NaCl 500, 1000, 1500 ppm. Also a control group (CON) with no disinfectant, was assessed. For each quantity three reps were made. Then they were washed out with saltwater, weighed and divided equally in 2lt water tanks. Salinity was 33 ‰ and temperature was 18 °C. Each tank had an oxygen supplier and a slight ascent to keep the eggs on the surface. Post-treatment the hatching rate of eggs (A), the percentage of dead larvae (B) and the percentage of egg compared to larvae in each tank (C) were recorded. For the observation, a stereoscope was used. All data were analyzed using "SPSS 17 Statistical Package" while differences were considered significant at $p<0,05$ using chi-square tests

Results

The results (Table I) were positive for use of natural substances as disinfectants, since the percentage of dead larvae was statistically significant higher in the control group compared to oregano oil (500ppm), tea tree oil (100,500 ppm) and NaCl (500,1000,1500 ppm). In contrast, the hatching rate was not statistically significant smaller than the control group. Also, it's obvious that the percentage of egg is statistically significant smaller in the control group, compared to others treatment groups.

Table I. Egg hatching parameters after disinfection

	OR 100	OR 250	OR 500	TTR 100	TTR 250	TTR 500	NaCl 500	NaCl 1000	NaCl 1500	CON
A	22±7.1^{a}	28±2.1^{a}	19±10.1^{a}	38±7.5^{a}	46±8.1^{a}	43±8.9^{a}	39±14.3^{a}	37±14^{a}	36±23.5^{a}	35±6.2^{a}
B	26.3±16^{a}	27.9±27^{a}	12±12^{b}	11.8±4.6^{b}	14.92±7^{ab}	8.16±8.4^{b}	1.95±1.9^{b}	9.26±1^{b}	6±4.8^{b}	24.4±8.3^{a}
C	43.6±18.9^{b}	66.9±18.4^{b}	32.7±17^{b}	40.3±18.6^{b}	56.7±19.6^{b}	22.5±11.5^{b}	44.3±6.3^{b}	32.2±0.2^{b}	46.0±12.5^{b}	4.8±3.34^{a}

All data are presented as mean of percentage ± SE

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Discussion and conclusion

The main disinfectants that are used in aquaculture are chemical. Iodine is mostly used for eggs of fish of the Salmonidae family, whereas for marine species, glutaraldehyde and ozone is used (Animal Health Standards Commission, 2009). The effects of essential oils are well known. Oregano oil has antibacterial, antifungal, antioxidant properties (Mabrok et.al 2016; Baydar et al.,2003) and tea tree oil is used as antibacterial agent (Souza et.al, 2016). In this research there are positive results for replacement of chemical disinfectants. The hatching rate was higher for tea tree oil compared to other disinfectants. Also, the natural substances increased the survival of larvae after the hatching. In control group, more dead larvae were observed. It is important to mention the fact that there has been a delay in egg hatching after the use of natural substances. Although no other researches about essential oil for disinfection of eggs have been carried out, it is important to have in mind that the subject requires further research.

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INCREASING DISEASE RESISTANCE OF FISH FOR PARASITES THROUGH BREEDING: THE CASE OF THE EUROPEAN SEA BASS AGAINST THE MONOGENEAN *Diplectanum aequans* AND THE COPEPOD *Lernanthropus kroyeri*

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Introduction

Parasites infestations are expected to be the major health risk of Mediterranean fish farming in the years to come. Variability of parasitic infestations is due to different life cycle strategies, host-parasite interactions and constantly changing environmental impacts, all of which require different defense practices. The opportunities to minimize the impacts through non – invasive strategies such as the selective breeding should be examined thoroughly. The aim of the present research was the investigation of the resistance of the European sea bass to parasites of high occurrence at the Mediterranean fish farming, namely the monogenean *Diplectanum aequans* and the copepod *Lernanthropus kroyeri*.

Materials and Methods

Natural exposure challenge tests were executed from September 2017 to January 2018 and from August 2018 to December 2018, by introducing naïve populations of seabass juveniles produced in a disease-free Breeding Hatchery into infection prevalent cage farms. In total four trials were conducted; two trials at each period, at two different sites Nafpactos and Sagiada, of confirmed heavily infected environment by the *D. aequans* and the *L. kroyeri* respectively. For all four trials, European sea bass juveniles were used, which originated from Nireus Research Center. Every year, 25 offspring coming from different full-sib and half-sib families were individually pit tagged at 15g and transferred to the selected open sea farming area sites. Offspring members of the same families were farmed also in a third site (Palairos) where the two parasites of interest were not dominant, to be used as the reference/control samples.

Individual weight recordings were conducted to the experimental fish every 2 months. The final weight measurement was conducted 6 months after the transfer to the open sea farming area. At the termination of the experiments, fish were sampled in small groups over a period of 2-3 weeks and the target parasites were counted by a team of experts. The number of parasites was counted on each gill arch on both sides of the fish with the use of microscopes in case of *D. aequans* and stereoscopes in case of *L. kroyeri.*

The growth performance of the populations at the two trial sites was compared to the population of the reference/control site. The average z-scores of average family growth was estimated using pedigree information from Nireus breeding program. Information regarding genetic variability of the parasite counts was revealed via the genetic parameters estimation. The genetic parameters were estimated using an animal model with the site and the year (in the case of *D. aequans* trial) as fixed effects and the animal as random effect. The VCE 6.0 (Geonevelt *et al*., 2010) software was used for the analysis.

Results

For the *D. aequans* infection trials, satisfactory survival rates were achieved in both years. The average number of parasites at family level for 2017 and 2018 was 19 and 47 parasites, respectively, ranging from 3 to 45 parasites and from 12 to 100 parasites per fish, respectively. The average family growth has negligible phenotypic correlation with parasite count (-0.03 and 0.15 for two trial periods). Nevertheless, the parasite count for *D. aequans*, was found to be a medium heritable trait (h^2=0.2) which does not seem to affect bodyweight (phenotypic correlations -0.03 to 0.05) but it is slightly affecting its genetic potential (genetic correlations 0.2 to 0.3 with bodyweight at different ages and 0.37 with growth at sea).

For the copepod *L. kroyeri* trials, satisfactory survival rates were achieved only in 2018. The average number of parasites per family ranged from 12 to 44 parasites. The heritability estimated for *L. kroyeri* presence was 0.28, a quite high value for a disease resistance trait. However, parasite count exhibited larger phenotypic correlation with bodyweight at different ages (0.14 to 0.32), but similar value with growth at sea cages compared to *D. aequans* (0.00 and 0.05, respectively). Moreover, the number of *L. kroyeri* parasites on the fish seems to slightly affect bodyweight and growth genetic potential (genetic correlations 0.09 to 0.4).

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Growth performance in three different sites shows that the possible impact of parasitism in fish growth is evident due to the different values of thermal growth coefficients (TGC) presented in the three different areas. The ranking of the families, from the best to the worst, was based on the average z-score of offspring growth, and the comparison of ranking of female breeders based on offspring's growth in different sites, provided helpful insights about a potential genetic component of parasites impact in growth of fish.

Discussion and conclusions

Heritability of parasite resistance in the case of *D. aequans* was medium and more or less equal with heritabilities recorded for other disease resistance trials. On the other hand, the high heritability of *L. kroyeri* recorded in the current trial has to be verified further with a larger number of fish. The results are indicative of the potential to increase resistance to parasites through selective breeding.

The ranking of the families in the three different sites in terms of growth can provide an indication about the severity of the effect of parasitism in the selection process and more specifically in the characterization of an individual as preferable or non-preferable. High correlation between the evaluation among different sites for both female breeders and families, provides us a certainty that the effectiveness of selection process will not be disrupted by the abundance of the specific two parasites and the selected offspring will continue to grow faster even under these special environmental conditions, compared to their non-selected counterparts. Additionally, the inclusion of parasite resistance as a selection trait will not impair selection on growth.

Nevertheless, the results of the present study look quite promising in terms of the existing potential for genetic improvement for a resistance trait. Families showing high variability in parasite count and phenotypic measurements are going to be genetically screened through a powerful genomic tool developed in the PerformFISH project, a SNP-array encompassing both European sea bass and gilthead sea bream markers, and Genome Wide Association Studies (GWAS) are expected to shed light into the genomic regions linked to disease resistance.

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SPERM CRYOBANK DEVELOPMENT FOR THE CONSERVATION OF THE MARBLE TROUT *Salmo marmoratus*

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Introduction

The marble trout (*Salmo marmoratus*) is an endemic species of North Italy and represents one of the most important faunal entities in the European panorama, threatened by numerous human activities and by the hybridization with brown trout. The strong contraction, both numerical and areal, led to the inclusion of this species in the European Union Habitat Directive and in the 2013 IUCN Red List, classifying it as a "Critically Endangered" (CR). The genetic analyses and the sperm cryobank have proved to be two important tools for a more efficient management of conservation of endangered species (Zuccon et al., 2017). The aim of the research was the development of a cryopreservation protocol to create a cryobank for the conservation of genetic material of pure breeders.

Materials and methods

The purity was verified following genetic method, amplifying the mitochondrial D-loop marker for the identification of the main trout haplotypes (Atlantic, Marble, Adriatic, Mediterranean). The identification of hybrid individuals was carried out by analyzing the LDH-C1* nuclear marker using the RFLP technique (Restriction Fragment Length Polymorphism) and the Ldhxon3F/ Ldhxon4R primers. Before freezing, sperm was analyzed for motility and concentration. Then, after a short equilibration at 4°C, sperm was addicted to cryopreservation solution (DMSO 10% v/v, according to Horvath et al., 2014), and loaded into French straws (0.5 ml). Semen-freezing was conducted in a programmable freezer (Micro Digitcool IMV) using a freezing curve (from +4°C to -140°C, 30°C/min). In order to verify the motility, the thawed sperm was activated by Actifish (IMV), according to Hor **á**th et al., 2015.

The frozen semen of 5 breeders was tested at 3 different concentrations (Test 1: 0.109 ± 0.05. 10^9, Test 2: 0.219 ± 0.01. 10^9, Test 3: 0.438 ± 0.02. 10^9 sperms/ml) of mobile spermatozoa, using batches of about 500 pooled eggs recovered from 28 females. The controls (C) consisted of batches of eggs fertilized by fresh milt of the 5 breeders. The fertilized eggs were incubated in vertical hatcheries and monitored (hatching time for marble trout: 420 day-degree). Fertilization rate was determined according to Bromage and Cumaranatunga (1998). The hatching rate was estimated by daily larval counting.

Results

The taxonomic diagnosis carried out on 66 individuals by genetic analysis showed the presence of 49 pure marble trouts and 17 hybrid individuals, which were discharged from the cryobank.

After thawing sperm, motility resulted 35.5% ± 7.6 (ranging from 20 to 50%) with an average duration of 89 ± 12 sec. The fertilization rate was similar for all the tests performed (average 96.6% ± 0.7) and not statistically different from the control carried out with fresh semen ($p > 0.05$). The hatching rate resulted 26 ± 5% in the Control, 8 ± 4.2% in Test 1, 10 ±1.9% in Test 2 and 13 ± 0.8% in Test 3. Tests carried out using frozen semen show an average drop in hatching of about 55% compared to the control.

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Discussion and Conclusion

The data obtained in this work showed a high-quality variability of the of frozen semen, which would seem to be breeder-dependent. The genetic analysis has proved a useful tool for verifying the purity of individuals of marble trout, really important for conservation management plans. Furthermore, a strong discrepancy between fertilization data and hatching results was found out. Preliminary data seem to show a relationship between the number of sperms used in fertilization and the hatching rate.

Further experimental design in field is necessary in order to confirm and optimize the cryopreservation protocol and the use of the semen fertilization techniques.

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A EUROPEAN SURVEY ON ZOONOTIC HELMINTHS REVEALS A NEGLIGIBLE RISK OF INFECTION FROM FARMED FISH TO HUMANS

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Introduction

There is an extensive range of helminthic parasites of fish, but only a moderate number of species are capable of producing foodborne diseases in humans. These diseases are either caused by an infection following ingestion of viable parasites, or by an allergic reaction against parasite antigens which occurs, so far, due to nematodes of the family Anisakidae. *Anisakis simplex* is the species most frequently associated with human disease, followed by *Pseudoterranova decipiens* (Adams, Murrell et al. 1997) and *Contracaecum osculatum* (Strom, Haarder et al. 2015). In fact, *A. simplex* is the second most predominant biological hazard, constituting 33% of the reported hazards in 2009 (Kleter, Prandini et al. 2009). Other fish helminths of zoonotic concern are cestodes and trematodes (especially Diphyllobothriids and Opisthorchioidea, respectively). They have received less attention despite of the reported human cases in Europe caused by these freshwater fish-borne zoonotic parasites Thus far, the available epidemiological data for farmed freshwater fish are scarce and necessary. For these main reasons in 2010, EFSA recommended studies to evaluate the effects of different farming practices on the prevalence of parasites in aquaculture (EFSA 2010). As a matter of fact, one of the conclusions of this report suggests that Atlantic salmon (AS), reared in floating cages and fed compound foodstuffs, are unlikely to contain live zoonotic parasites and therefore the risk of infection is negligible. However, a later survey detected anisakid and raphidascarid larvae (*A. simplex* and *Hysterothylacium aduncum* respectively) in AS runts (Mo, Gahr et al. 2014) which contrasts with a number of previous studies that confirmed no anisakids larvae in sea-farmed salmonids (Inoue, Oshima et al. 2000; Skov, Kania et al. 2009; EFSA 2010; Wootten, Yoon et al. 2010). These discrepancies were solved in a very recent study were 4,184 farmed AS, including runts, were sampled and examined in 2014/15. The fish were collected from 37 different salmon farms and confirmed that the presence of zoonotic parasites was restricted to runts and occurred in a very reduced number of runts (3/657); suggesting that the risk of any parasitic nematodes to occur in the flesh of farmed Norwegian salmon intended for human consumption is very low (Levsen and Maage 2016). In addition, a qualitative risk assessment analysis, developed in 2016, remarked that attending to the current knowledge of the biology of the system, and the practices adopted in the AS farming, the overall risk of commercialization of product infested by viable larvae appears to be very low (Crotta, Ferrari et al. 2016). Another conclusion of EFSA report stated that, apart from farmed AS, sufficient monitoring data are not available for any other farmed fish and therefore it is not possible to identify which fish species do not present health hazard with respect to the presence of parasites (EFSA 2010). The only work that shed light on this matter is a survey focused on farmed European sea bass (ESB), turbot (TB) and gilthead sea bream (GSB) bred in Spanish farms. This survey remarked that, similarly to AS, the presence of viable larvae appears to be indeed very low and therefore these species do not present a significant risk due to the presence of zoonotic parasites (Apromar 2012)

Concluding, it is generally assumed that farmed fish products have a very low or null prevalence of these helminths. However, this assumption has not been demonstrated scientifically and even less globally for the main European farmed fish species. The objective of the current work is to clarify the potential sources/routes of infection and design management strategies to decrease the occurrence of zoonotic helminths in farms.

(Continued on next page)

Material and Methods

From spring 2016 to spring 2017 a total of 9,333 farmed fish have been examined from the following species: GSB, ESB, TB, AS (including smoked fillets), Marine Rainbow Trout (MRBT), Rainbow Trout (RBT) and Common Carp (CC); obtained from a representative number of fish farms located in Spain, Italy, Greece, Denmark, Norway and Hungary. A random polietapic and stratified sampling plan was selected with a confidence level of 99%. In addition, primary processed products (fresh ESB and GSB from Greece, Croatia and Turkey) from supermarkets in Italy and Spain were also included in the study. As the first round resulted to be negative for zoonotic helminths in all the species and locations, a second round in 2018 was focused in i) sampling fish that are normally discarded for commercialization (runts), ii) in sampling farms close to marine environments with abundance of cetaceans (involved in the life cycle of the zoonotic helminths) and iii) common carp farms with incidence of other helminths. In the second round, a total of 1,480 GSB, ESB, CC and RBT fish runts were also collected from Spanish, Danish, Hungarian and Italian farms in 2018. The sampling of smoked AS was carried out in local supermarkets in the Basque region (Spain and France) and Italy during 2016. Sub samples size was split per suppliers, reflecting their AS commercial production volume in 2015 in the countries under study. In addition, from spring 2016 to spring 2017, 13 samples of smoked fillets of wild sockeye salmon, 2 from supermarkets in Spain and 11 from Italy, were also collected as control samples. Identification of parasites was carried out with different methodologies depending on the type of sample (viscera, fillet, etc.) and fish species including visual inspection, UV-press method, artificial digestion, candling, muscular compression/artificial digestion followed by microscopic examination and PCR. Species identification of parasites were done by sequencing

Results

No zoonotic parasites were found in any of the examined marine fish at the level of confidence of 99% with a margin of error of 4-8%. Only one L4 specimen of the raphidascarid nematode *H. fabri* encapsulated on the surface of the liver in one ESB from one Italian farm has been found. Conversely, 10 (76.9%) out of 13 smoked fillets of wild sockeye salmons were positive for Anisakis larvae.Similarly, no zoonotic parasites were found in any of the examined freshwater fish at the level of confidence of 99% with a margin of error of 4-8%. However, we have found muscle samples harbouring metacercaria of *Holostephanus spp*. in CC with an overall prevalence of 10.64% (114/1122) from Hungarian fish farms. During the first yea , 36 of 258 carp fingerlings (13.9%) were infected in the Northeastern farm. In addition, in the second year survey of the infected farm, heavy infection was found again, with prevalence ranging from 100% (30/30, in one-year-old) to 70% (21/30, in two-year-old) and 90% (27/30, in three-year-old carps). When the metacercariae from this CC were fed in an infection experiment to rodents (mice and hamsters), the trematodes were not able to develop in mammals. Therefore, it seems that *Holostephanus* species does not have any zoonotic potential based on both this negative results from experimental infections and literature review. As expected, the already known zoonotic helminth, the *Metagonimus* sp. used as positive control, were found in the intestines of rodents, 22 metacercariae and 5 adult *Metagonimus* were isolated from two hamsters.

Conclusions

In conclusion, no zoonotic helminthic parasites were detected in 10,813 marine and freshwater samples, even in runts, thus the prevalence is zero. Attending to the results obtained from different surveillances across Europe, besides the current work, we can establish that the overall risk of parasite infection in the selected farmed fish species is negligibl

Acknowledgments

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A NOVEL AND RAPID METHOD FOR THE IDENTIFICATION OF EUROPEAN MUSSEL SPECIES BASED ON REAL TIME PCR MELTING CURVE ANALYSIS

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Introduction

The abundance in coastal waters and the growing demand for human consumption has made mussels a target species for aquaculture. On a global scale, Europe is a major contributor of mussels, supplying over a third of the total production, being *Mytilus edulis* and *Mytilus galloprovincialis* the two main species harvested and cultivated here (FAO, 2019).

Mussels are widely distributed along the coast of Europe and are known to create extensive hybrid zones in the regions where they coexist (Bierne et al., 2003). In particular, *M. galloprovincialis* occurs throughout the Mediterranean and the Atlantic coasts from Portugal to France, creating a hybrid zone with *M. edulis* between the South West France and the Basque coasts. Mussel farming is an emerging activity in the Basque coast (DDEC, 2016), and the experiences of mussel culture carried out in long-lines in the Southeastern Bay of Biscay have shown very promising results.

Given the ecological and economical importance of these species, and the importance of seafood products labelling for traceability, there is a need for clarifying the distribution of farmed mussel species populations around Spain. Species identification based on morphological characteristics is controversial due to their plasticity, and even more problematic for *Mytilus* species from hybrid zones (McDonald et al., 1995). The most common PCR (Polymerase Chain Reaction) molecular methods for mussel species identification targets the polyphenolic adhesive protein gene using the specific primers Me15-16 (Inoue et al., 1995, Santaclara et al., 2006). However, these conventional methods are laborious and time consuming due to the number of steps needed for result visualization.

For this reason, there is a need to apply fast, reliable, and cost-effective methods for species verification. In this study, a real-time PCR assay with a SYBR Green post-PCR melting curve analysis was successfully developed and validated for mussel species identification.

Material and Methods

From autumn 2018 to winter 2019 a total of 244 samples of farmed mussels were collected at five production areas in Spain: Basque Country (Mendexa and Mutriku), Galicia (Ría de Arousa and Ría de Betanzos-Sada) and Catalonia (Ebro Delta); and 59 samples were collected in Algarve (Sagres) and Túnez (Lagoon Bibane). In addition, 37 mussel samples labeled as *M. edulis* were also collected from local markets. DNA from 340 samples was isolated with Wizard® Genomic DNA Purification Kit (Promega). Real-time PCR amplification was performed on a LightCycler® 480 Instrument II (Roche Diagnostics). The 10 µL reaction mixture contained 5 µL of Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent), 20 ng of template DNA and 300 nM of each primer previously described by Dias et al., (2006). The reaction conditions were 3 min at 95 °C, followed by 45 cycles of 5 s at 95 °C and 60ºC for 10s. The dissociation curves of the PCR products were monitored on the same instrument, from 65ºC to 95ºC with 0.11 ºC increases. The validation of the method was performed using the PCR-RFLP (Restriction Fragment Polymorphisms Pattern) Me15-16 *Aci*I method as described by Santaclara et al. (2006).

Results

The real time PCR assay developed showed specific peaks in dissociation curves with unique melting temperature (T_m) values for each species. The T_m obtained were the following: 78.87 **ºC ± 0.11 for** ***M. galloprovincialis*** (n=60) and 80.48 **ºC ± 0.19 for** *M. edulis* (n=25). The hybrids (n=10) showed a double peak which correlated with the T_m of both species analyzed. These results were successfully validated by analyzing samples using the conventional PCR-RFLP Me15-16 *Aci*I method. All collected mussel samples were analyzed using the developed methodology, obtaining the following results: from 303 samples analyzed, 289 were identified as ***M. galloprovincialis***, one sample as ***M. edulis*** and 13 samples as *M.galloprovincialis* x *M. edulis* hybrids. Hybrids where found in five of the production points, specificall , 1.5% of the samples from Catalonia, 6% of the samples from Galicia and 6.98% of the samples from the Basque Country were identified as hybrids.

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Conclusions

In conclusion, the real-time PCR assay with a SYBRGreen post-PCR melting curve analysis developed is suitable for the identification of most important commercial mussels species in Europe, as well as their hybrids. This method has proven to be fast and cost-effective in comparison to other methods previously described, holding the potential to become a routine methodology.

Acknowledgments

Our study was carried out thanks to the financial support of the SEA-TRACES (EAPA_87/2016) INTERREG Atlantic Area EU funded project; ORIME FEMP Project (00001-1NA2018-33) from the Department of Aquaculture and Fisheries from the Basque Government. Ane del Rio is the recipient of a PhD grant from the Education Department of the Basque Government. Samples were kindly provided by Consello Regulador Mexillón de Galicia, IIM-CSIC and Fepromodel.

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PATHOLOGY ASSOCIATED WITH *Pseudomonas sp.* IN *Pseudoplatystoma fasciatum*

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Introduction

Pseudoplatystoma fasciatum is a native fish from the continental water in the Amazonian Región from South America, which rearing is increasing for production. The objective of the present research was to contribute for improving fish production in the Amazonian by identifying the pathology and the causal agent of *Pseudoplatystoma fasciatum* mortality in a stuary from the IIAP, located in Pucallpa, Ucayali Region, Peru.

Materials and methods

Pseudoplatystoma fasciatum fingerlings 3.5 months old, 15 cm long and 65 g weight average were evaluated clinically in the estuary; hematocrit, and hemoglobin levels were determined. At the necropsy, anatomic changes were evaluated and ascitic fluid from the celomic cavity, gills and natatory bladder inocula were obtained and streaked on blood and Mc Conckey agar and the growth was replicated on TSI and cetrimide agar.

Results and discussion

Clinically, fish were floating on the water surface showing breathlessness and loss of equilibrium. Hematocrit and hemoglobin levels were 2.8% and 1.8g/dl respectively. At necropsy, pale color and edema in gills, abdominal bloating containing abundant yellowish bloody fluid in the cavity were found. Peritoneum showed a bloody coloration and edema and the natatory bladder a whitish fluid content. The liver was yellowish and the pancreas whitish-gray, and both showed increased size. In lumen of the intestine, abundant fluid was found. In the blood, Mc Conckey, TSI and cetrimide agars, *Pseudomonas sp* was identified.

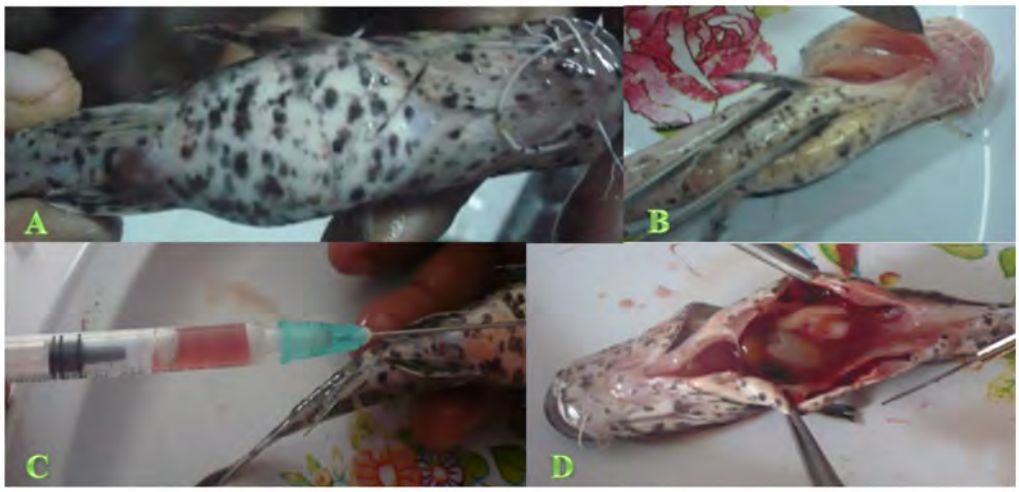

Fig. 1: *Psudoplastystoma fasciatum* fingerlings A: Distended abdomen, B: edema in gills, C: Bloody ascític fluid in celomic cavity, D: Bloody and edematous mesentery.

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Several infectious diseases in fish have been associated with haamorrhage, anaemia and ascites similar to those found in the present study. Viral agents as cat fish disease virus and the iridovirus from the siluronids (Boom and Huisman, 1996; Plumb and Hanson, 2011) have been reported worldwide. As bacterial agents associated with these infectious diseases, isolation and identificati n of Pseudomonas sp. as *Pseudomonas anguilleseptica, P. putida* and *P. fluorescens*, *P. aeuroginosa*, *P. plecoglossicida* have been reported. Those bacteria have aquatic environments as natural habitats and they are part of healthy fish gastrointestinal microorganisms, but they can cause mortality in fish when poor environmental conditions arise (Austin and Austin, 2007). Similar diseases pictures have been described in the infection by *Edwardsiella ictalury* in catfish (Plumb and Hanson, 2011) and *Aeromons hydrophila* in Surubin hibrido (*Pseudoplatystoma corruscans* x *Pseudoplatystoma fasciatum*) (Correa da Silva, et al., 2011). Conclusively, in the mortality of *Pseudoplatystoma fasciatum* fingerlings, haemolitic anaemia and ascites associated with *Pseudomonas sp*. infection was identified. *Pseudoplatistoma fasciatum* mortality resulted from a hemolitic anemia and ascitis associated with *Pseudomonas sp.* infection.

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THE NEIGHBORING FLOW FIELD OF STOCKED FISH CAGE: LABORATORY EXPERIMENT AND FIELD SURVEY

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Introduction

Offshore aquaculture fish cage uses the neighboring water current to purify the water in the fish cage. A change of the neighboring flow field can change the efficiency of the purification work. The influence of the empty fish cage on the neighboring flow field has been widely investigated (Løland, 1991). However, the study on the influence of the stocked fish cage is sparse (Gansel et al., 2014).

In the present study, Pseudo Fish school Structure (PFS) was developed to simulate the neighboring flow field of a stocked fish cage by Circulating Water Channel (CWC) tests. The result of the CWC tests was compared with the field survey for qualitative verification

Materials and methods

The study site, Kurose fish farming area, is a part of Shibushi Bay in Japan. A target cage had 8.0 m height with a square base of 10.0 × 10.0m. The cage had wire netting, and so it could not show major deformation. The stocking density of the cages ranged 30 – 40kg/m^3. About 10 000 of 1.5-year-old yellow-tails (*Seriola quinqueradiata*) were cultured in the cage.

An overall 1/25 length scale model cage of the full-scale fish cage was used for the CWC tests. It had 0.3m height with a square base of 0.4 × 0.4m. The model cage had PE nettings and a steel frame. Therefore, the model cage also did not show major deformation. The netting of the model cage was made following the Tauti's similarity law (Tauti, 1934).

The stocked fish were observed through the underwater video camera. Their fork length was assumed to be 560mm. Two horizontal swimming patterns were observed: swimming randomly (Fig. 1); swimming together (Fig. 2). Their instant swimming speed was roughly estimated from the video record (about 0.2m/s).

Fig. 1 Stocked fish swimming randomly

Fig. 2 Stocked fish swimming together

Fig. 3 Pseudo Fish Structure (PFS)

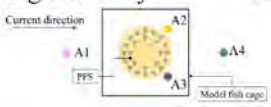


Fig. 4 velocity measuring points around the model cage

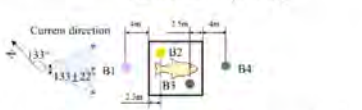
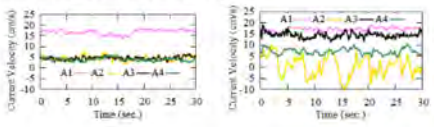
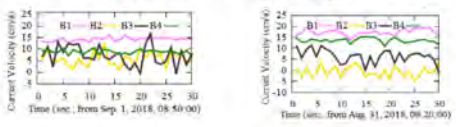


Fig. 5 velocity measuring points around the stocked full-scale cage



(a) motionless PFS (b) revolving PFS

Fig. 6 Times series velocities at the neighboring points of the model cage



(a) weak motion of fish school (b) revolving fish school

Fig. 7 Times series velocities at the neighboring points of the full-scale cage

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The fish were imitated by PVC pipes. The displacement of PFS was 3.76% of the whole volume of the model cage. Two types of the moving patterns were imitated by PFS: maintaining the position; revolving in counterclockwise.

The model tests were completed in the Horizontal Circulating Water Channel with Wave Maker and Wind Tunnel (24m long × 1.8m wide × 1.0m depth) in The University of Tokyo. The current velocities at neighboring four points (Fig. 4) of the model cage were measured by a current meter (ACM2-RS, JFE Advantech) in CWC.

The time series current velocities at four neighboring points (Fig. 5) of the target full-scale fish cage were measured from Aug. 29, 2018, to Sep. 3, 2018, by four COMPACT-EM current meters (JFE Advantech). The periods that showed the time-averaged velocity directions at points B1 and B4 ranged 133 ± 22° from the North were chosen; the direction from the North can be referred to Fig. 5.

Results

Time series velocities of the four points in CWC test show qualitative consistency with the field data (Fig. 6 and 7). When PFS was motionless, the average velocities at A2, A3, and A4 showed similar value (Fig. 6a). During the period of Fig. 7a, the average velocities at B2, B3, and B4 showed similar value as well. Therefore, it can be inferred that the stocked fish was swimming relatively randomly and gently during the period.
When PFS was revolving, the velocities at A2 and A3 showed significantly different aspect (Fig. 6b). Because the pipes of the PFS moved against the main current next to the point A2, the velocity at the point shows a significant decrease. However, the PFS moved with the main current next to the point A3, the velocity at the point shows only a slight decrease. During the period of Fig. 7b, the velocity at B2 is significantly lower than that at B3. It can be inferred that the fish school revolved in counter clockwise.

Conclusion

The qualitative similarity between the neighboring flow fields of a stocked model fish cage and those of full-scale fish cage was observed. The flow field inside of a stocked fish cage is highly depending on the moving patterns of the stocked objects. And the flow field was non-uniform and complex. Further research to obtain the quantitative similarity between the laboratory test result and the field survey result can deepen the understanding of the neighboring flow field of the stocked fish cage

Acknowledgement

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INTERACTION BETWEEN DIETARY COMPOSITION AND SEASONAL TEMPERATURE CHANGES IN GILTHEAD SEA BREAM *Sparus aurata*: EFFECTS ON GROWTH, FAT DEPOSITION, PLASMA BIOCHEMISTRY, DIGESTIVE ENZYME ACTIVITY AND GUT BACTERIAL COMMUNITY

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Introduction

The optimization of feeding strategy in relation to the environmental condition needs further investigation in order to maximise performance, fish health and fish quality of Mediterranean farmed species. Environmental temperature during seasonal changes may affects fish metabolism, digestive enzymes activity and gut bacterial community which may exert an effect on performance and tissue composition (Couto et al., 2012; Guerreiro et al., 2016). Data relating interaction between different dietary protein and energy ratio and water temperature changes on digestion condition, gut bacterial community and fat deposition of gilthead seabream fed current aquafeed formulations are scarce. For this reason, the purpose of this study was to evaluate the effects of two dietary protein and dietary energy ratio (DP/DE) during temperature changes on growth, feed efficienc , fat deposition, plasma biochemistry, digestive enzyme activity and gut bacterial community of gilthead sea bream (*Sparus aurata*).

Material and Method

Two experimental practical extruded diets formulated with 15% fishmeal and with different protein energy ratio (44/16; 44/21, protein/lipid, %) were tested in triplicated fish groups of 30 individuals (initial weight: 67.5g) and raised at two different water temperatures (23 °C and 17°C) in the same recirculation system over 119 days. Fish were fed manually to visual satiation twice a day. After 58 days fish were exposed to a switch in temperature (fish kept at 23°C were transferred to 17°C and the fish kept at 17°C were transferred to 23°C, 23/17 and 17/23, respectively) while continued to receive the same diet in each group. Specific growth rate (SGR), feed intake (FI), feed conversion rate (FCR), somatometric indexes and nutritional indices were performed in the intermediate periods and at the end of the trial to assess growth performance during seasonal changes. At the same times digestive enzyme activity (n=9 per treatment), plasma biochemistry (n=9 per treatment), and gut microbial community by Next-generation sequencing were determined. Data were analysed by Two-way ANOVA followed by a Tukey's multiple comparison test. Differences among treatments were considered significant at $P < 0.05$.

Results

At the end of the trial no significant diet effect on final body weight and SGR were detected in fish firstly exposed to 23 °C (23/17) compared to those firstly exposed to 17 °C (17/23). Similarly, no significant differences in FI and FCR were observed. In the intermediate periods, low water temperature negatively influenced SGR, FI and FCR under both dietary treatments. In those periods similar ($P > 0.05$) growth and feed utilization were detected between diets in fish reared at the same temperature even if in the second intermediate periods (days 58-119 after the temperature changes), FI was higher ($P = 0.01$) in 44/16 diets compared to 44/21. No significant dietary effect on perivisceral fat and HSI was detected at the end of the trial. Fat index was significant reduced in fish firstly exposed to 23 °C (23/17) under both dietary regime while HSI showed an opposite trend.

Discussion and conclusion

This study provided novel insight on the effects of DP/DE ratio on fat deposition, digestion condition and gut bacterial community of gilthead sea bream fed in summer before entering winter and fed in winter before entering in spring. Preliminary results suggest that different dietary lipid level did not improve growth performance and feed efficiency during seasonal changes, while a reduction in water temperature from 23 to 17 °C reduced feed intake and feed utilization under both dietary regimes. The increase in dietary lipid level from 16 to 21% seems to not affect lipid deposition.

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GROWTH OF MOROCCAN MINT (*Mentha spicata*) IN THREE HYDROPONIC SUBSYSTEMS (DRF, RAFT, NFT) UNDER DECOUPLED AQUAPONIC PRODUCTION OF *C. gariepinus*

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Introduction

Mint (*Mentha* spp.) is known for good performance under different aquaponic conditions and has a high economic potential due to essential oils and short growing periods (Knaus, 2016; Kotzen and Appelbaum, 2010). However, comparative studies of mint cultivation in different hydroponic subsystems are scarce. Best plant weight, length and number of leaves were obtained by using the nutrient film technique (NFT) and floating rafts with root aeration by air diffusers, while insufficient results were achieved in a gravel decoupled aquaponics system without additional aeration (Zimmermann, 2017).
The type of hydroponic aeration might play an important role for the provision of nutrients at the root inner space and surface allowing good plant growth. Beside active aeration with the help of air diffusers or an increased direct oxygen supply through the water surface by circulating the solution, the passive root contact to atmospheric oxygen was described by Silva et al. (2015) as the "dynamic root floating" technique or DRF. In this case, a small aerated space e.g. 5cm is left between the plant trays and the nutrient enriched water. The DRF system results in the formation of "oxygen roots" or "hair roots" in pak choi (*Brassica chinensis*) combined with Nile tilapia (*Oreochromis niloticus*) under coupled aquaponics conditions (Silva et al., 2015) and can minimize investment costs by the elimination of energy consuming active aeration.

The present study compared the growth of mint (*Mentha spicata*) in different hydroponics subsystems with active aeration in raft, active aeration by circulating the aquaponic nutrient solution in NFT and the passive root aeration in a DRF hydroponic subsystem under conditions in northern Germany.

Material and Methods

The experiment was performed in the FishGlassHouse, an experimental aquaponic facility in Northern Germany (Mecklenburg-West Pomerania, University of Rostock) during spring 2018. African Catfish (*C. gariepinus*) were fed with Alltech Coppens Special Pro 4.5mm (42.0% protein, 13% fat, 1.5% crude fibe , 7.6% ash, 1.02% total P) and *C. gariepinus* was held in an intensive aquaculture unit (104-156 fish tan $^{-1}$; max. 200kg m^3).

The hydroponics cabin contained one collecting tank and 9 individual hydroponic sub-systems with 7 plants, respectively. The aquaponic principle was decoupled and used effluents from *C. gariepinus* production without additional fertilizer. Three techniques were tested in triplicates and randomized block design:

a) dynamic root floating technique (DRF): 25 days after the plants were introduced into the system, a 5 cm air space was installed for root aeration (passive root aeration) and oxygen diffusers were taken out;
b) floating raft culture (raft): polystyrene rafts held the plants on the water surface and the water was enriched by oxygen diffusers (active aeration);
c) nutrient film technique (NFT): plants were embedded in a tube (12.5cm) where a constant flow of water supplied the roots with oxygen (active aeration).

The water of the collecting tank (537 L) was exchanged twice a week with the nutrient enriched water. The experiment lasted for 55 days and during this period the plants were examined for the relevant growth parameters (dry and wet mass). These also included the number of leaves (No), total length (cm), shoot length (cm), root length (cm), total weight (g), shoot weight (g), root weight (g) and the two leaf lengths and widths of the second shoot node from above. Physical water parameters were measured daily and chemical parameters biweekly.

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Table I. Comparison of *Mentha spicata* growth parameters cultivated in three different hydroponic subsystems (DRF, raft, NFT) under decoupled aquaponics conditions with effluent water of *C. gariepinus*, different letters show significant groups ($p < 0.05$).

Parameter	DRF	RAFT	NFT
Number of leafs (No)	482.3 ± 163.4^{b}	532.2 ± 180.3^{ab}	639.7 ± 207.7^{a}
Total plant weight (g)	139.4 ± 81.1^{b}	200.4 ± 97.2^{ab}	220.6 ± 113.5^{a}
Total plant length (cm)	109.2 ± 19.6^{b}	132.0 ± 17.8^{a}	128.1 ± 21.7^{a}

Results and Discussion

The best growing results with mint were achieved in the NFT system. The number of leaves and the total weight were higher for NFT compared with DRF (Table I, $p < 0.05$). Total plant length was lower for raft and NFT compared with DRF ($p < 0.05$). The shoot axis length and leaf weight were better for raft compared with DRF ($p < 0.05$). Total dried plant biomass and root mass were higher for NFT than for DRF ($p < 0.05$), with raft and NFT not differing from each other ($p > 0.05$). The growth potential of *M. spicata* showed the following order: NFT > raft > DRF. With regard to the economically most important parameters number of leaves and total weight, results by Zimmermann (2017) for *M. spicata* in the NFT system were confirmed. It could be verified that the hydroponics subsystem NFT is best suitable for the cultivation of *M. spicata* under decoupled aquaponics conditions.

The passive aeration in the DRF system was not suitable for the cultivation of *M. spicata* and showed brown colouring and lignification of the oxygen roots, and a reduction in root growth activity. This can be explained by the reduced air humidity indoors and higher temperature fluctuations at the sensitive hair roots, caused by season and location in northern Germany. We suggest that further studies should increase the DRF air space gradually by automatic control of the water level without using energy to minimize operational costs or the present setup should be repeated during the warmer summer season.

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ICTHYODIVERSITY AS A DRIVING FORCE TO PROMOTE NEW AGRO-ECOLOGICAL APPROACH FOR *Sander lucioperca* REARING

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Introduction

Nowadays, aquaculture provides up to 50% of the world's aquatic food consumption (FAO, 2018). However, its development has been seen as unsustainable because the current production model jeopardises wild environments, human food security, and economic prospect (e.g. Martinez-Porchas and Martinez-Cordova, 2012). This places a premium on the improvement of sustainability of the aquatic food production. In this context, the research program, ICHTYOSERV, aims at applying an agro-ecological-like approach on fish farming. Indeed, environmental-based alternatives could be considered to design innovative and agro-ecological production systems (Dumont et al., 2013). In such systems, special attention should be given to biodiversity since it could allow enhancing the resilience and the efficiency of aquaculture. To this end, ichtyodiversity should be considered according to a functional approach, with the study of fish traits and of their relationships (Violle et al., 2007). Such data are being compiled into the *Traits OF Fish* (TOFF) database that lists morphological, phenological, physiological, and behavioural data coupled to ecological characteristics (Thomas et al., 2017). Exploitation of this database allows defining different scenarios, which take account the compatibility among the fish species (in terms of living environment) and the absence of competition for used resources. The aim of this work is to explore a specific scenario from TOFF database. It consists to compare monoculture of *Sander lucioperca* with a polyculture option combining the latter species with one or two other species in recirculated aquaculture systems (RAS) so as to determine how ichtyodiversity could impact some key processes in agro-systems and improve the efficiency of fish rearing systems.

Materials and methods

The selected scenario involves pikeperch *Sander lucioperca* (Percidae), associated or not with sterlet *Acipenser ruthenus* (Acipenseridae) and tench *Tinca tinca* (Cyprinidae). Fish were obtained at juvenile stage from fish farmers. After the acclimatisation period, the mean weights of fish were 58.1 ± 11.2 g, 40.8 ± 6.2 g and 17.6 ± 4.6 g respectively for *S. luciorperca*, *A. ruthenus* and *T. tinca*. Then, fish were reared for two months in a 300 L tank with aerated recirculated water according to four modalities. In more details, it consists to keep one, two, or three species together, systematically with 36 fish per experimental unit. It corresponds to 36 pikeperch (for the modality P), 18 pikeperch and 18 sterlet (PS), 18 pikeperch and 18 tench (PT) or 12 pikeperch and 12 sterlet and 12 tench (PST). All these modalities have been tested in triplicates. Fish were fed daily with commercial pellets, at a rate of 1.5% body weight per day. The physico-chemical parameters of the water were checked three times a week (temperature: 20.5±0.7°C; dissolved oxygen > 6.1 mg/L; pH 7.5±0.2; $N\text{-}NH_4^+$ < 0.2±0.2 mg/L; $N\text{-}NO_2^-$ levels < 0.1±0.1 mg/L). During the experimental phase, the light / dark period was 10h / 14h, using white neon light (20 lux). All fish of each experiment unit were measured and weighed after 0, 29, and 60 days of rearing to calculate the following zootechnical parameters: percentage of survival, final body weight, weight heterogeneity, specific growth rate, Fulton's condition factor, biomass gain, and feed conversion ratio. Behavioural responses are also recorded according to two rounds of video recording (i.e round 1 between days 5 and 11; round 2 between days 31 and 35). Videos concern the fish spatial repartition in the water column, the group structure (distance from the nearest neighbour, mean distances between all individuals of the group and variances of these distances), fish activity (swimming), and relationship (contact) between fish with media player software. For the data analysis, we used ANOVA after checking that they were normally distributed (Shapiro's test) and that their variances were homogeneous (Leven's test).

Table 1. Recorded zootechnical parameters for pikeperch reared in monoculture (modality P) or in polyculture: pikeperch with sterlet (PS), pikeperch with tench (PT) or pikeperch with sterlet and tench (PST).

Modalities	Initial body weight (g)	Final body weigth (g)	Weight hetero-geneity (%)	Specific growth rate (%)	Fulton's condition factor	Biomass gain (%)	Percentage of survival (%)
P	58,1 (11,2)	99,5 (21,1)	21	0,83	0,756 (0,069)	71	100
PS	58,3 (10,3)	109 (25)	23	0,93	0,755 (0,049)	83	98
PT	57,5 (10,9)	107 (26,6)	25	0,96	0,764 (0,064)	86	100
PST	59,6 (10,2)	115 (25,8)	22	1,01	0,751 (0,077)	93	100

Results

Overall, our results indicate that it could be interesting to raise *S. lucioperca* in polyculture compared to monoculture. From the zootechnical aspects, survival rates for pikeperch are always higher than 98% whatever modalities. On the other hand, final body weight, specific growth rate, and biomass gain appeared higher when pikeperch are associated with sterlet, tench, or both compared to monoculture of pikeperch (Table 1).

Concerning the behavioural approach, some differences are also recorded between pikeperch alone or associated with one or two other species. Thus, *S. lucioperca* are swimming the entire well volume in monoculture whereas they are living at the bottom of tank in polyculture options. In the same way, distances from the nearest neighbour are higher when *S. lucioperca* is reared alone. Finally, pikeperch developed more direct physical contacts of intra-specific type compared to inter-specific nature

Discussion

The relevance of these new options of polyculture approach by experimental approach is fundamental to determine the best combination between pikeperch and other fish species. These first results demonstrate a complementary utilization of trophic resources between the three taxa in relation to a best exploitation of spatial resources.

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THE EFFECT OF LIGHT ON THE CHOICE OF FOOD OF REARED SEA URCHIN *Paracentrotus lividus* IN LAND-BASED SYSTEMS

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Introduction

The sea urchin *Paracentrotus lividus* (Lamarck, 1816) was selected as new candidate for aquaculture in European countries. Wild seaweeds are harvested to feed sea urchins, but this practice should be replaced by farmed seaweed or prepared feeds to be economically and environmentally sustainable (Carboni et al, 2013). Formulated diets were effective in increasing gonad size but sometimes failed to produce gonads with optimal organoleptic characteristics (Robinson et al, 2002). For these reasons, the addition of macroalgae in the diets, as a mix or included in the formulation, had been detected to increase the palatability and efficiency of prepared feed (Cyrus et al, 2014). However, all these previous studies were conducted on wild specimens. As far as we know, nobody has studied the attraction or the palatability of diets nor the feeding behaviour of reared sea urchins. The aim of this study was to evaluate the first choice of food and the feeding behaviour of reared *P. lividus* among two prepared diets and a macroalgae (*Ulva sp.*) considering light and dark experimental conditions.

Materials and methods

A total of 120 sea urchins (test diameter 22.3 ± 2.6mm) were collected from the stock of reared sea urchins by the experimental hatchery of the University of Cagliari. Before the experiment they were kept in starvation for 7 days. Sea urchins were subjected to the choice of three diets: A, fresh green-macroalgae *Ulva sp.*; B, experimental diet manufactured by SPAROS Lda; C, commercial feed known as Nofima diet (Prato et al, 2018). The experimental unit consisted on a white circular tanks of 150l of volume, provided by a digital camera set in the "time lapse function" (60 picture.h.-1, definition 12 megapixel), which was used to monitor the behaviour of sea urchins. Four sea urchins were positioned in the centre of the tank and the diets were positioned equidistant from the centre. Each experiment lasted 24h and was replicated thirty times. For each experiment were calculated the first choice of food, which was the first food selected by each sea urchin, and the feeding behaviour. The feeding behaviour was determined by measuring the number of sea urchin in contact with different diets as categorized above (A, B, C). When sea urchins were not in contact with any diet, were categorized as N. The number of urchins in each feeding behaviour category was calculated every hour under Light (15 replicates from 11 am to 4 pm) and Dark conditions (15 replicates from 11 pm to 4 am).

Results

The first choice of food was 45% for the diet A, 25% for the diet B and 30% of diet C. Over the total of sea urchins, the 40% of reared sea urchins did not choose any diet. Considering the light and dark behaviour, the average number (± se) of sea urchins in contact with the different diets in light and dark conditions is reported in Figure 1. Significant differences occurred between the behaviour categories in Light and Dark conditions.

Discussion and conclusion

The results confirmed our assumption, suggesting that the feeding behaviour is not affected by the captivity or by the food history of reared sea urchins. Indeed, results of the first choice showed that reared sea urchin preferred the macroalgae *Ulva sp.* instead of prepared feeds. This confirm that the choice is controlled mainly by the food shape, morphology and texture, rather than the nutrient content as observed for wild specimens (Vergés et al, 2011). Regarding the feeding behaviour, a significant increase of sea urchins interaction with the feeds occurred during the night hours. It is also interesting to highlight that the video recording showed a high number of specimens that never interacted with the feeds. These specimens with lack appetite were probably the sea urchins known as small "outliers" where the numbers ranged from 11% to 35% of the total sea urchin produced in a hatchery (Grosjean et al, 2001). These sea urchins growth was apparently inhibited by the other specimens, which, probably, interacting more quickly with the feed grew faster. The results suggested some practical recommendations for feeding practice in echinoculture: 1) it would be advisable to administer food at sunset, which had the advantage to give to sea urchins a more palatable food because it not may remain uneaten for several hours; 2) don't use fresh macroalgae and prepared feeds as mixed together in the tank because sea urchin eat first the macroalgae and in the meanwhile the prepared diets could dissolve in the water; 3) split frequently the "small outlier" sea urchins in separate tanks or cages for better growth performances.

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MODELLING MUSSEL LARVAL DISTRIBUTION FOR OPTIMAL SITE SELECTIONS OF MUSSEL FARMING

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Introduction

Eutrophication is one of the largest threats to the Baltic Sea manifested by algal blooms, turbid waters, loss of submerged vegetation and hypoxic and anoxic conditions at the sea bottom in large areas (Holmer et al., 2015). The potential of using bivalves such as blue mussels to mitigate effects of eutrophication in the coastal zone has been proved environmental effective in Danish waters (Timmermann et al., 2019). Mussels remove nutrients from the water through filtration of particles, which are incorporated into mussel biomass and removed from the system by harvesting. However, it is still a challenge to optimize mussel farms and mussel production. The farm design and locations need to be adapted to different environmental conditions in order to handle e.g. high predation pressure, low salinity, exposure to high wind, waves or ice coverage, but also in terms of efficient mussel larvae settling on the long-lines. Spawning takes place in the natural mussel beds in spring and the resultant larvae are spread by the water currents to other areas before settling on the bottom or on the long-lines in the mussel farms. In the following study, we use 3D ecosystem modelling to estimate the mussel larval distribution on fine spatial and temporal scales in a local set-up of the Limfjorden

Materials and methods

We couple a 3D physical Limfjord model with an agent based model (ABM) using the Flexsem system (Larsen et al., 2017). The hydrodynamic model has been validated against observations. Mussel larvae are defined by two main biological parameters: the pelagic larval duration (PLD) and a main spawning event in spring. To simulate the dispersion of mussel larvae we use numerical particles released from the mussel bed sampling stations acting like a source area (figure 1). The mussel densities in the stations are considered for the amount of larvae released. The individual trajectories of the particles are stored for the temporal extent of the pelagic phase. No random vertical or horizontal movements of the particles are included. At the end of the pelagic larval duration, settling occurs once for particles ending anywhere within the model domain. The model provides maps of mussel larvae distribution and connectivity between sub-areas, which can be used for site-selection processes of mussel farming in the Limfjorden.

Results and Discussion

Preliminary results on larval connectivity in the area show that there is a high probability of larvae to be recruited in the same location where they were originally released (self-recruitment). This is the case in all areas except for 1, 2, 4, 6 16 and 17 where no mussel were released. Self- recruitment is higher for short PLDs, and as we increase the number of days that the larvae remain in the water column, larvae will move to other areas within the Limfjorden (results not shown). Main donor and receiver areas can also be identified by the model for different scenarios. For 2010, area 5 corresponding to Kås Bredning is the main donor area and areas 10 and 11(Løgstør Bredning) and 13 (Risgårde Bredning) the main receiver areas. These connectivity results can be explained by the circulation current patterns in the area. Current mean speed and standard deviation in the studied month was calculated and it was observed to be higher in area 5. This area is a strait and therefore we expect higher current speeds that disperse the larvae to the inner parts of the Limfjorden (not shown).

Conclusion and Outlook

Larval dispersal is a complex process mediated by several factors acting at different scales. In this study, we are able to identify the main donor and receiver areas, as well as the areas with high self-recruitment and isolated areas for the specific year and season modelled. Note that the model does not account for post-settlement survival. Other factors such as bottom trawling, oxygen depletion and marine protected areas are also important to consider when modeling the spawning areas and potential settlement sites. Thus, this variability in density of larvae newly settled is caused by complex interactions between hydrodynamics, habitat structure, predation and species-specific traits. Further work will include (1) annual variability in mussel larvae distribution, (2) changes in climate affecting the physical conditions and biological traits of the species and (3) model validation against mussel beds densities, genetics and larval sampling. The tool developed in this study can provide useful information for decision makers to be used for management applications and aquaculture purposes in the inner Danish waters.

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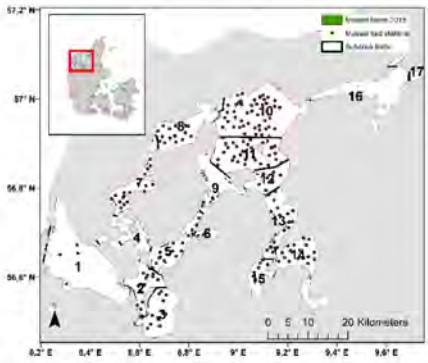

Fig. 1. Map of the sampling stations (red dots) in the mussel beds existing in the Limfjorden. The Limfjorden is divided into 17 areas for the connectivity study (subarea limits shown in black).

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TOWARDS THE ECOLOGICAL INTENSIFICATION OF EUROPEAN AQUACULTURE: THE GAIN PROJECT

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Introduction

The Green Aquaculture INtensification in Europe (GAIN) project is a 42 month long collaborative research programme funded by the European Union (EU), through Horizon 2020 (Grant Agreement 773330) (www.unive.it/gainh2020eu). GAIN started in May 2018 and is designed to support the ecological intensification of aquaculture in the EU and European Economic Area (EEA), with the dual objectives of increasing production and competitiveness of the industry, while ensuring sustainability and compliance with EU regulations on food safety and environment. Eco-intensification of European aquaculture is a challenge that requires the integration of scientific and technical innovations, new policies and economic instruments, as well as addressing social considerations, in order to promote the implementation of the principles of circular economy in aquaculture.

Materials and methods

GAIN aims at enhancing eco-intensification by pursuing the specific objectives listed bel .

1. Design and test an innovative range of finfish feed
2. Develop and test a platform for supporting the implementation of precision aquaculture by combining sensors, Big Data analysis, and predictive models;
3. Add value to cultivation of both finfis and shellfish by means of innovation in co-products from improved re-use of secondary materials, thus increasing profit and minimizing the environmental footprint of aquaculture
4. Support integrated policies concerning aquatic food production, by addressing current barriers to the circular economy;
5. Promote market access and help consumers, both in Europe and elsewhere, to understand the true value of quality production from European waters;
6. Provide guidelines for sustainable ecological and economic intensification of European aquaculture, and disseminate and exploit these findings and recommendations to farmers, managers, and policy-makers

Results

During the first year, GAIN has already obtained several relevant results, which are described in detail in seven public deliverables and summarized below.

Implementation of Precision Fish and Shellfish Farming: Innovations and decrease in costs of sensors are now making it possible to implement the framework of "Precision Fish Farming" (PFF) (Fore et. al., 2018), which in GAIN will be extended to "Precision Shellfish Farming" (PSF) (Brigolin et al. 2017). GAIN instrumented ten pilot sites with state-of-the-art sensors for real-time detection of both environmental and animal variables, e.g. fish length/weight distribution. Data will be processed using a range of process and data driven models.

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Design and performance assessment of novel feeds: novel feeds for salmon, trout, seabream and turbot combining emerging sustainable ingredients, e.g. insects, heterotrophic microalgae, and fisheries/ animal production byproducts, were designed and tested on the above species.

Valorisation of secondary products of the aquaculture supply chain: optimal conditions (e.g. pH, temperature, hydrolysis time ...) for the production of Fish Protein Hydrolysates (FPHs) from salmon and rainbow trout heads and trimmings were determined.

Valorisation of wastewater from land-based farms: an innovative process, combining sono-electro flocculation with vacuum filtering is being tested. Preliminary results indicate that the end-product can be used as a fertilize .

Valorisation of mortalities: an innovative process combining using mechanical fluidization and superheated steam is being tested, in order to reduce economic and environmental cost of disposal of fish farm mortalities

These components of GAIN will allow farmers to grow more and grow better within the space currently available, and an economic analysis of the performance of farms will help build trust in the innovative products that GAIN will make available.

Discussion and conclusion

The results outlined above provide early indications that there is considerable scope for reducing the environmental impact of European aquaculture and simultaneously enhancing its profitabilit . Crucial to that is the reuse of co-products and side-streams which, to some extent, is still limited by current EU and national legislations. GAIN is inventorying and analysing such legislation, in order to identify barriers to a full-scale implementation of the circular economy also in the aquaculture sector. The sustainability of innovative processes should additionally be demonstrated: to this aim, a new, comprehensive sustainability index, based on Life-Cycle Analysis (LCA) and including also welfare indicators, is being designed and will be applied to the supply chains of the main GAIN target species, i.e. salmon, rainbow trout, seabream and seabass.

GAIN has direct links with partners in the US, Canada, and China, as well as strong connections to other parts of the world. Our Affiliate Farm Programme is designed to broaden the reach of the innovations we develop, and to ensure a strong and continuing legacy.

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INVESTIGATION OF SPERM AGGLUTINATION AND A NEW METHOD FOR MEASURING SPERM CONCENTRATION IN COMMON CARP (*Cyprinus carpio*)

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Introduction

In this work, two independent experiments were carried out to improve the circumstances and the results of sperm cryopreservation in common carp (*Cyprinus carpio*). Sugar based extenders, which are required as extracellular cryoprotectants (Horváth et al. 2003, Bernáth et al., 2016, Marinović et al. 2017) cause jelly-like agglutination after thawing in common carp sperm (Horváth et al. 2003). Although Horváth et al. (2003) pointed out this agglutination does not reduce the fertilization or the hatching rates, Bernáth et al. (2017) found that it can negatively affect post-thaw motility and also the fertilization rate. In our research we tested whether removal of the seminal plasm inhibits agglutination or not. In the second experiment, the concentration of common carp sperm was measured by spectrophotometric analysis based on the research of Ciereszko et al. (1992) who found linear correlation between sperm concentration and absorbance in yellow perch. The main goal of this experiment was to find a fast and high-throughput method to estimate the concentration of common carp sperm.

Materials and methods

In the first experiment, fresh progressive motility from six individuals was measured by a CASA (Computer Assisted Sperm Analysis) system. After that 1 mL from the samples was put into 1.5 mL Eppendorf tubes and was centrifuged at 500 g at 4 ˚C for 10 minutes. Subsequently, the seminal plasma was removed and replaced with grayling extender (200 mM glucose, 40 mM KCl, 30 mM Tris, pH 8.0). This procedure was repeated 3 times. After that the samples with and also without seminal plasma were cryopreserved in the presence of 10% methanol in 500-µL French staws and in the vapour of liquid nitrogen 3 cm above its surface for 3 minutes. After cryopreservation the samples were plunged into liquid nitrogen. The samples were thawed (for 13 sec, at 40 ˚C) and post-thaw progressive motility was measured by CASA.

In the second experiment, sperm concentration of the same six individuals was measured with two different methods. Firstly, 10 µL of each sperm sample was diluted in 990 µL grayling extender (100-fold dilution) and the absorbance was measured at 505 nm by a spectrofotometer. At the same time, 100 µL from the pre-diluted sample was diluted further into 900 µL grayling extender (1000-fold dilution) and spermatozoa were counted using a Bürker-Türk type hemocytometer. Results coming from the same individual were correlated.

Results

In the first experiment we found that removal of the seminal plasma and its replacement with grayling extender did not inhibit the agglutination process. However, it significantly affected the post thaw progressive motility of the groups ($p= 0.018$): it was 28±13% in the group without seminal plasma and 44±16% in the group with seminal plasma (group B). The fresh progressive motility was not significantly different ($p=0.22$) in the two groups (group without seminal plasma: 86±6%, group with seminal plasma: 80±11%; Figure 1).

In the second experiment, we found a linear relationship between sperm concentration and optical density (at 505 nm) and spermatocrit which can be described with the following equation: $Y = 3.416\times10^{11}X - 6.658\times10^{9}$ ($R^2=0.7027$).

Discussion and conclusion

In the first experiment we proved that replacement of the seminal plasma with grayling extender did not inhibit agglutination of the sperm during cryopreservation, which means a process occurring in spermatozoa (and not in seminal plasma) leads to agglutination of sperm. In addition, replacement of the seminal plasma with grayling extender caused a significant reduction in progressive motility. Consequently, replacement of the seminal plasma does not offer a solution to post-thaw sperm agglutination.

In the second experiment we found that there is a linear correlation between the concentration and the absorbance of common carp sperm which was not described before. The linear relationship will enable easier and faster assessment of the concentration of common carp sperm in the future which will be useful for further research and even for breeding process.

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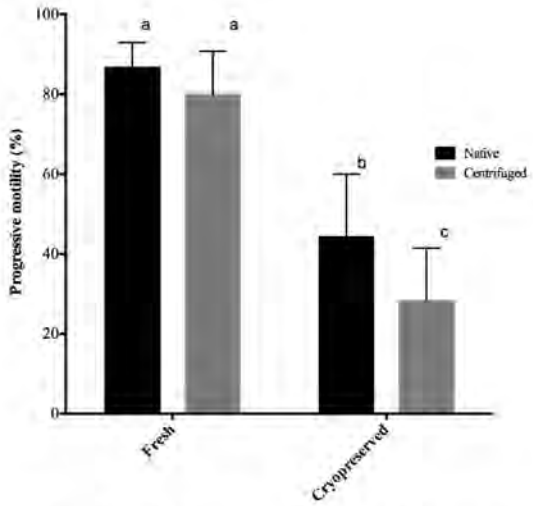

Fig.1. Progressive motility of the fresh and cryopreserved sperm with and without seminal plasma in common carp (*Cyprnius carpio*). Significant differences can be found between the fresh and the cryopreserved groups, as well as between the two cryopreserved groups.

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EFFECTS OF MICROPLASTICS TO THE CORAL *Zoanthus sociatus* MIGHT BE DRIVEN BY POLYMER TYPE IN SHORT-TERM EXPOSURE SCENARIOS

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Introduction

Coral reefs have a substantial social, economic and environmental value (Birkland, 2015). However, climate change and the degradation of the environment, due to pollution and other anthropogenic pressures, are seriously threatening these ecosystems. In the last years, the rising interest on corals for ornamental aquaria, pharmaceutical or biotechnological purposes are placing additional pressure on wild reefs. Coral aquaculture is raising as a sustainable solution to avoid harvesting of wild populations and to assist in the process of reef restoration. However, the rearing of corals brings in numerous challenges to optimize production and maintain (or even increase) corals' performance and appearance. The presence of microplastics (synthetic polymers with size < 5mm) in coral farms is a real threat since *in situ* facilities may be contaminated with microplastics present in seawater and *ex situ* farms, even operating with synthetic seawater, mostly rely on plastic material that likely undergo processes of fragmentation and deterioration over time (Lusher et al., 2017). Nevertheless, studies on the potential adverse effects of microplastics on corals and other ornamental organisms remain scarce. That said, this study wanted to evaluate the effects of two important polymers used in aquaculture facilities (low density polyethylene – LDPE, and polyvinyl chloride – PVC) in zoanthids, considering relevant concentrations in natural (Cheang et al., 2018) and aquaculture (Lusher et al., 2017) environments. For that, ingestion and epidermal adherence of microplastics, photobiological and biochemical biomarkers related with oxidative stress were evaluate.

Material and methods

Zoathus sociatus (Anthozoa: Hexacorallia) was selected as test species due to their farming plasticity, endosymbiosis with zooxanthellae, and quick regeneration. Two commercially available polymer type (LDPE, PVC – irregularly shaped, size between 63-125 µm) and two concentrations (1 and 10 mgL^{-1}; corresponding to ~0.5 x 10^5 - 4 x 10^5 LDPE or ~0.7 x 10^5 - 1.5 x 10^5 PVC) were used, resulting in a total of four treatments plus a control, in triplicate. Each replicate consisted of five colonies of three polyps distributed centrally (to receive the same light intensity) in glass aquaria containing 38 L of synthetic seawater. The test endured 96h at 25 ºC, pH~8, salinity 35, with no food supply. The evaluated endpoints included the ingestion and epidermis-adhesion of microplastics, initial (T_0) and final (T_{96h}) maximum photosynthetic efficienc (chlorophyll *a* fluorescence - Fv/Fm), oxidative damage (LPO – lipid peroxidation), antioxidant and detoxification capacities (Catalase activity – CAT; glutathione S-transferase - GST), and energy consumption (via electron transfer system – ETS).

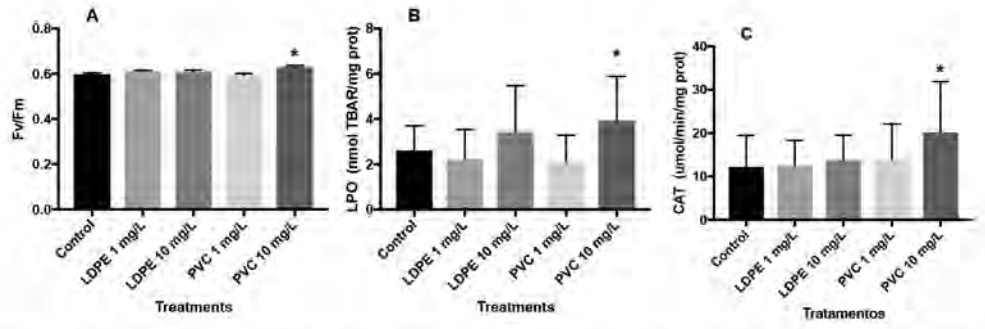

Figure 1: Effect of short-term exposure (96h) to 1 and 10 mgL^{-1} of low-density polyethylene (LDPE) or polyvinyl chloride (PVC) in the photochemical efficiency (A), lipid peroxidation (LPO – B) and catalase (CAT) activity (C) of *Zoathus sociatus*.

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Results

Zoathus sociatus polyps were able to ingest few particles of LDPE and PVC microplastics, independently of the tested concentrations and polymer type (nr. of particles up to 5). The number of microplastics adhered to the skin was dose- and polymer- dependent (nr. particles up to 85). Significant effects at photobiological and biochemical levels were only observed for the highest tested concentration of PVC microplastics (Figure 1). A 96h exposure to 10 mgL^{-1} caused a significant increase in the maximum photosynthetic efficiency (Fv/Fm; Figure 1A), induced oxidative damage (LPO; Figure 1B) and increased antioxidant defenses (CAT; Figure 1C). Yet, there were no effects in terms of energy consumption (via ETS) and detoxification capacity (via GST).

Discussion and conclusion

Short-term exposure to environmental-relevant concentrations of LDPE microplastics did not alter photochemical efficiency or induce oxidative stress or damage in *Z. sociatus*. Conversely, the presence of 10 mgL^{-1} of PVC microplastics caused a significant increase on the photochemical efficienc , probably related with the adhesion of a high number of particles to the zoanthids' epidermis (characterized by the presence of a surface mucus layer), which likely blocked some of the light that would otherwise reach the endosymbiotic zooxanthellae, as also observed in scleractinian corals (Polystyrene, 50 mgL^{-1}, Tang et al., 2018). Zoanthids exposed to PVC revealed a higher number of microplastics adhered to their epidermis compared with the similar concentration of its less dense counterpart (LDPE microplastics), which likely explains the lower effects in the latter. In addition, at 10 mgL^{-1} of microplastics, a significant lipid damage (i.e. lipid peroxidation) and increased catalase activity was observed once more for PVC, suggesting an increment of the reactive oxygen species (ROS) in the corals caused by such particle. Such ROS increment could result from direct effects, such as the ingestion followed by an alteration in the digestion processes or by an induced inflammatory response. However, due to the differences between both polymers, such results may be due to indirect effects such as the PVC polymer surface chemistry or due to the presence of potential PVC plasticizers (e.g. phatalates) that could have leachate during exposure. Although our results provide the first evidence for the potential short-term effects of realistic concentrations of microplastics (particularly PVC) in aquaculture facilities or even in the ocean to *Z. sociatus* populations (or potentially other corals), it is of extreme importance to investigate such scenarios in long-term exposure.

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MONITOR THE ENVIRONMENTAL IMPACTS THROUGH ARTIFICIAL INTELLIGENCE APPLIED TO METAGENOMICS

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Monitoring the health status of aquatic ecosystems is of crucial importance in a context of sustainable development of aquaculture activities. Different groups of organisms sensitive to changes in their surroundings are used as bio-indicators for monitoring environmental impacts. However, their morphological identification requires a lot of time and expertise. The metagenomics makes it possible to quickly and accurately describe the biological communities inhabiting an environment. However, a large proportion of the metagenomic data cannot be used to conduct large-scale environmental health diagnoses because many DNA sequences are not referenced in existing databases.

Here, we present a new innovative approach that combined metagenomics and automatic learning tools to assess the environmental impact of marine aquaculture. Our tool was developed for benthic monitoring of salmon farming in Norway, but it can be applied to any kind of finfis farms biomonitoring worldwide. It consists in predicting ecological status from metagenomic data using artificial intelligence tools, such as machine learning. Comparing to conventional methods, our approach allows (1) improving sensitivity of bioassessments by expanding the range of potential bioindicators, (2) enabling wider and more frequent applications by reducing the costs and time of analyses, and (3) limiting the uncertainties by standardizing protocols for genomic data acquisition and analysis. Based on cutting-edge technological and analytical advances, this new approach significantly increases the observation capacity of ecological impacts associated with finfis farming, offering a very efficient tool for future routine biomonitoring programs

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RISKS AND OPPORTUNITIES OF CLIMATE CHANGE TO EUROPEAN AQUACULTURE: ADVANCES MADE IN THE EU CERES PROJECT

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The EU project CERES (Climate change and European aquatic RESources, www.ceresproject.eu) is designed to advance a cause-and-effect understanding of how climate change (CC) will influence Europe's most important fish and shellfish resources and the economic activities that depend on them. Integrating physical, biological, economic and social science, the project highlights both the risks as well as the opportunities of CC to the European aquaculture and fisheries sectors. The role of aquaculture in supplying fish and shellfish for human consumption is projected to continue growing in the future. Understanding and potentially mitigating the effects of CC on aquaculture will be critical to ensuring the sustainable growth of this industry to maintain global food security. We summarize i) the socio-political scenarios developed for the European aquaculture industry, ii) work being conducted on both the direct (physical to physiology) and indirect (disease to food-web) effects of CC on Europe's seven most valuable aquaculture targets, and iii) the CERES approach to quantifying climate vulnerability through the exposure and sensitivity of aquaculture key targets and adaptive capacity of production systems.

The CERES Work is focused on the most valuable European finfis and shellfish to the aquaculture sector (Atlantic salmon, trout, sea bass, sea bream, carp, mussels, oysters and clams). We focus on species/production types which offer logical contrasts in the projected vulnerability and opportunities of CC in terms of coastal shellfish and finfis farms (e.g. in southern versus northern European waters and in fresh versus marine waters). Special emphasis in this presentation is on our efforts to rank the vulnerability of species / production types across European waters. Our vulnerability ranking is based on projected changes in 2050 in the exposure to abiotic stressors (e.g. higher temperature, lower dissolved oxygen, decreased rainfall, increased storminess) as well as the sensitivity of specific species (e.g. physiological tolerance) and results of a GAP analysis of the direct effects of CC. Indirect effects of CC such as the risks of specific diseases are also included. Adaptive capacity is also discussed in terms of stakeholder perceptions of available mitigation measures to help prevent unwanted consequences of climate change and the likelihood that those measures are adopted.

INNATE IMMUNE STATUS AND OXIDATIVE STRESS IN SENEGALESE SOLE (*Solea senegalensis*) POST-LARVAE FED MICRODIETS WITH NANNOCHLOROPSIS OR ISOCHRYSIS INCLUSION

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Introduction

Senegalese sole (*Solea senegalensis*) is a highly valuable flatfis species targeted for aquaculture in Southern-European countries. This species, as most farmed fish, is potentially subjected to stress and pathogens due to environmental factors. Now, more than ever, the industry and science have focused their attention on the development of diets that promote the welfare and health of farmed fish, as well the fish final quality. Microalgae has been included in diets for farmed aquatic animals due to their antioxidant capacity, high-quality protein content and bioactive compounds, promoting an optimal growth and health. For these reasons, this study intended to evaluate the effects of dietary microalgae inclusion in both health status and growth performance of Senegalese sole post-larvae.

Materials and methods

Senegalese sole post-larvae with 34 days after hatching (DAH) were randomly distributed by 9 tanks with an initial density of 2650 post-larvae/m^2 and three isonitrogenous (60% crude protein) and isolipidic (17% crude lipids) diets were randomly distributed by triplicate groups of tanks. The experimental diets comprised a control (CTRL) diet based in marine protein sources (i.e. fish meal, squid meal and krill meal) and 3 % of cellulose fille , whereas two other diets were based on the control diet but replaced cellulose by 3 % *Isochrysis galbana* (ISO) or *Nannochloropsis sp.* (NANO). The experimental diets were supplied through automatic feeders set up to supply 8 meals in a 24 h period. At 45 and 65 DAH, 30 post-larvae/ tank were sampled for analyses of parameters related health status. The total length, dry weight, feed conversion ratio, relative growth rate and survival were also assessed.

Homogenates of 10 individuals were performed for the analyses of immune (i.e. lysozyme and bactericidal activities) and oxidative stress (i.e. catalase, total glutathione, lipid peroxidation and superoxide dismutase activities) related parameters.

Results

Survival, relative growth rate and feed conversion ratio were not altered by the dietary treatments at 65 DAH. However, post-larvae fed ISO presented lower dry weight compared to the other dietary treatments whereas the total length of post-larvae fed NANO dietary treatment increased compared to those fed the ISO diet.

Regarding immune status, it was observed a decrease in lysozyme levels and bactericidal activity against *T. maritimum* of whole post-larvae from 45 DAH to 65 DAH. However, Senegalese post-larvae fed NANO showed a clear tendency to increase lysozyme activity at 45 DAH. Similar to parameters related to immune status, catalase and total glutathione activities decreased from 45 to 65 DAH in whole post-larvae, whereas the opposite trend was observed for superoxide dismutase activity. Total glutathione activity decreased in post-larvae fed NANO dietary treatment at 45 DAH. However, a drop in this antioxidant tripeptide did not translate in differences regarding lipids peroxidation, which was similar among experimental groups and sampling times.

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Discussion and conclusion

Results from the present study seem to be in line to the widely known recognition of microalgae in terms of antioxidant capacity and growth promotion, which usually translates into a good welfare of farmed fish. Senegalese sole post-larvae fed experimental dietary treatments presented normal ranges for growth performance and survival. Moreover, fish fed NANO dietary treatment seem to improve their immune status at 45 DAH while maintaining an excellent growth performance and survival. According to these results, *Nannochloropsis sp.* Seems to be a promising candidate for inclusion in microdiets for Senegalese sole post-larvae.

Acknowledgements

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EFFECTS OF REARING DENSITY ON GROWTH, WELFARE INDICATORS AND DIGESTIVE CONDITIONS OF GILTHEAD SEA BREAM (*Sparus aurata*) FED DIFFERENT FISH MEAL AND FISH OIL DIETARY LEVELS

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Introduction
To maximize productivity, sustainability and fish welfare of Mediterranean aquaculture, research needs to focus on interaction between feeding and rearing condition, paving the way to a wise fish farming management. To this aim, the present study explored the effect of high and low rearing density on growth, plasma biochemistry, gut microbiome structure, humoral immunity of skin mucus and digestive enzymes activity in gilthead sea bream (*Sparus aurata*) fed low and high fishmeal (FM) and fish oil (FO) dietary leve

Materials and methods
Two isoenergetic diets with high and low FM and FO percentages, (FMFO30/15 and FMFO10/3, respectively) were tested in triplicated fish groups until reaching a final high density (HD 30kg/m^3) and low density (LD 10kg/m^3). Fish (initial body weight: 96.2g) were fed to satiation twice a day (10% overfeeding) over 97 days. At the end of the trial, growth performances, feed utilization and carcass composition were estimated. The activity of pancreatic and intestinal brush border digestive enzyme extracts (3 fish per tank) was determined as in Gisbert et al. (2018); while microbiome structure (5 fish per tank) was analysed by Next Generation Sequencing as described in Parma et al. (2016). Skin mucus (8 fish per tank) and plasma biochemistry (5 fish per tank) were analysed according to Guardiola et al. (2014) and Bonvini et al. (2018) respectively. All gained data were analysed by Two-way ANOVA followed by a Tukey's multiple comparison test. Differences among treatments were considered significant when value was lower than 0.05.

Results
No significant effect on growth (final body weight FBW, weight gain and specific growth rate SGR) were detected between high and low rearing density for both dietary treatment while fish fed FMFO30/15 displayed higher FBW, weight gain and SGR compared to FMFO10/3. Feed intake (FI) was lower in HD compared to LD (density effect P = 0.002) with more marked differences in FMFO30/15 than FMFO10/3. No significant diet effect on FI was detected. No significant effect of density on FCR was detected. Survival rate was lower at LD. No significant effect of density on skin mucus peroxidase and protease levels were found in FMFO10/3 individuals. Concerning pancreatic enzymes activity, trypsin was higher in fish fed FMFO30/15, while lipase had the opposite reaction. Activity of pepsin was significantly influenced by density which interacted with diet as well, while chymotrypsin was influenced only by density. Alkaline phosphatase and aminopeptidase in the anterior intestine brush border were reduced in FMFO10/3 compared to FMFO30/15, while maltase was lower only in FMFO10/3 reared at HD. The posterior intestine brush border alkaline phosphatase and maltase were negatively affected by FMFO10/3 while leucine alanine peptidase (LAP) was reduced at HD. Density had no significant effect on parameters analysed in plasma, while diet influenced total protein, urea and creatinine levels.

Discussion and conclusion
Rearing density did not show negative impact on growth and feed utilization at both high and low FM and FO dietary level. Similarly, density did not affect digestion condition except for trypsin, pepsin, chymotrypsin, maltase and LAP. At the end of the trial high density did not compromise fish welfare in terms of mucus enzymatic activity and plasma biochemistry. Though high vegetal dietary levels reduced growth efficienc , stocking density up to 30kg/m^3 did not show negative effects on performances, digestive capacity and animal welfare at both feed regimes. Moreover, the absence of feed intake differences between high and low density at high vegetal dietary concentration could indicate a reduced competitiveness among fish under these feeding regimes. At the light of this study, at both considered FM and FO dietary levels, fish can be reared at both 10 and 30kg/m^3 without compromising performance and health status.

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Acknowledgements
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DEVELOPMENT OF A COMBINED SPECIES SNP ARRAY FOR THE EUROPEAN SEA BASS AND THE GILTHEAD SEA BREAM

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Introduction

SNP arrays are enabling tools for high-resolution studies of the genetic basis of complex traits, and for incorporating genetic markers to expedite genetic gain in selective breeding programmes (Huang *et al.*, 2015). The European sea bass (*Dicentrarchus labrax*) and the gilthead sea bream (*Spaurus aurata*) are the two primary species of importance for Mediterranean aquaculture. While selective breeding programmes increasingly underpin stocky supply for this industry (Janssen *et al.*, 2015), genomic selection is not yet widespread. Genomic selection has major potential to expedite genetic gain, in particular for traits practically impossible to measure on selection candidates, such as disease resistance. Commercial-scale application of genomic selection requires relatively low-cost genome-wide genotyping platforms. Combining SNPs derived from two species onto a single SNP array can significant y reduce genotyping costs when ordering higher volumes, which would facilitate the uptake of the technology by the industry and researchers. The aim of our study was to design a combined-species, 70K SNP array for sea bass and sea bream, and to test its performance on farmed and wild populations from multiple locations throughout the Mediterranean.

Materials and Methods

For the purpose of generating a combined SNP array with extensive application, widespread sampling of both wild and farmed populations from the Mediterranean was carried out (21 populations of sea bass and 27 populations of sea bream). In total, over 500 individuals per fish species were sampled for SN discovery.

A pooled whole-genome resequencing (Pool-seq) approach was followed to maximize the number of variants detected in a cost-effective manner. DNA was extracted from ~25 individuals per population and combined in two duplicate pools. Library pools were prepared using a PCR-free protocol and sequenced using an Illumina HiSeq X (for sea bream) or Illumina HiSeq 4000 (for sea bass) technology at a minimum coverage of 50x per pool. The obtained raw sequencing reads were quality filtered and trimmed to remove adapter sequences. Cleaned paired-end reads were aligned to the European sea bass (Tine *et al.*, 2014) and the gilthead sea bream (Pauletto *et al.*, 2018)2018 genomes using BWA (Li and Durbin, 2009) England. Only uniquely mapped reads that had a MAPQ>20 were kept for analysis. Variants were called from the pools with the software Freebayes v1.20 (Garrison and Marth, 2012) if (i) at least three reads supported the alternate allele or (ii) the SNP allele frequency in the pool was above 0.05, whichever condition was met first.

From this initial list of variants, SNPs were kept if they had: a sequence coverage between 40x-100x, supporting reads on both strands, at least two reads “balanced” to each side of the variant site, and >90% of the observed/alternate alleles supported by properly paired reads. Additional filters were applied to the dataset based on SNP minor allele frequencies (MAF). SNPs with a MAF >0.45 or MAF <0.05 were removed, as they likely represent artefacts. Finally, evenly distributed SNPs were selected along the chromosomes of both sea bass and sea bream. A subset of high priority markers (e.g. SNPs annotated to genes) will be selected as candidates for inclusion in the combined-species, 70K SNP array.

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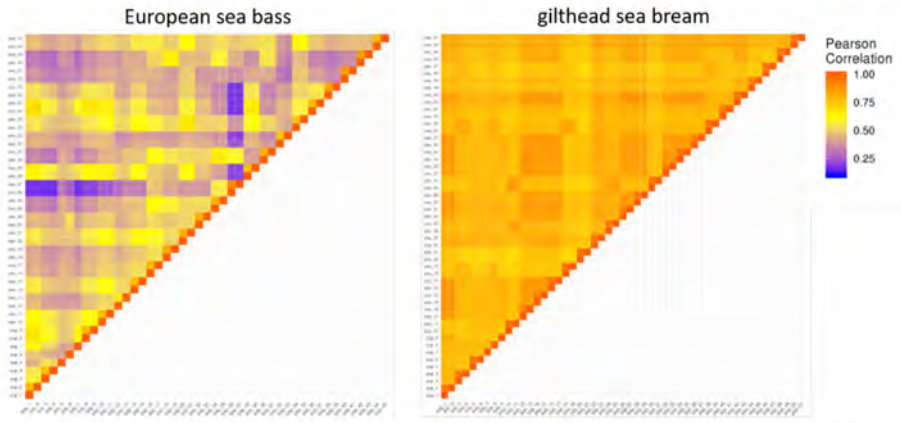

Figure 1. Pairwise correlation values of the MAFs of SNPs discovered on the sea bass and sea bream

Results and Discussion

The pooled sequencing of the diverse Mediterranean populations resulted in the discovery of 17 million SNPs for the European sea bass and 34 million SNPs in the gilthead sea bream genome.

In a preliminary analysis, Pearson correlation coefficients were calculated for the MAF of SNPs for all pairs of sea bream and sea bass populations. The results show a high correlation between technical replicates, as expected. Additionally, a higher structure was observed for the sea bass samples compared to the sea bream populations (Figure 1).

The discovered SNPs will be filtered to approximately 70K SNPs which will be used in the final combined species SNP array design. The newly developed SNP array will then be used to genotype a diverse set of wild and farmed sea bass and sea bream populations to assess its performance.

Acknowledgements

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MICROALGAL MIXOTROPHY AS A SOURCE OF FEED FOR *Mytilus edulis* LARVAE

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Mytilus edulis aquaculture represents an important section of the UK and EU economies. Current production mechanisms rely on the supply of wild larvae to be settled upon longlines and subsequently on-grown. This leaves larval collect and production of adult blue mussels reliant upon larval supply and so is one of a number of limiting factors in upscaling production. In Scotland 7000 tonnes of mussels were produced in 2015 and mussel growers aim to double production by 2020. To do so, the limiting factor of larval supply must be overcome. One method of doing this is the creation of a *M. edulis* larval hatchery. The costs associated with microalgal culture, for use as feed, in a larval hatchery are currently around 40% of total costs. As a result the creation of a larval hatchery is not economically viable (Hickman, 1992). This is due to the requirement for live microalgae, mussel larvae do not feed on dead cells. This study aims to address this limiting factor through use of mixotrophically cultured microalgae as a feed source.

Microalgae are classically considered to be photoautotrophic and are cultured in the light, in seawater with additional nitrate and phosphate. Mixotrophy is the ability of a microalga to uptake organic nutrients, such as carbon or nitrogen, in addition to, or as an alternative to, photoautotrophic fixation of carbon. This ability to be cultured mixotrophically is considered to be ubiquitous in the marine environment (Borowitzka, 2013) and has a number of advantages. Mixotrophic culture often results in greater cell numbers and dry weight, as well as an increased culture stability (Morales-Sánchez & Martinez-Rodriguez, 2015). Furthermore, dependent upon the method of culture and the carbon source utilised, the biochemical profile can be tailored to create a "designer" feed.

Over the course of three feeding trials this study takes current "industry standard" microalgal species and compares larval growth and survival to mixotrophic "designer" microalgal feeds, then subsequently optimises the diet used. The "designer" feed can be considered to be as effective as the standard benchmark feed and subsequent optimisation shows that a diet that is tailored towards the development performs the most effectively in terms of larval growth. Mixotrophic culture requires an additional organic carbon source which has a significant cost implication. Therefore using first order modelling, the economics of use of mixotrophy as an alternative to photoautotrophic culture reveals a more complex picture in terms of the optimal methods of culture. Culture optimization, a move to semi-continuous culture and alternative, low cost carbon sources potentially reveal a way forwards.

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EXPLORING THE BENEFITS OF NATURAL ANTIOXIDANTS IN DIETS FOR EUROPEAN SEABASS UNDER A CIRCULAR ECONOMY CONTEXT

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Introduction

Consumers increasingly show interest in a shift from synthetic to natural antioxidants in their food. Annual production of plants and aromatic herbs has passed 600 million tonnes per year, generating a high amount of by-products that are mostly wasted. But such discarded by-products can still be valuable source of nutrients, antioxidants and bioactive compounds that may have a positive effect on fish health and flesh quality traits. This study evaluates the potential of vegetable by-products in diets for European seabass (*Dicentrarchus labrax*). The effects of such natural antioxidants on immune system, fish growth, and fillet quality traits were evaluated.

Material and methods

Five isoproteic and isolipidic experimental diets were formulated. A commercial-based diet supplemented with a regular dose of vitamin E (100 mg/kg) was used as negative control (CTRL) and compared with a diet with a higher dose of vitamin-E (500 mg/kg) as positive control (VITE). Dried biomass from two fresh legumes and one aromatic herb (carrot, tomato and coriander, respectively) were added to the control diet at 2% resulting in three experimental diets: carrot (CA), tomato (TO) and coriander (CO). Experimental diets were fed to triplicate groups of European sea bass (initial body weight 114 ± 0.2 g) during 85 days, twice a day until apparent satiety. At the end of the trial (Day 0), growth performance, body composition, immune status and flesh quality traits (instrumental and sensory consumer panel) were evaluated. Catalase activity, total glutathione content and lipid peroxidation (TBARS) were determined as indicators of the liver oxidative stress. Fillet flesh quality traits were evaluated in terms of lipid peroxidation, muscle and skin colour, and flesh texture immediately after sampling the fish (Day 0) and after storing the whole fish in ice for 8 days (Day 8). Fish from day 0 were evaluated by a sensory consumer panel of 60 naïve consumers in order to assess the sensorial properties of fillets, using a classic 9-point hedonic scale (like extremely-dislike extremely).

Results and discussion

All diets were well accepted by fish that doubled their initial size reaching a final body weight between 237 and 248 g without significant differences among treatments. The specific growth rate (0.89 ± 0.06), feed conversion ratio (1.3) and whole body composition of fish remained unaffected by the experimental diets. Tissue lipid level was similar among diets, but the viscerosomatic index (VSI) of fish fed TO was significantly higher than those fed the CO diet (7.1 vs 5.7). Innate immune status of fish, determined by plasma lysozyme, peroxidase and ACH50 activities, as well as the adaptive immunity evaluated by total immunoglobulin M (IgM) content, were not significantly affected by the diets. Likewise, total red and white blood cell number, haematocrit and haemoglobin concentration were similar among treatments.

The antiperoxidative activity of catalase in the liver did not differ among treatments, but total glutathione levels were significantly higher in CTRL compared to CA fed fish. Moreover, fish fed the TO diet displayed significantly higher rates of lipid peroxidation in the liver than those fed the positive control (VITE). But the muscle lipid peroxidation was similar among all fish, irrespectively of the experimental diet or the storage time.

The experimental diets did not affect skin color. However, muscle color showed a significantly higher yellowness (b*) in fish from CTRL compared to those fed with TO, CA and CO. Muscle chroma (C*) values were significantly higher in CTRL, compared to CO, but hue angle (h*) was significantly lower in CTRL, in comparison to CA. Storage time impacted both skin and muscle colour: b* and C* significantly decreased in both tissues whereas h* increased. In muscle there was also an increase in the lightness (L*) values after 8 days of storage in ice.

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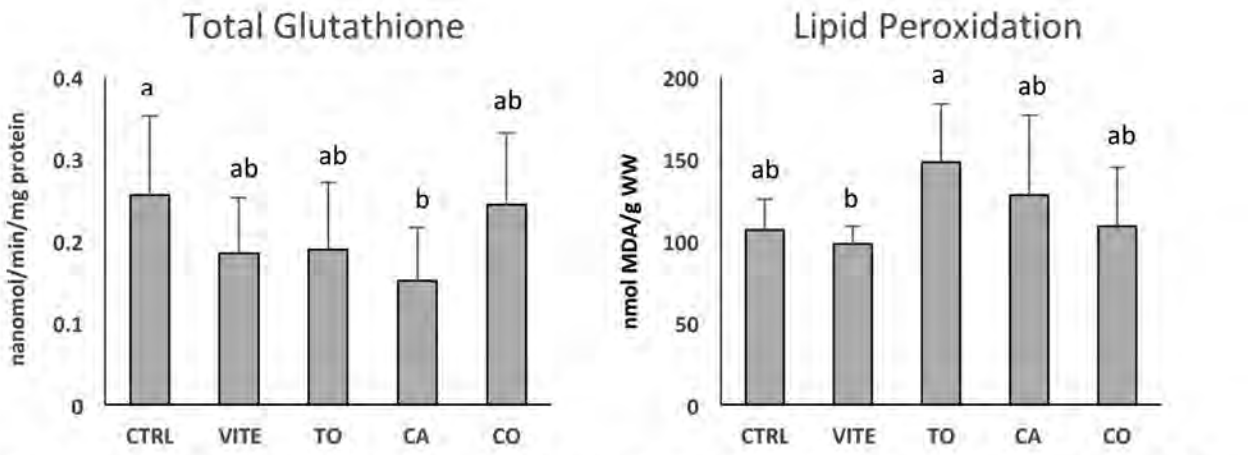

Figure 1 – Total glutathione (nmol/min/mg protein) and lipid peroxidation (TBARS, nmol MDA/g) in *D. labrax* liver after sampling (Day 0). Different letters indicate statistical differences ($P < 0.05$). Values are presented as mean ± SD. N= 12 per dietary treatment.

The fillets from fish fed CO showed significantly higher gumminess values compared to those fed TO. Storage in ice significantly affected all textural parameters: a decrease in hardness, adhesiveness, cohesiveness, gumminess and chewiness was observed whereas springiness and resilience increased. However, by the end of the experiment, the sensory consumer panel could not perceive any significant differences in fillets overall liking, as all samples were positively evaluated in terms of visual attractiveness, odour, texture, taste, juiciness and colour.

Conclusions

The natural antioxidants evaluated in this study did not affect European sea bass growth performance, immunological status or muscle lipid peroxidation, even though liver oxidative stress seems to be affected with higher lipid peroxidation caused by TO conversely to an antioxidant effect (i.e. increase of glutathione concentration) caused by CA. The supplementation of diets with a higher vitamin E level (500 mg/kg) does not seem to have any impact on the parameters evaluated. The sensory consumer panel could not perceive significant differences in global acceptance of fillets among dietary treatments.

This work was supported by Project MOBFOOD (POCI-01-0247-FEDER-024524) Mobilização de conhecimento científico e tecnológico em resposta aos desafios do mercado agroalimentar", cofounded by PORTUGAL2020, Lisb@a2020, COMPETE 2020 and the European Union.

CULTIVATION OPTIMIZATION OF THE DIATOM *Haslea ostrearia* AND APPLICATION OF MARENINNE IN AQUACULTURE

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Introduction

In the CAMAFAN project (Characterization and industrial Application of Marennine as Aquaculture Feed Additive and Nutraceuticals), we are focusing on developing industrial application of the marine diatom *Haslea ostrearia* and maximizing its blue-green pigment marennine for possible uses in aquaculture. This blue-green pigment is divided in two forms: the intracellular mareninne (IMn) and the extracellular mareninne (EMn) (Neuville and Daste, 1978). Apart from the widely known colouring capabilities, recent research has shown that this pigment has antibacterial, antiviral, antiproliferative and antioxidant (Pouvreau et. al., 2018) properties as well. Considering the optimization of the culturing protocols of *Haslea ostrearia* for marennine production, we conducted series of experiments focusing on important parameters e.g. light spectra, nutrients N:P ratio, photoperiod and salinity. These parameters have great influence and impact on the overall yield of this blue-green pigment production. By achieving these improvements, we have been producing blue water (culture medium enriched with EMn) to be applied on some parallel experiments that test the applicability of the pigment in improving health and overall quality of marine aquaculture species. In addition, no information in literature about the process of maintenance and storage of marennine has been found. Thus, a new set of experiments are currently in progress to identify the best/right conditions for marennine to be stored for long-term without any loss of concentration, quality or biological effect.

Material and methods

We have grown *H. ostrearia* cultures using North Sea water F/2 medium in a total volume of 2000 mL per flask, in a climate-controlled room at 18°C with aeration and CO_2 supply to maintain pH between 7.8 and 8.2. The photoperiod was kept at 16:8 light/dark. The different light treatments were obtained by using LED lights of different spectra, warm light with emission peaks of 450 nm and around 600 nm, and blue LED with emission peak around 450 nm. We choose blue and warm light based on literature on the effect of different light spectra on *Haslea* and based on our experience. Apart from spectra, we also tested intensity, from 80 to 200 µmol photons*m^{-2}*s^{-1}. Additionally, we have tested different light/dark photoperiods to determine their impact on marennine production. To test the effect of different salinities, we prepared North Sea water F/2 media with different degrees of salinity: 15, 20, 25 and 28 psu. The influence of light quality on marennine production by cultures of *H. ostrearia* was investigated in the laboratory. In the first series of experiments in the laboratory, a clone of *H. ostrearia* was cultured under light of different colors (white, blue, green, yellow, and red) and at two irradiances ('low' and 'high', 20 and 100 µmol photons m^{-2} s^{-1}, respectively).

Results

We have identified that marennine production is increased when using white LED's at 200 *µmol* m^{-2} s^{-1} (when compared with lower intensity blue or white light) (Figure 1). The effect of photoperiod in the production of the blue-green pigment indicated that its concentration is higher in groups cultured under 12/12 light/dark conditions then 18/6 and 20/4 light/dark treatments (Figure 2). There were noticeable separations in terms of marennine concentration between 18/6, 20/4 and 12/12 already after 10 days of cultivation period. Results in experiments on oysters and storage availability of marennine are still pending. Our expectation is that the process of maintenance and storage of mareninne being currently developed will be implemented as a new and standardized method in industry.

Discussion and conclusion

It should be possible to scale-up these positive findings to the industrial scale for indoor marennine production. We aim to present the preliminary data of this set of experiments at the conference where the best set of parameters to obtain the most efficiency production of marennine from *H. ostrearia* as well as the effects of this molecule on oyster health, growth and in the process of maintenance and storage.

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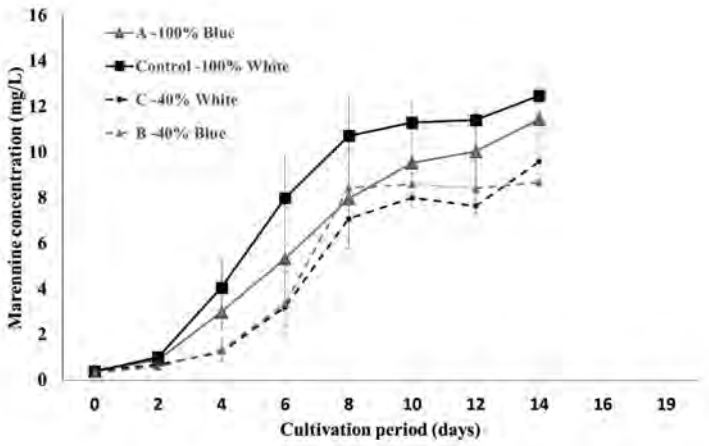

Fig. 1. Marennie production (concentration mg/L) under different light sources.

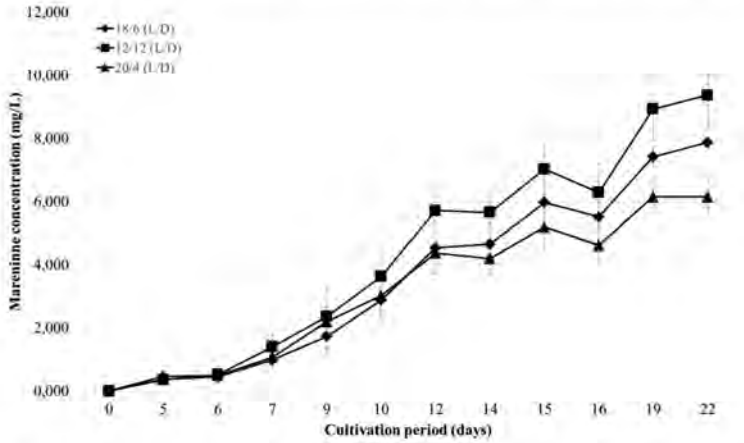

Fig. 2. Marennie production (concentration mg/L) under different photoperiods.

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ANTIOXIDANT AND IMMUNE-MODULATORY POTENTIAL OF MARINE ALGAE IN DIETS FOR EUROPEAN SEABASS

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Introduction

Marine algae are natural sources of several bioactive molecules that can have health promoting benefits. Both macro and microalgae have demonstrated high potential in stimulating antioxidant defences, and can also have an immune-modulatory effect on fish. The aqua feed industry can take advantage of such properties of marine algae potentially improving the defences of farmed fish against reactive oxygen species (ROS) and consequentially preventing the occurrence of peroxidative damage. This study selected one microalgae (*Nannochloropsis sp*.), one macroalgae (*Gracilaria sp.)* and a blend of both to include in diets for European sea bass (*Dicentrarchus labrax*) juveniles.

Materials and methods

Three practical isoproteic (53%) and isoenergetic (22 kJ/g) experimental diets were formulated with 8 % *Gracilaria sp.* (GRA), 8% *Nannochloropsis sp.* (NAN) and a blend of 4% *Gracilaria sp.* and 4% *Nannochloropsis sp.* (NANGRA) to partially replace fish meal. An algae-free diet was used as control (CTRL). Extruded diets were fed to triplicate groups of European sea bass (initial body weight 29.7 ± 3.5 g) during 140 days, three times a day until apparent satiety. At the end of the trial, growth performance and feed efficiency were evaluated. Humoral non-specific immune parameters (lysozyme, peroxidase and the alternative complement pathway (ACH50) activity and antiperoxidative activities (catalase, glutathione-peroxidase and glutathione S-transferase activities) were determined in liver. In terms of non-enzymatic parameters, total glutathione content, as well as lipid peroxidation using the TBARS method for malondialdehyde (MDA) formation were also measured in liver.

Results

By the end of the experiment, fish tripled their initial size, reaching a final body weight between 78 and 81g, without significant differences among treatments. Specific growth rate (0.9) and feed conversion ratio (1.6) remained unaffected by the experimental diets. The viscerosomatic index did not vary among treatments, but the hepatosomatic index of fish fed the CTRL diet was significantly higher compared to those fed the GR diet.

Humoral lysozyme and peroxidase activities did not show significant differences among dietary treatments, but the ACH50 activity was significantly lower in fish fed NANG compared to those fed the CTRL diet.

Catalase and glutathione s-transferase activities in the liver did not vary among treatments. Glutathione-peroxidase was significantly lower in fish fed NANGRA compared to those either fed the CTRL or GRA diets, whereas total glutathione content was significantly lower in fish fed NANGRA than in those fed the CTRL. Liver lipid peroxidation values were significantly higher in fish fed NAN when compared to those fed the CT (Figure 1).

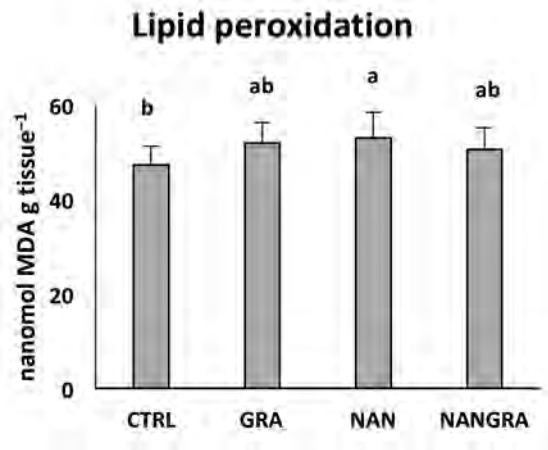

Figure 1 - Values are means + standard deviation: n = 12. Means in columns without a common superscript letter differ significantly (P<0.05).

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Conclusions

The experimental diets evaluated in this study did not affect fish growth or feeding efficienc , so both algae can be considered good alternative sources to fish meal in diets for European sea bass. Results from ACH50 showed that fish fed with the NANGRA displayed significantly lower values when compared to those fed with CTRL. This result suggests that this blend of algae may have provided antibacterial protection, but this will require further confirmation by submitting fish to a challenge trial.

The NANGRA diet induced both significantly lower glutathione content and glutathione peroxidase activity compared to the CTRL diet, but did not result in any differences in fish lipid peroxidation

Glutathione peroxidase acts in association with total glutathione and catalyses the conversion of hydrogen peroxide, therefore avoiding lipid peroxidation. The obtained results suggest that the antioxidants provided by the dietary algae blend seem to lessen the need for endogenous biosynthesis of glutathione and glutathione peroxidase. In terms of future perspectives, a growth trial with a pro-oxidant and/or bacterial challenge might provide further insight on the beneficial properties of the selected algae in European sea bass diets.

This work was supported by Project MARINALGAE4aqua (ERA-NET COFASP/004/2015) “Improving bio-utilisation of marine algae as sustainable feed ingredients to increase efficie cy and quality of aquaculture production”, funded by the ERA-NET COFASP.

STEAROYL-COADESATURASE-1ISEPIGENETICALLYREGULATEDBYBROODSTOCK NUTRITION IN GILTHEAD SEA BREAM *Sparus aurata*

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Introduction

High substitution of fish oil (FO) by vegetable oils (VO) in aquafeeds is still a challenge for marine fish. VOs have low content of *n-3* LC-PUFA such as EPA and DHA, and instead, they are rich in fatty acids (FA) such as ALA and LA. High VO feeding leads to increased hepatic fat accumulation in gilthead sea bream (*Sparus aurata*) and other fish species, increasing the risk of steatosis. Different studies in fish have provided evidence of the effect of broodstock nutrition on the lipid metabolism of the progeny (e.g., Izquierdo et al., 2015; Turkmen et al., 2017). In higher vertebrates, enriching maternal diet with ALA prevented fatty liver in the offspring (Hollander et al., 2014) by decreasing both SCD1 and FADS2 gene expression (Leikin-Frenkel et al., 2017). We used a 'control' and an 'ALA-rich' diet to study the effect of parent's nutrition on the promoter methylation and gene expression of *fads2* and *scd1a* in *S. aurata* offspring.

Materials and Methods

Two broodstock diets were used with a similar composition but a 19-fold difference in ALA content. ARA, EPA and DHA were kept similar to avoid confounding effects. The diet for juvenile offspring was a low FM (5%) and low FO (3%) diet. The genomic database of gilthead sea bream (http://nutrigroup-iats.org/seabreamdb) was used to obtain the gene sequences of *fads2* and *scd1a*. *In silico* analysis included predictions for gene structure, core promoter, transcription factor binding sites (TFBS), and CpG islands. DNA methylation was examined by bisulfite conversion and pyrosequencing. The pattern of CpG methylation of the *fads2* promoter was confirmed in a group of fish subjected to normoxia or moderate hypoxia. Gene expression was assessed by qPCR.

Results

The gene organization of *fads2* and *scd1a* was conserved respect to other vertebrates. Key TFBS were conserved in proximal promoters of both genes, including PUFA response regions. Methylation within key TFBS in *fads2* proximal promoter were unaffected by parent's diet or oxygen availability. CpGs within a regulatory SREBP site were particularly hypomethylated. Conversely, ALA enrichment of parent's diet increased methylation in the distal promoter of *scd1a*, mainly in CpGs associated with specificity protein 1 (SP1) and hepatic nuclear factor-4alpha (HNF4α) binding sites. DNA methylation at these sites (mostly CpG7) correlated inversely with *scd1a* expression of the offspring (Fig. 1). Liver fat content suggested that this might favor less fat storage in offspring. This would be more important under lipogenic conditions, as we found that the highest transcriptional response occurred for *scd1a* when fis fed on deficient or low n-3 LC-PUFA diets, exhibiting signs of hepatic fat accumulation.

Discussion and conclusions

CpG sites at regulatory regions in the proximal promoter of *fads2* are insensitive to the ALA content of parent's diet in 7 months old juveniles if nutritionally challenged with a low *n-3* LC-PUFA diet. Conversely, methylation of different CpGs, neighboring key regulatory sites (SP1 and HNF4α) of the *scd1a* promoter, is susceptible to ALA enrichment of parent's diet and correlates with offspring *scd1a* gene expression. These TFs have been related in other animals with SCD1 regulation (Mar-Heyming et al., 2008; Mauvoisin et al., 2010). These epigenetic changes may favor a more controlled upregulation of *scd1a*, and ameliorate hepatic fat accumulation, in fish fed with high levels of VO. This study provides the first evidence of epigenetic programing of a gene involved in lipid metabolism in a marine fish through broodstock nutrition. Further studies are needed to understand the true adaptive (or practical) value of *scd1a* epigenetic programing in marine fish

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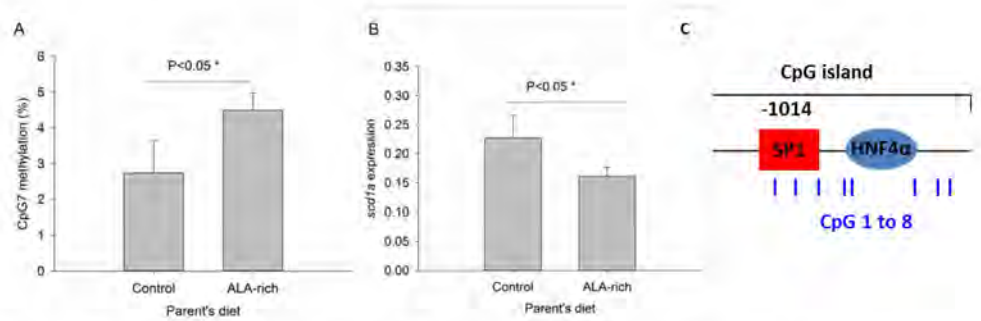

Fig.1. Effect of parent's diet on CpG7 methylation (A), *scd1a* gene expression of the offspring (B), and location of analyzed sites within the promoter of the *scd1a* gene (C).

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The study has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement no 727610 (PerformFISH).

THE EFFECT OF TOTAL REPLACEMENT OF FISH OIL WITH RAPESEED OIL ON GROWTH, BODY COMPOSITION AND GUT HEALTH OF JUVEILE LUMPFISH (*Cyclopterus lumpus*)

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Introduction

Commercial production of lumpfish has grown rapidly since 2012 and has reached 30 million fish in 2017 (Norwegian Directorate of Fisheries, 2018), making lumpfish the second largest aquaculture species in Norway. The boost in lumpfish production is due to their grazing efficiency on sea lice at low temperatures (Nytrø et al. 2014) combined with a relatively uncomplicated and short production period. They are introduced to salmon farms already four months post hatching. However as a new species, the lumpfish farming industry is facing several knowledge gaps that need to be overcome. One of these areas that needs more focus includes the tolerance to plant feed ingredients. Hence, the present experiment aimed to investigate the lumpfish performance when they were fed rapeseed oil replacing 0 – 100% of the fish oi

Material and methods

Four iso-nitrogenous and iso-energetic diets were formulated to contain 54% crude protein and 13% crude lipid (on dry matter weight). The control diet and three experimental diets were based on soy and pea protein concentrate in 1:1 ratio replacing 50% of the fishmeal (FM) as the main protein source. Diets were varying only in their alternative lipid source, rapeseed oil (RO) replacing FO at 25% (RO25), 50% (RO50) and 100% (RO100). The experiment was carried out in triplicates at Mørkvedbukta research station, Nord University (Bodø, Norway) and juvenile lumpfish were obtained from a commercial hatchery (Mørkvedbukta AS, Norway). In August 2017 approximately 2400 of juveniles with a mean weight of 4 g were randomly allocate into twelve 500 L circular tanks with continues feeding and dimmed light setting according to best commercial practice. Biometrical data (weight, total length and height) of all fish was recorded at prior to and at the end of the experiment. In addition, samples for chemical composition (whole fish), fatty acids and gut histology was taken to study the dietary effects on growth, chemical profile and digestive health of the juvenile lumpfish

Results and Discussion

Body weight of fish increase of approximately six fold within 42 days and there was no mortality during the experimental period indicating that the fish were in good health. The overall growth was similar among groups, except for the fish that had been fed on RO100% that was significantly lower compared to other groups ($p<0.05$). Other Analysis mentioned are currently being carried out and will be presented.

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AGE- AND SEASON-MEDIATED CHANGES IN DNA METHYLATION AND EXPRESSION PATTERNS OF SIRTUINS IN GILTHEAD SEA BREAM *Sparus aurata*

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Introduction

Sirtuins (SIRTs) are a conserved family of enzymes that couple protein deacetylation of histone and non-histone substrates with the energy status of the cell via the cellular NAD^+/NADH ratio. This protein family has been largely conserved through evolution, and the SIRT counterparts of higher vertebrates have been identified and molecularly characterized in gilthead sea bream (Simó-Mirabet et al., 2017). Transcriptional studies have also revealed a ubiquitous *sirt* gene expression that is tissue-specific for each *sirt* and highly dependent on feed intake and growth potentiality (Simó-Mirabet et al., 2017, 2018). This agrees with an epigenetic role of SIRT related with developmental, differentiation and genome environment interactions. Nevertheless, there are few studies assessing the DNA methylation pattern of SIRT promoters, and the aim of this study was to determine if changes of *sirt* gene expression in liver and white skeletal muscle of one- and three-year old fish during winter and summer are due, at least in part, to changes in DNA methylation of *sirt* promoters. The study also included the gene expression profiling of markers of oxidative metabolism (*cs, cpt1a, pgc1a*) and mitochondrial respiration uncoupling (*ucp1, ucp3*).

Materials and methods

The exon-intron organization of the seven gilthead seabream *sirts* was determined by blat searches in our genomic gilthead sea bream database (http://nutrigroup-iats.org/seabreamdb). This i*n silico* analysis included predictions of transcription start sites (TSS), core promoter regions, transcription factor binding sites (TFBS) and CpG islands (CGIs). For transcriptional and DNA methylation assays, tissue portions of liver and white skeletal muscle were processed for RNA and DNA extraction in fish of two class of age (+1, +3) at two different times (winter, February 2018; summer, June 2018). DNA methylation was examined by bisulfite conversion and pyrosequencing of CGIs close to TSS of *sirt1* (22 CpG sites) and *sirt3* (4 CpG sites). Gene expression patterns of *sirt1-7* in addition to that of *cs*, *cpt1a, pgc1α*, *ucp1,* and *ucp3* were analyzed by a PCR-array designed for the simultaneous gene expression profiling of all genes in the analyzed tissues.

Results

The number and length of exons-introns are highly conserved for each *sirt* variant through the evolution, despite of the variable length of introns across species (humans/sea bream/zebrafish) (Fig. 1A). Clear CGIs were only found at the 5'-flanking region of *sirt1* and *sirt3* (Fig. 1B). The analyzed CpG sites of *sirt3* remained highly hypomethylated regardless of age, season and tissue. By contrast, the methylation level of the 22 analyzed CpG sites of *sirt1* varied with age in skeletal muscle. Thus, in comparison to age +3, young fish (+1) sampled in summer, but not in winter, showed a two-fold increase in the methylation level of CpG sites close to TSS, reaching values close to 4% in the first four CpG sites. This feature was accompanied by a decreased expression of *sirt1,* which reduced the difference of expression between fish of the age class +1 and +3 when comparisons are made in summer. This was especially evident at the position CpG3, as it was evidenced by correlation analysis of *sirt1* gene expression and methylation level (Pearson Correlation Coefficient = -0.7; P = 0.007) (Fig.1D). Intriguingly, skeletal muscle also appeared more responsive than liver to changes in *sirt* gene expression with a statistically significant interaction of age and season for *sirt1*, *4, 5* and *7*, which was extensive to *cs* and *cpt1a*. This was reinforced by discriminant analysis (PLS-DA), which identified *cs, cpt1a* and *ucp1* as the most important variable loads in liver, whereas *sirts* contribute to better discriminate the energy status of skeletal muscle.

Discussion and conclusions

The presence of CGIs close to TSS did not match well with the level of *sirt* gene expression (*sirt5*>*sirt2*>*sirt1*>*sirt 3/4/6/7*). However, the overall hypomethylation of *sirt1* and *sirt3* CGIs would provide a transcriptionally permissive state that ensures their basal expression in relation with their central role in mitochondrial and intermediary metabolism. Moreover, methylation changes at specific CpG positions of *sirt1* promoter were tissue-specific (e.g. skeletal muscle) in response to a particular energy situation (e.g. summer). These results corroborate the high value of *sirts* as energy sensors through life cycle. This is the first study analyzing the DNA methylation at CpG islands located at the promoter region of *sirts* genes in a marine fish, and provide new insights into *sirt* regulation as key markers of energy status.

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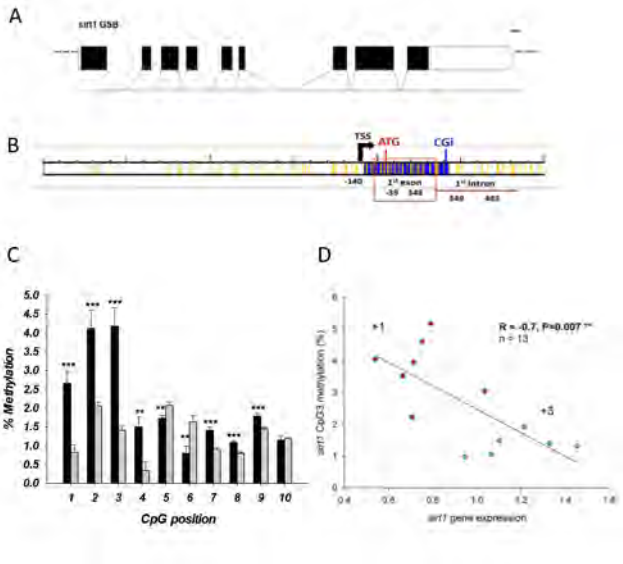

Fig.1. A) Gene structure of GSB *sirt1*. Non-coding exons: white boxes, coding exons: black boxes, introns: lines. B) CGIs of *sirt1* (yellow). Numbers indicate position respect to the ATG. C) Effects of age (+1, black, +3, grey) on DNA-methylation in WSM. D) Correlation of CpG3 methylation and *sirt1* expression in WSM during summer.

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Simó-Mirabet P., Perera E., Calduch-Giner J.A., Afonso J.M., Pérez-Sánchez J. 2018. Co-expression analysis of sirtuins and related metabolic biomarkers in juveniles of gilthead sea bream (*Sparus aurata*) with differences in growth performance. Frontiers in Physiology, 9:608.

The study has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement no 727610 (PerformFISH).

DEVELOPMENT OF A LOW-POWER UNDERWATER RFID-ENABLED DATA ACQUISITION SYSTEM TO CLASSIFY FISH BEHAVIOUR

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Introduction

The need to maintain health and welfare of livestock is necessary to ensure seafood product quality, minimize the impact on the environment and optimize the production system. The most common method of measuring stress in fish – which is a common welfare indicator –is blood sampling (Huntingford et al. 2006). This can itself cause stress, altering the results of the measurements. This problem can potentially be solved by using biotelemetry: the remote measurement of physiological, behavioural and environmental variables (Cooke et al. 2004). This is accomplished by equipping the animal with an electronic tag which contains a sensor and a transmitter that sends the measurements wirelessly to a receiver for analysis.

Objective

This work proposes a system that combines Accelerometry and data analytics to measure and classify fish behaviour to identify stress. The miniaturized tag transmits back the measurements via magnetic induction, and could potentially be used with PIT tag readers to monitor and evaluate fish health continuously and in real time

Background

Behavioural parameters such as detailed activity, movement, locomotion, and body pose are usually measured with an Inertial Measurement Units (IMU), usually comprised of a tri-axial accelerometer, and optionally a magnetometer and a gyroscope (Brown et al. 2013). It can be used to classify animal behaviour and patterns (Shepard et al. 2008), such as fish swimming modes, feeding and escape behaviours, spawning, post-tagging and handling behaviours. It can also provide estimates of swimming speed, tail-beat frequency and size-at-age and energy expenditure (Broell 2016)”genre”:”Thesis” ,”source”:”dalspace.library.dal.ca”,”abstract”:”Recent advancements in tracking technology have increased the ability to unravel key parameters affecting behaviour patterns among marine animals where direct observations are scarce. Within the suite of biologging techniques, tri-axial accelerometers are particularly promising for providing data that can link physiological and ecological processes in the context of movement. The objective of my thesis research was to determine how the analysis of accelerometer data can provide reliable and complex information on fish locomotion and behaviour that are relevant for advancing the informed management of commercially and recreationally valued fish. To reach this objective, a high-frequency accelerometer data logger was developed. Based on a series of controlled-environment and field experiments using this technology, a library of automated signal-processing algorithms was developed that relate acceleration signals to rates of activity, swimming speed, size-at-time and behavioural states in a variety of fish species. The algorithms are efficient in extracting behavioural states (feeding, escape, swimming. The main limitations of an accelerometer electronic tag are: its size and power consumption, as it requires battery for continuous measurement and logging; the method of attachment of the tag to the fish, which can be invasive; and data recovery, as wireless transmissions consume more power and archiving data inside the tag requires it to be retrieved.

As the recapturing event to recover data is a stressor for the fish, the ideal solution would transmit the measurements wirelessly and in real time. Propagating signals in water pose some challenges, especially in shallow water. Acoustic, optical and radio-frequency all have downsides, such as attenuation, fading, multipath propagation, Doppler effects and variable channel characteristics (Gussen et al. 2016)particularly in the sea, has ignited the development of many technological advances in the domains of environmental monitoring, oil and gas exploration, warfare, among others. In all these domains, underwater wireless communications play an important role, where the technologies available rely on radiofrequency, optical, and acoustic transmissions. This paper surveys key features inherent to these communication technologies, putting into perspective their technical aspects, current research challenges, and to-be-explored potential.”,”DOI”:”10.14209/jcis.2016.22”,”ISSN”:”19806604, 19806604”,”note”:”00018”,”journalAbbreviation”:”JCIS”,”language”:”en”,”author”:[{“family”:”Gussen”,”given”:”Camila M. G.”},{“family”:”Diniz”,”given”:”Paulo S. R.”},{“family”:”Campos”,”given”:”Marcello L. R.”},{“family”:”Martins”,”given”:”Wallace A.”},{“family”:”Costa”,”given”:”Felipe M.”},{“family”:”Gois”,”given”:”Jonathan N.”}],”issued”:{“date-parts”:[[“2016”]]}}],”schema”:”https://github.com/citation-style-language/schema/raw/master/csl-citation.json”} . This makes the deployment of these technologies challenging.

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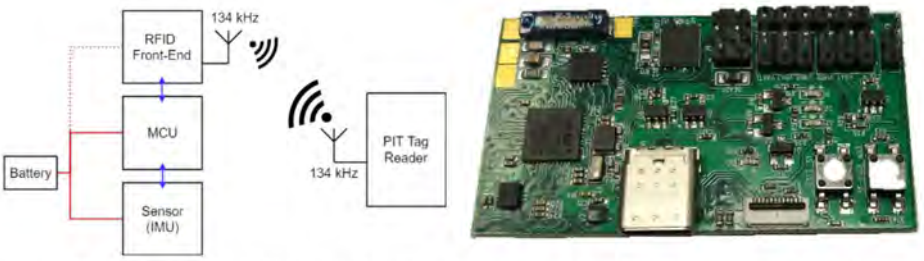

Figure 1: (a) Block diagram of proposed system and (b) photo of evaluation board

System Design

The solution proposed consists of a device with an accelerometer, microprocessor and RFID front-end with coil antenna. To test this, an evaluation board was developed, measuring 3 cm x 5 cm, with additional components for testing. The final solution is planned to be a third of the current size.

Low-frequency RFID was chosen due to its immunity to some adverse effects that other communication methods exhibit underwater, such as multipath propagation. The coil antenna can harvest energy from the field generated by the reader, powering up the tag. An added benefit is the compatibility of the system with existing PIT tag readers already used in aquaculture.

The evaluation board is currently being tested and results will be presented at the conference.

Future Work

The next steps consist of miniaturizing the system and developing the data analytics algorithm. The algorithm will use methods from machine learning to analyse the data gathered from the accelerometer to classify the fish s behaviour and identify states of stress.

Acknowledgments: This work is part of the IMPAQT project (presenting at booth 15). This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 774109.

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EVALUATION OF LACTIC ACID BACTERIA EFECT ON THE PROBIOTIC *Shewanella putrefaciens* PDP11 AND PATHOGENIC STRAINS

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Introduction

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The inhibition test has been made using pathogenic strains (*Shewanella putrefaciens* SH12, SH4, SH6, SH16, SH19), the probiotic strain Pdp11 and probiotics lactic acid strains (*Bifidobacterium lactis* Bb12, *Enterococcus faecium* Tehobak, *Lactobacillus johnsonii* La1, *Lactobacillus casei* Shirota).

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Enterococcus faecium Tehobak	++++	+	++++	+++	+	+
Lactobacillus johnsonii La1	++++	++	++	++	+	++
Lactobacillus casei Shirota	++++	++	+++	+	+	++

(Continued on next page)

Results

Table 1. Inhibition halos produced by the lactic acid strains tested on the pathogenic strains SH12, SH4, SH6, SH16, SH19 and the probiotic strain Pdp11 in the double-layer agar technique. The radius of the halo corresponds to: ≤1 mm (+); 1 mm <1.5 mm (++); 1.5mm <2 mm (+++), ≥2 mm(++++).

Discussion and conclusion

The lactic acid present in extracellular products are important in the inhibition assay, and to demonstrated this composition the ECPs were neutralized and used in the inhibition of the SH strains and it was evidenced that the inhibition was lower. Previous studies with lactic acid strains show that different concentrations of lactic acid produced by probiotics such as *Pediococcus acidilactici* induced the production of quorum quenching by the inhibition of homoserine lactone (Kiymaci *et al.*, 2018). This would indicate that the production of acids is an important element in the inhibition of pathogenic strains.

In base on the results about the higher susceptibility howed by Pdp11, other analysis were carried to check molecular difference between probiotics and pathogenic strains. In previous studies, 21 genes have been identified in the SH pathogenic strains absent in the Pdp11 probiotic. One gene encoding for two-component regulatory system (SO_4623) and others two genes (Sbal195_4327, Sput200_4303) codify for histidine kinases, which are intermediates in phosphotransfer pathways. In *Shewanella oneidensis* those genes functions are essential in responses to changes in acidic environments (Leaphart *et al.*, 2006).

Although the production of lactic acid is a relevant factor in the inhibition of pathogenic strains, it is important not to forget the influence of other elements that are part of the ECPs as the bacteriocins

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SUITABILITY OF FOXL2 GENE AS SEXUAL MARKER IN WRECKFISH (*Polyprion americanus*) BROODSTOCKS

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Introduction

The wreckfish *Polyprion americanus* is a promising aquaculture species with late sexual maturation, around the ten years of life (Wakefield et al. 2013), and no sexual dimorphism, involving the use of techniques such as cannulation or ultrasound imagery in the management of the captive broodstocks. However these techniques requires specialized training of the technical staff, and requires an advanced gonadal development of the animal to acquire clear results. The expression levels of Foxl2 gene during gonadal development has been studied extensively in vertebrates as sexual marker due to its female-specific expression profile (Bertho et al., 2016). Foxl2 is one of the earliest sexually dimorphic genes during ovarian development, regulating the activity of Cyp19 aromatase, which converts androgens into estrogens (Guiguen et al. 2010). Foxl2 expression is no restricted to the gonads, being also detected in other tissues like pituitary, brain, spleen, liver, eyes and gills (Wang et al. 2012). In the present study we have identified the Foxl2 sequences in wreckfish and its expression levels has been measured in branchial and gonadal tissues from wild and cultured wreckfish in order to evaluate its suitability as sexual marker in this species.

Material and methods

Thirty five wreckfish caught by commercial vessels and twenty four captive fishes from two stocks located in Galicia(NW Spain) were used in the present study. In all cases, branchial and gonadal samples were obtained (by cannulation in the live animals from the broodstocks) and preserved on ethanol and RNAlater at 4ºC for 24 hours and then transferred to -80ºC storage. Total genomic DNA was extracted from ethanol-preserved tissues using the FENOSALT method (Pérez and Presa, 2011). A 655 bp sequence was amplified using the primer pair f*oxl2F/R* designed by Alam *et al* (2008) to amplify *Epinephelus merra*forkhead transcription factor L2 (Foxl2).The Foxl2 amplicons were cloned using the pGEM-T Easy Vector System II (Promega). The cloned sequences were checked with Chromas Lite software (Technelsyum) and aligned using BioEdit Sequence Alignment Editor Software.Their homology with other teleost species obtained from GenBank, was compared using the BLAST software. The RNA from 30 gonadal and 27 branchial genetic samples were extracted individually with Trizol, and 1↑ g of total RNA from each sample was reverse transcribed to cDNA using a NZY First-Strand cDNA Synthesis Kit (NZYTech)PamFox148F and PamFox68R primers were used for perform the qPCR reactions. Differences in gene expressions between both sexes and both tissues analyzed were determined with one-way analysis of variance (ANOVA) following by Tukey´s HSD test

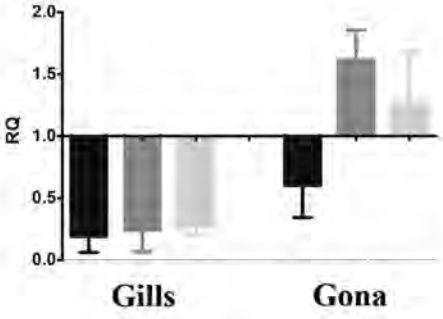

Fig. 1 Relative expression levels of Foxl2 for males (black bars), females (grey) and undetermined (light grey)

(Continued on next page)

Results

The application of these primers for the qPCR experiments was successful for both tissues (Fig.1). The relative expression of Foxl*2* were significantly higher in gonads than in gills ($p<0.001$), and differs between sexes ($p=0.042$) showing their maximum values on females followed by undetermined individuals (those which a sex cannot be assigned by macroscopic examination), while it is under expressed in males (fig.3). For the branchial tissue, all groups shows under expression comparing with the reference sample with no difference between sexes ($p=0.780$)

Discussion and conclusion

The amplificati n of Foxl2 was achieved successfully on both tissues analyzed. Our data in gonadal samples shows significant difference between sexes for the presence of Foxl2, detecting the highest levels on gonadal samples from females of the live broodstocks, over the 90 cm. of total length. The studies on wild populations shows that this size correlates with the age of first maturation, at the 10 years old in the southwestern Atlantic groups (Peres et al. 2003) and at 7 years in the Ionian Sea (Carbonara et al. 2003).The males showed a low expression profile regardless of its maturation status, as it has been recorded in most of vertebrates (Bertho et al. 2016). Five of the wild wreckfish sampled could not be sexed macroscopically due to its low gonadal development, however, their levels Foxl2 leads us to classify two of them as potential females, and as males the remaining three animals. On the branchial tissue, all samples shows a low level of expression with no differences between males, females nor undetermined individuals. Our conclusion is that Foxl2 is a suitable marker for determining the sex of the wreckfish starting from gonadal tissues samples, avoiding the traditionally used histological protocols. However, in other more accessible tissues such as the gills, the search for an appropriate marker is still required.

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EVALUATION OF LACTIC ACID BACTERIA EFECT ON THE PROBIOTIC *Shewanella putrefaciens* PDP11 AND PATHOGENIC STRAINS

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THE PHYSIOLOGICAL BASIS FOR INTER-FAMILY AND INTER-INDIVIDUAL GROWTH VARIABILITY IN THE SPAT OF CLAMS *(Ruditapes decussatus)*

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Introduction

In bivalve populations reared either under controlled laboratory conditions or under environmental conditions, inter-individual growth rates attain an outstandingly high variability (Gaffney and Scott, 1984; Brown, 1988, Tamayo et al., 2011, 2014). This variation indicates the existence of endogenously determined differences in the physiological capacity of individuals to acquire and metabolize food energy. Sibling specimens (families) constitute an excellent biological material to test the genetic basis of such dissimilarities. The present study was designed to identify the physiological parameters responsible for a) intra-familiar and b) inter-familiar differences in the growth potential of the clam *Ruditapes decussatus*.

Materials and methods

Four different families (F1, F2, F3 and F4) from the same cohort of the clam *Ruditapes decussatus* were produced simultaneously. The larvae resulting from each crossing were reared in separate water tanks, maintained under identical temperature and feeding conditions. After 4 months, individuals from the four families were divided into four different categories representing differences in growth capacity: larger than 8 mm (L), between 5 and 8 mm (M), between 2 and 5 mm (S), between 1 and 2 mm (SS), and a group under 1 mm in size. Samples of different size-categories from each family were selected to determine the main parameters underlying the energy balance of individuals: i) routine oxygen consumption (VO_2:$mlO_2 \cdot h^{-1}$) and ii) clearance rate (CR: $L \cdot h^{-1}$), which represent the main parameters of energy loss and gain, respectively. Experimental values of physiological parameters (Y: VO_2or CR) obtained in individuals from different size-categories in each family were used to calculate intra-familiar allometric relationship between physiological parameters and body weight (W) expressed as $Y = a\ W^b$.

Intra-family differences. Recorded mass-exponents in the intra-family allometric

Table I: Allometric relationship obtained for the individual weight, following the expression $Y = a \cdot LW^b$. A: oxygen consumption; B: clearance rate.

A	Family	n	a (±SD)	b (±SD)	R^2	p	F
	1	16	-4.741 (±0.125)	1.033 (±0.065)	0.948	< 0.0001	253.766
	2	17	-5.130 (±0.179)	1.201 (±0.092)	0.919	< 0.0001	170.380
	3	18	-4.837 (±0.097)	1.083 (±0.048)	0.969	< 0.0001	503.931
	4	16	-4.880 (±0.087)	1.184 (±0.048)	0.978	< 0.0001	613.575
B	Family	n	a (±SD)	b (±SD)	R^2	p	F
	1	16	-3.729 (±0.106)	1.033 (±0.054)	0.967	< 0.0001	414.245
	2	17	-3.602 (±0.176)	1.201 (±0.091)	0.900	< 0.0001	134.474
	3	20	-3.748 (±0.106)	1.083 (±0.050)	0.958	< 0.0001	413.726
	4	16	-3.086 (±0.106)	1.184 (±0.057)	0.926	< 0.0001	175.089

relationships for VO_2 and CR were close to 1 (with the exception of 0.753 obtained for

(Continued on next page)

Results

All the intra-family allometric relationships of VO_2 and CR showed high statistical significance (see Table I). Regarding the oxygen consumption, the analysis of covariance (ANCOVA) disclosed no significant differences ($F=1.5096$; $df=3, 59$; $p>0.05$) amongst the mass exponents (slopes, b) of the four families. Thus, a common slope ($b_c=1.124\pm0.034$) was calculated. Concerning the elevations (a), significative differences were observed between the second and the forth families (F2vs. F4: $q=4.3689$; $df=3, 35$; $p<0.05$). As regards the clearance rate, statistically significant differences were obtained for the comparison amongst slopes ($F=6.3891$; $df=3,61$; $p<0.05$) and the Tuckey test revealed that the F4 family had a significantly lower mass exponent for CR than the other families (F1 vs. F4:$q=5.5127$; $df=3, 61$; $p<0.05$; F2 vs. F4: $q=4.5706$; $df=3, 61$; $p<0.05$; F3 vs. F4: $q= 4.5268$;$df=3, 61$; $p<0.05$).

Discussion and conclusion

Intra-family differences. Recorded mass-exponents in the intra-family allometric relationships for VO_2 and CR were close to 1 (with the exception of 0.753 obtained for

the CR in F4). This indicates that both measurements follow an isometric relationship with body size. Besides, intra-family mass exponents for VO_2 and CR were not significantly different between them. This result contrasts with the literature that systematically shows lower mass-exponents for CR than for metabolic rate (e.g., Ibarrola *et al.*, 2012).The equivalency of scaling-exponents indicates that the capability to acquire energy (CR) is not comparatively diminished more than the metabolic expenditure (VO_2) with rising body size; thus, in mass-specific terms, the energy balance is virtually independent of body-size. Therefore, individual siblings with faster growth-rates are those in which clearance rate appears to be less constrained by rising body-size.

Inter-family differences. Recorded significant differences in the elevation of intra-family allometries of VO_2 with body size between F2 and F4 families indicate the existence of significant inter-family differences in the metabolic efficiency of individuals: the mass-specific oxygen consumptions of the individuals belonging to the F2 family are lower than those from F4. In addition to metabolic differences, the results of the F4 family show a significantly lower mass-exponent for CR ($b=0.753\pm0.057$) than for metabolic rate ($b=1.184\pm0.048$), denoting the existence of inter-family differences in the constraining effect that increasing body-size imposes to the filtering activit .

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INDUCTION OF METAMORPHOSIS IN THE LARVAE OF THE JAPANESE CARPET SHELL CLAM, *Ruditapes philippinarum* (ADAMS AND REEVE, 1850)

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Introduction

Producers and researchers have been trying to improve the production of bivalve mollusc spat for many years. Difficulties in the culture of bivalve molluscs are mainly associated with the settlement and metamorphosis processes. Bivalve larvae must develop competence and recognize appropriate exogenous morphogenetic cues to settle and metamorphose (Degnan and Morse, 1995).

The molecular mechanisms underlying the metamorphosis of larvae are not well understood although several inducers have been identified (Boettcher and Targett 1998; Pires et al. 2000). Competent molluscan larvae from different species can be induced to settle and metamorphose using analogues of natural inducers such as γ-aminobutyric acid (GABA) (Bryan and Qian 1998; Rahmani and Ueharai 2001; García-Lavandeira et al. 2005; Alfaro et al. 2011; Mesías-Gansbiller et al. 2008, 2013) and the catecholamine L-DOPA (Coon et al. 1985; Estupinan and Waite 1988; Pires et al. 2000; Dobretsov and Qian, 2003). In this study we examined the effect of several inducers on the larval metamorphosis of the clam *Ruditapes philippinarum* (Adams & Reeve 1850).

Materials and methods

Two experiments of induction of the metamorphosis at laboratory scale have been developed following the protocols previously described in García-Lavandeira et al. (2005) and Mesías-Gansbiller et al. (2013). Competent larvae of *R. phillipinarum*, supplied by the CIMA of Ribadeo, were treated with several neuroactive compounds: GABA, the catecholamines epinephrine and norepinephrine, L-DOPA, IBMX, serotonin and acetylcholine. To determine the optimum concentration of the possible inductor, three different concentrations were used: 10^{-4}M, 10^{-5}M and 10^{-6}M and the exposure time to the inducers was 72h. Experiments were performed in triplicate in Petri dishes of 90mm, for each one of the concentrations mentioned above at a density of 4 larvae/ml. Each experiment included a control with FSW with no potential inductor. Metamorphosis has been monitored with a Nikon SMZ-2T microscope. The percentage metamorphosis was calculated as 100 (total number of larvae metamorphosed/total number larvae). A larva was considered to have undergone metamorphosis when it lost its velum and was using its foot to crawl. Results were analysed by means of SPSS 20.0. Percentages of both metamorphosis and mortality were analysed by ANOVA. The results were considered to be significantly di ferent when $p<0.05$.

Results and discussion

After 72h, maximum percentages of settlement were induced by 10^{-4}M acetylcholine and serotonin. Exposure to 10^{-5}M and 10^{-6}M acetylcholine and serotonin also induced significant levels of metamorphosis in *R. philippinarum.* In contrast, GABA, epinephrine, norepinephrine, L-DOPA and IBMX failed to increase the metamorphosis rates comparing to the control larvae. In both experiments, the proportion of larvae that successfully exceeded metamorphosis in control, in the absence of inducers, was also very high, around 80%. Even in these conditions it could be observed that acetylcholine and serotonin in the three concentrations tested were effective inducers of the metamorphosis, increasing the percentage of success significantly to values close to 100%. Urrutia et al. (2004) previously reported that acetylcholine and serotonin induced larval metamorphosis of *R. philippinarum* larvae. Furthermore, García-Lavandeira et al. (2005) identified GABA and epinephrine as active inducers of metamorphosis of in two clam species *V. pullastra* and *R. philippinarum*. However, the percentages of metamorphosis were very low compared with those obtained in the present study. Differences in the efficacy of various chemicals in inducing metamorphosis may be due to several factors, such as species differences, larval age and qualitative differences among batches of cultures of the same species, resulting in a different degree of larval competence and responsiveness to chemical inducers.

Mortality of *R. philippinarum* larvae was not affected by acetylcholine, serotonin and other inducers except by the highest concentrations of L-DOPA which increased the percentage of mortalities.

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AEFishBIT: SMART DEVICE FOR TRACKING FISH BEHAVIOUR AND ACTIVITY

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Introduction

The use of new technologies for individual and non-invasive monitoring of farmed fish is becoming an urgent demand of the aquaculture industry (Føre et al., 2018). This offers the possibility of the measurement of a range of variables that are directly or indirectly related with metabolic condition, health and welfare status (Metcalfe et al., 2016; Neethirajan et al., 2017). The solution proposed within the AQUAEXCEL^{2020} EU project is a smart device named AEFishBIT, developed and validated to provide reliable and simultaneous measurements of physical activity and respiratory frequency. The device is attached in the operculum using a fairly invasive procedure, and initial testing has been conducted in gilthead sea bream and European sea bass, the two most important farmed fish of Mediterranean aquaculture

Materials and methods

The AEFishBIT is a tri-axial accelerometer for recording and processing acceleration data from x-, y- and z-axes. Records of operculum breathing (z-axis acceleration) served as a direct measurement of respiratory frequency, whereas estimation of fish activity was derived from the x- and y-axis signals. The device also includes a passive RFID tagging device for rapid identification. The final weight of the full packaged device is less than 1 g in air. The autonomy of the system in stand-alone mode is 6 h of continuous data recording with different programmable time schedules.

Algorithms to convert accelerometer measurements in physical activity and respiratory frequency (breaths/s) have been validated with exercised juveniles in a 10-L swim tunnel respirometer (Loligo® Systems). Fish were submitted to controlled speeds from 1 body-length per second (BL/s) until exhaustion by 0.5 BL/s increase steps, with measurements of O_2 consumption rates (MO_2). AEFishBIT was programmed for 2-min time window at different intervals. Examples of use: 2 min each 15 min for 2 days; 2 min each 60 min for 8 days; 2 min each 180 min for 24 days.

Measurements in free-swimming fish have been conducted in juveniles and adult fish for tracking the effects of a number of biotic and abiotic factors (age, season, stocking density, oxygen concentration, disease progression, etc).

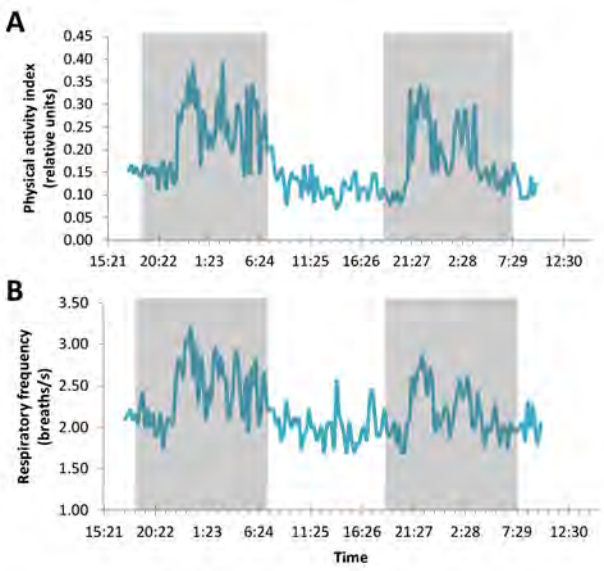

Fig.1. Activity pattern of a "nocturnal" sea bream measured with AEFishBIT, with different behaviour in the light (7:00-19:00 h, white area) and dark (grey area) phases. **(A)** On-board measurements of physical activity index. **(B)** On-board measures of respiratory frequency.

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Results

The close parallelism between the inferred respiratory frequency and MO_2 highly validates the use of the proposed algorithm to infer respiratory frequency in exercised sea bream and sea bass (1-4 BL/s speed range). Likewise, the physical activity index increased exponentially rather than linearly with the increase of swimming speed, as the total jerk magnitude only reflects fish-related accelerations. Regarding fish species differences, it is important to note that, at the same swimming speed, MO_2 and the calculated respiratory frequency were consistently higher in sea bream than in sea bass.

AEFishBIT measurements in free-swimming fish evidenced different chronotypes for a given fish population, termed as "diurnal", "nocturnal" or "arrhythmic" on the basis of the moment of the day when the maximum activity pattern (light phase, dark phase or undifferentiated, respectively) is achieved (Fig.1). AEFishBIT measurements also offer the possibility to discriminate different personality traits when fish are challenged with aquaculture stressors (e.g. hypoxia, stocking density).

Discussion and conclusions

The present work represents the proof of concept of a miniaturized device for individual fish phenotyping of metabolic condition and welfare. The different behavior patterns of free-swimming fish can be associated with a better performance or differences in stress and disease resilience. These monitored features also evoke changes in season, age, development or nutritional condition, remaining to establish the involvement of genetic and epigenetic factors on the measured parameters. This opens new research opportunities for the individual fish phenotyping of productive traits through the production cycle, but also for selective breeding in combination with other now promising *omics* approaches (e.g transcriptomics, methylomics, metabolomics, microbiomics).

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THE EFFECT OF ALTERNATIVE FEED INGREDIENTS ON GROWTH AND STRESS RESPONSE IN JUVENILE TURBOT (*Scopthalmus maximus*) KEPT IN RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

Aquaculture is the fastest growing food production sector in the world and plays a key role in human nutrition. In framework of this increasing intensification in aquaculture and expanding world population, which is expected to rise to 9.8 billion by 2050, rendering aquaculture practices more sustainable is a huge challenge. This implies that the global food production needs to increase by 25-70 % by 2050, whereby the global aquaculture production needs to increase to 109 million tons in 2030 (Ahmed et al., 2018; FAO, 2018).

Aquaculture production is depending on a variety of environmental as well as managementbased factors such as climate, water availability, space, etc. Hereby, feed, in particular fishmeal, represents a high cost for the farmer and is not sustainable as wild fish stocks are under threat and long distance transport to the aquaculture farm is in most cases needed. Subsequently, aquaculture feed producing companies as well as research institutes are urged to find alternative ingredients for fishmeal (Kobayashi et al., 2015). Most of these alternatives consist of plant-based ingredients, whereby these alternative protein sources often lead to a decreased digestion rate, health and welfare of the fish across species (Booman et al., 2018; Pelletier et al., 2018). Studies have demonstrated a reduced digestibility for turbot (*Scophthalmus maximus)*, when fishmeal protein was replaced with plant and insect protein (Burel et al., 2000; Fournier et al., 2004; Kroeckel et al., 2012). In our study, we investigated the effect of alternative feed formulation on growth characteristics, feed utilization and stress response in juvenile turbot.

Material and Methods

Experiments were conducted with juvenile turbot in a recirculating aquaculture system (RAS) at the Alfred Wegener Institute (Bremerhaven, Germany) between April and August 2019. Fish were kept at standardized conditions (16 °C, 90-100 % oxygen saturation, salinity of 32 ppt, pH of 7.7) in 700 L tanks (1 m² bottom area). Hereby, a total of 50 fish (initial weight of 20.0 ± 0.6 g and standard length of 8.0 ± 0.1 cm were housed per tank.

Four experimental diets (Sparos Ltd, Olhão, Portugal) were tested with five replicate tanks: (i) control [CTRL] consisting of > 50 % fish meal; (ii) no processed animal protein (NOPAP) consisting of approx. 35 % fish meal and 5 % insects and algae; (iii) processed animal protein (PAP) consisting of 35 % fish meal, no algae and some animal proteins (poultry meal); and (iv) MIX consisting of 25 % fish meal and a mix from plant, algae, animal and insect ingredients. In addition, an identical number of treatments was included, whereby each of the above mentioned diets were spiked with cortisol (CORT), as a positive control for stress.

Fish were fed twice a day *ad libitum* with extruded pellets for 6 days a week. Every four weeks, six fish per tank were weighted, measured, and sampled for blood, soft fin ra , and otoliths.

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Preliminary Results and Discussion

Preliminary data indicate that fish provided with cortisol-spiked feed eat less than non-stressed fish and have a slower growth curve, indicative for the negative effect of long-term elevated cortisol levels on fish performance. It was further observed that the intake of MIX is less compared to other diets, while control fish showed no difference in feed intake between the more plant-based diets.

No significant differences at the end of the trail between control diet and plant-based diet could result in a reduction of costs for the farmer. The stressed induced fish (with cortisol-spiked food) show that stressed fish feeds less and therefore shows a depressed length increament compared to the non-stressed food. This could be due to the fact that indicators for stress in farms need to be identified in more detail to increase growth.

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MITIGATING EFFECTS OF EUTROPHICATION USING MUSSEL FARMING: STATUS ON CURRENT RESEARCH AND FUTURE CHALLENGES

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Introduction

Ecosystem services provided by marine bivalves in relation to nutrient extraction from the coastal environment have gained increased attention provided the adverse effects of excess nutrient loading from human activities, such as agriculture, finfis aquaculture and sewage discharge. In combination with the increasing expenses of further reducing nutrient input from land or the difficulti s with nutrient emission from off-coast or off-shore finfis aquaculture mitigation in the receiving water body has become an attractive alternative to ordinary abatement measures. Nutrient extraction using shellfish farming is further attractive as it can boost blue economy in countries with little activity within the area.

Results and discussion

A number of national, e.g. the Danish MuMiPro project (http://www.mumipro.dk), and EU funded projects, like EU BONUS OPTIMUS (http://www.bonus-optimus.eu) and EU Interreg Baltic Blue Growth are currently developing the scientific background for implementing mussel farming as a tool for mitigating effects of eutrophication. The projects encompass various topics like development of culture practice in regions with adverse conditions for mussel farming, e.g. low salinity, environmental impact of mussel farming, mussel farming and coastal zone management and social acceptance. All topics of relevance for implementing mussel farming as a mitigation tool.

Here we report on recent achievements across disciplines within optimization of mussel production in terms of biomass at lowest possible costs, effects of mussel farming in terms of nutrient regeneration and particle depletion, processing and use of mussels for other purposes than human consumption. The results are put in a context of ecosystem services provided by mussel farming and how these can be rewarded by nutrient emitters or government agencies.

WASHINGTON STATE'S POLLUTION IDENTIFICATION AND CORRECTION PROGRAM'S WORK TO PROTECT AND RESTORE SHELLFISH BEDS AND PUBLIC HEALTH

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Introduction

Puget Sound, located on the Pacific Northwest of the continental United States (specifically Washington state), has 1,332 miles of shoreline. The Puget Sound watershed is home to twenty federally recognized treaty tribes, 12 counties, over four million people, a mixture of rural and urban areas (including Seattle and Tacoma), agricultural and dairy farms, and shellfish. Subsistence, recreational, and commercial harvesters take advantage of the region's vast availability of wild and farmed shellfish. Washington's commercial shellfish industry generates over $150 million every year from the harvest of clams, geoduck, mussels, and oysters.

To protect this resource, state agencies, the Environmental Protection Agency (EPA), tribes, county governments, academics, industry leaders, and citizens work together. Due to Washington's rainy climate, resource practitioners consider the whole watershed for management and planning decisions as the rain transports any fecal waste into streams that flow into to shellfish growing areas. Specificall , the Washington State Department of Health and county governments test freshwater and marine water for concentrations of fecal coliform to protect the public from waterborne illnesses. Counties use a methodology called Pollution Identification and Correction (PIC) to find and fix pollution hot spots. To correct and prevent high concentrations of fecal coliform, state agencies and local governments work with farmers, wastewater treatment plants, and pet and onsite septic system owners. Farms are a source of fecal waste from cows, chickens, pigs, and other livestock and from the land application of manure as a fertilizer. Onsite septic systems can contribute fecal waste if they are not routinely inspected and maintained or properly designed.

Materials and Methods

My study design aimed to understand challenges and barriers that PIC programs face and how Washington State agencies can improve oversight and build capacity for these programs. To complete this, I collected and reviewed previous, current, and planned work related to PIC programs around the Puget Sound. Additionally, I interviewed the practitioners from the twelve Puget Sound counties over a two month period. We discussed water quality sampling, education and outreach programs, enforcement protocols, inter-agency coordination, sustainable funding, and more. After the interviews, I transcribed the interview to analyze the responses.

Results

Results highlighted areas for future work to improve the stability and certainty of consistent funding sources, improving citizen trust and understanding of the importance to restore and protect marine water quality, and foster communication and knowledge sharing between programs. The programs highlighted their need for Washington State to improve access to laboratory services, define regulatory backstopping responsibilities and expectations, and provide continual political and moral support to programs for continued success.

Discussion and Conclusion

It is clear the PIC approach to improving water quality is widely effective, but there are structural differences between programs that lead to varied outcomes and impacts that improve marine water quality. Counties with effective program are recognized and trusted by citizens, utilize and communicate with partners, adapt their messaging to meet their audiences' values, and more. Effective programs dedicate resources to build working relationships with farmers, to educate home owners about maintenance of their septic system, and to establish cross county and international working groups. Over the last decade, PIC programs have improved water quality around Puget Sound leading to an increase in approximately 6000 acres of shellfish bed viable for a mix of commercial, recreational, and subsistence harvest. As a result of this work, state and county agencies will continue coordination to find sources of poor water quality and find ways to fix them. This work is done to protect public health, preserve property values, and improve water quality.

A SURVEY TO IDENTIFY OPERATIONAL WELFARE INDICATORS (OWIs) IN FARMED EUROPEAN SEA BASS *Dicentrarchus labrax* AND GILTHEAD SEA BREAM *Sparus aurata*

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Introduction

Welfare of farmed fish is a key aspect of sustainable and ethical aquaculture. At European level, the EU Commission has recently published studies on welfare of farmed fish and has reported on fish welfare during transport and at slaughter to the EU Council and Parliament (SANTE, 2017; SCAR FISH, 2018; COM 87 final (2018). The European Aquaculture Advisory Council has adopted in 2017 the position paper 'Farmed Fish Welfare During Slaughter in the European Union' and in June 2019 has provided a new recommendation for optimising fish welfare at slaughter (AAC, 2019). Producers' Associations, certification bodies and NGOs are searching for suitable operational welfare indicators (OWIs) for measuring the welfare status of farmed fish and improving standard and welfare friendly practices.

The PerformFISH consortium (http://performfish.eu/) is working on welfare of European sea bass and gilthead sea bream and is validating a set of OWIs and a welfare scoring system specifically designed for sea bass and sea bream, in collaboration with fish Producers' Associations and Mediterranean aquaculture companies. This paper presents the results of the PerformFISH *Fish Welfare Survey* for the selection of most suitable OWIs for sea bass and sea bream aquaculture.

Materials and Methods

The PerformFISH consortium has adopted in 2017 a first set of Welfare key performance indicators actually in use by 20 farm companies participating in the benchmarking of performances of sea bass and sea bream across the Mediterranean area. The welfare indicators are: i) total fish mortalities; ii-iii) fish mortalities after 3 and 10 days after transport and stocking; iv) vaccinated fish by disease; v-vi) number of antiparasitic and antibiotic treatments; vii) fish feed intake; viii) fish stocking density; ix) number of oxygen depletion persistence days

The *Fish Welfare Survey* was designed to identify additional OWIs for sea bass and sea bream to be used during grow-out farming in sea cages and land-based tanks, transport and at slaughter. The set of OWIs was chosen following an analysis and systematization of knowledge on welfare of sea bass and sea bream available in literature. About four hundred scientific publications were consulted, together with OIE and EU recommendations, EFSA opinions, Technical guidelines and Codes of conduct (FAO, FEAP), Benchmarking systems (GAPI, GSSI), Certification schemes (RSPCA, ASC, Global GAP, BAP GAA, FOS, Organic), Welfare Handbook for Atlantic salmon (Noble et al., 2018) and expert judgments. The approaches to assess animal welfare in terrestrial livestock production (e.g. Welfare Quality; AWIN) have been further considered.

The PerformFISH consortium (Scientific partners, Producers' Associations, Industry) participating in the *Fish Welfare Survey* is requested to score the suitability of OWIs on the basis of the following indicator's attributes: informative, reliable, cost and labour effective, repeatable, comparable and easy to apply.

Results

The 48 OWIs included in the survey cover 9 different areas of fish welfare: health, growth, behavior, housing, transport, harvest, stunning/slaughter, staff training and compliance with recommendations (OIE and EU). The overall set of OWIs includes a 46% of animal-based indicators and a 54% of indirect indicators related to environment and husbandry management and practices. The 47% of OWIs are quantitative indicators providing numerical values for welfare scoring and 53% are qualitative indicators reporting on the use of best management practices during farming, transport and slaughter. Although most OWIs proposed in PerformFISH survey are included in aquaculture certification schemes, only few quantitative indicators are available for farmed sea bass and sea bream.

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Discussion and conclusion

Although the general aspects of health and the welfare of farmed fish are well known by farm managers, a set of suitable OWIs for analyze welfare status of sea bass and sea bream is still missing and reference values for most of quantitative indicators are completely lacking. A Welfare Scoring System based on OWIs can thus improve the measurement and certification of fish welfare status. The set of OWIs will be a simple and friendly tool for farmers and health managers to measure fish welfare, helping them to find room for improvement in husbandry practices. Welfare indicators will be further used to inform retailers, consumers, NGOs and policy makers on welfare-friendly farming practices and standards in use in marine fish farming secto .

Acknowledgments

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TOWARDS SUSTAINABLE AWARENESS: IMPLEMENTATION OF AN AQUAPONIC CODE OF PRACTICE

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Introduction

Aquaponics (AP) is considered an innovative and sustainable source of food due to the high production and low environmental impact. However, these features are hardly known by the consumers which consider low environmental impact in food production a principal factor of choice. The main objective of the project is to develop a system able: to define and ensure the quality of AP products; to provide to the final consumer a comprehensive, clear and instant explanation of the various aspects and parameters that make an AP product a product of quality. This type of solution has never been made in Europe so far.

Development of the project

Adopting AP certification, allow the registration at Network of European Commercial Aquaponic Producers (NECAP).

Step 1 - put in writing CP. Every producer interested in being part of NECAP and commercializing, beyond their logo and label's product, with our logo (described in Step 3) must respect our values summarised in the following guideline.

Sustainability indicators

- **More than 50% of energy must to come from renewable sources.**
- **Less than 40% of water can be replace during whole cycle**. Modern RAS alone have a high degree of water reuse, i.e. 95–99%, Dalsgaard et al. (2013).
- **At least 75% of total Nitrogen and 70% of total Phosphorous retains by fishes and plants**.
- **Floating systems (RAFT) should be prefer to Media-Filled Beds Systems (MFBS)** which is more impacting due to the large use of inert material to sustain vegetables, Forchino et al. (2017).
- **Product Carbon-footprint and water footprint must be calculated.** Our producers must calculate them with software that they can freely choose.
- In order to **circular economy**, our project encourage the use of recycle materials and the recycle of any waste for the realizations of the system (ex: the reuse of abandoned greenhouses or manufactured) or use biodegradable materials.
- **Waste.** Use **solid waste** to obtain compost which can be used to fertilize plants instead of chemical fertilizers; cleanse **water waste** with a natural technique named phytoremediation.
- **To prefer local fish or seedling.**

Fish welfare indicators

- **Oxygen dissolved (DO) at least 8mg L^{-1}.** Although in literature is common to found suggested values ranging between 4-6mg L^{-1}, Yildiz et al. (2017).
- **Temperature (T) 18-30°C.**
- **pH at last 6 and not higher than 7.5.**
- **Ammonia (NH_3) at least 1mg L^{-1}.**
- **Nitrite (NO_2^-) at most 1mg L^{-1}.** High levels of Nitrite leads to the oxidation of Fe^{2+} in haemoglobin with a reduction in the transport of oxygen by blood cells, Yildiz et al. (2017).
- **Nitrate (NO_3^-) 5-150mg L^{-1}.** Concentrations higher than 300mg L^{-1} cause toxic lesions for fish, Yildiz et al. (2017).

Moreover, producers must give guarantees of taking under control the traceability and safety of food products. With respect to **traceability guarantees** we consider necessary maintaining an upgraded supplier list and monitoring data production and store (described in Step 2). The adoption of disinfection plants such as UV light (Elumai et al., 2017) or other sterilization tools (such as ozone) and biosecurity measures (like GMP) will be considered **guarantees** of **food safety**.

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Step 2- Creation of "Network of European Commercial Aquaponics Producers" (NECAP), a web platform in which producers upload production data. Data from NECAP are also used as link for smart labelling. Before yearly audit control body could be require data from NECAP in order to verify the accuracy of the information. NECAP could be integrated with digital tools, such as Ponics® app (https://ponics.it/) to upload data real time or could be uploaded manually.

Step 3- Creation of app for consumers, which is available for free download and allow to consumer to know products and provides a direct contact with producers. Eco-labelling has become increasingly popular a means of encouraging more sustainable consumer choices, Madin et al. (2015). In our project too, sustainability will be communicated through smart-label with QR code containing all information about product. In addition to the legal information which accompany food products, in certified product label will be a logo with the slogan "SUSTAINABILITY ON HAND", associated to a QR code. The QR code can be scan by consumer's devices, giving back a lot of additional information about the products.

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GUIDELINES ON DEVELOPING CLIMATE ADAPTATION PLAN FOR FISHERIES AND AQUACULTURE SECTORS: CO-CREATION APPROACH

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Introduction

The effects of climate change are already being widely observed in fisheries and aquaculture in Europe and will continue to have major implications on these sectors. The impacts of climate change on the seafood production sector will vary significantl , from negative to positive effects and both in its form and severity. In order to maintain responsible, sustainable and profitable seafood production under future climate change, development of adaptation plans or strategies is becoming increasingly important (FAO, 2015). The adaptation to climate change aiming at making Europe more climate-resilient was included in the EU strategy in 2013. The goal of sustainable fishery and aquaculture is also mentioned under the new Common Fisheries Policy (European Commission, 2016). This ambition is challenged by a range of climate-induced uncertainties.

National Action Plans (NAP) are currently under development in most European countries. To date, 23 European countries have adopted national adaptation strategies; whereas eight are reported to be in the process of development. However, these NAPs are mainly focused on transport, cities, and agriculture. Fisheries and/or aquaculture are not included in these plans.

This paper aims to develop a guideline on making Climate Adaptation Plan (CAP) within the fishing and aquaculture industry. To our knowledge, there are no such guidelines available today. The paper therefore is exploratory. We illustrate the guideline with an application to the two case studies built in the EU H2020 ClimeFish project.

Literature review on climate adaptation plans for fisheries and aquaculture sector

The analytical framework of CAP guideline is based on the three following documents: (1) The most comprehensive assessment of climate change undertaken by the IPCC, particularly the synthetic report of Climate Change (2014): Impacts, Adaptation, and Vulnerability (IPCC, 2014); (2) The work done by FAO on assessing climate change vulnerability in fisheries and aquaculture. Available methodologies and their relevance for the sector (Brugère, C., & De Young, C. 2015); (3) Sustainability and sustainable development framework introduced by Circular Ecology.

A vulnerability assessment is considered an important step in adaptation planning for climate change. Existing frameworks for conducting vulnerability assessments have been provided by authors such as FAO (2015), Johnson et al. (2016) and Grafton (2010), and in all cases, the process includes estimating sensitivity, exposure and adaptive capacity to yield vulnerability of a species, community, country or other entity under assessment. The ClimeFish CAP guidelines follows these existing frameworks by estimating these factors to weigh the climate-related vulnerability of all key components of the production system.

Discussion and conclusion

Communicating with stakeholders is an important component of the decision making process to increase a general acceptance of management decisions. The fact that each of stakeholders has different interests, agendas and roles in the process. They differ in their sense of urgency of the problem, their approach to the problem, their language and knowledge levels, and their rationality regarding potential solutions. Participation of stakeholders in decision making process is advocated for following reasons. First, both individuals and society are enriched through the encouragement of social and individual learning. Second, multiple views would help improves understanding of the issues and selection of the appropriate solutions. Third, the success of policy implementation is promoted by the encouragement of collaborative relationships. Thus, in order to involve the stakeholders in an efficient way, it is necessary to define rules of engagement, promote a shared vision and the planning to achieve it, extended peer review process.

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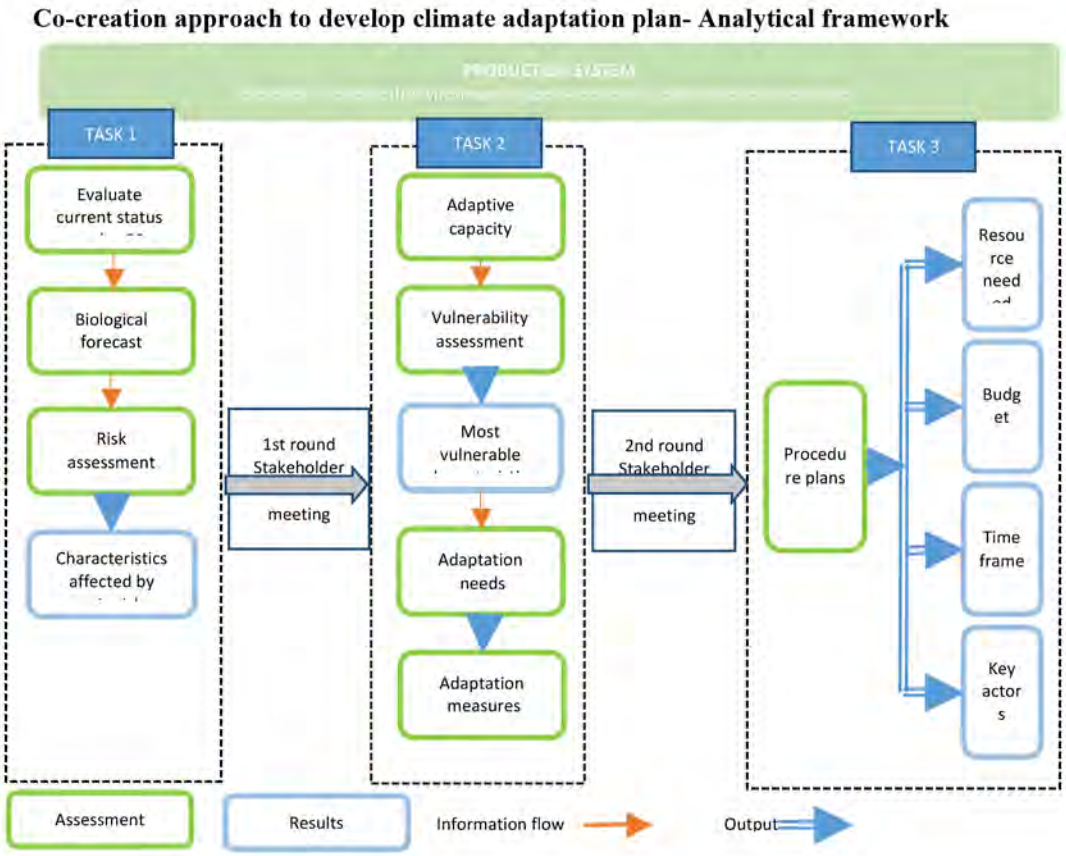

Figure 1: Analytical framework of developing CAP

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EFFECT OF DIETARY MACRONUTRIENT VARIATION ON ENERGY UTILISATION EFFICIENCY IN SNAKEHEAD (*Channa striata*)

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Introduction

Fish energy demands for maintenance and growth has been assessed using the factorial approach on a digestible basis (Glencross and Bermudes, 2012). In such an approach, the efficiency of digestible energy utilisation for growth (k_{gDE}) is the slope of the regression between retained energy (RE) and digestible energy (DE) intake with assumption that the feed composition does not affect k_{gDE}. However, the various k_{gDE} values in barramundi (Glencross *et al.*, 2017), Nile tilapia (Schrama *et al.*, 2012) and rainbow trout (Rodehutscord and Pfeffer, 1999)</style><style face="italic" font="default" size="100%">Oncorhynchus mykiss</style></title><secondary-title>Aquaculture</secondary-title></titles><periodical><full-title>Aquaculture</full-title></periodical><pages>95-107</pages><volume>179</volume><number>1</number><keywords><keyword>Rainbow trout</keyword><keyword>Digestible energy</keyword><keyword>Partition</keyword><keyword>Maintenance requirement</keyword><keyword>Energy utilisation</keyword><keyword>Multiple regression</keyword></keywords><dates><year>1999</year></dates><isbn>0044-8486</isbn><urls><related-urls><url><style face="underline" font="default" size="100%">http://www.sciencedirect.com/science/article/pii/S0044848699001556</style></url></related-urls></urls><electronic-resource-num><style face="underline" font="default" size="100%">https://doi.org/10.1016/S0044-8486(99 fed various macronutrient profiles reflects the effect of the dietary macronutrient profile on k_{gDE}. This effect maintains to be validated for other fish species. This study therefore aimed to investigate effect of dietary macronutrient variation on energy utilisation efficiency in snakehead *i.e.*, a carnivore).

Materials and methods

A total of 2400 snakehead (mean weight 29.1g) were fed a total of four diets, which were formulated based on a 2×2 factorial design with 2 dCP-to-dLipid ratios (7:1 and 2:1) and 2 dCP-to-dstarch ratios (3:1 and 1:1) with the gross energy ranging 18.4 – 21.3kJ.g^{-1}DM, and applied at 2 feeding levels to have a 2×2×2 factorial design with a total of 8 treatments in triplicate. This design aimed to achieve the large contrast in digested energy intake among the 4 diets. This was conducted to run regression analysis of retained energy (*i.e.*, growth response) as a function of digestible energy intake. The experiment includes a total of twenty four 500-l tanks (100 fish/tank)

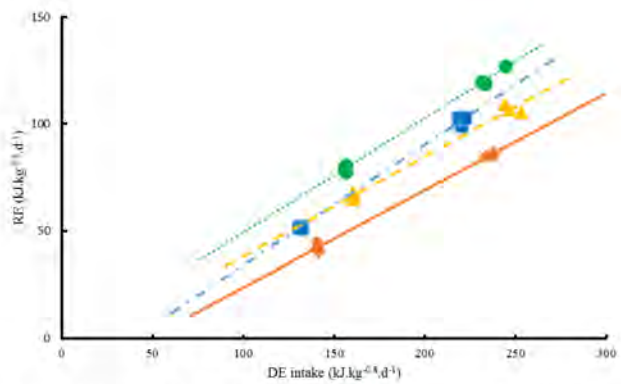

Fig 1. Relationship between retained energy and digestible energy intake for snakehead (■ Diet 1: RE= -22.3 (SE 2.55) + 0.56 (SE 0.01) DE (R^2=0.99), ♦ Diet 2: RE= -21.6 (SE 2.90) + 0.45 (SE 0.01) DE (R^2= 0.99), • Diet 3: RE= -3.8 (SE 2.80) + 0.53 (SE 0.01) DE (R^2= 0.99), ▲ Diet 4: RE= -8.1 (SE 5.80) + 0.46 (SE 0.03) DE (R^2=0.99)).

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Results

At both feeding levels, snakehead fed the carbohydrate-rich diet had the lowest feed efficiency while the highest value was found in fish fed the lipid-rich diet (Table I). By conducting regression between retained energy and digestible energy, the k_{gDE} was determined in a range from 45% to 56% with the highest value found in the protein-rich diet and the lowest value found in the carbohydrate-rich diet (Fig 1).

Discussion and conclusion

The observed k_{gDE} was macronutrient-specific, which is in agreement with previous studies on barramundi (Glencross *et al.*, 2017), Nile tilapia (Schrama *et al.*, 2012) and rainbow trout (Rodehutscord and Pfeffer, 1999)</style><style face=”italic” font=”default” size=”100%”>Oncorhynchus mykiss</style></title><secondary-title>Aquaculture</secondary-title></titles><periodical><full-title>Aquaculture</full-title></periodical><pages>95-107</pages><volume>179</volume><number>1</number><keywords><keyword>Rainbow trout</keyword><keyword>Digestible energy</keyword><keyword>Partition</keyword><keyword>Maintenance requirement</keyword><keyword>Energy utilisation</keyword><keyword>Multiple regression</keyword></keywords><dates><year>1999</year></dates><isbn>0044-8486</isbn><urls><related-urls><url><style face=”underline” font=”default” size=”100%”>http://www.sciencedirect.com/science/article/pii/S0044848699001556</style></url></related-urls></urls><electronic-resource-num><style face=”underline” font=”default” size=”100%”>https://doi.org/10.1016/S0044-8486(99. This can also indicate the k_{gDE} dependent on the trophic level because of the metabolic mechanism specilised differently among carnivore, omnivore and herbivore. High values of k_{gDE} in the protein-rich diet and the lipid-rich diet confirm snakehead to utilise these macronutrients (i.e., protein and lipid) as efficiently as rainbow trout (Rodehutscord and Pfeffer, 1999)</style><style face=”italic” font=”default” size=”100%”>Oncorhynchus mykiss</style></title><secondary-title>Aquaculture</secondary-title></titles><periodical><full-title>Aquaculture</full-title></periodical><pages>95-107</pages><volume>179</volume><number>1</number><keywords><keyword>Rainbow trout</keyword><keyword>Digestible energy</keyword><keyword>Partition</keyword><keyword>Maintenance requirement</keyword><keyword>Energy utilisation</keyword><keyword>Multiple regression</keyword></keywords><dates><year>1999</year></dates><isbn>0044-8486</isbn><urls><related-urls><url><style face=”underline” font=”default” size=”100%”>http://www.sciencedirect.com/science/article/pii/S0044848699001556</style></url></related-urls></urls><electronic-resource-num><styleface=”underline”font=”default”size=”100%”>https://doi.org/10.1016/S0044-8486(99 and barramundi (Glencross *et al.*, 2017). It is worth noting that dietary macronutrient variation affects energy utilisation efficiency in snakehead. Snakehead has limited capability to handle the dietary carbohydrate which is similar to barramundi (Glencross *et al.*, 2017). This study also reflects the drawback of factorial approach to assess the k_{gDE} when various ingredients are used to formulate fish feed instead of only fish meal and fish oil included.

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IMPACT OF DIETARY ZINC LEVEL ON THE ZINC STATUS OF ATLANTIC SALMON PARR, SMOLT AND POST-SMOLT FED LOW FISH MEAL DIETS

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Introduction

Reduction and replacement of marine raw materials with plant ingredients have affected the availability of minerals in Atlantic salmon feeds. Subsequently, the dietary mineral levels essential to meet the requirement have been altered with changing feed formulations. Zinc (Zn), an essential micro-mineral to fish, is low in plant ingredients and exhibits poor availability in plant ingredient-based fish feeds (Antony Jesu Prabhu et al., 2016). Therefore, efforts to optimize the inclusion level of zinc in salmonid feeds low or devoid of fish meal has gained importance (Antony Jesu Prabhu et al., 2019; Read et al., 2014). In this regard, the present study aimed to investigate the impact of dietary zinc levels in low fish meal diets to Atlantic salmon parr in freshwater (FW), smolt and post-smolt in seawater (SW).

Material and Methods

Experimental diets, comprising of plant ingredients and low in fish meal were produced at Skretting ARC and the feeding trials were conducted at Lerang research station. Atlantic salmon were fed 6-7 graded levels of dietary Zn (40 to 280 mg kg^{-1}) in two feeding trials following a regression design. In trial 1, the Atlantic salmon parr (43 g, mean weight) were fed 6 dietary Zn levels, in duplicate groups for 8 weeks in FW, transferred to SW and fed the respective diets for another 4 weeks. In trial 2, Atlantic salmon post-smolt (125 g, mean weight) were fed 7 graded levels of Zn in triplicate groups for a period of 9 weeks in SW. The fish were fed twice a day to apparent satiation and feed intake monitored. During the start and at the end of the feeding periods, length and weight were recorded; samples collected for whole-body and tissue (plasma, vertebrae and liver) mineral status and feces samples were collected by stripping to assess apparent availability. The mineral analyses of the feed and fish samples were performed by ICP-MS.

Results

Dietary Zn did not have a significant impact on the growth of Atlantic salmon parr and post-smolt in trial 1 and trial 2, respectively. In trial 1, the fish grew to a mean weight of 71 ± 4 g after 8 weeks in FW and reached 87 ± 6 g at the end, 4 weeks post SW transfer. In trial 2, the mean weight across groups was 304 ± 12 g at the end of 9 week feeding. The data presented in the Figure 1 corresponds to the Zn status in the whole-body (A) and in the vertebrae (B) of Atlantic salmon post-smolt as affected by dietary Zn levels. Test of linearity showed that, both whole-body and vertebrae Zn status in Atlantic salmon post-smolt showed highly significant linear trend with increasing dietary Zn levels ($p<0.0001$), with no indication of non-linearity ($p>0.5$). The Zn status of Atlantic salmon parr in FW and smolt 4 weeks after SW transfer from trial 1 are being analyzed.

Discussion and conclusion

Despite the lack of growth differences, the Zn status in the whole-body and vertebrae of post-smolt Atlantic salmon in SW was significantl influenced by dietary Zn levels. However, neither of the Zn status indicators showed signs of saturation in the range of dietary Zn level examined. Atlantic salmon fed the basal diet, with lowest dietary Zn supply were able to cope without compromising on growth despite their whole body and vertebrae Zn status declining by 100%, compared to the fish fed highest dietary Zn level. The final whole-body Zn concentration of 33 mg kg^{-1} wet weight achieved with the highest Zn level (280 mg kg^{-1} feed) corresponds to the recent data reported by Åsgård et al. (2018), however, is lower than the level reported by Shearer et al. (1994). Considering the existing EU legislation on maximum permitted total Zn level of 180 mg kg^{-1} feed for salmonids (EC, 2016) and an EFSA (2017) scientific opinion suggesting a further decrease to 150 mg kg^{-1} feed, the present results are of high significance The findings will be presented in this context along with further data from ongoing analyses.

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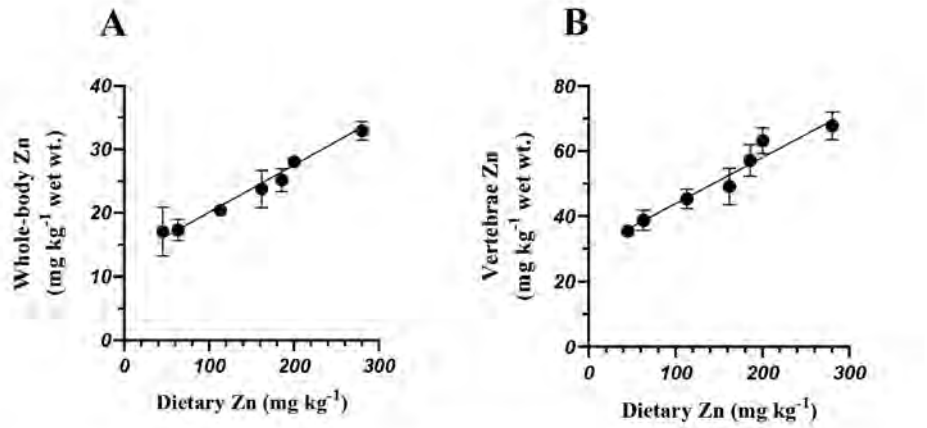

Figure 1: Zinc status in the whole-body (A) and vertebrae (B) of Atlantic salmon post-smolt in seawater, fed graded levels of dietary zinc in low fish meal diets.

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SEX, AGE AND BACTERIA: HOW THE INTESTINAL MICROBIOTA IS MODULATED IN A PROTANDROUS HERMAPHRODITE FARMED FISH

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Introduction

Intestinal microbiota is key for many host functions, such as digestion, nutrient metabolism and absorption, disease resistance and immune function. With the growth of the aquaculture industry, there has been a growing interest in the manipulation of fish gut microbiota to improve welfare and nutrition (Egerton et al., 2018). However, a long road lies ahead to establish the baseline parameters to guide this manipulation. Intestinal microbiota varies with many factors, including host species, genetics, developmental stage, diet, environment and sex. The aim of this study was to compare the intestinal microbiota of adult gilthead sea bream (GSB, *Sparus aurata*) from three groups of age maintained under the same conditions.

Materials and Methods

One-, 2- and 4-year old GSB (Y+1, Y+2, Y+4) were kept in the same open-flow system and fed the same diet for more than 6 months. After 2-days of fasting, 10 fish per group were sacrificed and the anterior intestinal portion was dissected, opened and washed to remove non-adherent bacteria. Intestinal mucus was scrapped off and immediately used for DNA extraction. The V3-V4 region of the 16S rRNA of each individual sample was amplified and sequenced by Illumina MiSeq. After quality filtering, taxonomic assignment was performed with a custom-made pipeline using the RDP database. Alpha diversity was calculated using Phyloseq and beta diversity using PERMANOVA and PLS-DA models. Metagenome prediction and pathway analysis were performed using Piphillin.

Results

All Y+1 individuals were males, while the ones belonging to Y+2 and Y+4 age classes were females. A total of 686461 high quality reads (22882/sample) were assigned to 846 OTUs (97% identity). Almost 30% of the OTUs were classified up to the level of species.

Microbiota diversity and richness did not differ among age groups; however bacterial composition did (Fig. 1), highlighting the presence of *Photobacterium* and *Vibrio* only after 2 years of age and a higher presence of *Staphylococcus* and *Corynebacterium* in Y+1 animals. The core microbiota was defined by 14 OTUs and the groups that showed more OTUs in common were Y+2 and Y+4. PLS-DA analysis (Fig. 1) showed a clear separation by sex (component 1) and age (component 2), with bacteria belonging to the phyla Firmicutes, Proteobacteria and Actinobacteria driving the separation. Pathway analysis performed with the inferred metagenome showed significant differences between Y+1 and Y+4, with

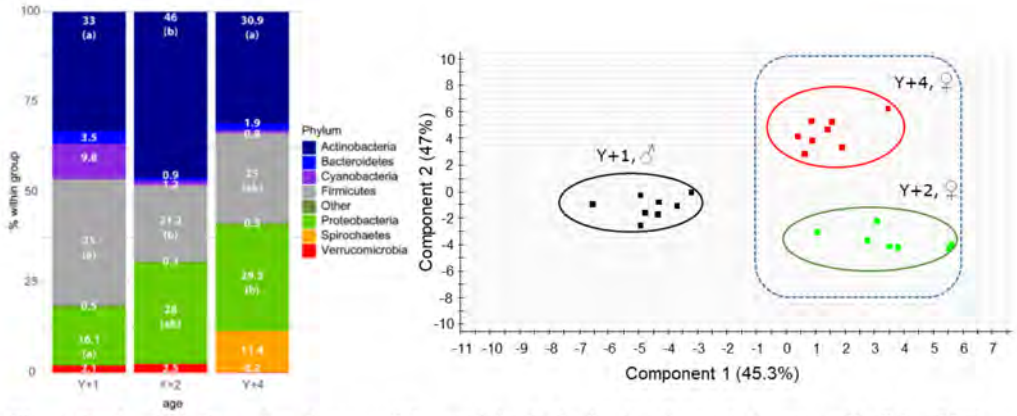

Fig. 1. Left: Relative abundance of bacterial phyla in 1-, 2- and 4-year old fish. Right: Discriminant analysis (PLS-DA) of the bacterial composition of the different groups.

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an increase with age of pathways related to cell death, motility, biofilm formation, environmental response, infection, metabolism of lipids, secondary metabolites, ansamycins biosynthesis and nitrotoluene degradation. Carbohydrate and lipoic acid metabolism and atrazine degradation were down-regulated with age.

Discussion and conclusion

The effect of host age on the intestinal microbial diversity has been previously studied in other fish species finding an increase in diversity with age (Zhang et al., 2018). However, these studies mainly focused on early life stages of the fish. Nonetheless, it is clear that the intestinal ecological niche matures with age leading to changes in the associated microbiota (Stephens et al., 2016). The dominance of the phyla Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria has already been reported in several studies on carnivorous marine fish, including GSB (Estruch et al., 2015). The current results highlight a gradual increase of Proteobacteria abundance with age with the consequent decrease of the other phyla. *Photobacterium* and *Vibrio* were previously described as the dominant genera in GSB posterior intestine (Piazzon et al., 2017) and it is noteworthy that in the current study these genera are absent in the anterior intestine of Y+1 fish, becoming more dominant with age. These two genera include many pathogenic and opportunistic species, but others are important symbionts, assisting in the breakdown of dietary components (Egerton et al., 2018). Overall, changes in microbial composition drive changes in metabolic pathways, as the up-regulation of infection-related pathways, nitrotoluene degradation and sphingolipid metabolism, and the down-regulation of carbohydrate metabolism pathways, which seem to indicate an inflammatory profile in the intestines of older fis

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SMART AQUAPONICS: DEVELOPMENT OF A TOOL FOR EDUCATION, DECISION SUPPORT & MONITORING FOR AQUAPONICS

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Introduction

Aquaponics is a technology combining both aquaculture and hydroponics. The first aquaponic systems has been developed in the late seventies. Then after, aquaponics has been rapidly adopted by education sector and urban communities. During the last decade, thanks the apparition of different aquaponic farms, aquaponics became a professional activity. Nevertheless, aquaponics is facing the drawback of innovative technologies. Firstly, the lack of advanced education dedicated to aquaponics limits the availability of technicians and engineers trained to aquaponics. Secondly, the absence of references and tools specifically dedicated to aquaponics makes the conception and monitoring of aquaponic system quite complex. In order to overcome these limitations and to foster the development of aquaponics among local communities and the corporate sector, the Interreg project Smart Aquaponics is developing an online training program, a decision support tool and a monitoring tool. These tools will be accessible within an application (smartphone and PC).

The training program will be composed of a serious game and several theoretical modules. The game will allow the user to handle virtual aquaponics systems with different levels of complexity and experiment an extensive range of events occurring in real aquaponics systems. The target groups are technical secondary schools, colleges, universities and local communities.

The decision support tool will allow users to compose virtual aquaponics systems and perform simulations. These simulations will estimate the yield, efficiency and stability of the systems and, finally allow fine-tune the design

The monitoring tool will monitor the status of the different component of an aquaponics system and propose some prediction of the future status of the aquaponic system in order to (i) anticipate potential problems (ii) maintain the parameters in an optimal range. The monitoring is based on connected sensors (pH, t°, nitrogen, …) and will be compatible with small and semi-professional systems.

Materials & methods

The three tools will be based on a model that predicts the evolution of different parameters (oxygen, nitrogen, plant and fish growth, …) of an aquaponics system. The specific nature of this model lies in its ability to model aquaponics systems of different sizes and designs. Beside, the project is also developing a data acquisition chain able to connect the sensors present both in the aquaculture and hydroponic compartment to the application.

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The calibration of the model, the evaluation of the data acquisition chain and the beta testing of the application will be achieved in three steps. First, in 2019, seven aquaponic systems belonging to the Smart aquaponics partners will be used to test the data acquisition chain. The data collected will enable a first calibration of the model. Secondly, in 2020, a panel of beta testers including professionals and hobbyists will be invited to test the data acquisition chain and the beta version of the application. The data collected with this panel will enable a second calibration of the model. The panellists will also provide a feed back on the application for improvement. Third, after the release of the application (2021), all the data collected by the application will enable further calibration of the model. In fine, the accuracy of the model and of the prediction of the application will increase with the number of users.

Results

In 2021, the Smart Aquaponics project will release an application including a training program, a decision support toll and a monitoring tool. Smart Aquaponics will also propose a data acquisition chain able to connect the sensors present in the aquaponics system to the application. Due to two years of testing, the prediction of the model will be accurate, the data acquisition chain reliable and the application user friendly.

In fine, these tools should facilitate both the design and the monitoring of new aquaponic systems. This associated with the online training program should facilitate the emergence semi-professional and professional aquaponics system.

NEW INSIGHTS INTO THE APPETITE GENE REGULATION IN COMMON CARP (*Cyprinus carpio*)

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Introduction

Stress is known to influence feed intake of fish. But the differential effects of different stressors on this essential process in detail are not known. To investigate effects of different stressors on the appetite gene regulation in common carp (*Cyprinus carpio*) brain, quantitative polymerase chain reactions (qPCR) were used.

Methods

Prior to the application of the different stressors, the fish were acclimatized in an experimental tank and were trained to a feed reward applied at the same time of the day for 6 weeks. After that the fish were reared in groups of two fish each and the different stressors were applied. These included the use of the feed reward as well as the exposure to air for 1 min. The blood and brain samplings were performed after 10, 30 and 60 min after the stress applications. Reference groups were included in the experimental set-up. All experimental procedures were conducted according to the regulative permissions for animal experiments. The individual blood samples were used for cortisol quantification by means of high-performance liquid chromatography. For the gene expression analyses, the individual brains were subdivided into telencephalon, optical tectum, cerebellum and hypothalamus to allow investigations of gene regulation in different brain areas. Relative quantification of appetite gene expression was achieved using a set of validated reference genes

Results & Discussion

The results showed that the highest blood cortisol levels were observed in fish 10 minutes after exposure to distress, and the plasma cortisol concentrations decreased over time. Thus, the experiments proved that the experimental design resulted in the expected stress response patterns. Compared to this the cortisol response in the feed-rewarded group and the sham-treated fish was much shorter than in the distressed fish. Different regulation of appetite gene expression was also observed in the fish exposed to the di ferent stressors and will be discussed during the presentation.

AQUAPONIC PRODUCTION OF *Salicornia ambigua* AND PACIFIC WHITE SHRIMP IN BIOFLOC SYSTEM UNDER DIFFERENT SALINITIES

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Introduction

Aquaponics has been presented as an innovative and sustainable alternative as it combines aquaculture with hydroponics in a system that recirculates water and nutrients in order to promote the growth of aquatic organisms and plants in an integrated way. However, to apply this system to marine crops, it is necessary to use plants that have commercial value and are tolerant to salinity so that they can develop in the saline effluent (Buhmann and Papenbrock, 2013). Many species of halophytes are able to have their growth stimulated by the presence of salt and retain high Na^+ and Cl^- concentrations (between 25% and 30% of their dry weight), especially in shoots (Kudo and Fujiyama, 2010). However, some species differ in their degree of tolerance to salinity, and the ideal concentration for the development of these plants is that equal to or less than that of seawater (Ventura et al., 2014). Thus, the aim of this study was to evaluate the relationship of salinity to the integrated performance of Pacific white shrimp (*Litopenaeus vannamei*) and *Salicornia ambigua* in an aquaponic system using biofloc technolog .

Material and Methods

The experiment consisted in evaluating four different salinities in the cultivation of shrimp *L. vannamei* and *S. ambigua* in aquaponics: 8psu, 16psu, 24psu and 32psu, with three replicates. Each experimental unity was constructed and operated according to Pinheiro et al. (2017), and consisted of a BFT culture tank of 800 L, with titanium heaters, aeration and artificial substrates. It was coupled to this tank a settling chamber with 40 L volume and a hydroponic bench for 12 plants (40 plants.m^{-2}). Each tank was filled with 400 L water from a biofloc matrix tank (salinity 20psu) and then stocked with 240 shrimps with average weight of 1.6±0.1g (300 shrimp.m^{-3}). Posteriorly, the shrimp were slowly acclimated, and tap water and filtered seawater were added to the tanks to reach the desired salinity and the useful volume in each treatment at a rate of 2psu per hour. Shrimp were fed 4 times a day with commercial feed with 38% crude protein and was supplied according to the feeding table proposed by Van Wyk (1999). After 57 days of cultivation the production indexes of shrimp and *Salicornia* were calculated. All data were submitted to second-order polynomial regression analysis (α=0.05), and the maximum and minimum points were estimated by deriving the quadratic equation.

Results

The shrimp production data are presented in Table I. Salinity affected only the survival of the shrimps, with maximum values found at salinity of 25.7psu. The results of final mean weight and weekly weight gain were not affected by the salinities evaluated. For the production indexes of *S. ambigua* no salinity relationships were detected with any of the analyzed parameters (p>0.05). Productivity was 0.46±0.12kg.m^{-2}, 0.61±0.12kg.m^{-2}, 0.46±0.05kg.m^{-2} and 0.38±0.05kg.m^{-2} in the treatments 8psu, 16psu, 24psu and 32psu respectively.

Discussion and conclusion

Litopenaeus vannamei inhabits natural environments with variation of salinity of 1 to 50psu (Davis and Roy, 2013). In this experiment, a direct relationship was found between the reduction of salinity and the increase in shrimp mortality. This low survival may be related to the mineral composition of the water since, although it is an eurihaline species, to ensure satisfactory survival and growth of *L. vannamei* in low salinity the proportions of ions such as sodium, potassium and magnesium should be close to those found in seawater (Roy et al., 2010). Also, the maximum survival point was found at salinity 25.7psu, and is near the isosmotic point of *L. vannamei* (24.7 to 26psu) (Castille and Lawrence, 1981).

Although there was no significant relationship between plant performance and salinity, the highest yield was achieved in the 16psu treatment and the maximum productivity and final weight were estimated at salinities close to that (17.5psu and 16.4psu respectively). Halophytes of the Amaranthaceae family, such as *S. ambigua*, have their growth stimulated in the presence of NaCl, with the salinity being between 150 and 300 mM NaCl ideal for their development (approximately 8 to 17psu) (Ventura and Sagi, 2013).

The cultivation of *L. vannamei* integrated with *S. ambigua* can be performed between salinities of 16 and 24psu since the performance of the shrimp is not impaired and the growth of the plants are favored in this salinity range.

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Table I. Production indexes of *Litopenaeus vannamei* cultured in aquaponic system under different salinities during 57 days.

Parameter	Treatment				*p*-value
	8psu	16psu	24psu	32psu	
Survival (%)	56.3 ± 4.7	83.3 ± 1.2	82.6 ± 4.3	84.0 ± 4.0	0.0389
Final average weight (g)	12.7 ± 0.3	11.5 ± 0.4	11.6 ± 0.2	11.8 ± 0.5	0.3082
Average weight gain ($g.week^{-1}$)	1.4 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.1	0.5242
Productivity ($kg.m^{-3}$)	2.3 ± 0.3	2.7 ± 0.2	2.9 ± 0.2	3.0 ± 0.1	0.5978
FCR	2.0 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	0.8335

Data are mean ± standard deviation. **ns**: not significant. Quadratic effect for survival: $y = 26.47 + 4.674x - 0.091x^2$; $R^2=0.8427$.

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KNOWLEDGE FLOW WITHIN AQUACULTURE CLUSTER PROJECTS: THE HIDDEN ADDED VALUE

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Introduction

Aquaculture, as one of the pillars of European Blue Growth agenda, should aim at sustainability in a broad way, both reducing the impact on natural stocks and environment while keeping the economic and social growth. Aquaculture commercial production usually includes a culturing step in natural conditions, such as shellfish facilities in coastal areas and lagoons, or fish cages offshore. This practice reduces the costs associated to the production of food (i.e. microalgae for shellfish) and keep the water quality in indoor facilities, but exposes the production to environmental changes and threats. The production activity also impacts the natural area in which it is located.

Managing all these layers of interactions, crossed impacts and goals requires knowledge-based strategies. Cluster projects, in which the triad government, academia and industry are closely involved from the preparation stage, allow the convergence of different knowledges and actors able to provide outcomes beyond the specific subject, activity or area of the project. The challenge is to overcome communication barriers, design information flows and boost the interaction between all the stakeholders. This type of intervention is in favour of the sustainable management of fish resources to limit and contain their overfishing. Fisheries policies must guide activities to reduce the level of exploitation of biological resources on levels that guarantee renewability in the medium and long term, also to protect the strategic functions of fishing (food, economy, employment, conservation of traditional activities and cultures, etc.). The definition of the technical measures must be the result of a process of sensitive evaluation of the local realities in the dimensions often evoked (ecological, economic, social, juridical), realities that have different criteria of judgment and that also require an integrated treatment.

Materials and methods

A local ecologic knowledge (LEK) approach (Azzurro 2018) has been included in the communication and technology transfer activities planned in two cluster projects, "Valorizzazione della produzione sostenibile delle ostriche nel sistema produttivo della molluschicoltura in Sardegna – (Enhancement of the sustainable production of oysters in the mollusc farming production system in Sardinia)" (OSTRINNOVA) and "Trasferimento alle aziende operanti in laguna delle tecniche di riproduzione e di allevamento in ambiente controllato di *Mugil cephalus* – (Transfer to the companies operating in the lagoon of breeding techniques in a controlled environment of *Mugil cephalus*)" (TECNOMUGILAG). Both projects have been funded by Sardinian Region as part of its regional development strategy, and Sardinian fishermen cooperatives working in coastal lagoons are the main Industry partners' profile. OSTRINNOVA aims at developing *Crassostrea gigas* culture in Sardinian lagoons, while TECNOMUGILAG is based on transferring the aquaculture production of *Mugil cephalus* for restocking its natural populations. The LEK approach is based on semi-structured questionnaires that will be conducted by vis a vis interviews with local fishermen. The interview protocol consists of a successful methodology that has been widely used across several Mediterranean countries (Azzurro et al., 2018).

Knowledge flow (Figure 1) is based on: 1) Group meetings organized by Academia participants convening Industry participants, 2) one-to-one meetings between an Academia and an Industry participant, and 3) participant-tailored LEK interviews.

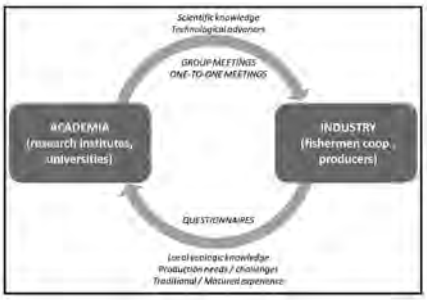

Figure 1. Knowledge flow between Academia and Industry participants in cluster projects. Italic capital letters: communication activity; Italic minor letters: knowledge type flowing.

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Results

Group meetings allow the exchange between participants of similar profile, and prompt the debate within the project, but are difficult to implement because they should fit with Industry economic activity, calendar and workload. Despite several attempts to find suitable meeting days, the final success, in terms of number of participants, is scarce: 6% of OSTRINNOVA Industry and 42% of TECNOMUGILAG Industry has participated. One-to-one meetings require more organization and time investment but have been more effective, with 100% of both cluster Industry participating; these meetings allow a more active interaction between Academia and Industry, the quality and quantity of information is higher, and facilitate the implementation of LEK approach.

Discussion and conclusion

Cluster projects are aimed at transferring the scientific knowledge to the productive sector. The knowledge flow has been usually identified as unidirectional, the transfer of scientific knowledge and technological advances from academia to industry. The reverse, from industry to academia, has usually been overlooked. Knowledge gathered from industry, ranging from matured experience at larger scale production to local, even traditional knowledge on the specific area in which aquaculture production is planned, is a unique and too often unexpected outcome. The integration of this knowledge with that generated from academia is the most valuable result for government stakeholders in order to design and implement a knowledge-based management of coastal areas and aquaculture production development. The potentialities of LEK have just started to be explored in the Mediterranean area and its importance for environmental monitoring and natural resources management is expected to grow, empowering the voices and the observational potential of people living in intimate relationship with the natural environment (Azzurro 2018).

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INFLUENCE OF WATER TEMPERATURE AND FEEDING REGIME ON THE INCIDENCE OF EARLY SEXUAL MATURATION IN ATLANTIC SALMON (*Salmo salar* L.) POSTSMOLTS DURING THE FRESHWATER STAGE

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Introduction

The current trend in salmon aquaculture towards intensification and Closed-Containment Systems (CSS) brings along some undesired effects, among which male early sexual maturation in the freshwater stage has been identified as one of the most challenging (Good and Davidson, 2016). During maturation salmon stop feeding, resulting in poor FCR and reduced growth rate (McClure et al., 2007). In addition, mobilization of resources for gonadal development highly affects flesh quality, making fillets not marketable (Aksnes et al., 1986). Finally, mature postsmolts cannot be easily identified in freshwater and often end up in the sea stage (Good and Davidson, 2016). Overall, this leads to high economic losses for the industry (McClure et al., 2007) and compromise the economic feasibility of these production systems. Factors identified as likely causes for early maturation in freshwater include photoperiods, temperature, diets, growth rate or size, among others (Good and Davidson, 2016). This study aimed to assess the effect of temperature and diet on early sexual maturation.

Materials and methods

Experimental design

This experiment consisted on a 3x2 factorial design that combined three temperatures (8, 12.5 and 18°C) and two feeding rations (Full and 67%), producing six experimental groups that were reared in duplicate at the flow-through facilities of the Department of Biological Sciences (BIO, Bergen). On 27 September 2018, 1800 Atlantic salmon parr (mean weight 26g), were transferred from a commercial RAS (Bremnes Trovåg, Norway) and randomly distributed among 12 freshwater tanks of 0.5m^3 (n=150/tank). Conditions set included water temperature 12.5°C, specific flow rate 0.5 $l.kg^{-1}.min^{-1}$ and LD24:0 photoperiod. Initially all tanks were fed a full ration and were allowed to acclimate for a week, after which temperatures were gradually adjusted to 8 and 18°C in two pairs of tanks per feeding group, while the third pair was maintained at 12.5°C. When temperatures were stable, feeding ration was reduced in six tanks to 67% by fasting every third day, while the remaining six were maintained at a daily full ration. Fish were fed appropriate commercial feeds (Biomar®) provided by Bremnes Seashore AS (Norway). The LD24:0 photoperiod was maintained for the first four months to ensure maximum fish growth, after which, on 4 February 2019, was reduced to LD12:12 to provide the fish with a winter signal for smoltification. After 5 weeks winter signal, photoperiod was set back to LD24:0 and maintained until the end of the experiment.

Samplings and statistical analysis

Seven samplings were performed until submission of this abstract, in which six males per tank were collected to record fork length, body weight, sex, gonad weight and liver weight. Gonadosomatic Index *GSI(%) = Gonad weight*100/Body weight* was used to assess maturation status. Four categories of maturity were established based on literature (Peterson and Harmon, 2005; Thorpe, 1994) and gonad visual inspection: "Immature" (GSI =< 0.06), "Early stage" (0.06 < GSI =< 0.1), "Maturing" (0.1 < GSI =< 1) and "Mature" (GSI > 1). In order to find differential trends in early gonadal development among groups, GSI during the first six months with no maturation was modelled as a function of temperature, feeding regime, time and their interactions. This analysis was performed by fitting a GLMEM using the package "nlme" in the statistical software R. A significance level α=0.05 was used

Table I. Mean body weight (g), GSI (%) and percentage of maturation found for groups *18°C-Full* and *18°C-67%* during samplings 6 and 7. Body weights and GSI (%) are displayed ± standard deviation. Maturity categories have been defined as follows: "Immature" (GSI =< 0.06), "Early stage" (0.06 < GSI =< 0.1), "Maturing" (0.1 < GSI =< 1) and "Mature" (GSI > 1). Percentages are calculated per row (experimental group).

				Maturity categories (%)			
Sampling	*Group*	*Body weight (g)*	*GSI (%)*	*Immature*	*Early stage*	*Maturing*	*Mature*
6	18°C-67%	280.1 ± 78.1	0.153 ± 0.278	53.3	20.0	20.0	6.7
	18°C-Full	336.3 ± 93.7	0.168 ± 0.133	21.4	7.1	71.4	0.0
7	18°C-67%	343.8 ± 109.4	2.480 ± 0.661	33.3	16.7	25.0	25.0
	18°C-Full	410.8 ± 117.7	2.820 ± 0.807	25.0	8.3	41.7	25.0

(Continued on next page)

Results

First obvious signs of sexual maturation appeared in males from groups *18°C-Full* and *18°C-67%* during the 6th sampling in early April. Maturation skyrocketed two weeks later in the same groups during the 7th sampling, with an increase in mean GSI greater than 15-fold (Table I). Mean GSI for immature fish across all samplings was consistently low 0.035 ± 0.009%. However, the GSI model for early development showed a significantly higher GSI in both 18°C groups during the period without maturation (p-value< 0.01), regardless of feed ration. Also, a significant positive effect on GSI was found due to the interaction 18°C*Time (p-value< 0.001), occurring from almost sampling 2 in mid-December. Feeding regime showed no significant e fect on GSI.

Percentages per maturity category displayed in Table I show that in both samplings, the proportion of immature fish and the proportion of individuals caught at early stage of maturation was higher in 67% ration group. In contrast, the percentage of individuals engaged in advanced stages of maturity was much higher in the Full ration, although advanced stages were clearly present in both groups.

Discussion and conclusions

Our results clearly point at high temperature as the main driving factor for early maturation of salmon postsmolts in freshwater. Although no maturation occurred for the first 6 months, results from the statistical analysis performed on the first five samplings suggest that individuals in 18°C may have already started to physiologically develop for sexual maturation as early as late December, three to four months before any signs were observed. This is in accordance with authors stating that the decision to mature may occur very early (Berrill et al., 2003; Thorpe, 1994) and warns the industry that high temperatures during an uncertain period in early development might be problematic for aquaculture production. The fact that maturation occurred in both 67% and Full ration groups, although not to the same extent, suggests that the feeding regime did not play a crucial role on sexual maturation while still having some influence. The percentages shown in Table I suggest that feeding restriction partially delayed sexual maturation and decreased its incidence, although this restriction was not enough to impair sexual development. The mechanisms by which high temperature promotes early maturation are not well understood and will be the main aim of this project.

It is uncertain whether maturation would have occurred to the same extent or at all if no winter signal had been given. Many authors have stated that a photoperiod cue is required for Atlantic salmon to commence reproductive development (Berrill et al., 2003; Good and Davidson, 2016; Peterson and Harmon, 2005; Thorpe, 1994), which is consistent with the timing in which the first signs of maturation appeared in our experiment (4 to 6 weeks after the end of 12:12 winter signal). Further research may help elucidate whether high temperature alone without photoperiodic cue could trigger maturation.

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"ULVAPRO" PROJECT: SHEDDING LIGHT ON THE *ULVA* HOLOBIONT: THE ROLE OF LIGHT IN QUORUM SENSING AND IN MICROBIAL INTERACTIONS WITH IMPLICATION IN IMTA-RAS

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Introduction

Among IMTA techniques, the integration of fish and seaweed cultures in recirculating systems (IMTA-RAS) is currently one of the most promising strategies, in order to achieve diversification and sustainability in aquaculture activities. Macroalgae of the genus *Ulva* (*Ulvales*, Chlorophyta) have shown to be especially suitable for use as biofilters in IMTA-RAS systems. Combining *Ulva* spp. with sole (*Solea senegalensis*) is based on the matching of ecophysiological requirements for both cultures, the simplicity of cultivation and the high growth rate of *Ulva*, as well as the commercial interest of sole, whose cultivation on an industrial level has been recently developed and mainly performed in inland facilities.

The natural populations of *Ulva* seem to be well adapted to conditions of light heterogeneity and this population behaviour could suggest the involvement of quorum perception mechanisms. Since the bacterial communities associated with *Ulva* spp. play an important functional role both in the morphogenesis and in the reproduction of *Ulva* -considering the macroalga and its associated microbiota a singular functional entity or holobiont- it is important to know the role that microbiota plays in the mentioned mechanisms. On the other hand, light intensity can have influence on the maintenance of *Phaeobacter* biofilms in *Ulva*, as well as on the production of the antagonist compound (TDA), regulated by quorum-mechanisms.

Thus, the hypothesis of the ULVAPRO research project, funded by the Spanish government, is that the intensity and heterogeneity of light can affect both the physiology and the population behaviour of *Ulva*, its associated microbial communities and the colonization by *Phaeobacter* bacteria, and that quorum-sensing chemical signalling could mediate the response to those factors.

Material and methods

The verification of the hypothesis will be approached from a multidisciplinary perspective, including di ferent objectives:

1) Demonstrate the existence of biochemical communication by quorum sensing in *Ulva* populations in response to light heterogeneity and to identify the chemical signals which would mediate that communication, and the possible role of epiphytic microbiota on it.
2) Define the responses of *Ulva* epiphytic microbiotal comunities to light intensity and heterogeneity.
3) Determine the influence of light intensity and heterogeneity on the experimental colonization and maintenance of *Phaeobacter* bacteria on *Ulva* and the production of TDA, oriented to its application as a bacterial control strategy in IMTA-RAS systems.

Results

Verifying this hypothesis from a multi-disciplinary approach (from -omic techniques to the engineering of IMTA-RAS systems) will allow i) to understand the mechanisms underlying the behaviour of *Ulva* populations both in nature and in culture (main Objective of SubProject 1) and ii) to provide important guidelines for the design and operation of *Ulva* culture, towards the optimization of production and capacity of biofiltration as well as bacterial control in IMTA- RAS, as well as the safety and nutritional quality of the biomass produced (main Objective of SubProject 2).

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IMPROVING MICRODIET TECHNOLOGY FOR FIRST-FEEDING GILTHEAD SEABREAM (*Sparus aurata*) LARVAE

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Introduction

Feeding protocols for gilthead seabream (*Sparus aurata*) larvae normally envisage the introduction of live-prey during the first 30 days after hatching (DAH), with full weaning to commercial microdiets occurring during the second month of development. Despite challenging, developing a microdiet to offer at first-feeding would be extremely advantageous for hatcheries, although at this stage larvae have reduced preying and digestive capacities. Since microdiets tend to be poorly accepted and less digestible than live-prey, especially in what concerns complex proteins, it is imperative to account for these constraints when formulating a microdiet. For instance, these factors can be attenuated by including protein hydrolysates in microdiet formulations, enhancing its attractability and leading to a further maturation of larval digestive tract (Cahu and Infante, 1995). A microdiet for first-feeding seabream needs to be comprised of small particles (approx. 100 µm) with a high surface-to-volume ratio, what leads to excessive leaching nitrogen compounds resulting from protein hydrolysis to the rearing water. Microencapsulation offers the possibility of controlling leaching of these water-soluble nutrients, increasing the nutritional value of microdiets upon ingestion. This control seems paramount for a successful introduction of microdiets in first-feeding seabream.

This study aimed at developing a microencapsulated prototype able to reduce leaching of a protein hydrolysate commonly used in microdiets for fish larvae. This prototype was subsequently added to the formulation of a commercial microdiet at 8.5 and 30 % and its biological efficacy was tested by evaluating gilthead seabream growth and survival from the onset of exogenous feeding until 34 DAH.

Materials and methods

A protein hydrolysate and a protein concentrate mixture was spray-dried, with resulting microencapsulated powder being evaluated for leaching of water-soluble proteins.

Three dietary treatments were tested: in the Control treatment, a commercial microdiet was used. In the remaining treatments, the microencapsulated prototype was used at 8.5 (CAP8.5) and 30 % (CAP30), replacing the protein fraction of the microdiet used in the Control treatment. All microdiets were introduced in the feeding regime of gilthead seabream at first-feeding (3 DAH), being offered close to satiation and maintained until the end of the experiment (34 DAH). Along with microdiets, larvae were initially co-fed with enriched rotifers until 13 DAH. Compared to a standard feeding regime comprised by live-prey only, the quantity of rotifers provided to all treatments was initially reduced by 20 %, gradually decreasing to 40 and 85 %, from 7 to 13DAH, respectively. From 12 to 23 DAH, a residual amount (0.3 nauplii mL^{-1}) of *Artemia* AF was maintained in the rearing tanks, with larvae feeding on the microdiets alone from 24 to 34 DAH. Larvae were reared at an initial density of 120 larvae L^{-1} in triplicate 100 L conical-cylindrical fibreglass tanks set in a partially-closed water recirculation system. Larvae were sampled at 2, 12, 20, 27 and 34 DAH for dry weight and total length. Larval survival was determined the end of the trial. Microdiet particles and live-prey present in larval digestive tract were assessed by visual inspection throughout the experiment.

Results

Following two hours of immersion in seawater, the novel microencapsulated prototype released only 25 % of its content in water-soluble protein, 65 % less than values obtained in the reference curve (90 % of leaching during the same period).

At 12 DAH, a higher number of seabream larvae was observed with microdiet in the digestive tract than with live-prey. At this age, a significantly higher larval dry weight was observed in CAP8.5 and CAP30 than in the Control treatment (Fig. 1). At 34 DAH, larvae from the CAP8.5 were significantly larger than larvae from the CAP30 treatment. No significant differences were obtained between the former treatments and the Control. Larval survival was not influenced by the microdiets (4-7% in all treatments).

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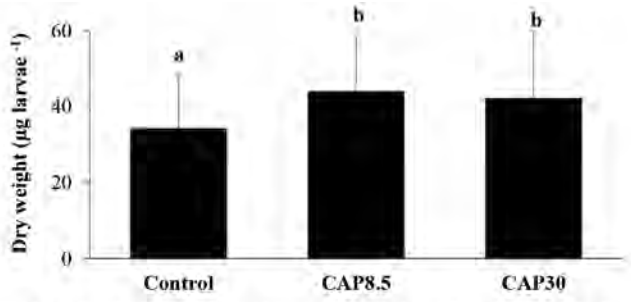

Fig. 1. Dry weight of 12 day-old seabream reared under different dietary treatments. Results are expressed as means ± standard deviation ($n = 60$).

Discussion

This study showed that seabream larvae benefited from the dietary inclusion of a microencapsulated prototype able to reduce leaching of water-soluble proteins during the first days of development (CAP8.5 and CAP30). Yet, in comparison with the Control, only larvae from the CAP8.5 treatment maintained a good performance at the end of the trial. These findings suggest that high dietary protein hydrolysate levels may not be adequate for seabream following the first two weeks of development, as reported previously for Senegalese sole larvae (Canada et al., 2017). Furthermore, growth in all treatments was very similar to findings published by Mata-Sotres et al. (2016) for seabream fed on live-prey only. Therefore, although there is still room for technological improvements in commercial diets, an early weaning and high live-feed replacement during the first weeks of development may now be as an avenue to explore in gilthead seabream hatcheries.

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LESIONS OBSERVED IN WILD AND CULTURED *Crassostrea angulata* AND *C. gigas* IN PORTUGAL

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Introduction
Oyster culture is one of the most economically productive sector of Portuguese aquaculture (INE, 2019).

The primary species, of oyster, cultured in Portugal is the Pacific oyster, *Crassostrea gigas*, and the most common methods of culture are mesh bags attached to tables (Conte and Moore, 2001). The portuguese oyster, *Crassostrea angulata*, was historically locally abundant in portuguese estuaries, but now it is present only in relatively few locals of the Iberian Peninsula (Ruano, 1997; Fabioux, 2002), like Mira and Sado estuaries. This species is mainly found in wild beds, with distinct edaphoclimatic conditions, than those found in production nurseries. Those wild beds are usually found in areas upstream of estuaries where oysters are subject to particular unfavorable ecological conditions. In contrast, in ocean-influenced farms, oysters are subject to other unfavorable conditions, typical of production sites

There was a significant change in bivalve mollusc production. Initially, in Portugal, it was choosed exclusively for semi-intensive production in large areas, at this moment there is a intensification of production in less land area, increasing the animal density. This option took to changes that were described in other places, which had result in lower growth rates, lower product quality, lower fertility rates and epizootic diseases (Grizel et al., 1987).

Materials and Methods
Two population of oyster, Portuguese oyster (n=30) from Sado estuary and Pacific oyster (n=30) from Alvor lagoon, were surveyed. An anatomohistopathological examination was performed. The tissue samples for histopathology were fixed in Davidson's fixative for 48h, dehydration, embedding in paraffin and cutting with a microtome in sections less than 5 µm thick, then stained with Hematoxylin and Eosin (H&E).

Results
The macroscopic lesions were recorded in the specimens collected in wild beds (*C. angulata*) and farmed oysters (*C. gigas*). In samples of wild beds oysters there was observed 20% with mud blisters, mainly caused by *Polydora sp*., 33% with gills lesions and 10% with foot diseases. In farmed population there was observed only 10% with mud blisters. The histopathology showed, that of portuguese oysters sampled, 30% had *Trichodina sp*. in gills and mantle epithelium. Similarly, 20% contained *Ancistrocoma sp*. in the digestive gland tubules. Of the farmed oysters sampled, 23% had *Trichodina sp*. and 27% had *Ancistrocoma sp*. Copepods (*Mytilicola sp.*) were observed within the intestine of oysters at prevalences of 17% and 3% for wild and farmed oysters, respectively.

Concerning tissue lesions and morphological alterations, 27% of wild oysters showed metaplasia in digestive gland and 36% hemocyte inflammation, mainly in connective tissue. Regarding to farmed oysters, 6% showed metaplasia and 13% showed hemocitosis. No other serious morphological changes or lesions were detected.

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Discussion and conclusion

In the present study, two *Crassostrea sp.* population were sampled with the objective to evaluate the sanitary condition of these population. The lesions can be a consequence of factors such as pathogens, seasonality, temperature, reproductive cycle, host density and general state of the hosts. Most of the parasites found among 60 oysters examined are commonly observed in oyster populations worldwide and are usually of negligible or minor significance as pathogens, but when there are massive infestations of these parasites, they can lead to host weakness (Ruano and Dias, 1994; Ruano, 2008). No organisms potentially pathogenic to humans were identified

Organic lesions, such as metaplasia and hemocytosis, may reflect physiological stress, contaminants or the presence of large parasitic loads. (Ruano and Dias, 1994)

The identification, the characterization and the registration of pathologic processes in oysters constitutes important measures for sanitary control.

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HISTOPATHOLOGY OF *Crassostrea gigas* FROM A PORTUGUESE COASTAL LAGOON WHERE MORTALITIES OCCURRED DUE TO SERIOUS OUTBRAKES OF HERPES VIRUS

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Introduction
The culture of bivalve molluscs is an activity with high expression in Portuguese aquaculture. It represents 55% of whole production, being the main species, clams, oysters and mussels. Oyster culture is an important production in Portuguese aquaculture, accounting for about 17% of the total production in this sector (INE, 2019). The primary species cultured is the Pacific oyster, *Crassostrea gigas*, and the most common methods of culture are mesh bags attached to tables (Conte & Moore, 2001).

Materials and Methods
We surveyed a population of Pacific oyster from Alvor lagoon, south coast of Portugal, cultivated in a strong ocean-influenced environment. Those farms use high densities during the production cycle which could induce unfavorable conditions for animal welfare.

We took a sample of this population (n=30) for gross lesions and histopathological examination. The tissue samples for histopathology were fixed in Davidson's fixative for 48h, dehydration, embedding in paraffin and cutting with a microtome in sections less than 5 µm thick, then stained with Hematoxylin and Eosin (H&E). Morphological changes and pathogens were recorded.

Results
In the population sampled we observed 10% with mud blisters (mainly caused by *Polydora sp.*). The histopathology showed the presence of protozoan ciliates, 23% *Trichodina sp.* and 27% of *Ancistrocoma sp*. Copepods (*Mytilicola sp.*) were also present in the intestine lumen at prevalences of 3%.

Concerning tissue lesions and morphological alterations, 6% of the animals showed metaplasia in digestive gland and 13% showed hemocitosis, indicating an inflammatory process, mainly in connective tissue. We also observed that most animals sampled had destruction of the cover epithelium of diverticula of digestive gland and necrosis of hemocytes.

Discussion and conclusion
In the present study a population of *C. gigas* were sampled with the objective to evaluate the sanitary condition of this population with an historical of severe mortalities. The lesions can be a consequence of several biotic and abiotic factors such as pathogens, seasonality, temperature, life cycle, host density, and general state of the hosts. The parasites found are common in oysters and usually are not a risk, unless in massive infestations that can lead to host weakness (Ruano e Dias, 1994; Ruano, 2008). Lesions of the internal organs, such as metaplasia, may reflect physiological stress, contaminants or the presence of large parasitic loads (Ruano, 1994). The destruction observed in the epithelium of the diverticula of the digestive gland, the hemocytic infiltration and necrosis are usually associated with an inflammatory process that could be related with the presence of the virus and associated bacteria, such as *Vibrio splendidus* and *Vibrio aestuarianus*.

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ASSESSMENT OF FOULING COMMUNITIES ON OFFSHORE AQUACULTURE STRUCTURES IN MADEIRA (EASTERN ATLANTIC)

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Introduction

The archipelago of Madeira (Portugal) is located in the Eastern Central Atlantic and belongs to the biogeographical region of Macaronesia (Madruga et al. 2016). The aquaculture industry is an emerging activity in the island, accounting for 60% of national sea bream production. Given the physical and oceanographic features of this outermost region, and the lack of information regarding environment and ecosystem management, there is a need to characterise and update the faunal database. Therefore, the present study serves as a prior assessment to identify the epifaunal communities on offshore aquacultures structures.

Materials and methods

The study was performed in two aquaculture facilities rearing gilthead sea bream (*Sparus aurata*), located in the south coast of Madeira. Experimental units consisted in PVC plates (10x10cm) hung at the seacages, facing down to the bottom and sustained by bricks. All replicates (n=36) were submerged at -1m depth on the external ring of the floating structure, avoiding any contact with the net and surrounding ropes. Sampling was repeated monthly, by means of photographs, in order to assess the structure and dynamics of fouling communities. After four months, the remaining sets were translated to the laboratory to sort and identify macrofaunal organisms.

Results

Organisms were sorted and counted into main phyla, including arthropods, annelids, molluscs and other fauna. Preliminary results suggest significantly larger abundance of arthropods in both aquaculture operations. Major groups within arthropods were identified as amphipods, copepods, decapods, isopods, tanaids and pycnogonids. Amphipods were considered the outstanding group, since their abundance was represented by ~87% of the overall. However, non-significant differences were found between installations, even if the geographical location and oceanographic conditions at each location differ.

Discussion and conclusion

Epifaunal assemblages, namely amphipods, play an important role as trophic resources for fish populations (Vázquez-Luis et al. 2009). In this sense, amphipods respond to habitat alterations and can be used as indicators of environmental impacts (Png-Gonzalez et al. 2014). Moreover, the periodic sampling of the fouling community helps to understand the dynamics and detect further impacts (Greene and Grizzle 2007), such as aquaculture pollution (Sliskovic et al. 2011), by the presence/absence of sensitive species.

Given the different location of both aquaculture facilities (closed bay *vs.* open sea) and, therefore, the oceanographic characteristics at each location, significant differences in the fouling abundance were initially assumed. The lack of proper evidences suggests good management practices according to the fish production and the carrying capacity of the local environment.

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SALINITY REDUCTION BENEFITS EUROPEAN EEL LARVAE: A REVIEW

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Introduction

European eels (*Anguilla anguilla*) are euryhaline species that undertake a catadromous reproductive migration resulting in eel offspring naturally occurring in a hypo-osmotic environment in the ocean. Here, plasma osmolality is lower than the environment and offspring need to maintain osmotic balance through desalting processes to counteract osmotic water loss. Interestingly though, reducing salinity during early life history rearing in aquaculture results in better growth and survival (Okamura et al., 2009; Politis et al., 2018). Here, we overview three studies on effects of salinity reduction on captively produced European eel larvae, in the quest to elucidate functionality and timing of osmoregulation related processes affecting larval performance and assess optimal conditions for rearing European eel larvae.

Materials and Methods

First investigations focused on European eel offspring reared in 1L beakers throughout the yolk-sac stage. Here, larvae reared at 36 psu (control), representing the salinity occurring in the natural spawning area in the Sargasso Sea, were compared to larvae reared under conditions where salinity was decreased on 0 or 3 days post hatch (dph) and at rates of 1, 2 or 4 psu/day, resulting in seven different salinity treatments (con, 01, 02, 04, 31, 32 and 34). Secondly, at actual hatchery level, eel larvae were reared in replicated 8L Kreisel tanks connected to Recirculating Aquaculture Systems (RAS) under four different salinity reduction protocols: i) 4 psu/day step-wise (fast treatment) or ii) 24h drip-wise (slow treatment) reduction regimes as well as iii) an extreme reduction on day6 from 36 to 18 psu (drastic treatment) *vs* iv) a control treatment, where salinity was kept constant at 36 psu. As such, also the possibility of applying a more efficient salinity reduction, requiring only two stable RAS engines, was further explored. Finally, a third study investigated at which stage this salinity change should be implemented in order to counteract the high mortality observed during the first 6 days post hatch (dph). Here, larvae reared in constant 36 psu (control) were compared to larvae experiencing an extreme change in salinity from 36 to 18 psu either on day 1 (drastic 1), day 2 (drastic 2) or day 3 (drastic 3), respectively.

Results and Discussion

In all experiments, larval mortality was highest when salinity was kept at 36 psu (control), which resembles the salinity regime larvae would naturally encounter in the oceanic spawning area in the Sargasso Sea. Earliest results show that the earlier and faster salinity is reduced, the higher the larval survival. Reducing salinity, especially on 0 dph and at 4 psu (fastest reduction) resulted in reduced mortality (Fig. 1), increased body area, higher growth, reduced oil drop utilization and improved growth efficienc . However, starting salinity reduction on 3 dph can result in a more balanced mortality/deformity ratio. The applicability of the gained knowledge was then tested on an actual hatchery level. Here, larval survival was improved up to 50% when salinity was reduced slow, representing a physiologically gradual and gentle salinity reduction, for both eel larvae and the RAS biofilte . Fortunately, larval survival was equally improved when salinity was reduced fast, which represents a salinity reduction protocol in line with previous experimental findings. Considering a full-scale hatchery production, this is a more realistic and cost-efficient approach - reducing the need for dedicated RAS for each batch produced. Thus, the fast salinity reduction approach can be applied using a few RAS engines already set at the desired salinity steps, ensuring the water quality stability of each system and microbial equilibrium of each biofilte , respectively. Moreover, the larvae of the drastic treatment showed similar survival to the control, as they were also reared at 36 psu until day6, but were astonishingly able to cope with the extreme physiological change when they were then drastically moved into 50% reduced salinity. As such, a new question raised was at which stage or age this drastic change should be applied in order to counteract the high mortality observed during the first 6 dph. Here, the final study showed that the earlier the drastic salinity regime was introduced, the higher the larval survival. These results prove that a drastic salinity reduction can be applied, also resulting in 50% increased larval production by applying the most cost efficient salinity reduction, requiring only 2 stable RAS engines. Results relating to larval performance will be presented including morphological and physiological measurements substantiating insights and defining optimized rearing protocols.

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Conclusion

We conclude that salinity reduction benefits European eel larvae in terms of lower mortality and improved growth efficienc , which is likely facilitated by an energy surplus associated to lower osmoregulation demands. At the same time, we established the technically most efficient salinity reduction methodology from a hatchery cost-benefit point of view. Hence, the overall knowledge gained adds to our understanding of underlying biological mechanisms during early life history of European eel and provides a promising step towards optimized rearing conditions in the strive for sustainable aquaculture of this species.

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DOES THE DIET OF A *Holothuria tubulosa* BROODSTOCK INFLUENCE VIABILITY AND LARVAL DEVELOPMENT?

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Introduction

The majority of harvested sea cucumbers are exported to Asian seafood markets, primarily as a dried product called *beche-de-mer*. The intensive exploitation of this resource has contributed to deplete natural populations of several species (Meloni and Esposito, 2018; Purcell et al., 2018). Aquaculture production of sea cucumbers has expanded rapidly in recent years but mainly in Asian countries. In Europe, the aquaculture production of these echinoderms is non-existent, reflecting the low scientific production on the topic. In Portugal, like in Mediterranean countries, there are native species with high commercial value, which can be potential candidates for aquaculture. However, there are very few studies of larval development of European species from broodstock harvested in the natural environment (Domínguez-Godino et al., 2015; Domínguez-Godino and González-Wanguemert, 2018; Rakaj et al., 2017) and none for matured broodstocks in captivity. The control over the reproductive cycle is an important tool for rearing success, which, in turn, decreases the pressure over natural stocks. The present work studied the influence of three diets administered to *Holothuria tubulosa* Gmelin, 1971 broodstocks on the viability of larval development.

Materials and Methods

Holothuria tubulosa (N=100) were harvested in January 2018 in the coastal zone of Arrábida (38°28.011'N08°59.374'W). The sea cucumbers were randomly distributed in three tanks (N=30 per tank) in a recirculating aquaculture system (RAS) at 15.8±1°C and salinity 33±1. Afterwards, *H. tubulosa* were fed for 120 days with different diets: diet 1 - frozen microalgae mix; diet 2 - live microalgae mix; diet 3 - live microalgae and dry extruded feed. The gonadosomatic index (GI, % ± SE) was assessed at the beginning (N=10) and at the end of the trial (N=10 per diet). Histological analysis was performed to evaluate the gametogenic development stage of *H. tubulosa* fed with each diet. At the end of the trial, the spawning of *H. tubulosa* was induced by thermal stimulation, raising the water temperature by 5°C for 2 hours (Domínguez-Godino et al., 2015; Domínguez-Godino and González-Wanguemert, 2018; Rakaj et al., 2017). The larval development, from all batches, occurred at the temperature (mean ± SD) of 21±1°C and salinity (mean ± SD) of 33±1. The larvae were fed with a mix of *Isochrysis galbana* and *Phaeodactylum tricornutum* during 27 days. Daily, samples were collected to evaluate the survival and the larval development.

Results

At the beginning of the trial, the GI was not quantified because the gonads were not present. In the end of the trial the GI was higher in individuals fed with the diets 2 (9.9 ± 2.5%) and 3 (9.5 ± 2.5%) than in *H. tubulosa* fed with the diet 1 (4.1 ± 1.2%), although without statistically significant differences (p>0.05). At the end of the trial, the broodstock fed with diet 1 were immature (30%) and mature (50%). The broodstock fed with diet 2 and diet 3 were immature (50% in both batches), mature (40% e 10%, respectively) and spawning (10% in both batches). The embryonic development occurred in the first 72 h post fertilization in larvae from all the broodstocks. In the larval rearing from broodstock fed with diet 2, total mortality was observed after the onset of mid auricularia stage at day 7. The late auricularia and doliolaria stages appeared first in larval rearing from broodstock fed with diet 1 (on day 17 and 25 post fertilization, respectively) while in larval development from broodstock fed with diet 3 appeared two days later (on day 19 and 27 post fertilization, respectively). The first larvae in the pentactula stage were observed on day 27 in larval rearing from broodstock fed with diet 1 and diet 3.

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Discussion and conclusion

The histological analysis allowed evaluating the gonadal maturation stages in captivity. Broodstock fed with diet 2 and diet 3 presented *H. tubulosa* in the spawning stage while the broodstock fed with diet 1 presented only immature and mature individuals. Even so the thermal shock treatment used was an effective method that allowed obtaining larvae from the different broodstocks fed with the three diets. The larval cycle *H. tubulosa* was consistent with that of holothurians reared previously; however, the duration of the larval stages differs from other species (Domínguez-Godino et al., 2015; Domínguez-Godino and González-Wanguemert, 2018). In this study, with a controlled temperature of 21°C, the late auricularia stage was achieved 3 days earlier than previously recorded in a study regarding the same species, with a controlled temperature of 24°C (Rakaj et al., 2017). This was observed in the larval development from the broodstock fed diet 1. However, Rakaj et al. (2017) achieved the doliolaria and pentactula stages one day before us. The pentacula stage was obtained from broodstock fed with diets 1 and 3 suggesting that this species can accept different types of diets as frozen microalgae mixes or live microalgae and extruded feeds. These diets can be used for *H. tubulosa* broodstock conditioning with hatchery production purposes. Nevertheless, varying the broodstook diet can yield differences in larval survival and development of *H. tubulosa*.

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GONAD YIELD AND NUTRITIONAL QUALITY OF WILD AND ENHANCED SEA URCHIN *Paracentrotus lividus* (LAMARCK, 1816)

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Introduction

Sea urchin roe is becoming an extremely appreciated delicacy in occidental gastronomy. Besides that, it also has a high nutritional value, being rich in of protein, lipids, fatty acids (mainly ω3-PUFA), carotenoids, vitamins and minerals. The high commercial value and the continuously growing market demand are starting to cause the decline of sea urchins natural stocks in Europe, namely of *Paracentrotus lividus* (Lamarck, 1816). Thus, the interest in sea urchin aquaculture has increased in the last decade and it is possible to do gonadal enhancement. For this purpose, harvested adult sea urchins are maintained in captivity and fed with artificial diets, to improve gonad yield and quality. This study aimed to assess the gonad yield and nutritional quality of *P. lividus* maintained in captivity and fed with a jellified diet in comparison to wild individuals. This assessment was performed with different duration of the gonadal enhancement period.

Materials and Methods

Adult *P. lividus* (N=120; test diameter >35mm) were collected in intertidal rocky pools from the coast of Peniche (Portugal). Initially, 40 animals were sacrificed, in order to collect biometric data and determine their gametogenic stage through histology. The remaining sea urchins (N=80) were maintained in a recirculating aquaculture system (RAS), at ambient temperature, with a 12h photoperiod. They were fasted for a week. Afterwards, *P. lividus* were fed *ad libitum*, with a jellified diet (maize, spinach, pumpkin and agar), during two different periods: 6 weeks (Aqua1) and 12 weeks (Aqua2). At the end of these periods, sea urchins' test diameter (TD), total wet weight (TWW) and gonad wet weight (GWW) were measured, the gonadosomatic index (GI) and the gametogenic stage were evaluated, as well as the total protein (Lowry et al., 1951), lipid (Bligh and Dyer, 1959), carotenoid content (Symonds et al. 2007) and fatty acids profile (Lepage and Roy, 1986) of the gonads. The same analyses were done also to wild individuals (N=40) collected from the same original population, at the end of each gonadal enhancement period (Wild1; Wild2).

Results

Regarding somatic growth of the sea urchins, it was verified an increase in TD (Aqua1: 6.40% and Aqua2: 3.33%) and TWW (Aqua1: 18.70% and Aqua2: 8.51%). However, biometric results showed that GI was higher for wild *P. lividus* (10.17%) compared to enhanced individuals from both periods (Aqua1: 8.59%; Aqua2: 4.90%), even though Aqua1 did not present statistical differences when compared to Wild1 (Aqua1 *vs* Wild1: p-value>0.050; Aqua2 *vs* Wild2: p-value<0.001). The ideal gametogenic stage for consumption is the pre-maturation, which was achieved by half of the sea urchins in Aqua2 and only 30% of those from Aqua1, in comparison to 40% in Wild2 and 35% in Wild1. In general, biochemical composition of gonads was similar between wild and enhanced *P. lividus*, with protein content (Aqua1: 67.93; Aqua2: 83.54; Wild1: 61.39; Wild2: 106.69 $mg.g^{-1}$) higher than lipid content (Aqua1: 14.65; Aqua2: 21.67; Wild1: 12.88; Wild2: 16.02 $mg.g^{-1}$). The exception was the ω3:ω6 ratio, which was higher in wild sea urchins compared to each gonadal enhancement period (Aqua1 *vs* Wild1: p-value<0.050; Aqua2 *vs* Wild2: p-value<0.050). Comparing both periods, Aqua2 reached a higher lipid content (p-value<0.050), but a lower ω3:ω6 ratio (p-value<0.050). In terms of carotenoid content, an approximate mean value of 10mg per 100g of gonads was assessed for enhanced and wild sea urchins, without significant differences (p-value>0.050).

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Discussion and conclusion

The lower GI values obtained in enhanced sea urchins could be related with the fact that the temperature was not maintained constant during the trial. In further investigation, it should be maintained a constant temperature (13-15˚C), in order to increase gonad yield without promoting maturation (Spirlet et al., 2000). Regarding nutritional quality, the results were in accordance with other authors. The protein content was much higher than lipid content (Prato et al. 2018; Rocha et al. 2019). In terms of fatty acids profile, it was obtained a lower ω3:ω6 ratio for enhanced sea urchins, which might be related with dietary input of PUFA resulted from the jellified diet. Even so, the mean values of the ratio were always higher than the recommended value of 1 (Chow, 2007). For that, it could be concluded that wild and enhanced *P. lividus* gonads have a good lipid quality and consequently it can be considered beneficial for human nutrition. Since sea urchins are not capable of *de novo* synthesis of carotenoid pigments (Matsuno, 2001), it can be assumed that the vegetable-based diet used during the trial, which contained low (but detectable) levels of carotenoids, was capable of producing gonads with an acceptable carotenoid content and, consequently, a suitable colour.

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EFFECT OF ARTIFICIAL DIETS ON THE COLOUR AND CAROTENOID CONTENT OF THE SEA URCHIN *Paracentrotus lividus* GONADS

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Introduction

The sea urchin *Paracentrotus lividus* (Lamarck, 1816) represents an important economic resource, since its reddish-orange gonads are long considered a luxury food item, with a high nutritional content, particularly in carotenoids. Given the overexploitation of the natural stocks and the high market demand for roe throughout the year, the development of a sustainable and reliable echinoculture is mandatory. In this context, aquaculture is turning to alternative products to be used in formulated feeds, in particular land vegetable products or by-products, allowing the recycling of unprocessed agricultural discards into biomass of high commercial value. Gonads of wild *P. lividus* range from pale yellow to dark brown, both colours considered inappropriate for consumption (Symonds et al., 2007). A bright red-orange range represents the ideal colour for market acceptance and high-priced gonads, as an indication of a high-quality product (Shpigel et al., 2006). The present work studied the influence of three diets on the colour and carotenoid content of the *P. lividus* gonads.

Materials and methods

Wild *P. lividus* adult individuals were collected in Peniche, Portugal (39°22'12.7"N, 9°23'08.2"W) and were starved for 30 days. Three jellified diets were developed, using agar as binding agent: maize and spinach (diet A); maize, spinach and the macroalga *Laminaria digitata* (diet B); maize, spinach and the pumpkin *Cucurbita maxima* (diet C). These diets were given to three different groups of sea urchins for 90 days. Gender identification was made through histological analysis. For colour evaluation, 12 individuals from each diet (6 males and 6 females) were randomly selected. Total carotenoids were extracted in duplicate using acetone (10:1, v:w). Total carotenoid content was determined by UV-Vis spectrophotometry, at a wavelength of 454 nm (Guzman et al., 2010), using an extinction coefficient ($E^{1\%}_{1cm}$) of 2200 (Pocock et al., 2004). For colorimetric analyses, one gonad from each individual was photographed in controlled conditions and photos were converted to CIE L*a*b* (CIELAB colour space, defined by the International Commission on Illumination in 1976). The a* parameter was used to define the "redness" of each gonad, which is usually highly related to carotenoid concentration (Hatlen et al., 1998).

Results

Total carotenoid content in gonads was highest for diet C, considering all individuals (387.3 ± 147.0µg.g^{-1} DW) or males (382.0 ± 199.0µg.g^{-1} DW) and females (392.6 ± 88.6µg.g^{-1} DW) in separate. Diet B led to the lowest carotenoid content for the whole set of individuals (330.2 ± 85.4µg.g^{-1} DW) and for females (343.8 ± 49.4µg.g^{-1} DW), with an intermediate result for males (316.7 ± 114.8µg.g^{-1} DW). Diet A promoted an intermediate carotenoid content, regardless of gender (345.6 ± 108.5µg.g^{-1} DW), as well as for females alone (386.8 ± 72.8µg.g^{-1} DW), but registered the lowest value for males (304.3 ± 128.4µg.g^{-1} DW). In general, females presented higher total carotenoid content (374.4 ± 71.3µg.g^{-1} DW) than males (334.3 ± 147.8µg.g^{-1} DW). Regarding the gonads colour analysis, individuals fed with diet C, with an average a* parameter

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of 143.9 ± 6.8, presented more reddish gonads than diet A (139.6 ± 5.9) and B (139.4 ± 6.3). Diet C also promoted higher values than other diets, when considering only males (142.2 ± 8.9) or females (145.6 ± 3.8). Overall, females presented more reddish gonads (143.1 ± 3.6) than males (138.7 ± 8.0). A strong positive correlation (r = 0.8) was found between a* parameter levels and total carotenoid content, when considering all individuals.

Discussion and conclusion

Gonad colour in sea urchins derives mainly from carotenoid pigments (Vizzini et al., 2015), in particular, echinenone and β-carotene (Symonds et al., 2007). Increasing echinenone content is normally correlated with an intense and acceptable gonad colouration (Shpigel et al., 2006). In maize and spinach, predominant in diet A, lutein represents the major carotenoid source (Janick-Buckner et al., 1999; Bunea et al., 2008). In *Laminaria* spp., present in diet B, β-carotene only accounts for 4-6% of total carotenoids, with all-trans fucoxanthin representing 80% (Haugan and Liaaen-Jensen, 1994). The main carotenoid in pumpkin (>80%) is β-carotene, with lower levels of lutein, lycopene and α-carotene (Seo et al., 2005). These factors may partly explain why diet C was the most successful in promoting higher *a values (gonad redness) and carotenoid levels in all analyses. Since echinenone is not commercially available to be used in a formulated feed (Symonds et al., 2007) and there is the need to reduce the dependency from wild macroalgae, this study emphasized the efficacy of accessible vegetable sources, particularly pumpkin, for colour enhancement.

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SEA URCHIN LARVAE DEVELOPMENT WITH DIFFERENT MICROALGAE DIETS: IMPACTS IN GROWTH AND SETTLEMENT

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Introduction

Controlling the planktonic larval development represents one of the key bottlenecks for commercial echinoculture. Particularly, the transition from planktonic larvae to benthic juveniles, the metamorphoses, the settlement rates and survival are the most critical stages for full cycle production. For this reason, significant improvements are needed regarding the rearing of early life stages (Carbonara et al., 2018). The provision of suitable food and larval nutrition are key factors for successful larval culture. The diet selected have high impact in larval plasticity, competency, metamorphosis, development rates, deformations and mortality. Moreover, the selection of specific cues will improve settlement rates of competent larvae, decrease mortality and improve the growth of the post-settlement juveniles. The present study was carried out aiming to 1) optimize the larvae survival during rearing phase through the choice of an optimal diet; and to 2) identify of reliable metamorphosis-inducing cues to induce settlement of *Paracentrotus lividus* larvae.

Material and Methods

The larval culture methods were adapted from Castilla-Gávillan et al. (2018) and Carboni et al. (2012). Larvae were cultivated in closed aquatic systems, at a density of 4ind.mL^{-1}, using 50L conical tanks in triplicate. During the experiment, larvae were kept at a salinity of 35, an average temperature of 19.5°C, continuous light and gentle aeration. Three experimental diets were tested: 1) *Rhodomonas baltica* (diet R), 2) *Phaedactylum tricornutum* (diet P) and 3) a mixed diet of these two species (1:1; diet C). Microalgae were fed to the larvae every two days, during their exponential growth phase. The diet ration was calculated according to the larval stage of development: larvae with two, three, four pairs of arms and rudiment were fed with 1000, 3000, 6000 and 12000cells.mL^{-1}, respectively. The microalgae rations were standardized for equivalence between cell volume and number of cells for each treatment, according to the known microalgae biovolume. Larval body length (BL), body width (BW), post-oral arm length (PAL) and rudiment length (RL) were measured to characterize growth and morphology of larvae fed with different diets. Larval survival was assessed volumetrically every two days.

The settlement experiments were conducted by testing the effectiveness of Detrital Urchin Shell Particles solution (SUP), Ground Ulva Solution (GUS) and Sea Urchin Waterborne (SUW) as metamorphosis-inducing cues. The three metamorphosis-inducing cues were tested with competent larvae reared in each of the diet treatments (diet R, P and C) in a balanced orthogonal experimental design. When the rudiment was equal or larger in length than the stomach, larvae were considered to have reached competence for settlement and were submitted to settlement tests. For each tank, 30 larvae were transferred into petri dishes containing the tested metamorphosis-inducing solutions. The petri dishes containing the competent larvae were incubated at 22°C in the dark and the experiment run in triplicates. Larval settlement and metamorphosis rate were checked at 24h and 48h. Thus, attached larvae with well-developed spines and tube feet were considered properly settled.

Results

For all treatments, BL and BW showed a continuous increase until the age of competence, however in treatment R (BL: 724.74±102.88µm; BW: 664.31±128.29µm) were significantly higher than on the other diets. Diet C also showed body length and width (BL: 666.32±52.89µm; BW: 583.75±60.35µm) higher than diet P (BL: 599.26±48.57µm; BW: 583.75±57.21µm). The results also showed that for diet R and diet C, PAL increased until day 16 post-fertilization and then decreased. For diet P, the PAL increased until day 20 DPF.

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The rudiment was observed for the first time on day 14 DPF for diet R, whereas in diet C it was only observed by day 17 DPF, and finally in diet P, at day 20 DPF. The RL for diet P was significantly higher, reaching a maximum RL of 294.44±58.55µm, at day 25 DPF, when compared to the other diets. Competence was first reached by larvae fed with diet R at 20 DPF, followed by larvae fed with diet C at 23 DPF and diet P at 28 DPF, showing that the development of the rudiment was not synchronized between treatments. Larvae fed with diet P showed higher survival at the end of the experiment (17%), when compared to diets R (16%) and C (15%).

The percentages of metamorphosed larvae obtained in response to exposure to SUP, GUS, and SUW were different for larvae fed with different diets. For larvae fed with diet R, SUP showed metamorphosis rates of 46% after 24h of exposure, while diet C only 0.6% of the larvae were induced to metamorphosis. After 48h, SUP induce metamorphosis in diet P (<2%). For both diets R and P, GUS induce metamorphosis at least 7% (24h), while no metamorphosis was found in diet C (after 24h and 48h). For all diets, SUW was the cue with the lowest percentage of metamorphosed larvae (< 4%) after 24h and 48h. While, SUP produce settlement only in diet C (<2%) after 24h and 48h. GUS induce settlement for diet C at least 0,6% after 24h. No settlement was found in diet R and P for SUP and GUS. However, settlement was achieved for all diets for SUW but with a low percentage (<3%).

Discussion and conclusion

Larval survival rates obtained in this study (15-17%) were comparable with the results obtained by Castilla-Gávillan et al. (2018) (13-25%). In the present study, *P. lividus* larvae fed with the *Rhodomonas baltica* (R) diet showed better growth performances, with faster development and longer and larger larvae at 20 DPF. This difference may be explained by a high PUFA content (22:6n-3 and 20:5n-3) that is essential for larvae development (Castilla-Gávillan et al., 2018). It would be expected that *Ulva* sp. solution (GUS) would be the best metamorphosis-inducing cue, however it presented the lowest metamorphosis rates (<7%), both for the larvae of treatment R and P, which was a poor result when compared to 80% obtained for Carbonara et al. (2018). Adapting the use of an inorganic inducer of metamorphosis and settlement from Castilla-Gávillan et al. (2018), SUP showed the highest metamorphosis levels for the larvae fed with diet R. This study highlighted that the microalgae diet influences metamorphosis and settlement of *P. lividus* larvae. The low settlement rates obtained can be explained by the absence of a grazing substrate. Thus, further experiments must be carried out to optimize metamorphosis and settlement post-larvae, as well as metamorphosis-inducing solutions.

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THE EFFECT OF INORGANIC SELENIUM COMPOUNDS ON THE REPRODUCTIVE FUNCTION OF FISH

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Introduction

Among the biologically active substances with antioxidant and adaptogenic effects, which have a stimulating effect on metabolism, selenium can be distinguished. The use of selenium, which is part of a number of hormones and enzymes in the feeding of farm animals in recent years has become increasingly popular, as studies conducted in this direction in various sectors of livestock and fish farming, show positive results (Levina, 2017; Metallo , etc., 2013a, 2013b).

Selenium stimulates growth and development of animals, increases their productivity, fertility. The increase in productivity under the influence of selenium is a consequence of its adaptogenic e fect to various stresses.

Correction of selen – unprofitable states increases nonspecific resistance and normalize nitrogen metabolism, reduces morbidity.

Selenium compounds are able to protect the body from the toxic effects of heavy metals. The presence in the body of a sufficient number of substances involved in antioxidant protection is very important for fish rich in unsaturated easily oxidized fatty acids. Since selenium, vitamin E, methionine are essential nutritional factors and are not synthesized in the body, they should come with food.

An indicator of the security of the body of fish with selenium can serve as the activity of glutathione peroxidase, which it is part of. The maximum activity of glutathione peroxidase in trout plasma was found at the content of selenium in the diet in the amount of 0.15-0.38 mg/kg. Various combinations of selenium and vitamin E can prevent signs of selenium deficienc . Thus, trout has a high content of selenium (0.9 mg/kg) and a relatively small concentration of vitamin E (41mg/kg), or salmon against the background of low levels of selenium in the feed (0.1 mg/kg) increased vitamin E – 500 mg/kg. For channel catfish, the selenium level of 0.25 mg/kg was equivalent to 30 mg/kg vitamin E in feed. These norms were sufficient for normal fish growth and glutathione peroxidase activi .

In high amounts, selenium is harmful. 3- 15 mg/kg are called as toxic doses (Ostroumova, 2012). However, it is known (Lovell, 1996) that in some fish, such as trout, excess selenium is easily excreted through the gills and urine. Consequently, it does not accumulate in the body like heavy metals, but this apparently does not prevent to have a toxic effect on fish at high concentrations in the feed.

Despite the existence of a variety of information in the literature is not enough data on the impact of selenium on the physiological and biochemical processes of mature sturgeon in the reproductive period, in which there are significant hormonal changes.

Materials and methods

The research was carried out in the laboratory of aquatic bioresources and aquaculture on the basis of the experimental aquarium complex "Kagalnik" (Kagalnik, Rostov region) of the Southern scientific center of the Russian Academy of Sciences using a unique bioresource collection. The object of the study was the producers of the hybrid sterlet × beluga. Feeding of breeders was carried out with granulated feeds, introduction of selenium into the feed was carried out by irrigation, pre-diluting the drug E-selenium with water at a concentration of 1:50.

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Results

The dynamics of blood parameters was studied to assess the effect of vitamin and mineral supplements of E-selenium in the feed for sturgeon.

Hematological parameters during the entire period of the study were within normal limits. In females of the experimental group, the concentration of hemoglobin, serum protein and beta-lipoproteins at the end of the study was higher in comparison with the control group fish

Analysis of the data on the maturation of females showed that in the experimental group there was a positive dynamics of the transition to the final stages of maturity of gonads. Thus, in the experimental and control groups at the beginning of studies, only 5% of females were at the IV stage of gonad maturity. In the group of fish grown using feed with the addition of the drug E-selenium, for fish at the IV stage of maturity gonads was 30% of the total, in the control group this figure did not exceed 15 %.

Discussion and conclusion

Based on the above, it can be concluded that the use of sodium selenite in combination with vitamin E, first of all, has a positive effect on reproductive function in breeders who have a violation of lipid metabolism.

Additional studies will be conducted to better assess the effect of E-selenium on fish in the pre-spawning period

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BIOCHEMICAL ANALYSIS OF SERUM FROM AFRICAN CATFISH *Clarias gariepinus* RAISED IN BIOFLOC SYSTEMS

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Introduction

Biofloc, being new in aquaculture systems, has been found useful in recent years. Its principle involves the microbial conversion of nutrient waste in aquaculture systems (ammonia) into microbial biomass which can be reused by the cultured organisms as a food source (Crab et al., 2012). Apart from improvement in feed efficiency and reduction in the nutrient waste, studies have shown that biofloc systems improve fish immunity (Cardona et al., 2016). The study was carried out to assessed the effects of biofloc technology (BFT) application on African catfish (*Clarias gariepinus*) fingerling production with emphasis on its health status using heamatology and biochemical analysis as indices.

Materials and Methods

Two biofloc (Rice bran and Cassava flour C/N = 10) were prepared. The regular additions of organic carbon source (Rice bran and Cassava flour ratio of 10) was adopted to induce the growth of heterotrophic microbial biomass as constituent of biofloc, where no carbon sources were added is the control systems. Nine hundred (900) of *Clarias gariepinus* juveniles (8.89±0.3g) were purchased from Bond farm in Ibadan, Nigeria. Experimental fishes were divided into three groups with replicated as Control, Biofloc rice bran (BR) and Biofloc cassava (BC) respectively. Fishes were fed with 42% crude protein commercial pelleted feed at 5% body weight for 72 days. Water quality parameters in the culture tanks were monitored daily. At the end of 72 days, pooled blood serum was collected from the experimental fish for biochemical analysis. The biochemical parameters examined are Alanine phosphatase (ALP), Aspartase aminotransferase (AST), Alkaline aminotransferase (ALT), Albumin, Globulin, Total protein, Glucose and Cholesterol

Results

The results show that the biochemical parameters measured differed significantly ($p<0.05$) between the treatments (bioflucs and control). (Table 1).

Discussion and Conclusion

Aspartase aminotransferase was observed to be relatively higher in the serum of fishes reared in the control tanks than it is in the fishes raised in the biofloc system this implies that severe tissue damage must have occurred which results in more of the mitochondrial enzyme being released.Increased activities of aminotransferases indicated amplified transamination processes. This occurs due to amino acid input into the TCA cycle in order to cope with the energy crisis during toxicant-based stress (Philip et al., 1995). The significant changes in the activities of AST and ALT enzymes in blood plasma indicate tissue impairment caused by stress experienced in the control treatment (James et al., 1991; Svoboda, 2001). In this research, it was observed that the concentration of glucose in the serum of fishes raised in control tanks are higher than that of bioflocs tanks indication that biofluc improved the rearing stress. Therefore, this study revealed that fish raised in a biofloc systems are able to stand the stress of density and also revealed that fish immunity is improved through the use of biofloc

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Table 1: Biochemical composition of the serum from *Clarias gariepinus* raised in biofluc systems

	ALT	CHOL	AST	ALP
BR	0.81±0.01^{b}	121.73±3.35^{c}	1.29±0.01^{c}	1.65±0.03^{b}
BC	1.22±0.10^{a}	137.01±0.52^{b}	1.26±0.01^{b}	2.00±0.02^{a}
C	1.3±0.01^{a}	183.03±2.79^{a}	1.62±0.01^{a}	1.95±0.01^{a}
P-value	0.002	0.0001	0.0001	0.0001

	GLOB	GLUC	T. PRO	ALBU
BR	1.70±0.03^{a}	38.14±2.75^{a}	0.2±0.02^{a}	1.89±0.02^{a}
BC	1.29±0.08^{b}	31.26±0.18^{b}	0.14±0.01^{b}	1.43±0.08^{b}
C	0.63±0.02^{c}	42.66±0.09^{a}	0.10±0.01^{b}	0.74±0.02^{c}
P-value	0.0001	0.007	0.003	0.0001

Means within a row with the same superscript do not differ ($P > 0.05$). BR=Bofluc Rice bran, BC= Biofluc Cassava flour, ALT= Alanine aminotransferase AST= Aspartase aminotransferase ALBU= Albumin, ALP= Alkaline phosphatase, CHOL= Cholesterol, GLOB= Globulin, GLUC= Glucose, T. PRO= Total protein

USING SATELLITE DATA FOR ASSESSING THE RISK OF FEACAL BACTERIA CONTAMINATION IN MUSSEL FARMS

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Introduction

Earth Observation data represents an important and free source of information, which can be used in site selection of shellfish farm and AZA (Allocated Zone for Aquaculture) identification. For instance, as shown in Brigolin et al. (2017), Sea Surface Temperature (SST) and Chlorophyll a can be used for mapping potential biomass yield: these maps can be combined with other selection criteria and constraints by means of multicriteria methodologies, in order to obtain suitability maps. To this regard, mapping the risk of bacterial contamination in coastal areas in relation to the intensity and duration of precipitation can provide an important additional criterion for shellfish farm allocation. In fact, according to the current European legislation (Reg. 854/2004; Reg. 2073/2005; Reg. 2285/2015) areas where the harvesting of live bivalve molluscs is authorised are classified as type A if the product may be collected for direct human consumption. Samples of live bivalve molluscs from these areas must not exceed, in 80% of samples collected during the review period, 230 *Escherichia coli* per 100g of flesh and intravalvular liquid. The remaining 20% of samples must not exceed 700 *E. coli* per 100g of flesh and intravalvular liquid. Furthermore, the competent authority, based on a risk assessment, can decide to disregard an anomalous result exceeding the level of 700 *E. coli* per 100g of flesh and intravalvular liquid. For producers, it is therefore very relevant to operate in areas where the probability of exceeding the above threshold is very low and, should that happen, to be able to provide to competent authorities evidence that the sample was an outlier. In fact, should an A area be reclassified as B at the end of a review period, a producer would have to face depurations costs, leading to a marked decrease of profits. The aim of this study is therefore twofold: 1) to map the risk of faecal bacteria contamination related to rainfall events; 2) to include these maps as additional selection criteria and apply the Spatial Multi-Criteria Evaluation methodology for mapping the suitability of bivalve marine aquaculture (i.e.: *Mytilus galloprovincialis*) in the Veneto regions (North Adriatic Sea - Italy). This approach, if validated, besides providing a valuable input to the planning of aquaculture activities within the framework of the Maritime Spatial Planning Directive, will also allow the application of an early warning alert system, aimed at optimizing the sampling effort of the agencies to which the implementation of the above Regulations is entrusted.

Materials and methods

Faecal bacterial contamination has a terrestrial origin and is usually caused by a malfunctioning of waste water treatment plants, which may occur in presence of heavy precipitation. Ocean colour and temperature data could then be used for assessing the probability that a given location in a coastal area which, on average, is not affected by river plumes, can be reached by freshwater during such events: this probability can be taken as a proxy of the risk of faecal bacteria contamination. This methodology was tentatively applied to the coastal areas off the Veneto region, Northern Adriatic Sea, where mussel farming is a relevant economic activity. In this preliminary application, time series of the main river flow rates (*e.g.*: Po, Piave, Brenta and Adige) were analysed in order to identify discharge peaks after heavy precipitation events (95th percentile). Remotely sensed data detected just after these events were then processed for mapping Total Suspended Matter (TSM) and Sea Surface Temperature (SST). Landsat 8 images, with a resolution of 15, 30 and 100 meters, respectively for the panchromatic, multispectral and thermal bands, were used to estimate the TSM and SST. TSM maps were produced using the Gordon model (Gordon, 1988) for the Landsat 8 Band-4 (Red-Band), while for the SST analysis the Landsat 8 Thermal Bands (Band-10) was used. The satellite images were analysed using Google Earth Engine Code editor software and database, implemented with a Java-script to correct the images, masking the cloud presence over a 20% limit, and selecting the region of interest. The TSM and SST were mapped using QGIS and, in order to evaluate the river plume effects, the shellfish farms were mapped

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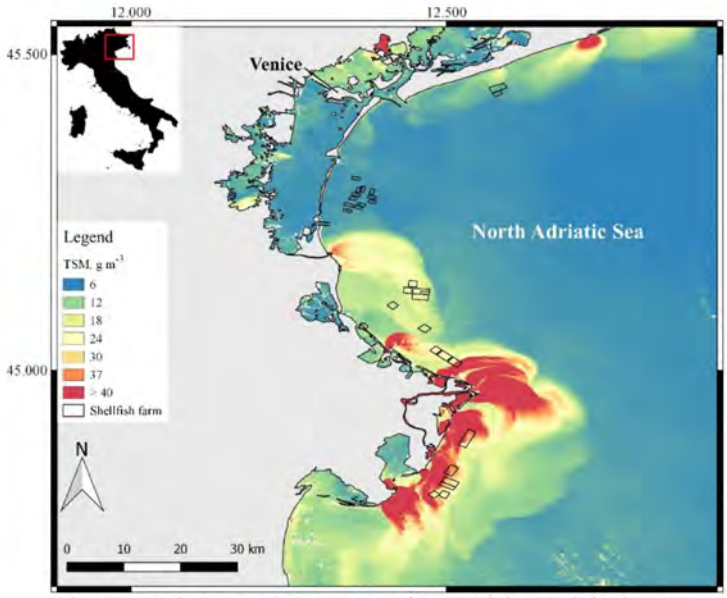

Fig 1. Total Suspended Matter after a high precipitation event (19/11/2014) in the study area.

Results and discussion

The spatial distribution of TSM 2 days after a high precipitation event occurred on 19/11/2014 is show in Fig. 1. As one can see, the river plumes reached shellfish farms located South to the Lagoon of Venice: these areas are more vulnerable to faecal bacteria contamination, with respect to the northern ones.

Based on these findings, satellite data available for the last decade will be processed, in order to estimate the frequency that a given farm is reached by a river plume. The methodology applied will be validated using data of faecal contamination collected in situ and sampled within the studied area immediately after high precipitation events.

After normalization, these frequencies will be used as proxies of faecal bacteria contamination risk. The resulting map will be added to the criteria used in Brigolin et al. (2017) in order to improve suitability maps.

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STATE-OF-ART OF AQUAPONICS PRODUCTION IN BRAZIL

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Introduction

Aquaponics is a system that integrates the productions of aquatic organisms and plants, of which the majority of the nutrients (> 50%) that contribute to vegetable growth comes from the wastes of fed aquatic organisms (Palm et al., 2018). Aquaponics represents a sustainable alternative to produce food in a more environmentally friendly manner due to the use of low volumes of water, the diversification of production and the offering of chemical-free products (fertilizers and antibiotics) (Diver and Rinehart, 2010; Hundley and Navarro, 2013; Pinho et al., 2018). In Brazil, aquaponics is still considered a new activity. No organization exists for commercial aquaponics activities or knowledge of the realities related to production. The knowledge of the economic status of aquaponics in Brazil is necessary to encourage the expansion of the activity. The characterization of the production system enables a better understanding of the activity, allowing the prediction of how this activity will evolve and identifying the distribution of the productions in the country and the time in which the aquaponics farms were established. This information can be used to identify the structures, products and processes appropriate for the particularities of each region. In addition, this information may reveal the constraints in the production process that are unknown to Brazilian aquaponic participants and may lead to improvements. This work aims to identify aquaponics production facilities in the Brazilian national territory and characterize the productions.

Material and Methods

An online questionnaire was developed using the Google Forms platform, with questions related to: producer identification; geographic distribution; major animal and vegetable species produced; monthly productions; destinations of production; motivation for production; purpose (research, subsistence, commercial, hobby etc.); amount of resources and infrastructure used. The questionnaire was made available from July to September of 2018 and distributed through social media, educational institutions, research, extension, and in all known professional platforms and discussion groups related to the topic. The data obtained were a descriptive analysis of the general characteristics of aquaponics in Brazil.

Results

The questionnaire received 41 responses, 39 of which completed the questionnaire. The groups of producers identified by the data survey are located mainly in the Southeast (35.9%) and South (30.7%) regions, with no records of this production system in the North (Figure 1A). They are inserted mostly in urban areas (64.1%), with the main purpose of subsistence (30.9%) and commercial/business (30.8%). The first aquaponics enterprise started operations in 2006 and, over a ten-year period (until 2016), there were only 16 new units of production created. However, from 2017 to 2018 there were 11 new aquaponics enterprises per year, with 56.4% being established in less than three years. Since these farms are operating for a short time, 71.8% of the properties perform some experimental trials to improve the production systems and to adapt it to their realities. The coupled production system is predominant (76.9%), the vegetable beds are mainly horizontal NFT (28.2%), semi-dry substrate (28.2%) and hybrid systems (23.1%). There were 39 groups/varieties of vegetables identified, out of which the more prominent were lettuce (84.6%), chives (51.3%), tomatoes (48.7%), arugula (35.9%), strawberry (30.7%), basil (33.3%), parsley (33.3%). There were 21 groups/species of aquatic organisms identified, consisting of the Nile tilapia (66.7%), common carp (28.2%), ornamental fishes (23.1%) and lambari *Astyanax* spp.) (23.1%).

Discussion and Conclusion

The first initiative to identify the groups working with aquaponics in Brazil was carried out between October 2015 and February 2016 by Emerenciano et al. (2016). From the publication of this article to the present day, 22 new aquaponics initiatives were created, with growing interest for commercial purposes, which have already matched those of subsistence. In other words, 61.7% of the aquaponics in Brazil is no longer for research or hobby purposes. Love et al. (2015) identified only one commercial aquaponics in Brazil, much different from the current reality. The high diversity of plant and animal species used in the systems indicate that producers seek to adapt their production for the different regional demands and characteristics, which may hamper the standardization of a technological package, since there is a need to meet the requirements of several species. However, the current scenario demonstrates the potential that aquaponics has as an integrated multi-trophic aquaculture system, to suit different realities.

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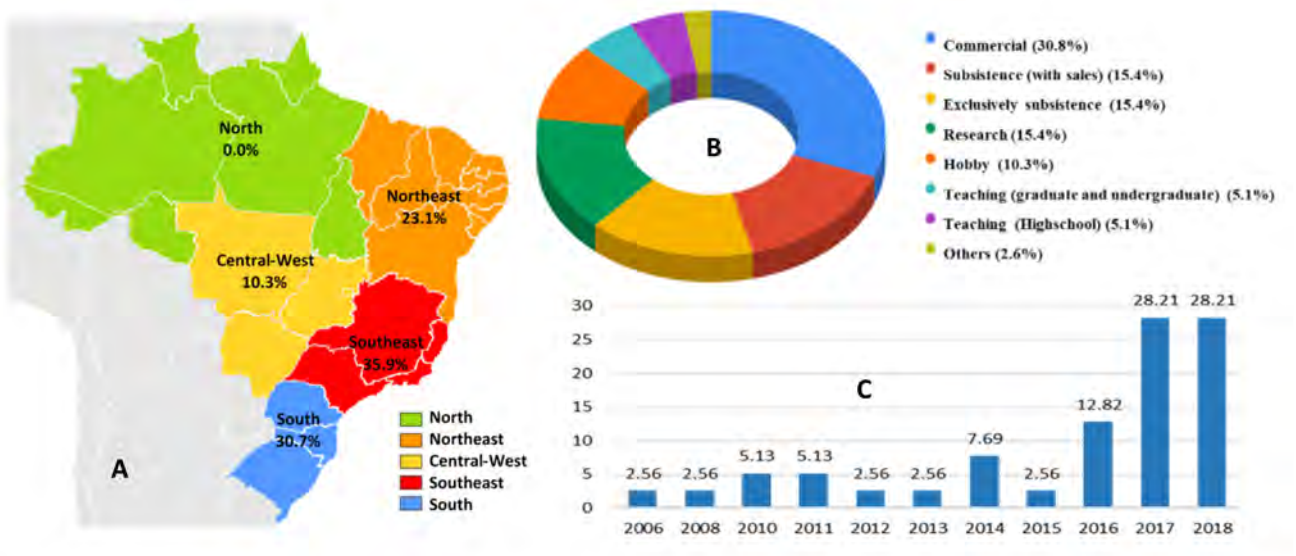

Figure 1 – Geographic distribution (A), production purpose (B) and year of beginning of the aquaponics enterprises in Brazil (C).

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OYSTER PRIMARY CELL CULTURE: DEVELOPING TOOLS TO UNDERSTAND DISEASE

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Introduction

Pacific oysters (*Crassostrea gigas*) are one of the most important aquaculture species globally. Sustainable production of oysters is hampered by outbreaks of mass mortality caused by Oyster Herpes Virus (OsHV) (Degremont et al. 2019. Understanding of OsHV pathogenesis is limited, and experimental replication of the virus requires serial passage through live oysters. This is partly due to the lack of a relevant immortalised cell line. Establishing an immortalised pacific oyster cell line is a key goal for several research fields including ecotoxicology, virology, immunology, and genetic resistance to disease (Yoshino et al. 2013). The aim of the current study was to establish primary cell cultures from multiple pacific oyster tissues using an optimised culture method, with a view to future cell culture and live oyster disease challenge experiments to better understand host response and resistance to OsHV.

Materials and methods

Live pacific oysters were obtained from a UK oyster farm. Tissues were dissected and sterilized using antibiotic and antifungal treatments under sterile conditions. Tissue explants between 2 mm and 10 mm across were transferred to cell culture well plates pre-coated with matrigel. Sterile filtered media for all cultures was prepared using Leibovitz's L15 media and artificial seawater with antifungal and antibiotic treatments. Plates were sealed and incubated at 22 ºC, and media was refreshed regularly to promote cell proliferation.

Results

Using this optimised method, axenic primary cell cultures from heart, mantle, gill, adductor muscle, gonad and hemolymph have been successfully established (e.g. Figure 1). The cell cultures demonstrate highly confluent and varied cell assemblages demonstrating that the culture technique can be successfully applied to all these tissues without modification. Successful culture of oyster muscle cells has been achieved, which has not been reported previously.

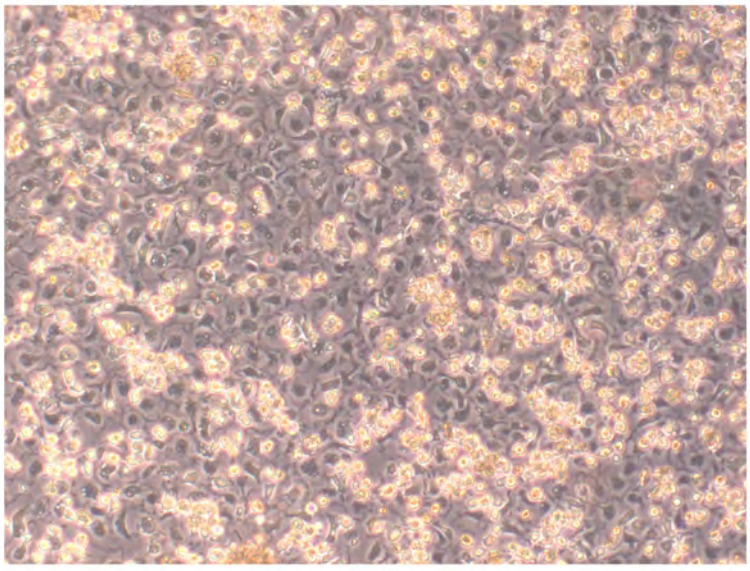

Figure 1) Primary culture of oyster heart cells with multiple morphologies and high confluency. Scale bar = 100 µm

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Discussion and future work

The methods developed have produced primary cell cultures that can potentially be used for a range of applications, including the establishment of an immortalised cell line in the future. There are multiple approaches for immortalisation, but these are generally optimised for mammalian cell culture. The immortal gastropod mollusc *Biomphalaria glabratra* cell line was established by careful maintenance and sub-culturing of primary cells (Hansen 1976), which is currently underway with the *Crassostrea gigas* lines. Furthermore, these primary cultures present an opportunity to replicate OsHV in the laboratory, which will be trialled in the future. It is anticipated that these cell cultures will provide tools to improve our understanding of host response and genetic resistance to OsHV in Pacific oysters

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Vibrio europaeus: OPPORTUNISTIC PATHOGEN FOR A WIDE RANGE OF BIVALVE SPECIES IN HATCHERY: A CASE REPORT

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Introduction

Hatcheries are nowadays the best alternative to sustain the aquaculture of bivalves, producing the seed needed, increasingly difficult to obtain from natural beds. Unfortunately, these installations are threatened by frequent episodes of larval and post-larval mortalities, which compromise the production and economic viability.

The aetiological agents of these outbreaks have been identified mainly as bacteria belonging to some species of the genus *Vibrio*, opportunistic pathogens naturally associated to the system and lethal when they proliferate under suitable conditions.

In this work, we report the recurrent detection of the pathogen *Vibrio europaeus* in a hatchery along the larval season, affecting five bivalve species cultured

Materials and methods

Different bivalve species are cultured along the year, following the procedures in Centro de Cultivos Mariños de Ribadeo (CIMA, Consellería do Mar, Xunta de Galicia): *Ensis magnus*, *Polititapes rhomboides*, *Ruditapes philippinarum*, *Ruditapes decussatus* and *Donax trunculus*. Microbiological protocols have been implemented for seawater and phytoplancton (food) circuits. A new protocol of microbiological control of larval cultures is being developed, in early stages yet.

Larvae were directly sampled from mesh sieves at the changes of seawater, using sterile loops, and spread on TCBS. Bacterial isolation, preservation and identification of isolates followed the methodologies described in Prado et al. (2014).

Results

The pathogen *Vibrio europaeus* was identified in di ferent cultures of five bivalve species along the season

In March, *V. europaeus* was simultaneously found in initial samples (2-3 days post-fertilization, dpf) of larval cultures of the sword razor *E. magnus* and the banded carpet shell *P. rhomboides*. None of the cultures survived, reaching only 13 and 17 dpf, respectively.

Two months later, the pathogen was isolated from Manila clam *R. philippinarum* larvae (3 dpf). This batch reached settlement, with 53% of larval survival.

In August, *V. europaeus* appeared associated to new species: the wedge clam *D. trunculus* and the grooved carpet shell *R. decussatus*.

In the grooved carpet shell, it was found in initial samples (5 and 1 dpf, respectively) of two batches obtained two weeks apart. Both cultures failed, though in the second one a few number of larvae completed the larval development, being later eliminated.

In the wedge shell clam, *V. europaeus* was found during the settlement of two simultaneous spawns, from broodstock with different geographical origin. Both batches were eliminated at days 34 and 62. In the first one, *V. europaeus* appeared suddenly 29 dpf and only 5 days later the culture failed completely. In the second one, it appeared in samples 31 dpf and it was detected again 36 dpf. The culture recorded heavy mortalities, being successively eliminated the sub-batches (38, 43 and 48 dpf) until the complete discard at day 62.

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Discussion and conclusion

Vibrio europaeus was present along the season in the hatchery, acting as a non-host-specific microorganism. The differences in the geographical origin of the broodstock, between and in each bivalve species, and the simultaneous emergence in different moments, pointed to the hatchery as a reservoir. This could also explain its recurrence in the installation.

Regarding to its effects, the bivalve cultures did not complete the larval/post-larval development, with the only exception of Manila clam. However, it should be noted that this bacterial species was initially isolated from episodes of mortality affecting larval cultures of *Ostrea edulis* and small spat of *R. philippinarum* in Galicia hatcheries (Prado et al., 2005; Prado et al., 2015).

Once the microbiological control of larval cultures has been established, and the batches are regularly monitored, complete information will be obtained, about the recurrence and dynamic of the pathogen in the installation, as well as the real effect of its presence in larval cultures.

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VIBRIOS IN PHYTOPLANCTON CULTURES OF A BIVALVE HATCHERY AND PERSISTENCE IN SURFACES

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Introduction

The bivalve hatchery, from a microbiological point of view, may be considered a whole, with a continuous flow of microorganisms between compartments. In many cases, the microbiota is innocuous or even beneficial/necessary for the development of cultures. However, there is a risk of proliferation of opportunistic pathogens. These are mainly bacteria of genus *Vibrio* (Prado, 2006). And, though only a few species cause mortalities in hatchery, and the genus is a common component of marine microbiota, protocols of control based on the presence of vibrios have been useful.

One of the "compartments" is the live food, the microalgae. These cultures are a direct source of bacteria, with a high and constant load of marine heterotrophic bacteria throughout the year. Vibrios may be occasionally detected, being their presence related with environmental and production factors (Dubert et al., 2015). Furthermore, high levels of vibrios seemed to be indicators of problems.

Minihatcheries are small, and relatively low cost, installations designed to obtain individuals suitable for pre-ongrowing. In this work, we present the results of microbiological control of phytoplancton cultures in a Minihatchery, the detection of vibrios and their persistence in surfaces of storage tanks.

Materials and methods

Routine microbiological control of phytoplancton is being implemented in the Minihatchery located on IGaFA (A Illa de Arousa, Galicia, NW Spain). Samples are taken weekly in different points of the circuit of production and distribution of live food. The bacteriological medium Thiosulphate-Citrate-Bile-Sucrose (TCBS) is used to detect the presence of vibrios, with the aim of preventing their transmission to bivalve cultures, as well as indicator of problems in the microalgae. Processing, bacterial isolation, preservation and identification of isolates were performed following Prado et al. (2014)

Results

Routine control showed the presence of vibrios in the phytoplancton stored in the "Harvest-tank" (HT). This tank contains the mixture of different species of microalgae from the continuous culture in 400 l. bags. The tank was cleaned, following the protocol of the installation.

In the next two weeks, the samples of HT continued to show growth in TCBS (presumptive vibrios), although in lesser numbers. The third/fourth weeks, the routine control showed a sharp increase of vibrios in HT and their distribution through the feed circuit. The bags in harvest were sampled. Cultures of *Phaeodactylum* were identified as the source and they were removed.

In the following controls, despite elimination of the sources, the samples of HT continued to show vibrios. Complementary samples of the phytoplancton, just before it reached the tank, demonstrated that the cultures were not the source. The possibility of persistence of vibrios associated to the surfaces of tanks was evaluated. In this case, TCBS contact plates, specially designed by us, were used. Samples were taken from bottom and walls of the tank.

Representative colonies from the samples were isolated and identified. The results showed that two different vibrios (type I and II), initially presented in the phytoplancton, were able to persist weeks associated to the surface of HT, even after their disappearance from the sources, the original cultures.

The Type I was related to the first episode of detection, while the Type II was found in the second episode and the *Phaeodactylum* cultures. Interestingly, both types were isolated from bottom and walls of the HT, after the elimination of vibrios from the harvested phytoplancton and the standard cleaning of the tank.

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Discussion and conclusion

The results showed the potential of phytoplancton cultures as source of vibrios in hatcheries. Moreover, the microalgae seemed not affected by these bacteria, being thus "asymptomatic carriers". If the appropriate microbiological controls are not established, the vibrios may be distributed through the installation with the risks implied.

By other hand, the persistence of vibrios in the surfaces of tanks should be taken into account. It is known that many microbes persist attached to surfaces with a structured biofilm ecosystem, where they function as an integral part of a population, obtaining ecological advantages, such as protection from desiccation (Ophir and Gutnick, 1994), or resistance to toxic substances such as antibiotics, chlorine and detergents (Costerton et al., 1987). The present study showed the persistence of vibrios, even after 6 weeks of the first detection in the case of Type I, associated to the surfaces of HT. During this period the tank had been regularly cleaned and treated with chlorine. Hence, the occasional presence of vibrios in the microalgae could lead to their recurrent distribution across the hatchery. Once again, routine microbiological protocols are imperative if we want to control the dynamic of potential pathogens in the installation.

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MORPHOLOGICAL PREDICTORS OF SLAUGHTER YIELDS USING 3D DIGITIZER AND THEIR UTILIZATION IN COMMON CARP BREEDING PROGRAM

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Introduction

Slaughter yields are traits of high economic value especially for fish species sold in the processed form. Common carp is now more and more offered as headless carcass or fillets. However, genetic improvement of slaughter yields is tricky and alternatives are either sib selection based on slaughtered fish, or indirect selection on correlated traits recorded *in vivo* (Kause et al., 2007; Haffray et al., 2013; Vandeputte et al., 2017). In a previous study we combined external (phenotyping, 2D imaging) and internal (ultrasound imagery) measurements to study their phenotypic and genetic relationship to the real slaughter yields of market size Amur mirror carp (Prchal et al., 2018). In the present study, the similar approach was applied but for external measures a 3D digitizer was used to collect body landmarks directly on the fish body, instead of 2D digitizing from images. Thus, the aim of this study was to i) determine best morphological predictors of slaughter yields using combination of 3D landmarks and ultrasound imagery, ii) estimate genetic parameters of slaughter yields predictors, and iii) predict and compare potential genetic gain based on slaughter yields predictors.

Materials and methods

The experimental stock was established by a partial factorial design of 20 dams and 40 sires of broodstock of Amur mirror carp, and 1553 progenies were assigned to their parents using 12 microsatellites. The stock was reared communally until market size under semi-intensive pond conditions. Fish were phenotyped for body weight, standard length, muscle fat content and slaughter yields (headless carcass and fillet yield). External measures were performed using 3D digitization (MicroScribe G2LX) and fifteen coordinates of morphological points were digitized. These coordinates were used to calculate lengths, heights, areas and volumes. Five internal measurements were collected through ultrasound imagery (Hospimedi LC1000, 7.5 MHz). Slaughter yields were calculated as log-log residuals of the regression with body weight, and their predictors were estimated using a multiple linear regression model including 3D external and ultrasound imagery internal morphometric variables using the reg.best function of the FactoMineR of R software. Heritabilities and genetic correlations were calculated using an animal model with software DMU with a fixed effect of sex. Genetic gains were calculated using breeder's equation (Falconer and McKay, 1996) for theoretical mass selection (MS), full-sib selection (FSS) and indirect selection (IS) with 10% and 30% proportions of selected individuals.

Results

The accuracy of the phenotypic prediction was high for headless carcass yield ($R^2 = 0.59$) and intermediate for fillet yield ($R^2 = 0.49$). Heritability of predicted slaughter yields (0.46 – 0.56) was high and highly genetically correlated with the real yields ($r_g = 0.86 – 0.88$). Besides, three individual predictors (P_1, P_2, and P_6) included in the prediction models showed high heritability estimates ($h^2 = 0.40 – 0.66$) and moderate to high genetic correlations to the slaughter yields ($r_g = |0.44 – 0.80|$). The expected genetic gain (with 10% and 30% proportion selected) per generation calculated for fillet yield using model-predicted fillet yield was 0.65% and 0.43%, respectively. The best individual predictors achieved expected genetic progress for fillet yield in range of 0.41% – 0.48% for a proportion selected of 10 % and 0.27% – 0.32% for a proportion selected of 30%.

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Discussion and conclusion

Heritability estimates of predicted slaughter yields and individual predictors using 3D landmarks were similar to previous study on common carp using 2D landmarks (Prchal et al., 2018) but higher than in other fish species (Haffray et al., 2013; Vandeputte et al., 2017). Genetic correlations of model-predicted yields and the best individual predictor (P_2) were slightly lower when compared to 2D-based predictors (Prchal et al. 2018) as well as regarding the expected genetic gains using indirect selection on predictors for fillet yield improvement. In conclusion, 3D predictors also have a solid potential for genetic improvement of slaughter yields in common carp. While such predictors are not better than 2D predictors, they are much more convenient and faster to collect in the field, as they do not imply post-processing of images. These practical aspects should be taken into account in the future carp breeding program.

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BLUE MUSSEL × GREEN MUSSEL HYBRIDS ALONG THE MOROCCAN COAST

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Introduction

The mussels *Mytilus galloprovincialis* and *Perna perna* are species of aquaculture interest distributed along the Moroccan coast. Despite interspecific hybridization is a naturally occurring process between congeneric species, barriers to hybridization are strong, numerous and often prevent the movement of genes from one species to the other (Arnold, 1996). DNA based molecular methods using polymerase chain reaction (PCR) allow nowadays the identification of those two species and their hybrids from overlapping zones. Hybrids are of upmost interest in mytiliculture for they gather properties of the parental species and are a priori genetically neutral to the environment.

Materials and methods

More than one thousand blue mussels (*M. galloprovincialis*) and green mussels (*P. perna*) were sampled from the Moroccan coast and their DNA was extracted and purified with the method FENOSALT (Pérez and Presa, 2011). Enzymatic digestions were carried out separately on genes 18S, ITS1, and ME, and reactions were incubated for 4h at 37ºC. Differentiation between both species and their hybrids was done upon diagnostic allele sizes for each species. Additional sequencing of mtDNA Cytochrome-C Oxidase subunit I gene was performed in an ABI Prism 3100 capillary sequencer using the BigDye Terminator Cycle Sequencing Standard (Applied Biosystems). Phylogeny and molecular evolutionary analyses were conducted using MEGA 5.1 software (Tamura et al., 2011) using maximum likelihood computation on 10000 bootstrap replications and applying the "General Time Reversible Model" for substitutions.

Results and discussion

Individuals belonging to genus *Mytilus* showed two bands from 18S-RFLP restrictions (68bp and 169bp) while just one band (237bp) from *Perna* mussels. Hybrids showed three bands (68bp, 169bp and 237bp). Such RFLP technique revealed 46% *M. galloprovincialis*, 33% *P. perna* and 21% hybrids from overlapping zones. The mitochondrial Cytochrome C Oxidase subunit I gene sequenced was also a powerful marker for species discrimination, i.e. 59% were *Mytilus*, 39% were *Perna*, and only 2% exhibited an unambiguous recombinant gene molecule between *Perna* and *Mytilus*. Interbreeding between *M. galloprovincialis* and *P. Perna* can take place at cytoplasmic level (mtDNA) as has also been observed at nuclear level (18S rDNA) but no mechanism explains satisfactorily the recombination of uniparentally or double uniparentally inherited mitochondrial genome of those species. Independent of mechanistic mtDNA genomic arrangements which need further hypotheses testing on in vitro crosses, hybrids can be of great interest in aquaculture for their novel properties and propagative safety.

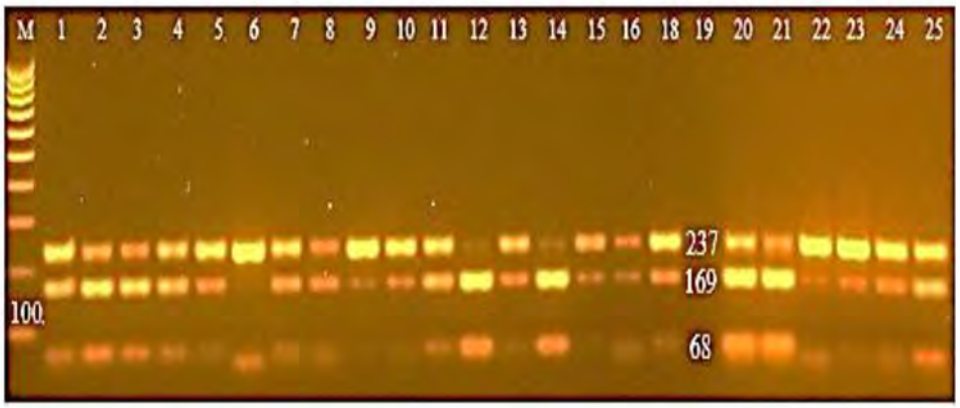

Fig. 1. Agarose gel pic showing profiles of 18S rDNA RFLP patterns on mussels from the *M. galloprovincialis* (68+169bp) - *P. perna* (237bp) overlapping zone and their hybrids (68+169-237bp).

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MOLECULAR IDENTIFICATION OF A NEW GROUPER SPECIES *Polyprion* spp FROM SOUTH AFRICA

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Introduction

The systematics of *Polyprion* spp in the Southern Hemisphere is far from resolved due to records of sympatric congeneric species. For example, early records of *Polyprion* in South Africa were assigned to *P. americanus* (Rowan and Rowan, 1955) but some evidence suggests that they might correspond to *P. oxygeneios* (Andrew et al., 1995). Preliminary distinctive mtDNA profiles and microsatellite genotypes allowed hypothesizing that a third species of *Polyprion* might exist in the Indian Ocean waters off South Africa (Ball et al., 2000). The clarification of *Polyprion* systematics is key for the management of wreckfish fisheries as well as for its aquaculture development (Matusse et al., 2016) provided its good flesh qualit , large size and high market price .

Materials and methods

Thirty-seven Cytochrome Oxidase I sequences from *Polyprion* spp. individuals sampled worldwide were obtained at CACTI facilities (University of Vigo). Maximum Likelihood phylogenetic tree (ML) were inferred upon the 37 sequences plus nine from *P. americanus* and six more from *P. oxygeneios* deposited in BOLD database. Additionally, the relationship between COI haplotypes from both species was appraised from a Neighbor-Joining tree built with the Recombination Detection Program – RDP4. The divergence time between species was inferred using a standard mtDNA-clock (Avise et al., 1998).

Results

The ML tree comprised 3 well-supported clades: one group comprised *P. americanus* from the Atlantic North, South America and the South Indian Ocean and Oceania. The second clade comprised all samples of *P. oxygeneios* and the third clade comprised all specimens from South Africa. The NJ tree based on eight COI haplotypes showed two main supported clades, one comprising three haplotypes of *P. oxygeneios* and the other one comprising four haplotypes of *P. americanus*. A specific haplotype from South African samples formed a well supported intermediate clade between the two major clades of *P. americanus* and *P. oxygeneios*.

Discussion and conclusion

While current NJ dendrogram is largely consistent with previous UPGMA on microsatellite variation (Ball et al., 2000), the position of the South African sample within the *P. americanus* cluster contrasts with its position outside *P. americanus* and *P. oxygeneios* using mtDNA COI phylogenies. Those contrasting results suggest that wreckfish specimens caught in South Africa belong to a distinct mitochondrial lineage within *Polyprion*. The support of the South African COI haplotype as phylogenetically intermediate between *P. americanus* and *P. oxygeneios* strongly suggests a hybrid origin of the South African wreckfish. Based on conventional molecular clock of 2% divergence among COI sequences per My (0.69% - 3.00% molecular fork in fishes, Avise et al., 1998), the South African *Polyprion* divergence from parental species would date back to 1.4 My (0.92My – 4.00My). Such temporal fork comprises the advent of glacial cycles and cold-water upwelling around South Africa some 2.5My ago (Shannon, 1985).

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INGESTION RATE AND NUTRITIONAL REQUIREMENTS OF JUVENILE RED SEA URCHIN Loxechinus albus FROM THE XII CHILEAN REGION

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Introduction

The Chilean red sea urchin *Loxechinus albus* (Molina 1782) is one of the world most important echinoidean species in terms of exportation and landings (Andrew y Agatsuma, 2002). Massive and viable production of juveniles is presented as the most suitable option for the recovery of the stocks, currently depleted by overfishing (Cárcamo et al., 2005). This work aims to progress on optimizing and dimensioning the production of early juveniles after metamorphosis. That aim is addressed both, through estimates of nutritional needs according to body size and by optimizing control of culture parameters regarding previous assays. A feed intake test was assayed in four body size-class urchins placed on benthonic *Nitzschia* diatoms films at 13°C and standard photoperiod (12h light) which are representative of the average latitudinal conditions in the range of *L. albus* and are also applicable to the aquaculture of the red sea urchin in the southernmost Chilean region.

Materials and methods

In order to determine the average environmental conditions in the range of the species distribution, the equinoctial photoperiod and the average value of 13ºC were chosen upon paleoclimatic and oceanographic records. Other culture conditions followed Bustos and Olave (2001). The selected food was a cultivated strain of *Nitzschia* sp. Three trials were carried out. First, a test of ingestion rate (FI) on 4 size classes (range 0.78 – 2.44 mm) was carried out to estimating daily intake upon the microalgae reduction regarding the control. Second, a quantitative (dry weight) and qualitative (bromatological) assessment was performed on the food supplied. In third place, an experiment of stabling and fattening of juveniles was enforced under the selected culture conditions to check for the system efficienc . Growth progress was assessed upon optimal health criteria (Hernández and Russell 2010) as well as upon both, the mortality rate and the effective growth of individuals.

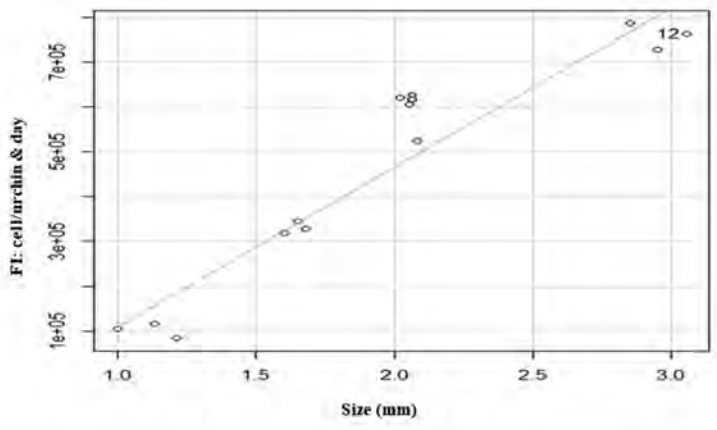

Fig. 1. Linear regression model between the cephalic perimeter and ingestion rate (FI)

(Continued on next page)

Results

Feed intake assessed per total dry weight, per nutritional component and per size-class, exhibited a linear regression model between cephalic perimeter and FI (r = 0.9175) in the size range assayed (figure 1).

Discussion and conclusion

The stabling test showed low mortality rates (<3% monthly), good health and a growth rate (GR) of 0.533mm/month, i.e. lower than that observed (0.7mm/month) on a similar body size (Zamora and Stotz 1994), a fact attributed to the use of an average range temperature during the pre-fattening period. Therefore, such environmental conditions are valid for the austral region but should be optimized upon latitude.

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IFISHIENCI: WILL INCREASED EFFICIENCY THROUGH THE USE OF BREAKTHROUGH INNOVATIONS AND PRINCIPLES OF CIRCULAR ECONOMY TRANSLATE INTO IMPROVED SUSTSAINABILITY FOR EUROPEAN AQUACULTURE?

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Introduction

The iFishIENCi project aims to contribute towards the sustainable development of European aquaculture by developing novel technologies based upon artificial intelligence and the Internet of Things. Additionally, new feed ingredients are being developed to replace the inclusion of fishmeal and oil, which are limited in supply. These novel ingredients, such as microalgae, are being produced using nutrient wastes from aquaculture as a substrate for growth; an approach in line with the EU strategy of adopting circular economic principles. However, it cannot be assumed that increases in efficiency achieved through these developments will translate into improved sustainability. For this reason, Life Cycle Assessment (LCA) is being used to explore how technological innovations developed by the iFishIENCi project may affect the sustainability of the aquaculture production species and systems to which they are applied. However, the use of LCA for aquaculture production is challenged by difficulties in comparing the sustainability of competing, alternative and eme gent species.

Method / Approach

Whereas most types of environmental assessment focus on one or only a few impacts of a specific economic activity, LCA estimates a variety of environmental impacts produced by all stages of a products value-chain. LCA plays a vital role in allowing us to identify situations where reductions in environmental impacts are achieved through innovation at the cost of increasing contributions towards other impacts. Utilising aquaculture effluent as a substrate for algal production may both reduce nutrient related impacts whilst reducing pressure upon limited fishmeal and oil resources. But doing so will introduce a new chain of economic activities, involving infrastructure production, electricity use, and possibly nutrient emissions from distal stages of the value-chain. By assessing the potential environmental impacts associated with the activities of this circular economic approach, the iFishIENCi project aims to understand if the reduction of aquaculture effluents and decreased dependence on wild fish stocks can be realised without unintended impacts in other areas of environmental concern. The project will use LCA to critically assess issues of sustainability relating to the adoption of Artificial Intelligence and the concept of 'Internet of Things': will these technological advances increase the efficiency and competitivity of European aquaculture at the expense of contributing to environmental problems such as climate change?

Clearly, the concept of sustainable aquaculture development is inextricably linked with food security (Prescott, 2017). Food security depends upon the availability of nutrition essential for good health. Thus, we need to compare the relative sustainability of different aquaculture products and systems within the context of their respective contribution to nutritional supply. To do this, the nutrient content per weight of species can be calculated. Then, the environmental impacts of producing the weight required to deliver a set quantity of nutrient content will be calculated for each species. The life cycle impacts can then be compared between different fish species produced in different systems and using various technology, within the context of food security. However, different species differ, sometimes drastically, in their nutrient profile, each containing different nutrients combined in different ratios. Thus, a variety of nutrient based units of comparison will be used for the comparative assessments.

Expected results

The LCAs will allow a critical evaluation of trade-offs between the various environmental impacts resulting from the application of technological innovations and circular economic principles, to produce several cultivated species. Comparing the environmental impacts of different products using various unit of comparison based upon nutrient content, will likely produce conflicting results. This will provide valuable insights and provoke challenging discussions about how we understand sustainability within a globally important food production sector. It should be particularly relevant for policy makers who seek to promote and shape the development of a competitive European aquaculture industry capable of delivering a sustainable contribution towards nutritional security.

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MUSSEL CULTIVATION IN THE BELGIAN NORTH SEA

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Introduction

A research project to examine the feasibility of growing mussels off the coast was initiated within the project named Edulis. The suspension culture (Danioux *et al.*, 2000; Morse and Rice, 2010) is considered for the study. The floating system consists of buoys, anchors and a long line that supports suspended ropes to which mussels are grown (figure 1). Two test setups were installed within the wind turbine park of Belgian North Sea. Bio line was installed in May 2017 to study the growth of mussel whereas Force line was installed later in November 2017 to measure forces and positions of the mussel line system. Numerical calculations were performed with a lumped-mass based open source software, namely MoorDyn (Hall and Goupee, 2015). Adaptations are made to the code, mainly to cope with 3 degree of freedoms cylindrical buoy, horizontal seabed frictions and environmental loads of wave and current. The results are validated and new proposed configuration will be proposed based on numerical calculations.

Materials and methods

A measuring device is installed in the Force line, logging the coordinates of two spar buoys at each end of the long line. Haversine formula (Van Brummelen, 2013) is used to calculate the relative positions of the buoys changing in time with South West (SW) anchor as the origin (0,0) in the Cartesian frame. On the other hand, adapted MoorDyn is used to perform numerical calculations. Tidal current of 13 hours range changing in direction, magnitude and elevation is used as the input for the simulation. The results are compared with measured buoys' positions of the same time frame as the input for the simulation.

Results

In figure 1, preliminary result shows that calculated positions as well as pattern of system displacements are comparable to those found in measured data (6% of error at the highest discrepancy of buoy's position). Both simulation and measurement logged new positions every 1 second. However, measured data shows more thickness in the plot, this is due to the wave induced load causing the system to oscillate in a higher frequency compared to the tidal current. The preliminary calculation only consider current induced load.

Discussion and conclusion

As a large portion of chains are laying down on the seabed, modelling of seabed contact gives a significant influence on the numerical model. The seabed friction model used in the simulation does not take into account soil displacement and accumulation. Simplification also made by assuming flat seabed. Model test will be conducted to obtain hydrodynamic coefficient of the mussel collector lines, which currently is based on a model test for a dropper line type (Plew, 2005). This will further improve the validation results as the drag force is mainly driving the displacement of the system. Further investigation will be conducted with wave input to compare force amplitudes. Validated software will be used to design a new proposed configuration consists of multiple mussel line systems

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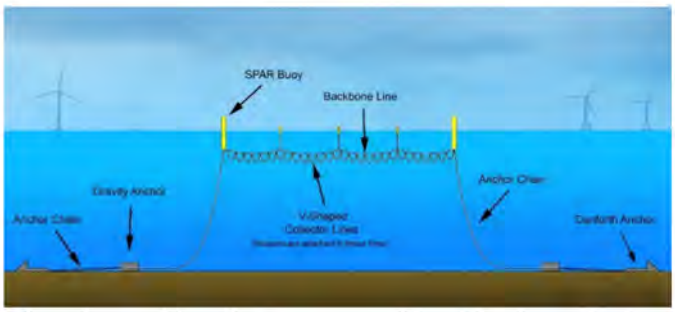

Fig. 1. Illustration of mussel longline system considered for the study (length not drawn to scale)

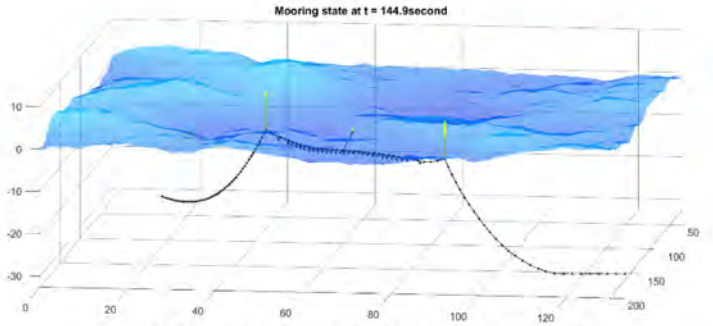

Fig. 2. Numerical model of Force line test setup

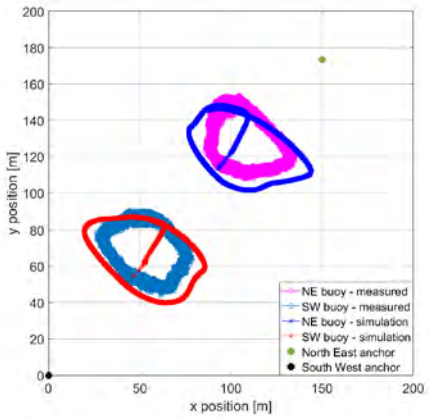

Fig. 3. Buoys' positions during one tidal cycle (13 hours range)

GROWTH IN THE MUSSEL *Mytilus galloprovincialis*, A BALANCE BETWEEN ENDOGENOUS AND ENVIRONMENTAL FACTORS

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Introduction

Growth in bivalves is influenced by both endogenous and environmental factors. It is well established that the temperature and the nutritional environment are the most influencing environmental factors in bivalves growth, whereas the interindividual differences in the growth rate in populations maintained under the same environmental conditions has a genetic basis. The physiological differences between fast (F) and slow (S) growing individuals living in the same environment relies upon a higher acquisition capacity, higher metabolic efficiency and/or lower costs of maintenance of the F specimens (Bayne et al. 1999). Recently, Tamayo et al. (2016) suggested that the environment conditions could determine the physiological differences between fast and slow growing individuals. In other words, the phenotype arising as fast grower is determined by the requirements of the living environment and the capacity of such phenotype to maximize growth. The present work aims to analyze the influence of the rearing environment in the physiological parameters determining fast growing. For that aim, mussels were reared under 5 different nutritional conditions and 2 distinct water temperatures. In addition, the gill transcriptome of F and S mussels reared in different nutritional conditions was analysed to ascertain the genetic basis of fast growing.

Materials and methods

Juvenile seeds of *Mytilus galloprovincialis* mussel were gathered from a natural population of an intertidal rocky shore. At the laboratory, mussels of similar live weight and shell length were selected and divided in seven groups of approximately 150-200 individuals. Each group was placed in a different rearing condition to test the effect of the nutritional environment and the temperature on the physiological basis of fast growing. 5 groups of mussels were maintained at 16 ºC and at different food availability conditions, as follows: i) Continuous immersion and continuous feeding of a diet dosed below the pseudofaeces threshold (1) ; ii) Continuous immersion and continuous feeding of a diet dosed above the pseudofaeces threshold (2) ; iii) Discontinuous immersion and continuous feeding of a diet dosed below the pseudofaeces threshold (3) ; iv) Discontinuous immersion and continuous feeding of a diet dosed above the pseudofaeces threshold (4) and v) Continuous immersion and discontinuous feeding of a diet dosed below the pseudofaeces threshold (5). The remaining 2 groups of mussels were reared at vi) 10 ºC (6) and vii) 20 ºC (7) under continuous food supply conditions, dosed below the pseudofaeces threshold.

After the rearing period the largest and the smallest individuals from each group were selected and denoted as fast (F) and slow (S) growers. Thus, 14 experimental groups were obtained (7 maintenance conditions * 2 growth conditions). Individuals belonging to these groups were used in a series of different feeding and thermal experiments and the physiological parameters determining the energy balance were measured. Once the experiments were performed, mussels were dissected and the gill-surface area was determined. In addition, the gill transcriptome of (1) and (2) mussel groups was analysed using a microarray.

Results

Feeding experiments showed that, when mussels were reared under good food availability conditions, faster growth is based on the significantly higher food acquisition rates and preingestive selection efficiencies of F individuals. However, when mussels were reared under severe food restriction conditions, the main physiological difference promoting a differential growth rate between F and S is the significantly lower standard metabolic rate of F individuals.

Thermal experiments showed that, in agreement with the feeding experiments, faster growth relies on the higher food acquisition rates of F mussels at both warm and cold temperatures. However, differences in the thermal compensation capacity between mussels from both rearing temperatures were found: F and S mussels reared at 20 ºC showed a similar thermal compensation capacity, but among mussels reared at 10 ºC, only F individuals were found to partially compensate the water warming effects on the physiological parameters.

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Irrespective of the maintenance condition F individuals were found to have significantly larger gill-surface areas (standardized to a common weight) than their slow growing counterparts.

Gene expression analysis revealed 117 genes differentially expressed by the fast and slow growing mussels. The F individuals overexpressed genes involved in i) growth; ii) maintenance of the structure and functioning of extracellular matrix; iii) filtration and ciliary activity; iv) aerobic metabolism and v) the immune-system. In contrast, S individuals overexpressed a different series of genes from the immune system along with genes involved in the response to cellular stress as well as anaerobic metabolism.

Discussion

The repeatedly observed existence of large interindividual differences in growth rate of juvenile mussels that were maintained in a broad variety of homogenous and stable nurture conditions indicates the existence of a strong endogenous (genetic) component in the growth rate variability usually found in the populations of the mussel *Mytilus galloprovincialis*.

The obtained results have evidenced that variations in the thermal or the nutritional regime can modify the physiological basis underpinning interindividual growth rate differences. Thus, it seems that rather than relying upon a single physiological process, interindividual differences in growth rate are caused by multiple physiological traits and that the contribution of each physiological trait to growth rate differences depends on the rearing environment.

The potential to develop a high gill-surface area is a key factor determining the interindividual growth rate differences in the mussel *Mytilus galloprovincialis*.

The transcriptomic results suggest that the gills of F mussels are well equipped to maintain higher filtering activities that enable fast growing mussels to maximize food acquisition and sustain fast growth rates. In contrast, slow growing individuals might need to spend higher rates of metabolic energy to fight against exogenous stressors, thus reducing the scope for growth of the organism.

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EFFECTS OF HUMIC SUBSTANCES ON GROWTH PERFORMANCE AND HEALTH STATUS OF JUVENILE *Clarias gariepinus* (BURCHELL, 1822)

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Introduction

Humic substances (HS), a class of compounds resulting from decomposition of organic matter, are the most common form of organic carbon (Islam et al., 2005) and comprise the majority of dissolved organic matter in freshwater ecosystems (Steinberg et al., 2004).

Recently, these substances have been used as alternative feed additives in animal husbandry practices to improve animal (dairy cows, pig, poultry, etc.) production (Islam et al., 2005). Beneficial effects of HS on growth rates, feed efficienc , health of gastro-intestinal tract, inhibition of bacterial and fungal growth, or reduced production of stress causing hormones were observed (Gao et al., 2017; Islam et al., 2005). Besides, HS are promising natural immunostimulants which may be an alternative to 'traditional' chemical therapeutants to fish (Lieke et al., 2019). However, the effects of dietary supplementation with HS have not been widely researched in aquaculture yet, and information in relation to aquatic animals is limited (Gao et al., 2017).

Therefore, the present study assessed the effect of HS feed additives on growth performance and health status of *Clarias gariepinus* (Burchell, 1822), as one of the commercially important fish species for intensive freshwater aquaculture

Material and Methods

A 56-day feeding experiment was conducted to determine the effect of dietary HS supplements (pure Siberian Leonardite containing of 80% humic acids) on growth performance and health status of juvenile *C. gariepinus*.

Fish (initial weight of 32.3±0.8g) were stocked into four separate recirculation aquaculture systems (RAS; 26.8±0.6°C, 61.3±13.4% O_2, 6.8±0.3 pH) and fed with four experimental diets (0, 1, 3, and 6% content of HS) in five repetitions (90 fish tank). Feeds (recommended ratio of 3–4%) were supplied to fish using automatic feeders twelve-times a day. Temperature, pH and oxygen concentration were checked twice a day. Each 14 days, fish growth (biomass gain, individual weight, body length) and mortality was measured in all experimental tanks (20 fish/tank).

At the end of experiment, blood and tissue samples were collected (10 fish/group) for biochemical analysis. Selected somatic and production indices were calculated. Data were statistically evaluated by the program Statistica 12.0.

Results

In the present study, growth performance including final weight (W), body size (TL) and specific growth rate (SGR) was not significantly affected ($P>0.05$) in juvenile *C. gariepinus* that were fed with different HS supplement (0, 1, 3, 6%) for 56-day period. However, coefficient of variation (CV) significantly varied among tested groups ($P<0.05$) after 56 days of rearing. The significantly highest CV (48.4±4.6) was observed in control group (0% HS) compared to other three groups (41.4±0.9) fed with HS supplements (1, 3, 6%). Condition factor (CF), hepato-somatic and splenic-somatic indices were not significantl affected with use of HS in this study. Also, mortality of fish was not significantly ($P>0.05$) affected with HS supply during whole experiment.

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Furthermore, biochemical analysis revealed no significant differences in ALT, AST, cholesterol, triglyceride, total plasma protein and LDH between groups after 28 and 56-day exposure. Only glucose level significantly differed between groups. It decreased from 6.2 to 3.9 $mmol.l^{-1}$ with higher HS feed supply (from 0 to 6%, respectively).

Discussion and Conclusion

In the present study, significantly positive effect of HS feed supplement on growth performance was not confirmed in juvenile *C. gariepinus* after two-month exposure, although other studies reported enhanced growth (Gao et al., 2017; Islam et al., 2005).

Generally, glucose is one of the most common stress indicators. Present results indicated that probably more-stress resistant fish were in HS supplemented groups, compared to control group where glucose levels were significantly higher. Meinelt et al. (2008) explained that, although HS are not too strong as 'traditional' therapeutics, they may show advantages in repairing secondary, stress induced damages in fish

Further research on longer exposure to HS feed supplements may reveal other, or deepen current, positive effects of HS on *C. gariepinus*. Therefore, we decided to prolong this experiment until fish will achieve market size, and evaluate also immunology, gut microbiology, mid-intestine histology, and sensory analysis as they may provide new information on the effect of HS on fish.

Acknowledgement

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CITYFOOD: ASSESSING AQUAPONICS WITHIN THE URBAN FOOD-WATER-ENERGY NEXUS

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Introduction

The world's cities are growing - by 2050, 68% of the global population will reside in cities (United Nations, 2018). By the same time, global food demand is projected to increase by 59-98% compared to 2005 (Valin et al., 2014). Combined with growing concerns about the destructive effects of climate change on wildfish capture and the significant role of the agricultural sector in perpetuating resource depletion, these figures paint a daunting outlook on impending urban food insecurity. Conventional food supply networks exert pressure on energy and water sectors through increasing demand for irrigation, storage, processing, transport and operating industrial equipment.

Aquaponics has been recognized as one of "ten technologies which could change our lives" by merit of its potential to revolutionize how we feed urban populations (Van Woensel et al., 2015). Funded through the Sustainable Urbanisation Global Initiative/ Food-Water-Energy Nexus call, the project CITYFOOD addresses the interdependence of urban food, water, and energy systems by evaluating the potential of aquaponic farms for urban integration and as a sustainable solution.

This investigation connects concepts of the Food-Water-Energy Nexus framework with best practices in aquaponics to evaluate aquaponic farms' potential performance in cities based on case study research.

Aquaponics as a food source

Aquaponics provides a protein source (fish) alongside fresh produce to satisfy diverse urban dietary needs regardless of climate. Urban aquaponic farms can compete with imported perishable vegetables and fish year-round without the need for pesticides and reduced fertilizer use.

For urban aquaponic farmers, two major benefits of urban locations are a growing consumer market with an interest in fresh, high-quality, locally grown produce and the reduced need for transportation. Within inland cities that rely on imported seafood to meet inhabitants' needs, aquaponic farms can provide a fresh alternative on the market, and achieve premium pricing for locally-grown status (Quagrainie et al., 2018). Additionally, urban farms may alleviate food insecurity in vulnerable populations; for example, from 1993 to 2018, Will Allen's Growing Power has famously provided access to fresh food and education to underserved communities in the Milwaukee area.

Aquaponics within the urban water cycle

In aquaponics, the use of recirculating water infrastructure reduces overall water consumption for the production of both fish and vegetables. Situated in cities, aquaponic farms are well-poised to take advantage of alternative water sources to increase water efficiency even further, using rainwater harvested from rooftops and foundation drain water. As an added benefit, aquaponically-grown produce strives to close the nutrient cycle, thereby reducing the production of nitrogen-rich run-off from aquaculture. Through smart resource management within major environmental systems, aquaponic systems may avoid excessive water consumption and eutrophication usually created by industrial agriculture.

Aquaponics and energy supply

Urban aquaponic farms can save operational energy demand by reducing transportation distance to the consumer and reducing the need for cold storage. Due to the heat island effect, urban environmental conditions can also be advantageous for aquaponic farms in cold climates - average temperatures in cities are higher than in rural surroundings, therefore reducing the demand for space heating during colder months. Aquaponic farms that are integrated with the building systems of a host building can further utilize urban resources such as waste heat and CO_2 in the exhaust air to benefit the growth of plants as an alternative to conventional CO_2 fertilization.

Energy demand is one of the most pressing challenges for urban aquaponics, since the mechanized nature of commercial-scale aquaponics requires energy for heating, cooling, ventilating, pumping, and illuminating the space.

(Continued on next page)

Results

The results of the food-water-energy analysis described above can be used to holistically juxtapose the environmental impacts of urban aquaponics and rural aquaculture. Based on this comparison within the FWE Nexus framework, CITYFOOD aims to identify synergies unique to the city that can improve the environmental sustainability of urban aquaponic farms.

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BUILDING-INTEGRATED AQUAPONICS: ARCHITECTURAL DESIGN CONSIDERATIONS FOR FUTURE FARMS

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Introduction

Techniques to extend the natural crop-growing season range from temporary modifications in soil-based fields to full environmental control in permanent facilities that allow year-round production. The latter strategy is also known as controlled environment agriculture (CEA) and includes greenhouses and indoor growing facilities. Most aquaponic farms are conceived as CEA since they combine fish with produce, which both require consistent growing conditions and protection from pathogens to achieve optimal yields. Additionally, CEA enables aquaponic farms to amortize high investment by providing produce to the market outside of the natural growing season.

The relationship between the aquaponic system and the surrounding envelope has the potential to be beneficial for both - an aquaponic farm can improve host building performance, while a well-designed envelope can raise farm productivity (Alsanius et al., 2017; Sanjuan-Delmás et al., 2018). Currently, aquaponic studies often omit the host building; however, including the enclosure is essential to evaluating aquaponic farms in temperate and colder climates. Understanding that the contribution of the building sector to global environmental impacts is comparable in magnitude to the agricultural sector, the deliberate architectural design for aquaponic farms becomes a critical opportunity to reimagine sustainable CEA.

Urban aquaponic farms can be designed from the built environment perspective to optimize multiple resource flows at once - primarily, the exchange of heat, CO_2, and water between the system and the enclosure (see Fig. 1). Designing aquaponic facilities with respect to the local climate and seasonal swings can create productive and sustainable farms that minimize dependence on fossil fuels (Graamans et al., 2018).

Methodology

The research team has compiled a database of 273 aquaponic farms using self-published documentation from practitioners and has developed a taxonomy of five enclosure types - passive solar greenhouses, medium-tech greenhouses, high-tech greenhouses, rooftop greenhouses, and plant factories. Each farm type is characterized by its reliance on climate control systems and envelope opacity.

The team has also selected nine plant factory case studies to analyze aquaponic and hydroponic farming through the lens of spatial arrangement (see Fig. 2).

Results

Analyzing the contextual conditions where each farm type is present, the team has found that indoor growing spaces are only favored by farms located in climates with greater seasonal variability, where the trade-off between better insulation to establish indoor air temperature and the need for artificial lighting is profitable. In almost all cases, greenhouses are preferred, signifying that using light and heat from the sun is still more sustainable and profitable than relying on artificial lighting arrays.

Using case study analysis, this project will lead to a series of spatial design guidelines with the recommendations for (1) the selection of enclosure types, (2) efficiencies of layouts of different enclosures, and (3) programmatic layout and space allocations for the different functional components of an aquaponic farm.

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GLOBAL AQUAPONIC PRACTITIONER SURVEY: PRELIMINARY FINDINGS AND TRENDS

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Introduction

Aquaponics is catching on; many farms have opened their doors since 2013, the year of the last international survey of the field (Love et al., 2014). The Global Aquaponic Practitioner Survey has been launched by the CITYFOOD team in April 2019 to assess the state of the aquaponic field. Using information gathered in this survey, the research team aims to connect and empower aquaponic farmers, researchers, and decision-makers worldwide.

The international project CITYFOOD investigates how aquaponics can be integrated into urban food, water, and energy networks to satisfy growing food demand and minimize the environmental impact of food. A preliminary search for aquaponic farms conducted by the CITYFOOD team at the University of Washington in July 2018 shows that the number of aquaponic operations has rapidly increased over the last six years. This focused online search for aquaponic operations identified 197 active aquaponic farms and research labs worldwide (see Fig. 1). 4 out of every 5 farms in this cohort have opened their doors in 2013 or later; only 39 aquaponic farms have been in operation for more than six years.

The Global Aquaponic Practitioner Survey (GAPS) is meant to follow up on earlier surveys of the field, while recognizing that aquaponic farming is gradually scaling up as interest in commercial urban agriculture grows (Love et al., 2014; Mchunu et al., 2018; Villarroel et al., 2016).

Methodology

The GAPS is structured as two branches of a single survey - one meant for commercial aquaponic farmers, and another for researchers in the field. Many of the questions overlap to maintain comparability between labs and farms. Unlike previous surveys, the GAPS gives more focus to built environment infrastructure that houses most farms - asking respondents to identify their building type, relative footprints of farm components, and climate control systems.

The GAPS shifts focus away from aquaponic hobbyists, and towards commercial-scale facilities. To reflect this shift in focus compared to earlier surveys, the GAPS gathers minimal demographic information about the respondent but increases the number of questions about farm equipment, infrastructure, initial design, and regulatory barriers.

The CITYFOOD team has used REDCap to program and administer the survey online. Snowball sampling was used - each respondent was asked to distribute the survey to colleagues within their aquaponic network.

Fig. 1 Aquaponic operations in the United States, identified by online search in July 2018 (red = for-profit, blue = research labs, green = non-profit and hobby operations).

(Continued on next page)

Results

The GAPS is scheduled to run until June 2019, at which point all responses will be consolidated and analyzed using Tableau Prep and Tableau by the CITYFOOD team at the University of Washington. Results will indicate the current scale of aquaponic farming worldwide, as well as existing preferences for various systems and farm configurations among practitioners that can inform further research. The regulatory barriers identified by the respondents will inform further advocacy work for shaping aquaponic policy. Additionally, the GAPS will be used to create a searchable Global Aquaponic Directory for public use.

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MICROBIAL COMMUNITIES IN BIOFLOC SYSTEMS FOR WHITELEG SHRIMP (*Litopenaeus vannamei*)

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Introduction

The global demand for aquatic food products is steadily increasing. As capture fishery production is not expected to increase further, the demand in aquatic foods must be supplied by aquaculture. With key resources for aquaculture production (e.g. water, feeds, and land) being limited, growth of aquaculture production must mainly be realized by intensification and a more efficien use of key resources (Avnimelech 2015). The biofloc-technology is a novel kind of aquaculture for the production of fish and crustaceans with minimal water exchange. Supplemental input of a carbon source (e.g. glucose) as energy source and high aeration creates a highly active microbial milieu with heterotrophic bacteria in the culture volumes (Avnimelech 1999). These bacteria are binding excreted nutrients (especially nitrogen and phosphorus) and form bioflocs which are submerged in the water and can be taken up by the cultured fish or shrimps species as an additional feed resource (Crab et al. 2010; Crab et al. 2012). Consequently, biofloc systems are described as nutrient efficient and water saving systems (Avnimelech 1999; Emerenciano et al. 2017).

But, the food production in such a highly active microbial milieu leads to the question of the hygienic conditions in these systems and whether the conditions within biofloc systems are enabling potential human pathogenic bacteria to establish and pose a food safety hazard to the consumers. Within this study, the focus laid on the production of Whiteleg shrimp (*Litopenaeus vannamei*) in biofloc systems as these systems are highly intensified and low level of postharvest processing of shrimp are common (e.g. no removal of head, legs and viscera; inadequate heating before consumption).

Materials and methods

A literature study was performed by screening the databases for scientific papers on the microbial communities in different, globally applied biofloc systems for the production of Whiteleg shrimp (*Litopenaeus vannamei*). Further, the methods for describing the biofloc microbiome were collected and grouped for their level of data quality. Special emphasis was put on the occurrence of potentially human pathogenic bacteria. In summary, about 122 scientific reports were reviewed.

Results and discussion

Globally, different biofloc systems are in use for the production of Whiteleg shrimp. These systems range from highly intensive indoor tanks to outdoor ponds (Avnimelech 1999; Avnimelech 2015). Further, they differ in the use of quantities and kind of organic carbon sources, the kind of aeration (gassing or mechanic circulation of water), water exchange rates, pre-treatment of water, removal of sludge, feeding regime etc. For the biofloc culture of Whiteleg shrimp, the amounts of feed and carbon source are adjusted to a C/N ratio of 20:1 (Emerenciano et al. 2017). To establish the biofloc systems, inoculation with water from other running systems or with a specific starter microbiome are often performed.

Among reviewed publications, a limited number of studies (38) report about bacteria in terms of abundance and/or community description, with 16 studies alone recording the presence of *Vibrio* spp.. Only very few of these publications (7) are determining whether certain families or species are present. Generally, reports about bacterial species composition are rather focusing on shrimp pathogens and less on the identificat on of potential human pathogens. The identified bacterial communities show high diversity, within and between systems, which might change over time as the feeding rate and consequently also the addition of carbon sources increase. The transfer of pathogens into the production sites via water, wind, and animals can often not be excluded.

The methods to describe the different microbial communities in biofloc systems are ranging from cultivation methods, microscopy, and molecular biology techniques to genomic methods. The microbiomes are generally determined down to the level of phyla.

(Continued on next page)

Conclusion

The presence of human pathogens in biofloc systems is described in several publications. But scientific assessments of the safety of the products of different biofloc systems are scarce. The establishment of human pathogens under such conditions has not been systematically assessed, yet. Further research is needed to assess the risk of microbial contamination with human pathogens in these systems by using highly sensitive and specific methods for their detection. To describe the microbiome and the presence of potential human/shrimp pathogens, it is recommended to use metagenome analysis applying next-generation sequencing techniques which may be followed by specific PCR methods for verification. The knowledge about the dynamics of potential human pathogens in biofloc systems is essential to develop best management practices to proactively mitigate and control these food safety hazards.

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BACTERIAL COMMUNITIES OF DIFFERENT BIOFILMS IN FRESHWATER AND SYNTHETIC SALT WATER RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

In recirculating aquaculture systems (RAS), understanding of bacteria-bacteria interactions and bacteria-fish interactions is essential for developing and optimizing practices that support fish health and economical operation of the system. Stable bacterial communities can carry out the nitrification process and maintain the balance between beneficial and the harmful strains (Rurangwa and Verdegem, 2015). Recent advantages of next-generation sequencing have made it possible to efficiently explore the genetic material associated to environmental samples, enabling characterization of the whole microbiomes. In this study, we examined bacterial community structures in the start-up phase of freshwater and salt water RAS. Our objective was to study the development the bacterial communities in different compartments of the freshwater RAS system using the 16S rRNA gene sequencing, and compare the freshwater RAS to an otherwise identical system where the water source was supplemented with 7 ppt salt.

Materials and methods

Two separate experimental scale RAS units were used, each consisting of a 500 l fish tank, waste feed collector, swirl separator, 60 µm drum filte , 125 l fixed bed bioreactor, 125 l moving bed bioreactor, trickling filter and a pump sump. Units were disinfected before the experiment using sodium hydroxide. Rainbow trout (*Oncorhynchus mykiss*) were stocked to both units (24 kg, average weight 249 g). Fish were fed constantly 240 g per day (Biomar Orbit 929) with constant make-up water of 120 l per day. Excess fish were removed weekly. Microbial samples were collected from the circulating water and biofilms from the fixed bed bioreactor, moving bed bioreactor, trickling filter and fish tank surface. The samples were collected eight times during the 23 week start-up period. DNA was extracted using commercial kits and sequencing was performed from the V4 location of the 16S rRNA gene using Ion PGM (Ion Torrent, Thermo Fisher Scientific). Water quality parameters were monitored using online probes and manual water sampling. The RAS units and online water quality monitoring system has been described more detailed by Pulkkinen et al. (2018).

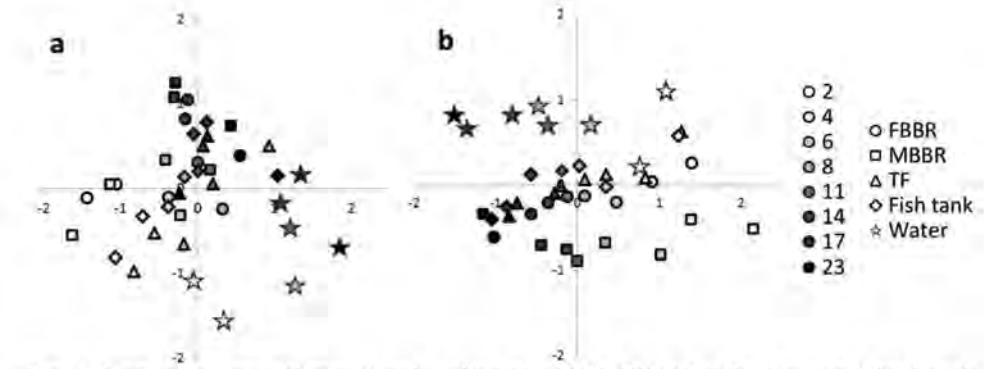

Fig. 1. Non-metric multidimensional scaling plots (NMDS) of a) fresh water RAS b) salt water RAS based on Bray-Curtis dissimilarities. Numbers refer to sampling weeks. FBBR = fixed bed bioreactor, MBBR = moving bed bioreactor, TF = trickling filter, Water = circulating water from the fish tank outlet.

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Results and discussion

Bacterial communities were different between freshwater system and salt water system, as well as between circulating water and biofilm samples (Fig 1.). The most distinct genus separating freshwater and salt water RAS was *Polynucleobacter*, which was more abundant in freshwater system. On the other hand, *Luteolibacter* was more abundant in the salt water system. In salt water, moving bed bioreactor had different communities than the other biofilm samples. Some water quality parameters may have affected the fluctuation of communities in freshwater RAS, whereas salt was a strong factor affecting the salt water RAS communities.

Nitrosomonas was the most abundant nitrifying bacterial genus in the salt water RAS. *Nitrospira*, typically classified as a nitrite-oxidizing bacteria were dominant nitrifying bacteria in the freshwater RAS. Low amount of ammonia-oxidizing bacteria might indicate that some species of *Nitrospira* observed in this system might have also ammonia-oxidizing potential (comammox) (Bartelme et al., 2017).

In the start-up phase of the freshwater RAS, development of characteristic bacterial took over 8 weeks, which should be taken into consideration when studying bacterial communities in RAS experiments.

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BLOOD BIOCHEMISTRY DURING SEAWATER TRANSFER: INVESTIGATION INTO THE CAUSES OF FAILED FISH SYNDROME IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

The term failed fish (or runt) syndrome is a synonym for a fish with impaired growth or substantially slower growth rate after sea-transfer (Skjelstad and Gu, 2016). Although observed throughout salmonid aquaculture, (typically ~10%) it is greatly increased for rainbow trout (*Oncorhynchus mykiss*) reared in full salinity where failure levels of >30% are often encountered. Rapid acclimation to increasing salinity through physiological modifications is crucial in euryhaline teleost (Guner et al., 2006). Thus, this study objective was to compare the clinical chemistry and tissue structure differences in rainbow trout pre and post seawater transfer to investigate the impacts and potential causes of this syndrome.

Materials and methods

The study took place in a fish farm, under aquaculture conditions. Blood samples were initially taken from random pit-tagged fish 4 weeks before seawater transfer. Second blood samples were taken 8 weeks post seawater transfer. Fish condition was measured and tissue samples were taken for supportive histopathological analysis in both time points. All serum samples were analysed within 3 months of freezing on the clinical chemistry analyser Daytona RX (Randox Laboratories Ltd., Crumlin, UK) using commercial kits. Routine histology was used to produce tissue sections for histological inpection of trout tissues. Statistical analysis was performed using the General Linear Model (GLM) and Linear Discriminant Analysis (LDA) to classify the two groups according to their biochemical profiles

Results

Fish serum biochemical analysis showed significant increase activity ($p<0.05$) of ALP (alkaline phosphatase) and concentration of ammonia, recorded in the fish with good condition after the transfer to seawater. In comparison, fish with retarded growth had significantly higher activity of LDH (lactate dehydrogenase), together with decreased activity of amylase and concentrations of potassium, while concentration of chloride was higher after the transfer to seawater. Interestingly, despite no statistical differences could be found for the majority of measured endpoints in freshwater environment, it is noteworthy that the parameters with potential effect on osmoregulatory balance and nutritional deficit, chloride, sodium and zinc concentrations, respectively, were identified as the variables that distinguish best these two groups at initial stage of growth. Moreover, no significant differences in fish condition were observed. Histopathological evaluation of stunted fish liver showed hepatocyte shrinkage along with marked glycogen reduction. Furthermore, tissue damage to the eye chamber was evident in form of exfoliation of cornea.

Discussion and conclusion

Results of the present study indicate that initial fish size does not have any particular role in predisposition to runt syndrome. Runt fish showed signs of poor nutritional status that was observed through serum metabolic endpoints, but also some evidence of osmoregulatory dysfunction. Moreover, there was a proof that fish from the freshwater, that later became failed in seawater, had disturbed osmoregulatory function and zinc deficienc . Cataracts induced by zinc deficiencies have been described wild and farmed salmon (Bjerkås et al., 2004). A study by Bjerkås and Sveier (2014) demonstrated that fluctuation in water salinity caused higher intraocular pressure. This could be due either to increased influx through the damaged cornea, or as increased water uptake in the whole body due to poor fluid regulation through periods of osmotic stress. In addition, another possible cause for this syndrome in trout could be reduced vision due to cataracts that prevents normal feeding and eventually led to trout emaciation and mortalities. Blood biochemistry shows excellent potential to investigate fish health status and was associated with organ/tissue damage. Further research to evaluate the genetic aspect of this syndrome and better bloodstock selection could add to improvements in sea trout aquaculture.

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A MINI REVIEW ON HIGH PRESSURE PROCESSING OF FISH AND FISHERY PRODUCTS: ALTERNATIVE TECHNOLOGY TO THERMAL PROCESSING

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High pressure Processing is a non-thermal process capable of inactivating and eliminating pathogenic and food spoilage microorganisms. Nowadays consumers have a growing demand for convenience food of highest quality in terms of natural flavour and taste, which are free from additives and preservatives. This demand triggered the need for the development of non-thermal approaches to food processing. Among other technology, HPP has proven to be very valuable. Pressure range between 300-600 Mpa inactivates yeast, mould and most vegetative bacteria including infectious food born pathogen and spoilage microorganisms. Thus, pressure leaves small molecule such as many flavour compounds and vitamins intact. Therefore, HPP treated food is very similar to that of fresh food products. Herein, the mini review article discuss the basic principle of HPP, pressure temperature effect, operation of the equipment, effects of HPP on microbial food safety and molecular biology. Specific opportunities are discussed that a food processor can consider to implement this new processing technology to increase their profit and product qualit .

Introduction:

High pressure processing (HPP) is one the best innovation in food processing sector. Its influence on microbial inactivation has been recognized since late 1800s and in 1900 the first commercial installation of HPP occurred. Consumers have a growing preference for minimally processed extended shelf life foods which are convenient, healthy and fresh (Aymerich et al., 2000). The sensory and nutritional characteristics of foods are best retained by HPP over thermal sterilization and pasteurization but it is currently more expensive to commercialize (Balasubramaniam et al., 2008). The use of the HPP offers novel gateway for food industry to fulfil the demand of consumers

In HPP, the intense pressure ranging between 100 – 1000 Mpa accompanied with or without heat which inactivates vegetative, pathogenic and spoilage microorganisms effectively. This processing method is used for liquid and high moisture content solid foods (Balny and Masson., 1993).

Basic principle of HPP:

According to Le – Chatelier principle: In any reaction, structural change and phase transition is accompanied by decrease in the volume by enhancement of pressure (Cheftel, 1995). The physical compression in food by pressure treatment results in volume reduction and increase in temperature and energy within food products which is sufficient to destroy the microorganisms. Since transmission of pressure is not time and mass dependent, the products are independently compressed of product size.

Fig 1: Represents high pressure processing equipment

(Continued on next page)

Pressure – Temperature effect:

At room temperature, for every increase in 100 Mpa, there is a 3^{o}C increase in the water temperature. Fat and oil in the food have a heat of compression value 100 Mpa at 8-9^{0}C and Protein, carbohydrates have intermediate compression values. The pressure and duration of HPP are the key factors of microbial inactivation. The pressure resistance of microorganisms is maximum at temperature 15-30^{0}C and significantly decrease at igher or lower temperature (Patazca et al., 2007).

Conclusion:

High pressure processing develops new "minimally" treated foods with high nutritional and sensory quality with increased shelf life. HPP is only alternative technology that has reached consumer with variety of new products. The main barrier for its commercialization is high cost. These can be overcome by research and studies to fill the gaps and to fully understand the process to reduce the production cost, which will eventually leads to reduction in the capital and operation cost.

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STUDY ON THE APPLICATION OF HURDLES IN POST HARVEST PRESERVATION

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Hurdle technology was developed as a new concept for the production of safe, stable, nutritious, tasty and economical foods. The basic principle is to combine different preservation technique in order to achieve multitarget, mild but reliable preservation effects. Nowadays, consumers interest on fresh like foods which can be made by applying mild preservation techniques. Mild preservation is achieved by combining already existing traditional or novel preservation techniques with its least intensity.

Hurdle technology also called barrier technology. Each preservative factor is considered as hurdle with which microorganisms present should not be able to overcome. Commonly used hurdles are temperature, water activity (a_w), pH, redox potential, preservatives and so on. If higher the hurdles, greater influenc on microbial stability and contributes to safety of food. This concept is used unconsciously in many traditional foods. But it was re-invented some 15 years ago in food industry to design effective process for different food with minimum changes in sensory attributes.

Types of hurdles for food preservation:

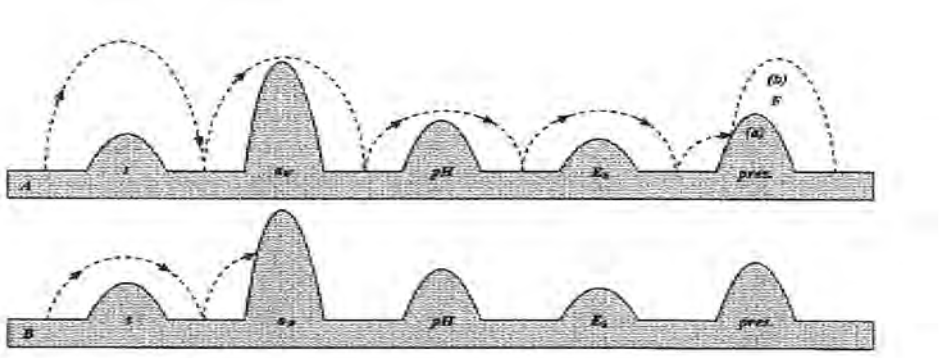
Types of hurdles	Examples
Physical hurdles	Electromagnetic energy(microwave, radiation) High temperature(blanching, pasteurization, baking) Low temperature (chilling, freezing) Packaging(active and vacuum packaging, edible film)
Physical non thermal hurdles	High hydrostatic pressure pulsed electric field and pulsed light.
Physicochemical hurdles	Co_2,O_2, O_3, Ethanol, Lactic acid, Lacto peroxidase, Low pH, Low a_w, Maillard reaction, Smoking, Nitrite/Nitrate, Sulphite and Spices
Microbiological hurdles	Competitive flora, Protective cultures and Microbial products

Mode of action:
Hurdle technology is a critical concept of mild preservation of foods. Hurdles will control microbial spoilage and food poisoning, leaving desired fermentation processes unaffected. Individual hurdles applied at lower intensities acts "synergistic" with one another in order to achieve safety and also maintains the sensory, nutritive and economic attributes of a food.

Fig.1 illustrates food containing six hurdles high temperature during processing, low temperature during storage, low water activity, acidity (pH), low redox potential (Eh) as well as preservatives. Some microorganisms can overcome a number of hurdles but none can jump over all hurdles. Finally food is stable and safe for long duration in theoretical cases.

Fig.2 illustrates hurdles are applied at different intensity like water activity and preservatives are main hurdles whereas pH, storage temperature, redox potential are minor hurdles. Intensity usage of individual hurdle is greatly reduced while combining preservative techniques. The number of hurdle application directly influence of product quality and safet .

(Continued on next page)



Conclusion:

Researchers have growing interest in designing and application of hurdle technology in food preservation. It is expected that this development will proceed in near future. Certain works on new hurdles like antimicrobial agents from plants, active packaging, processing technologies such as ultra-high pressure, high electric field pulses etc. paves the way for new invention in hurdle technology. This technology minimally affects sensory attributes of food. Innovation in hurdle technologies offer wide benefits to food industry with respect to increased product quality and assured safet .

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SHORT SUPPLY CHAINS OF THE MILICZ CARP *Cyprinus carpio* IN POLAND

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The carp market in Lower Silesia covers the area of 8493.265ha, where there are 48 breeding areas, including the biggest carp breeding center in Poland and even in Europe - Milicz Ponds (6114.64ha). The Milicz carp as the most recognized local product has become a key element of the economic, natural and cultural offer of the area.

Unfortunately, as a result of the intensifying processes of concentration of land and capital, strong competitive pressures and the demand for cheap food, the dominant form of carp distribution is based on long supply chains.

The factor that further stimulates the development of long supply chains is the negligible amount of local processing and the rapid development of transport as part of the global food industry network.

Shortening the supply chain and promoting year-round consumption of carp also in a new form (frozen, smoked, filleted fish, etc.) associated with the development of processing and recreational services may be crucial for maintaining the financial liquidity of farms.

The main goal of the research is to develop an optimal model of effective cooperation between producers and customers, based on short supply chains on the market of the Milicz carp.

The research was conducted on the basis of in-depth interviews among the participants of the supply chain on the market of the Milicz carp, i.e. on fishing farms, agritourist farms and among consumers

The research has shown that the effectiveness of this model requires a coherent and consistently implemented action plan, based on cooperation of participants in the supply chain and business environment institutions.

IDENTIFICATION, MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF GLUTAREDOXIN-1 FROM BLACK ROCKFISH (*Sebastes Schlegelii*)

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Introduction

Glutaredoxins(Grx) are versatile disulfide oxidoreductases with CXXC motifs that belong to the thioredoxin fold super-family. They can catalyze the reduction of protein disulfides, glutathione (GSH) and protein mixed disulfides via coupled reactions with NADPH, GSH and glutathione reductase. As these redox proteins serve as an inevitable member for the redox homeostasis, it is implicated as a cellular protector against oxidative stress conditions. Further, glutaredoxins involve in iron homeostasis and bind labile 2Fe–2S clusters. The present study was carried out to identify, molecular characterize, study the transcriptional modulation of rockfish glutaredoxin-1 (*SsGrx1*) upon immune challenges and ultimately examine the functional aspect of recombinant rockfish glutaredoxin-1(rSsGrx1)

Material and method

Phylogenetic tree and multiple sequence alignment were constructed to determine the evolutionary relationships and sequence homology respectively. The immune stimulation of *SsGrx1* was examined after challenging the healthy rockfish with three immune stimulants, Lipopolysaccharide, *Streptococcus iniae* and poly I:C. Blood and gill tissues were sampled from the challenged and unchallenged negative control fish at different time points. Various tissues were separately collected from five healthy fish for distribution of analysis *SsGrx1*. Those tissues were used for the RNA extraction. For both tissue distribution and immune modulation analysis of *SsGrx1*, qPCR assay was performed using the cDNA that were synthesized from the isolated RNA. The *SsGrx1* ORF was inserted into pMAL-c5x vectors and then it was transfected into BL21 expression systems. Recombinant protein was overexpressed by the IPTG treatment and the protein purification was carried out using maltose affinity chromatography. In order to study the protein activity, insulin disulfide reduction assay was performed.

Results

Amino acids sequence analysis indicated the presence of specific motifs and amino acids required for the GSH binding including $T^{69}V^{70}P^{71}$ motif, $G^{83}S^{84}D^{85}$ motif, K^{20} and Q^{58}. The phylogenetic tree unveiled that all the Grx family members were emerged from a single ancestor and affirmed the evolutionary conserveness of *SsGrx1* with the other Grx1 counterparts. *Larimichthys crocea* was identified as the evolutionary closest member. *SsGrx1* and other vertebrate Grx1 showed higher sequence homology, especially the two catalytic cysteine residues and the TVP motif for GSH binding were 100% conserved among the counterparts. Tissue distribution analysis confirmed an ubiquitous nature of the *SsGrx1* basal expression in naive rockfish tissues with the highest expression found to be in kidney. Immune challenge experiment showed a significant up-regulation of the *SsGrx1* mRNA expression in blood and gills with the injection of LPS, *S. iniae* and poly I:C. During the functional studies, rSsGrx1 was able to reduce the insulin disulphide bonds in a concentration dependent manner.

Discussion and conclusion

The conserveness of the amino acids and motifs including the TVP motif, K^{20} and Q^{58} implies the importance of the GSH mediated redox activity which was affirmed by the conservation in multiple sequence alignment. The tissue distribution of *SsGrx1* was found to have a wide range in the rockfish tissues illustrating its ubiquitous functions in different kind of cells and tissues. The significant upregulation of the *SsGrx1* after immune challenge denotes the immune relevance of *SsGrx1* to prevent the pathogenic attack. The functional study using the reduction of insulin by the recombinant protein was used as a method to assay the protein activity to show the redox potential of the purified protein. Collectively, the results obtained in this study plausibly suggest the immune relevance of *SsGrx1* and the relevance for oxidative protection.

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CELL MODELS FOR THE DEVELOPMENT OF VACCINES FOR AQUACULTURE FISH

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Introduction

Today, almost 50% of global fish demand is met by aquaculture (FAO, 2018). And, although its growth has decelerated, aquaculture still is a constantly growing market worldwide. However, with increasing production volumes in net enclosures, ponds or recirculating aquaculture systems (RAS), the risk of disease outbreaks also increases. Stress due to high stocking densities and the exchange of stocking fish can facilitate the spread of diseases. Viral pathogens, whether long known or newly discovered, are therefore a challenge because there are few effective treatment options and the development of efficient viral vaccines in aquatic systems has so far been difficult to grasp (Crane and Hyatt, 2011). Other factors, such as climate change, are also contributing to the spread of known viral diseases such as pancreas disease (PD), infectious salmon anemia (ISA) and the emergence of new diseases. Through imports of Koi fish the Koi herpes virus (KHV) has been shown to spread significantly and is now carried by warmer temperatures in 28 countries (OIE, 2017). In general, industry-wide losses from aquatic animal diseases summed to over US$6 billion per year (World Bank, 2014). For this reason, vaccine development will play a central role in limiting existing and emerging viral diseases in fish (Gudding et al., 2013). Depending on the fish species and mechanism of a specific virus, different cell models are required. The establishment of new cell cultures, especially of CCApin (Rakers et al., 2018) as part of the "KHV-Vacc" project is presented as an example.

Materials & Methods

For the establishment of new cell cultures, different protocols have been used, depending on the target tissue and culture medium used. One-year old carp (*Cyprinus carpio*) were anaesthetized with clove oil and immediately decapitated. Protocols for cell isolation have undergone an ethical review process by the German Animal Welfare Law §8a. In total, eight new permanent growing cell cultures have been established. Among these cell cultures, three cultures have already been characterised in detail, whereof the CCApin culture from fin turned out to be the most promising. Therefore, cross-reactivity of mammalian-specifi antibodies has been tested and carp-specific primers for gene expression analysis by PCR have been generated. To characterise KHV replication in CCApin cells parameters such as medium, fetal bovine serum content, time of infection (TOI, cell density at virus inoculation), multiplicity of infection (MOI, ratio of applied virus particles per cell) and time of harvest (TOH) were varied. Virus replication was monitored using the 50 % tissue culture infective dose assay ($TCID_{50}$) to determine the number of infective particles. Viral DNA content was assessed using real time polymerase chain reaction (qPCR).

Results

The most successful permanent growing cell culture was derived from a preparation of carp fin and termed CCApin (*Cyprinus carpio pinnae* (lat.)). This culture was characterized in detail by proliferation studies as well as by immunocytochemistry (ICC) and gene expression of certain markers. Besides the molecular technologies, the cells have also been tested by our consortia partners for their virus replication capacity. The highest titres of 10^7 $TCID_{50}$/mL were reproducibly detected when a TOI ranging from 2x 10^4 to 6 x 10^4 cells/cm^2 was used, independently of applied MOI (from 0.005 to 3). In comparison, using lower TOI ranging from 5 x 10^3 cells/cm^2 to 2 x 10^4 cells/cm^2 resulted reproducibly in maximum titres of only 10^6 $TCID_{50}$/mL, again with no correlation to used virus load. To achieve the highest virus yields of 10^7 $TCID_{50}$/mL, low MOI (between 0.001 and 0.01) in combination with an intermediate TOI of ~3 x 10^4 cells/cm^2 were used for inoculation.

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Discussion & Conclusion

Cell models are an important building block on the road to an efficient vaccine. They not only enable the production of viruses in suitable titers, but also the manageability of the pathogens, e.g. for the observation of host-pathogen interactions *in vitro*, which can lead to improved diagnostics. As an example, new cell lines from carp that have high virus-replication capacities are urgently needed as reliable diagnostic method for KHVD and also for vaccine development. With the new CCApin cells, application of higher TOI at inoculation resulted in higher virus titres. Comparison of KHV replication in CCApin and CCB cells showed that similar titres were generated, indicating that both cell lines are equally suited for KHV replication. Replication in other, newly developed cell lines might prove successful to enable higher KHV titres, and they will help to develop more effective vaccines in the near future.

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NOVEL BANANA BY-PRODUCTS IN SEABASS (*Dicentrarchus labrax*) DIETS

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Introduction

Banana production in Canary Islands is a well stablish industry that produces 400mT per year from which generates a 10% of residues, that causes in most of the cases a problem of waste accumulation directly in the plantation. In the Life BAQUA (LIFE 15/ENV/ES/000157) project, a pilot facility has been developed to extract the fiber from the banana organic residue (banana pseudostem) to using as biodegradable materials in packaging and as natural additive for high volume production sectors like automobile industry. During pseudostem fiber extracting process, an important by-product is generated: the pulp. Moreover, the banana crops also generate other important residues like banana flowers. Different studies show the opportunity and high interest of banana crops wastes due not only to the great amount generated, but also to its high content in antioxidants (Gonzalez-Montelongo *et al*., 2010; Bhaskar *et al*., 2011), but is not much still used in human or animal products.

Materials and methods

Two raw materials from canarian banana crops waste, banana pulp (BT) and banana flower (BF), were tested at increased dietary levels in isoproteic and isolipidic diets for seabass. The experiments were carried out in two different feeding trials: for BT trial 24 fishes per tank in 12 tanks were fed with control diet (no BT), BT2, BT4 and BT8 (percentage of BT inclusion) for two months. BF feeding trial was performed for one month were 30 fishes in 12 tanks were fed with control diet (no BF), BF1, BF3 and BF6 (percentage of BF inclusion). Growth and performance parameters were taken at the end of the trials apart from biochemical composition, fatty acid and digestive and liver histology.

Results

In both experiments, the fish with the higher final weight were the fish fed with BT4 diet (23.38±1.56 g) for the banana pulp and BF6 diets (25.58±0.89 g) for the banana flowe . All the obtained results will be presented and discussed.

Acknowledgments

This study was partially funded by the European project Life BAQUA (LIFE 15/ENV/ES/000157), which aims at implementing the circular economy through spanning the value of the banana crop ensuring the use of wastes in other industries, being aquaculture and aquaculture feeds an important target objective.

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ROLE OF ANTIBIOTICS IN SHRIMP FARMING

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Introduction

Antibiotics or antimicrobials are drugs that can kill or inhibit the action of microorganisms which is derived from either natural or synthetic source. Antibiotics should be safe to the animal, allowing their use as chemotherapeutic agents for the treatment of bacterial infectious diseases. An antibiotics are used to prevent the diseases by treating the water or fish before disease occurs. However, the heavy dosage of antibiotics in the fish/shrimp rearing systems could be leads to negative impact to the health of fish/shrimp, consumers and nature which will also increase the bacterial resistance to antibiotics. Hence more attention should be taken while using prophylactic antibiotics in fish/shrimp farming. At this circumstances role of antibiotics with respects to its positive and negative effects in culture systems were discussed for the benefits of farming communities.

Role of antibiotics in aquaculture

Among the animal protein, fish is the best source of nutrients, which flourished with PUFA, proteins, iron, calcium, zinc and etc. In the present situation, capture production is keep on decreasing, hence aquaculture is imperative to compensate the food demand. Aquaculture is the fastest growing sector meanwhile, to aiming the high profit, intensification came to existence in culture practices which leads to imbalance the host, pathogen and environment, this situation create the diseases and environmental impact. At this juncture use of antibiotics in shrimp/fish farming will minimize the diseases and maximize the production. Some of the antibiotics are being used with permissible level (to safe the animal as well as environment) in fish and shrimp farming and certain antibiotics are cause negative impact on animals as well as environment (Table 1). The antibiotics (oxytetracycline, florofenicol, sarafloxacin, and enrofloxacin) are most frequently used in aquaculture to combat bacterial diseases. Globally, other antibiotics such as chlortetracycline, quinolones, ciprofloxacin, norfloxacin, oxolinic acid, perfloxacin, sulfamethazine, gentamicin, and tiamulin are used

Certain farmers are using the antibiotics for prophylaxis and to treat the diseases, thus leads to negative effect. To conquer the problems, we have to promote the use of alternatives to antibiotics and create awareness among the farming communities regarding pros and cons of antibiotics. For sustainable aquaculture, we have to promote the organic farming and restrict the antibiotics usages.

S. No	Antibiotics	Maximum permissible level (ppm)
i.	Chloromphenicol & Neomycin	Nil
ii.	Nitrofurans (Nitrofurazone, Nifuratel, Nifuroxime, etc)	Nil
iii.	Oxytetracycline	0.1
iv.	Trimethoprim	0.05
v.	Oxolinic acid	0.3
vi.	Nalidixic, Fluroquinones & Glycopeptides	Nil
vii.	Chloroform	Nil

Table 1. Above mentioned antibiotics were banned in shrimp farming

LIFE AQUAPEF: EFFECTIVE IMPLEMENTATION OF THE PRODUCT ENVIRONMENTAL FOOTPRINT IN THE MEDITERRANEAN AQUACULTURE SECTOR

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Introduction

Aquaculture is playing, and will continue to play, a significant role in boosting global fish production and in meeting rising demand for fishery products. In 2013, fish accounted for about 17 % of animal protein consumed by the global population. A further shift towards a more pescatarian diet has the potential to reduce global agricultural greenhouse gas emissions and help prevent diet-related diseases. Such change is thus desirable from human and environmental health perspective, but this requires a sustainable growth of the fishery sector. A recent session of the FAO Committee on Fisheries stressed the increasingly key role of aquaculture in fish production for human nutrition and poverty alleviation in many rural areas. As such, there is a need to provide insights on how to ensure sustainable growth of the fishery sector but particularly of the aquaculture-fish secto .

Overall, the huge growth expected for aquaculture products makes necessary a more sustainable aquaculture development to mitigate the environmental impacts linked to this growth. Aquaculture can contribute to the overall objective of filling the gap between EU consumption and production of seafood in a way that is environmentally, socially and economically sustainable (COM (2013)229). Aquaculture is one of the pillars of the EU's Blue Growth Strategy and its development can contribute to the Europe 2020 Strategy. When practicing responsibly, fish farming can help provide livelihoods and feed a global population that will reach nine billion by 2050.

In 2013 European Commission proposes the Product Environmental Footprint (PEF) and Organization Environmental Footprint (OEF) methods as a common way of measuring environmental performance (COM2013/179/EU). To validate the methodology, a series of pilots for different products are developing, and Marine Fish was selected as one of the 11 food products. The package establishes the Life Cycle Assessment methodology methods to measure environmental performance of European Product and organizations.

This project aims to demonstrate that this integrative methodology can facilitate the evaluation and reduction of aquaculture environmental impact.

Methodology

AQUAPEF is going to promote the implementation of the PEF methodology into Mediterranean aquaculture sector by developing an integrative methodology to facilitate data availability, footprint calculation, verification, demonstration and communication. In the overall scheme of the project concept is presented.

- **Data availability:** Two main challenges could be distinguished regarding the data acquisition: a) collection of traceable primary data and b) representativeness of the background data. AQUAPEF project will ensure traceability of the primary data by i) developing a harmonized data acquisition protocol specifically oriented to the Mediterranean aquaculture value chain and by ii) collecting traceable data for marine aquaculture.

Regarding the representatives of the background data, LIFE AQUAPEF will collect main data regarding the feed types and ingredient or wastewater organic loads of the Mediterranean marine aquaculture. This challenge is fully in line with the needs of the PEF marine fish pilot working group

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- Calculation simplification and verification: AQUAPEF will provide the first software tool specifically designed for Mediterranean aquaculture sector that enables the calculation in an easy-way of a harmonized environmental impact, complying PEF methodology. The project demonstrates that the new software tool could provide to the aquaculture industry a reliable and representative ad-hoc tool to calculate their environmental impacts.

- Communication strategies: In relation to previous studies of consumer behavior associated to ecofriendly purchase, to date, several studies have pointed to the relationship between sustainability and the impact of knowledge, attitude and behavior on the consumption of sustainable or environmentally responsible products. As an example, some relevant studies carried out at European level are those of the Eurobarometer, such as the one on "European's attitudes toward the issue of sustainable consumption and production 2009". The AQUAPEF project will explore different communication strategies. However, the certification and label market has become ineffective and counterproductive. Consumers are confused by the stream of incomparable and diverse environmental information. About half of European consumers think it is not easy to differentiate between environmentally friendly and other products and only about half of them trust producers' claims about environmental performance. This also influences their readiness to make green purchase

Results and discussion

Three main results are expected from this project:

- AQUAPEF tool: A new SAAS (Software-as-a- service) is under developing to facilitate the implementation of PEF methodology in the Mediterranean aquaculture sector

- Improvement strategies: AQUAPEF analyses and tests potential uses of the PEF to reduce the environmental impact by a demonstration case-study in 3 seabream and seabass fish farms located in the Mediterranean regions: multitrophic aquaculture, new feeding systems and valorization of biowaste. This action will also demonstrate the need to include additional ecological indicators to complement Life Cycle Assessment results to obtain a better understanding of the system sustainability, as proposed by PEF recommendation

– Communication strategies: to ensure the added value and maximize the environmental and socioeconomic benefits of sustainable aquaculture products, action B4 focuses specifically in understanding consumer perceptions, raising awareness and informing consumers about the impact of their aquaculture-fish product choice with the aim of realizing a behavioral change towards more sustainable aquaculture product consumption.

Conclusion

AQUAPEF project starts in September 2018 and currently is undergoing. Final results of the tool demonstration and communication strategies are still under development. Thus, this is a presentation of the overall project concept.

Acknowledge

This project is co-funded by LIFE European Environment Programme (LIFE17 ENV/ES/000193), which is the EU's financial instrument supporting environmental, nature conservation and climate action projects throughout the EU. The implementation, updating and development of EU environmental and climate policy and legislation by co-financing projects with European added value are among its main priorities.

BROWN SEAWEEDS – A STATUS REPORT OF OFFSHORE CULTIVATION AND EXPECTED MARKET DEVELOPMENTS IN EUROPE

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A resource forgotten in Europe

Globally, the cultivation of brown seaweeds takes the largest share of marine aquaculture production, mainly due to extensive farming of kelps, like *Saccharina japonica* ('Kombu') and *Undaria pinnifatida* ('Wakame') in Eastern Asia. While a highly important food commodity in Asia, in Europe seaweeds have not been part of the mainstream culture, and often only known in the context of Sushi. For both 'Wakame' and 'Kombu', there exist European species with comparable qualities for food consumption, namely *Alaria esculenta* (Atlantic Wakame) and *Saccharina latissima* (Royal Kombu), respectively. The value of seaweeds for the culinary and for our diet has increasingly caught attention of European chefs and food industry players, and the present scenario suggests a potential uptake in the European food market. Meanwhile, cultivation of these species is still in a pre-commercial phase, due to the relatively small amounts required to date.

In addition, in some areas, kelp forests have been declining in a worrying pace. For these and other reasons there cannot be expected major volumes of wild harvested seaweed in the future, which is why the more expensive, but also more sustainable path of cultivation is the only likely supply option.

A cultivation industry in its beginnings

Initial considerations of cultivating brown seaweeds in Europe were in the context of very large-scale farms for biomass-for-fuel scenarios. However this would require huge investments and levels of automation, versus a very low achievable unit price. Since a few years ago, it has been understood that the path towards cultivation for food as main product, similar to most of the actual cultivators of brown seaweed. The combination of near-term market demand expectations and possible specific sales price that can be achieved has been identified as the most promising sector to yield commercial feasibility. Seaweed Energy Solutions (SES) built a pilot cultivation farm in 2014, in which the technical feasibility of harvesting 100-150 tons per year was demonstrated. Since then, several companies in Europe, in particular in Norway, have been increasingly active and planning to upscale production with similar, relatively conventional farming techniques, and similar target volumes. After initial experiences in the (more complex) cultivation process of seaweed for food – since the raw material must be stabilised very soon after harvest, similar to fish –, the break-even for cultivation activities in a typical brown seaweed farm in Norway is expected to be in the range of 200 to 500 tons per year, depending on the sales price achieved per kilogram of raw material.

This contribution will describe the chosen farming technique and process implemented by SES in order to yield break-even cultivation within a short time frame, and without major investment requirements, allowing for a modular upscaling.

At the present stage, the market is not defined to a point that specific stabilising/processing methods are identified to be the most relevant ones for the industry. The seaweed landed on dock is mainly frozen, for the sake of availability, reasonable cost and relative flexibility for further processing options

Opportunities and threats for brown seaweed in European food market

Like for other products – as long as there is no large-scale production but significant initial investments are required – there is a need of relatively high sales prices of early production years. The food market – especially in the premium and HORECA sections – has been identified as an enabler for this phase

Brown seaweed are increasingly recognised for their health benefits and valuable compounds. They are a natural source of iodine, lack of which in the European population has been a concern for the health departments. They are also a source of more the 50 different minerals and trace elements, and omega-3 fatty acids. They are prebiotics. They are a natural texturizer and natural preservative. Containing higher amounts of glutamate, brown seaweeds give the *umami* taste and a enhancement of flavours.

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Given the varying characteristics of the raw material, as well as the high standards of the target markets it is highly important to perform frequent analyses on composition and product quality, and also to improve and consolidate knowledge about these aspects, which is the target of several research projects and initiatives, in some of which SES has participated or is participating. The present status of the discussion will be presented.

Valorization of environmental benefits

The ongoing years have been and will remain a dynamic phase for seaweed-for-food in Europe, and consequently seaweed cultivation in general. Overall, seaweed is a highly sustainable and energy-efficient food resource, as no fresh water is used, no fertilisers or chemicals applied in the sea, and no significan footprint on land is caused. Therefore, its use as a food ingredient has tremendous impact for the environment.

There are even benefits of seaweed cultivation on several levels, which may or may not soon be taken into account on a policy level. They absorb significant amounts of nutrients (N, P) and increase the CO_2 uptake capacities of the water body. As such, brown seaweed may be a prime candidate for adding sustainability to aquaculture operation, be it in IMTA approaches. The cultivation of seaweed in the sea can actually be compared to planting a temporary forest, contributing significantly to mitigate such effects as water quality and biodiversity decline. The seaweed forest is a biodiversity enhancer, e.g. through increased shelter and maternity areas for juvenile fishes, crustaceans and others. Among other initiatives, in particular the 'Seaforester' organisation aims at turning the wide recognition of these values of seaweeds into action. The concept of seaforestation, as well as the knowns and un-knowns in relation to this approach, will be briefly presented

Potential threats to sustained large-scale cultivation like e.g. genetic deterioration from breeding and diseases are being targeted in research efforts, including the ongoing GENIALG project. The findings of this and other ongoing projects like MACROSEA, KELPPRO and SEABEST will be presented, with the aim to give a global insight into the actual status and future prospects of brown seaweed cultivation.

FEEDING MANAGEMENT OF JUVENILE PIKEPERCH *Sander lucioperca* UNDER PRODUCTION CONDITIONS

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Introduction

Pikeperch is a promising species for cultivation in recirculation aquaculture systems (RASs) (Wang et al. 2008). It is valued by consumers, offers a stable sales market and the attainable market prices are high (Knaus, et al., 2008). However, pikeperch production in RAS is currently still expensive and it is essential to optimize the use of resources. The use of fish feed, typically the most prominent variable cost factor (Alanärä et al., 2001), can mainly be optimized by the feeding management to reduce the amount of feed loss without compromising fish growth. Here we present a study on the effects of number, duration and period of feeding events in the feeding regime of juvenile pikeperch under production conditions.

Material and methods

The experiment was conducted in eight tanks with each 1.5m^3 water volume and water treatment systems. The feeding regimes included 1) continuous 24h/d feeding with 30min feeding intervals (C30), 2) continuous 24h/d feeding with 30min feeding intervals, whereas the feeding period was extended by 35% until the assigned amount of feed was fed (C30L), 3) continuous 24h/d feeding with 120min feeding intervals (C120), and 4) intermitted 12h/d feeding with 30min feeding intervals (I30) during daylight hours. Pellets were supplied with a drum feeder. Before the start of the experiment fish were graded by size (mean weight±SD: 104.6±3.63g; mean length±SD: 23.6±2.1cm). Each tank was stocked at a density of 16.7kg/m^3. After 42 days the study was terminated and 50 randomly chosen fish from each tank were measured for total length and weighed and biomass in each tank was assessed to calculate specific growth rate (SGR), Fulton's condition factor (K), coefficient of variation (CV), biomass gain, apparent feed conversion ratio (aFCR), apparent protein efficienc ratio (aPER), and apparent lipid efficiency ratio (aLER)

Results

Coefficient of variation showed significant differences between treatments whereas differences in SGR, K, biomass gain, aFCR, aPER, and aLER were not significant (Table 1). The CV was higher when feed was only supplied for 12h (I30) compared to when fish were fed over a 24h period (able 1).

Discussion and conclusion

The high CV for fish with an intermitted feeding regime (I30) indicates a more heterogenic growth compared to a continuous feeding. Fish with more heterogenic growth have to be graded more frequently to reduce mortality through cannibalism (Baras & Jobling, 2002) which increases working hours and expenses.

There was a trend that treatment C30L experienced highest SGR, K, biomass gain, and better feed efficiency (i.e. aFCR, aPER, aLER) although differences were statistically not significant. This could indicate a more sufficient feeding when feed is provided slower (Alanärä et al. 2001), but not too slow to support feed monopolization (Grant 1993). Thus, data of this work could provide additional support for the advantage in prolonged feeding events during a continuous 24h feeding regime. However, potential effects were not great enough to be depicted by the duplicate design due to its low power. Therefore, we encourage further studies with a specifically adjusted experimental design

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Table 1: Mean (±SE) specific growth rate (SGR), Fulton's condition factor (K), coefficient of variation (CV), biomass gain, apparent feed conversion ratio (aFCR), apparent protein efficiency ratio (aPER), and apparent lipid efficiency ratio (aLER) of four different feeding regimes. Additionally the corresponding test statistics are given. Feeding regimes include continuous 24h/d feeding with 30min feeding intervals (C30), continuous 24h/d feeding with 30min feeding intervals, whereas the feed was supplied over a period that was 35% longer compared to the C30 treatment (C30L), continuous 24h/d feeding with 120min feeding intervals (C120), and intermitted 12h/d feeding with 30min feeding intervals (I30) during daylight hours.

Variable	C30 (Mean±SE)	C30L (Mean±SE)	C120 (Mean±SE)	I30 (Mean±SE)	F-value; df; P-value
SGR [% d^{-1}]	0.709±0.061	0.794±0.003	0.746±0.046	0.679±0.027	F=1.533 df=3 P=0.336
K	0.752±0.0166	0.769±0.0004	0.746±0.0046	0.750±0.0032	F=1.334 df=3 P=0.381
CV [%]	24.941±0.789	26.128±0.041	25.339±2.772	34.862±2.073	F=6.776 df=3 P=0.048
Biomass gain [kg m^{-3}]	5.86±0.59	6.68±0.03	6.17±0.50	5.47±0.26	F=1.581 df=3 P=0.326
aFCR	1.70±0.17	1.48±0.01	1.61±0.13	1.80±0.09	F=1.400 df=3 P = 0.365
aPER	1.21±0.12	1.38±0.01	1.28±0.10	1.14±0.06	F=1.512 df = 3 P = 0.340
aLER	5.94±0.60	6.78±0.03	6.26±0.50	5.58±0.27	F=1.512 df=3 P=0.340

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EFFECT OF FOUR DIFFERENT COMMERCIAL FEEDS ON GROWTH PERFORMANCE AND SURVIVAL OF EUROPEAN PERCH *Perca fluviatilis* I N RECIRCULATING AQUAQUALTURE SYSTEM

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The aquaculture production of European perch is still limited by several bottlenecks. Predicting growth rates, feed intake and energy requirements of the farmed fish in different life stages are crucial for the viability of an enterprise. Especially the economic production of high quality and healthy meat depends essentially on the composition of artificial diets. There are different products on the feed market which may suit the production of European perch. It is known that the European perch tends to accumulate visceral fat in its cavity, which can lead to a lower filet yield and higher amount of carcasses. Taking this in consideration it is of great importance to test different commercial feeds on their performance for perch farming to optimize production costs.

We tested four different available commercial feeds (3mm), of which three were declared for percids (feeds A, B, D), in a practical approach during grow out from 100g to 200g fresh weight in a three month trial in triplicate at an initial density of about 20kg/m³ (157 fishes per treatment). The amount of crude protein ranged from 49 – 54% and crude fat ranged from 10% (feed C and D), 15% (feed A) and 20% (feed B). Further, D was floating feed in contrast to sinking feeds A, B and C.

At the end of the experiment we will compare survival, final fresh weights, specific growth rate, Fulton's condition factor (K), coefficient of variance, filet quantity and quality (fatty acid analysis), visceral somatic index and hepatosomatic index.

After 73 days of the experiment mean survival was highest with feed D (99.2%). Remarkable lower survival was overserved for feed A. At this stage of the experiment no differences between sinking (B, C) and floating feed (D), nor between feed declared for percids (B, D) and alternative feed (C) were ascertained.

The experiment is still in progress and the results are not available at time of abstract submission. The final results will be presented at the resubmitted version of this abstract and provided on the poster.

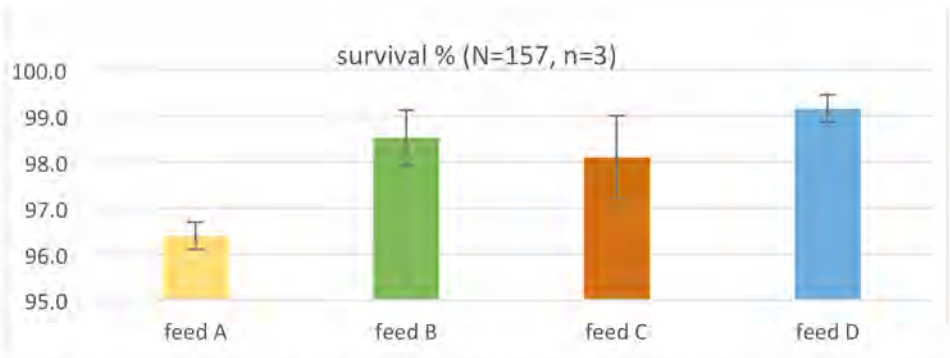

Fig. 1: Mean survival (%) of *Perca fluviatilis* after 73 days during growth out with four different commercial feeds.

GAMETE MATURATION OF THE POLYCHAETE *Nereis diversicolor*: EFFECT OF DIFFERENT TEMPERATURE AND PHOTOPERIOD CONDITIONS

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Introduction

Nereis diversicolor is a polychaete species which is suitable for industrial aquaculture, because of its adaptability to wide environmental conditions, feed flexibility (detritivorous, carnivorous) and high growth rates under culture conditions (Batista et al., 2003). However, reproduction control (or programming) is a bottleneck for *N. diversicolor* production and further research is needed, particularly regarding gamete maturation. The present study aims to investigate specific factors (temperature and photoperiod) as well as aquaculture procedures that influence gamete maturation for both reproduction management and improving and getting successful artificial fertilization of cultured *N. diversicolor*. In particular, since a massive reproduction event was observed in our aquaculture facilities after a steep fall in water temperature, we study whether a cold treatment can influence gamete maturation (experiment 1). In difference to this observation, we hypothesize that warm temperatures can influence gamete maturation as occurs during spring-summer natural conditions (experiment 2). In relation to experiment 1, Bagarrao et al. (2013) tested short-time temperature falls to induce spawning; hence, we investigate if this kind of punctual thermal shock can also influence gamete maturation (experiment 3). In addition, since photoperiod usually interacts with temperature for maturation, we analyze this effect on gamete maturation of *N. diversicolor* (experiment 4).

Material and methods

Experiment 1 (cold treatment): 150 worms (815±228 mg of initial body weight) were distributed into six tanks (25 worms per tank) after three summer months of acclimation under natural temperature (18.4±0.9ºC) and photoperiod (L:D, 15:9) conditions. A cold temperature treatment (11.7±0.3ºCand L:D 15:9) was applied to these worms (three tanks), in comparison to control groups (three tanks) maintained like in summer conditions (19.1±1.1ºCand L:D 15:9).

Experiment 2 (warm treatment): 180 worms (1053±272mg) were spread in six tanks (30 per tank), after being in artificial conditions of temperature (12.9±0.6ºC) and photoperiod (L:D, 15:9). Three of these tanks were subjected to warm temperatures (18.8±1.2ºand L:D 15:9) and compared with the other three tanks kept like in initial conditions (11.7±0.3ºCand L:D 15:9).

Experiment 3 (punctual cold treatment): 180 worms (689±125 mg) were distributed into six tanks, after being under artificial conditions of temperature (18.8±0.8ºC) and a winter natural photoperiod (L:D, 9:15). These worms were shocked with a short cold treatment (11.7±0.3ºCand L:D, 9:15) lasting six days, and then reverted to initial conditions for the remaining experiment. Control groups were always maintained like in initial conditions (19.5±0.9ºCand L:D, 9:15).

Experiment 4 (temperature-photoperiod): 360 worms from our stock were sampled (755±173 mg) and distributed into twelve tanks, after three months in natural winter conditions of temperature (11.2-13.0º) and photoperiod (L:D, 9:15).Three groups of these worms were changed to 19.2-20.8ºC and/or L:D 15:9 (9 tanks, 3groups x 3 tanks) to study the individual effect of temperature and photoperiod, as well as their interaction.

The maturation stage of the gametes (free sperm or tetrad stage for males, and oocyte diameter>175µm for females), survival and weight of the worms were recorded at the end of the experiments. The end of each experiment coincided with the time of gamete maturation observed in similar experimental tank/s of worms which were periodically sampled, being: five weeks (experiment 1), three weeks (e2), four weeks (e3) and two weeks (e4). Data were statistically compared by a t-student test for the experiments 1, 2 and 3, and by a 2-way ANOVA for the experiment 4. Also 1-way ANOVA and Tukey post-hoc tests were used for between-group comparisons for experiment 4.

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Results

At the end of experiment 1, mature females, mature males and total mature polychaetes were not significantly (P>0.05) different in the cold group (11.7ºC) of worms as compared to the control one (19.1ºC). However, total survival and final body weight of worms were both significantly (P<0.05) higher in the cold group. On the other hand, a significantly higher number of mature females (12, almost double) were retrieved from the warm treatment (18.8ºC), compared to the control group (11.7ºC) in experiment 2.The total number of mature polychaetes (13) showed the same pattern, though barely significantly (P=0.052). Mature male number, final body weight and survival were not significantly modified. In experiment 3, the punctual 6-day cold treatment (11.7ºC) did not increase the number of total mature worms nor mature females in comparison to the control treatment (19.5º). However, the punctual cold treatment influenced a significant low reduction in the number of mature males (from 1.3 to 0), while survival and final body weight were not significantly modified. Experiment 4 showed a significant effect of temperature, irrespective of photoperiod, on the number of mature females and total matures. A significant effect of photoperiod on the number of mature females and total matures was also obtained, irrespective of temperature. In consequence, the interaction effect was not found statistically significant. Between-group comparisons found significant differences for the higher number of mature females and total matures from the group treated with high temperatures (19.2-20.8ºC) and photoperiod L:D 15:9 against the control group.

Discussion

Results indicate that changes in temperature imposed on worms that have been under summer conditions (18.4º and L:D 15:9), by using a cold water treatment of 11.7º, did not have a significant effect on maturation. On the contrary, a warm temperature treatment (18.8ºC) did have a significant effects on female gamete maturation, as well as on the total number of mature worms, that had been previously subjected to "cold" artificial conditions (11.7ºand L:D 15:9), almost doubling both values. The final body weight of worms under cold treatments was also significantly higher in experiments 1 and 2, which suggests both gamete emission by worms under warm experimental temperatures and some worm degeneration related to the high maturation observed. Survival under cold treatments was only higher in experiment 1and it was probably related with the longer experimental time used in this trial since worms always die after spawning. It is also remarkable that the "punctual cold shock" treatment (experiment 3) significantly reduced the number of mature males; this is particularly noteworthy given the low proportion of mature males. In addition, this thermal shock did not improve any other of the measured parameters such as female maturation, total maturation, survival or final body weight. Experiment 4 indicates the importance of temperature for oocyte maturation, irrespective of photoperiod, as well as the significant effect of photoperiod for oocyte maturation, irrespective of water temperature. A treatment by treatment comparison suggests that the simultaneous treatment with long photoperiods and high temperatures should be recommended to achieve gamete maturation, although an increase in only one of these factors alone promotes gamete maturation.

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IMTA IN THE ATLANTIC AREA: DEFINITION, BEST-PRACTICE, STATUS AND NEEDS

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Whilst Integrated Multi-Trophic Aquaculture (IMTA) is conceptually appealing, it has nevertheless failed to become a part of mainstream aquaculture on an industrial scale. The European Project Integrate, funded by the ERDF through the Interreg Atlantic Area Programme has looked into reasons for this lack of uptake. As part of this process each project partner from the European Atlantic Area countries UK, Ireland, France, Spain, and Portugal, held a series of thematic roundtables asking questions aiming to define what IMTA best-practice might be, and how it might be achieved. For each of 5 areas, namely, environmental; economic; social; regulatory and technical, the expert groups were asked to define IMTA best-practice, to identify challenges and bottlenecks to its achievement and to recommend priority areas for research and development. These country by country analyses have been synthesised to provide an overview of the European Atlantic Area situation, including what is common across the board, and what is unique to countries or regions.

From these roundtables a common problem emerged: although the conceptual definition of IMTA was clear, a more utilitarian definition making some of the details explicit, was necessary. It was envisaged by IMTA stakeholders and producers that by addressing this need IMTA could be better regulated and commercialised and, in addition, the development of an IMTA technical standard could be facilitated. INTEGRATE addressed this by organising an 'IMTA Definition Event', that was hosted by the Interreg Atlantic Area Programme Managing Authority in Porto, Portugal, in May 2019. The event brought together 44 international experts and the structured discussion built on information gathered previously by questionnaire. In large part, consensus around a 'Europe-appropriate' IMTA definition was achieved, although some points remain in need of further clarification

Synthesised results from the expert roundtables, the questionnaire and the preliminary conclusions from the IMTA definition event will be presented.

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BUILDING KNOWLEDGE FOR A SUSTAINABLE AQUACULTURE INDUSTRY: THE DECISION-SUPPORT TOOL SEAGRID

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The aquaculture production in Norway has experienced an extraordinary growth in the recent years and it is expected to export more than 200 billion NOK per annum by 2030. While offshore locations are currently tested, the traditional Norwegian aquaculture industry has been mainly located in the coastal and fjord areas, thus interacting with other marine activities.

The main objective of the AquaAccept project was to explore the sustainable development of coastal area activities, with special focus on aquaculture, from an interdisciplinary perspective, drawing on expertise in biology, ecology, economics, sociology and computer science. One of the main project's goal was to develop a decision-support tool (SEAGRID) to aid stakeholders and decision-makers address conflicts and syne gies between marine activities.

The SEAGRID, a GIS-based, user-friendly tool, can be used to quantify environmental, social and economic suitability and it allows stakeholders and decision makers to make spatial choices that strike a balance between multiple ecological, economic and social objectives.

SEAGRID uses stress level and conflict score analyses to evaluate marine activities interactions, including environmental risk calculations as well as socio-economic evaluations. SEAGRID assesses conflict scores, generate matrices of interactions, environmental risk and social acceptability, plots maps, evaluates spatial and/or temporal interactions existing in a specific marine coastal area, calculates overlaps and ranks the effects of synergies and conflicts by the calculation of global stress levels.

Environmental data, available from regional and national authorities have been used to implement the environmental layers and score calculations. Socio-economic data were obtained from public databases (production costs and revenues) and from national and local surveys and interviews carried on in the AquaAccept project. The data have been synthesized, as quantitatively as possible, and managed inside the SEAGRID database. Their environmental, economic and social aspects in terms of induced stress levels and multiple layer matrices and stakeholder interactions were evaluated for the Boknafjord (Rogaland County, South-West Norway) case study.

The results from the case study analysis and the final implementation of the SEAGRID are currently being discussed with the stakeholders (local and national authorities, industries, interested commercial and private actors) to promote a bottom-up approach meeting the needs for a cross-sector and transparent management of the marine areas.

The results of the case study will be presented at the conference.

THE USE OF FULL-FAT SUPERWORM (*Zophobas morio)* MEAL IN GUPPY (*Poecilia reticulata*) NUTRITION

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Introduction

The interest of pet food market in insect meals increases, and in the most cases meets more liberal law regulations that other branches of feed production (Lahteenmaki-Uutela et al. 2017). However, in opposition to aquaculture, a very few studies are done on ornamental and laboratory fish species. It creates the need of development of sustainable feeds for non-edible aquaculture species seems to be important from nutritional, environmental and marketing point of view (Vargas-Abúndez et al. 2018, Zarantoniello et al. 2018, Vargas-Abúndez et al. 2019). Moreover, the initial studies showed that superworm (*Zophobas morio*) is an attractive feed component for guppies (*Poecilia reticulata*) kept in experimental conditions (Rawski M., unpublished data).

The aim of the experiment was to assess the effects of 100% inclusion of the *Zoophobas morio* full fat-meal into the diet of guppy in comparison to commercial fish feed.

Materials and methods

The experiment lasted 60 days. A 150 juvenile (14 day old) gupies from outbreed line maintained at Poznań University of Life Sciences were used in the experiment. At day 1 The mean total body length of the fish was 8,5 mm.. The fish were randomly allocated into two treatments: control – feed with commercial feed, and experimental: feed with *Zoophobas morio* full fat meal only. The fish were allocated to 6, filtrated and aerated 20l tanks (3 replications/ treatment, 25 fish/ tank. The temperature of water was: 25-27º C, filtration flow: 160 l/h, and applied photoperiod of 12h of light and 12h of darkness.

The insect meal was obtained from HiProMine S.A (Robakowo, Poland). *Zophobas morio* larvae were feed with plant origin by-products, they were dried in 50º C for 24h. The insect meal as well as commercial feed were finely grinded and stored in fridge through the time of experiment (4º C).

The animals were feed once daily at 8.00 until visual satiation. At day 1, 30 and 60 survival rate, total body length and its gain were assessed. All obtained data were statistically analysed with the use of T-student test, with significance level of $p \leq 0.05$.

Results

The results obtained in two month long experiment showed no difference in mortality, total body length, total body length gain. The fish from control group reached 13,5 mm of total body length while *Zophobas morio* feed 13,4 mm. Survival rate in both cases was high and comparable: 90,7% in control and 85,3%.

Discussion and conclusion

According to available scientific literature, the presented study is probably the first case of the use *Zophobas* morio full-fat meal in juvenile guppy rearing. It shows the possibility of the use of insect meal as the basal diet in omnivorous ornamental fish nutrition, which may be an alternative for conventional feed resources. The results confirm the possibility of partial or total inclusion of insects to ornamental fish diet (Vargas-Abúndez et al. 2018, Vargas-Abúndez et al. 2019).

However further studies on the insect effects on physiology and reproduction of ornamental fish should be performed due to variability of observed in other species effects on fish growth, gastrointestinal physiology and microbiota (Vargas-Abúndez et al. 2018, Józefiak et al. 2019, Zarantoniello et al. 2019).

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THE DEVELOPMENT OF INEXPENSIVE SALT MIXTURES FOR INLAND, INTENSIVE SHRIMP (*Litopenaeus vannamei*) FARMING

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Introduction

Various forms of recirculating aquaculture systems (RAS) can be used to grow marine shrimp practically anywhere. By locating these systems in greenhouses or insulated buildings fresh, large shrimp can be produced year-round near consumer markets, opening opportunities for branding a higher-quality, sustainable, local-grown seafood product. One of the major costs associated with growing marine shrimp inland is salt, and the current standard is to use complete, brand name sea salts. The purpose of this project was to evaluate the use of an inexpensive salt mixture at various inclusion levels for the production of shrimp.

Materials and Methods

An 84 day-long experiment was conducted using 20, 1m^3 tanks. Each tank had a 15 L external biofilte , a 15 L settling chamber, and an electrical heater. Five treatments were developed (Table 1) with combinations of a least cost salt formulation (Table 2), and a standard, store-bought, full sea salt Crystal Sea® Salt – CSS).

Each treatment was randomly assigned to four tanks which were located in a climate-controlled (20°C), insulated building with household fluorescent lighting kept on constantly. Shrimp were stocked at 4.3g mean weight and fed three times per day based on estimated FCR and survival. A repeated measures (RM) ANOVA was used to analyze water quality data over time, and one-way ANOVAs were used to analyze final dissolved mineral content and shrimp production data

Results

Because the RM ANOVA considers the entire data set over time, it did detect some subtle but consistent significant differences between treatments with regard to DO, pH, and nitrite levels. However, these differences do not correspond to the proportion of either salt mixture. There was significantly more sulfate, magnesium, and strontium in the CSS than the LCS mix and that was evident from dissolved mineral data.

There were no significant differences detected in any measured shrimp production metrics, which included average weight, growth rate, FCR, biomass (kg m^{-3}), and survival (Table 3).

The cost of salt per kilogram of shrimp was significantly lower in the 50% CSS/LCS treatment than the 100% or 75% CSS, and the 75% and 100% LCS treatments were significantly lower than any of the other three treatments (Table 4). The 100% LCS treatment was less than half the cost per kilogram than the 100% CSS treatment.

Table 1. The experimental treatments.

5 Treatments
1.) 100% CSS
2.) 75% CSS – 25% LCS
3.) 50% CSS – 50% LCS
4.) 75% LCS – 25% CSS
5.) 100% LCS

Table 2. The least-cost salt formulation (LCS).

15 ppt. Salt Solution @ 1m^3	
NaCl	11,310 (g)
$MgSO_4$	1,830 (g)
$MgCl_2$	855 (g)
$CaCl_2$	376 (g)
KCl	240 (g)
$NaHCO_3$	90 (g)

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Table 3. Shrimp production results in the five treatments using combinations of a Crystal Sea Salt (CSS) and a Least Cost Salt (LCS) formulation.

	Treatment				
	100% CSS	75% CSS	50% CSS/LCS	75% LCS	100% LCS
Average Weight (g)	22.9 ± 0.8	22.2 ± 0.5	22.4 ± 0.2	22.5 ± 0.6	22.5 ± 0.4
Growth rate (%)	1.6 ± 0.1	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.1	1.5 ± 0.0
FCR	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	2.1 ± 0.2
Kg/m^{-3}	3.8 ± 0.2	3.9 ± 0.2	3.8 ± 0.3	4.0 ± 0.2	3.2 ± 0.3
Survival (%)	67.2 ± 3.8	70.3 ± 3.9	68.3 ± 5.2	73.1 ± 4.2	57.2 ± 6.2

Table 4. The cost of salt per kilogram of shrimp produced in each treatment.

Cost of Salt per Kg of Shrimp	
Treatment	$USD
100% CSS	$6.66[a]
75% CSS	$5.50[a]
50% CSS/LCS	$4.57[b]
75% LCS	$3.20[c]
100% LCS	$2.87[c]

Discussion

This study demonstrates that using a simple, in-house made salt formulation can be an effective strategy to produce shrimp in intensive, indoor farming. The shrimp were grown to over 22g, which is a highly marketable size in the US, and can fetch a substantial price. Survival was lower than expected across treatments, but most notably in the 100% LCS treatment. This warrants further investigation and a follow-up project is looking at the effects of using lower levels of CSS, down to about a 5% CSS - 95% LCS blend to determine if a small amount of CSS is needed. Likewise, examining the effects of balancing some of the relatively deficient minerals in the formulation will be explored

Nonetheless, the production levels from the LCS-containing formulations are encouraging, especially when the cost per kilogram of shrimp is considered. This has potential to reduce the costs of production in intensive shrimp farming substantially. Reducing recurring costs in this manner may make this production strategy more financially feasible and allow farmers to produce shrimp close to consumer markets at inland locations.

MICROARRAY-PREDICTED MARKER GENES AND MOLECULAR PATHWAYS INDICATING COMBINED CROWDING AND THERMAL STRESS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Stressors in aquaculture cover a broad and diverse range of abiotic and biotic factors. The co-occurrence of multiple stressors has various physiologic consequences for the farmed fish ranging from moderate alterations at the molecular level to increased disease susceptibility, behavioural disorders or even mortalities (Schulte, 2013). The objective of the present study was to identify and evaluate informative indicators for the welfare of rainbow trout *Oncorhynchus mykiss* exposed to (A) a critical water temperature of 27°C and (B) acute crowding of 100kg/m^3 combined with water temperature of 27°C. To this end, we combined a broader and complementing panel of phenotypic, plasma- and transcript-based parameters, to understand whether the concurrent occurrence of thermal and crowding stress evoke additive or synergistic or even antagonistic effects at the molecular level.

Material & methods

Rainbow trout at the age of ~10 months were randomly distributed over six experimental tanks in a recirculating aquaculture system (RAS). The experimental procedures are illustrated in Fig. 1.

Further husbandry conditions are detailed by Rebl et al. (2017). Eight days after the start of the experiment, seven rainbow trout per tank were randomly sampled. Blood, head kidney and spleen of these animals were isolated and either used for profiling of plasma parameters or cell culture or nucleic acid extraction.

Microarray-based gene-expression analysis was performed on 8×60 K AgilentSalmon Oligo Microarrays (Agilent Technologies) following the Agilent 60-mer oligo microarray processing protocol, as described by Rebl et al. (2017).

Fig.1. **Schematic representation** of the stress experiment subjected to rainbow trout. In the reference tanks, temperature (16°C) and density (30kg/m^3) were kept constant. In the 'temperature stress' tanks, water temperature was gradually increased up to 27°C (stocking density: 30kg/m^3). In the 'temperature/crowding stress' tanks, rainbow trout were exposed to stocking densities of 100kg/m^3 and gradually increased temperatures up to 27°C.

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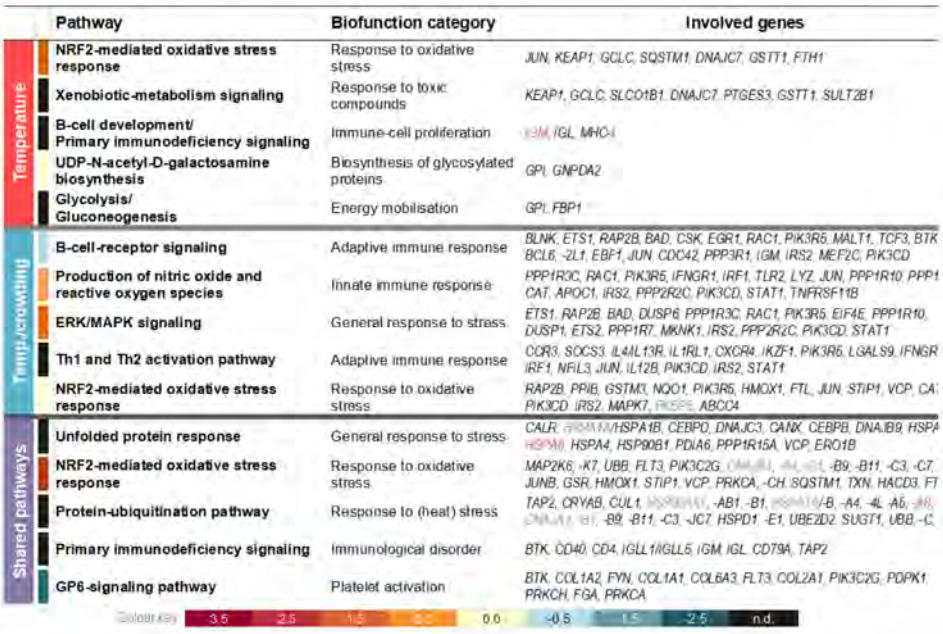

Fig.2. **Pathway analysis** of the DE gen sets exclusively affected by temperature stress alone (red label), by combined temperature/crowding stress (blue) and shared by temperature stress and combined temperature/crowding stress (purple). The 5 most strongly affected pathways are listed. Z-scores (colour code below the scheme) indicate activated or repressed pathways.

Results

We determined health index, spleen-somatic index and haematocrit and recorded, in addition, traditional plasma parameters of rainbow trout under challenging *versus* control conditions. However, each of these tests revealed non-significant differences between the two stress groups and the reference group. On the other hand, we detected a strong influence of both stress conditions on gene expression in the spleen. In total, the transcript abundance of almost 1500 genes was modulated at least 2-fold in rainbow trout stressed by temperature stress alone or combined temperature/crowding stress over the unstressed control fish. The complete microarray-data set has been deposited in the GEO database (accession #: GSE129271). Based on the sets of differentially expressed (DE) genes, we identified unique and common tissue- and stress type-specific pathways reflecting the crossroads between signal transduction, metabolic and immunologic pathways to cope with thermal and crowding stress (Fig. 2).

The immunologic aspects of the transcriptomic analyses were confirmed in primary-cell-culture experiments

Discussion and conclusion

The present study demonstrates that temperature and crowding stress share similar expression profiles. Nevertheless, we identified stress-specific pathways and thus contribute to improve the monitoring of the salmonid's welfare in aquaculture.

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CONSTRUCTION ON AN INTEGRATED GENETIC MAP OF BENEFIT TO THE *Solea senegalensis* AQUACULTURE

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Introduction

The Senegalese sole (*Solea senegalensis*) is a flatfis that is distributed throughout the Mediterranean Sea and in the Atlantic areas of Africa. Its culture has a high commercial value, with sole being a good candidate for diversification of the European markets, saturated because of the overproduction of seabream and seabass. A limiting factor in Senegalese sole aquaculture is the absence of methods for controlling reproduction in captivity due to problems with sole generations born and bred in captivity. This species is characterized by a karyotype with 42 chromosomes, and a preliminary physical map, with chromosomal markers for the 21 pairs has been constructed (García-Cegarra et al., 2013; Portela-Bens et al., 2016). A possible sexual proto-chromosome in the sole (Portela-Bens et al., 2016), originated by fusion of 2 acrocentrics has also been described (Merlo et al., 2017; García-Angulo et al., 2018). Results presented here show the updated integrated map in the Senegale sole and the characterization of relevant genes for aquaculture as some involved in sexual determination, differentiation and maturation, immunology and metamorphosis. In addition a comprehensive study of repeat sequences and miRNA location in the sequenced BACs will be presented.

Material and Methods

Cytogenetic methods and protocols used for mFISH, NGS sequencing and genomic comparison have been described in Portela-Bens et al. (2016) and García-Angulo et al. (2018).

The "MISA" program (Microsatellite identification tool) was used to determine the distribution of satellite DNA in *S. senegalensis* chromosomes. The data were distributed by BACs hybridized per chromosome and they were measured as number of loci (NL) per Mb of chromosome.

A nucleotide database was created containing 2981 sequences from BAC clones of a *Solea senegalensis* BAC library and interrogated using blastn command for potentially conserved miRNAs (Arias et al., 2018). The reference transcriptome for *S. senegalensis* (v4.1) was downloaded from Solea DB (Benzecri et al., 2014) and used for target prediction using miRanda v3.3a.

Results

An integrated genetic map with 97 BACs hybridized to the chromosomes of the karyotype of *S. senegalensis* has been constructed (Fig 1). BACs were isolated from a library by selecting genes involved in important processes in the sole aquaculture: reproduction, sexual determination / differentiation, immunology and metamorphosis. BACs showed a distribution in all the chromosomes not at random but a co-localization related with its functional activity was observed. Each BAC was sequenced and its sequences used to comparative genomic studies with other species.

A total of 5330 microsatellites were identified based on BAC sequences and comprising 1.27% of the genome analyzed. In average, 595,7 loci were found per Mb of Chromosome. The NL abundance ranged between 229.9 loci per Mb in chromosome 20 and 935,4 in chromosome 17.

The BLAST analysis of 2981 contigs yielded 272 miRNA matches. These matches resulted in 124 potential miRNAs organized in 45 groups. Of the 45 groups, 19 were assigned to the Senegalese sole chromosomes using BAC-FISH data, and 34 unique mature sequences targeted 12387 sequences from an *S. senegalensis* reference transcriptome. Of the 59514 sequences, present in the reference transcriptome, 12387 were putative targets according to miRanda, with some of them appearing up to seven times.

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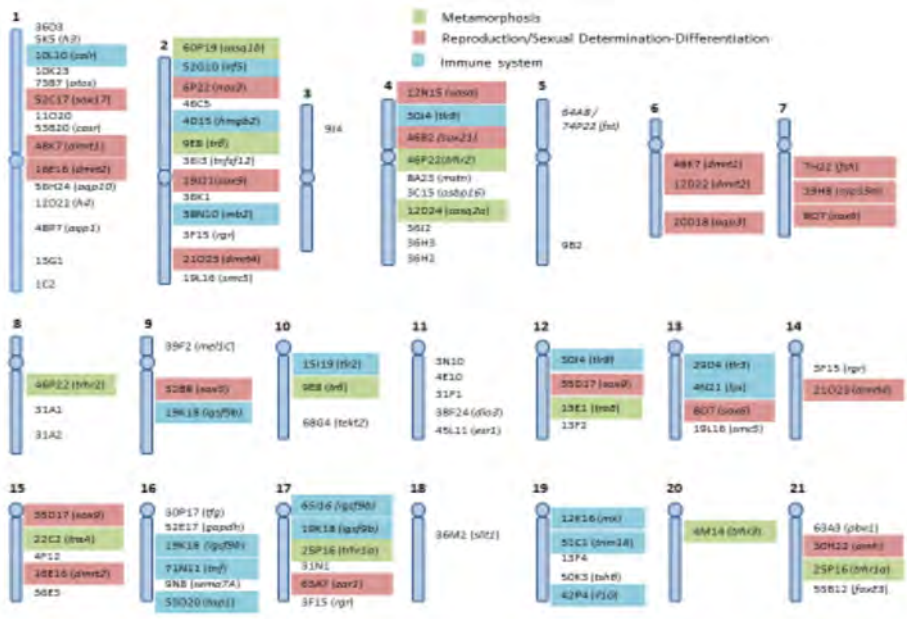

Figure 1. Integrated genetic map in *Solea senegalensis*

Discussion

The possibility of integrating the genetic and physical maps combining the linkage analysis information with the sequencing and its chromosomal location will facilitate the assembly of genome sequencing as well as obtaining very useful information for different aspects such as evolutionary studies through comparative cartography with related and model species or the identificatio and monitoring of quantitative traits loci and genes involved in sexual differentiation. Considering that Senegalese sole still lacks a useful genetic map for applications such as QTL screening and marker-assisted selection (MAS) additional genomic data are of special relevance.

SSRs contribute to the structure of DNA, the organization of chromatin, the regulation of DNA recombination, transcription and translation, gene expression and cell cycle dynamics. The abundance of SSR observed in *S. senegalensis* is similar to that found in the genome of the fugu puffer fish, *T. rubripes,* and slightly less than half in the green puffer fish, *T. nigroviridis*.

Thirty-four unique miRNA mature sequences targeted 12387 sequences from an *S. senegalensis* reference transcriptome. This suggests that miRNAs might modulate a wide range of processes in this species. These results might be helpful for understanding processes such as sexual differentiation, larval development, and disease susceptibility, which hamper the commercial utilization of this species in aquaculture.

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Acknowledgements

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DENITRIFICATION USING INTERNAL CARBON IN RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

Two emissions are of concern in state-of-the-art recirculating aquaculture systems: nitrate and fish faeces (sludge). Nitrate emissions can be reduced using a denitrification reactor. This, however, entails additional challenges that emerge from the use of an external carbon source, such as storage, safety or fish-health (Müller-Belecke et al. 2013). Sludge disposal and the use of an external carbon source for the denitrification account for up to 4% of production costs (Tschudi, 2013). In principle, these emissions could be reduced in a single step: partially digested sludge serves as a carbon source for denitrification.

The objective of this study was to determine the feasibility of a partial anaerobic digestion reactor (pADR). In the pADR organic solids (TVS) are degraded to easily degradable substances such as volatile fatty acids (VFA) and filtered using an ultra-filtration unit, to produce a solids free carbon source for use in an MBBR denitrification reactor (**Error! Reference source not found.**).

Material and Methods

A designated pADR, consisting of two separate tanks (T1, 45 l; T2 40 l, overall HRT 8.5d), was constructed and operated in two trials (durations of 15d and 8d respectively). In the second trial, an ultra-filtration system was added to the reactor and solids free permeate was produced. In a further trial, permeate from the sludge filtration was used as carbon source in bench-scale moving-bed denitrificatio reactors (V = 15 l), operated with synthetic wastewater. Additionally, the denitrification reactors were operated using ethanol and sucrose as carbon sources, to determine reference values to the permeate.

Results and Discussion

In the pADR trials, an average VFA production per gram of TVS in the inflow of 0.07mg/mg and 0.057mg/mg respectively, was determined, corresponding to an increase in VFA concentration of 35% and 43%. Compared with other studies, the achieved VFA production is two to three times lower (Conroy and Couturier, 2010; Suhr, Pedersen, and Nielsen, 2014).

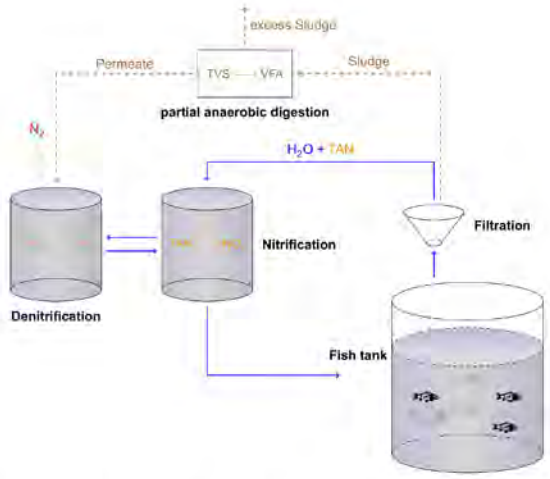

Fig. 1 RAS with an integrated partial AD process (compartments were omitted or simplicity). The supply of an external carbon source and the sludge disposal are substituted by the pADR, whereby the organic solids present in the sludge are degraded to a carbon rich, solids free permeate, which is used as carbon source for the denitrification.

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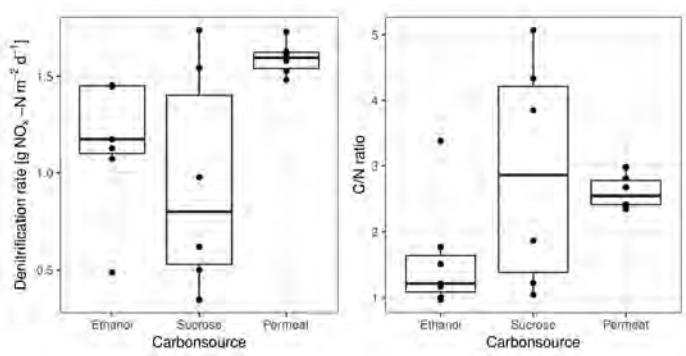

Fig. 1: Denitrification rates and C/N ratio achieved in the denitrification trials.

In the denitrific tion trials, reactors operated with permeate exhibited the highest average denitrification rate of 1.59 ± 0.09mg/m²/d, while ethanol and sucrose exhibited denitrification rates of 1.17 ± 0.34g/m²/d and 0.96±0.57g/m²/d, respectively. Utilized C/N ratios were 2.6±0.26 for permeate 1.57±0.85 for ethanol and 2.9±1.73 for sucrose (Fig. 1).

Based on a model calculation, it was determined that if using permeate as a carbon source (with an equivalent DONALD performance, achieved in this thesis), it was determined that nitrate emission could be reduced by 34.6%, water exchange by 24.2% and sludge disposal by 24%.

Conclusion

It was possible to produce a carbon-rich, solids-free carbon source from digested sludge, which additionally was a suitable carbon source for denitrification Sludge digestibility has to be improved to allow larger permeate production and therefore nitrate reduction. Nitrate emissions, water exchange and sludge disposal could be reduced substantially using the proposed technology.

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PERCEPTION ABOUT AQUACULTURE AMONG UNDERGRADUATE STUDENTS. INFLUENCE OF PERSONAL PROFILE AND PSYCHOGRAPHIC MODULATORS

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Introduction and objective

University students that are today following courses dealing with aquaculture will be in the future the professionals of the sector. Understanding their perception about the sector before starting the courses is interesting as a reference of a specific social segment (age, education profile). Information about aquaculture has been provided to evaluate changes in perception among European (Altintzoglou et al., 2010) and Spanish (Claret et al., 2016) consumers. In this case, the effect of a course on aquaculture on the students' perception is assessed. The aims of the work are (1) to explore the relation between demographic data, as well as some psychographics modulators, with the perception about aquaculture, and (2) to evaluate changes on the psychographic modulators and the perception on aquaculture, after having received information about the topic.

Material and Methods

A group of 69 undergraduate students from an engineering school at the Universitat Politècnica de Catalunya were asked to answer a questionnaire including a set of beliefs summarizing the perception about aquaculture, before having received any previous information on the topic. Data about their personal profile (age, gender), objective knowledge about aquaculture and fish buying and consumption habits (frequency and attributes driving fish buying and consumption) were also gathered. A questionnaire was posed to determine their psychographic profile (involvement as a consumer, domain specific innovativeness, subjective knowledge about fish, pessimistic bias, environmental friendliness, personal welfare, social representation of food, hedonistic traits and neophobic traits) (Reinders et al., 2016). The questionnaires for psychographic profile and beliefs, were repeated at the end of the course. The demographic data and the psychographic modulators were used to segment the group and assess potential relations with the beliefs in each segment. Results were analysed by means of ANOVA, plus Tukey test, to assess the relation between personal data and psychographic profile with the beliefs ($p \leq 0.05$). A paired sample t test was applied to compare responses before and after ($p \leq 0.05$). Consensus among students for each one of the beliefs was assessed by estimating the Interquartile range (IQR) of the responses. The salience of attributes driving fish buying and consumption was estimated by a Cognitive Salience index (CSI= frequency/number of time in each position, where max value is 1).

Results

The student population analysed is 21.4 years old as a mean, 47.9% of them were women and 52.1% men. A 56.3% of them is responsible for buying food at home. Their mean objective knowledge about aquaculture, before the course, scored 6.9 out of 10. Their mean fish consumption could be considered high since 49% of them are heavy consumers (once a week or more) while 25% are regular consumers (2-3 times a month). The highest CSI, i.e. the most salient attribute, is pleasure (0.82 out of 1.0), being origin the next one (0.725 out of 1.0). The brand and the price are the least salient attributes. Gender, responsibility for buying food at home and level of fish consumption promote significant differences in the agreement with some of the beliefs. Men show more agreement with topics as human health and fish welfare. Students that are responsible for buying food are more worried about welfare and environment. The level of fish consumption segments the agreement with topics related with fish welfare, i.e. the lower the consumption, the higher the concern about welfare in farmed fish

The analysis of the psychographic modulators, before and after the course, shows that the involvement as a consumer and the subjective knowledge about food have significantly increased, while the pessimistic bias and the neophobic traits have significant y decreased. They are closely linked factors since a higher level of knowledge may reduce the fear or the uncertainty associated to food consumption.

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Half of the beliefs posed undergo a significant change in the level of agreement after the course. Among the beliefs where there is a significant difference (10 out of 20), 6 of them imply a better image of aquaculture. The most significant change refers to the quality of the fish: the students move from agreement that wild fish quality is better to disagreement with the sentence. Before the course, wild fish was considered fresher, healthier and more controlled, and it was considered that farmed fish live crowded and not in their natural habitat. The beliefs referring to the image of aquaculture in society reach the highest level of agreement. After the course, two of these sentences have significantly increased their agreement, so students agree more with the belief that the society distrust the production processes in aquaculture and tend to reject its products, which are two sentences closely related.

When the students are segmented according to the psychographic modulators (high, ambiguous, low) there is only one belief, 'aquaculture is an abuse on the part of humans on fish', that show significant differences. The higher the innovative character, the lower the agreement with the sentence. Inversely, the higher the pessimistic bias and the neophobic trait, the higher the agreement with the sentence.

Discussion and conclusions

The present study reflects a very homogeneous group in their profile and perceptions (STEM students in a narrow range of ages). A similar study relating psychographic characteristics with consumer perceptions of farmed fish in five countries, found much more diverse profiles (Reinders et al., 2016). The psychographic profile of the students shows a group including mainly involved consumers, environment friendly, with high values for hedonic and personal welfare and with an optimistic bias. Nevertheless only one belief, in the field of ethics, was significantly related to psychographic modulators. The most pessimistic and with neophobic traits students considered aquaculture an abuse on the part of humans on fish, while this was not the case for students with a higher innovative character. This and other ethical approach sentences, related to environment and welfare, were the most sensitive ones, mainly for non consumers and occasional consumers, with negative opinions about farmed fish. These are important issues when dealing with campaigns directed to young people. Among consumers with a much broader profile, providing information on aquaculture has sometimes managed to change the perception (Claret et al., 2016), with less clear results when the previous opinion was already very positive (Altintzoglou et al., 2010). In our case, perceptible changes in specific topics occur. Nevertheless, before the course the consensus in the beliefs was higher than after it. This reflects a different sensitivity towards opinion changes within the group. Defining the main drivers for these changes is an interesting issue needed to build up efficient and positive messages

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IMPROVING AUTOMATIC FEEDING PROTOCOLS IN SEMI-INTENSIVE POND CULTURE OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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The continued success of shrimp farming will rely on improved feed management and reductions in labor costs. Shrimp are grazers eating many small meals with limited stomach capacity for food storage. Hence increased performance may be obtained by spreading feed through multiple meals. Previous works in similar conditions have demonstrated that moving from two feeding per day into multiple feeding systems increases growth rate and production. Further advances have been made with on-demand (satiation) feeding systems. Building on previous research, the goal of this work was to develop a standard feeding protocol (SFP) for automatic feeding systems to maximize growth rates in semi-intensive pond production of Pacific White Shrimp, *Litopenaeus vannamei*. For the first trial a 13-week trial was performed in 16, 0.1 ha outdoors ponds, stocked at a 26 shrimp/m^2, and fed a 35% protein soy-optimized feed. Four treatments including: three fixed feeding treatments of 130, 145 and 160% of a SFP (SFP130, SFP145, SFP160, respectively) were offered using automatic timer-feeders, and a fourth treatment utilized on-demand acoustic feeding system (AQ1). Results for this trial are summarized in Table1. No statistical differences were found between treatments for survival and FCR. increased feed inputs resulted in significantly higher production and higher product value. The best response was with the AQ1 acoustic feeding as animals under this treatment were offered higher feed inputs resulting in larger shrimp and consequently yields.

Following the results of the first trial, a second pond production trial with four treatments is being conducted. Including three timer feeder feeding treatments (12hr daytime, 12 hr nighttime and 24h/day), as well as a fourth treatment utilized on-demand acoustic feeding system (AQ1). Results will be reported.

SYSTEMIC IMMUNE RESPONSES OF GILTHEAD SEABREAM *Sparus aurata* JUVENILES FED MICROALGAE-DERIVED BETA-GLUCANS

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Introduction

Nutritionally balanced diets are paramount for fish growth and welfare, particularly when fish are subjected to stressful conditions. To enhance fish disease resistance and general health, fish diets are often supplemented with immune-modulators such as β-glucans. These compounds are known to have beneficial effects in fish innate immune response (Guzmán-Villanueva et al., 2014). β-glucans show repeating patterns on their structure that are recognized in the gut by cell pattern recognition receptors (PRR), leading to the activation of the host´s innate immune cells enhancing its immune response (Dalmo et al., 2008). The present work aimed to evaluate the effects of both short- and long-term dietary supplementation with algae (*Phaedactylum tricornutum*) extracted β-glucans on immune related genes expression, oxidative stress biomarkers and plasma immune parameters in gilthead seabream juveniles.

Materials and methods

The trial comprised four isonitrogenous (63% crude protein) and isolipidic (17% crude fat) dietary treatments. A high quality, practical diet was used as CTRL, whereas 3 experimental diets based on CTRL were further supplemented with a constant dose of β-glucans, derived from *Saccharomyces cerevisiae* (diet C+) and different extracts of *P. tricornutum* (diets PH21 and PH37). Diets were randomly assigned to quadruplicate groups of 200 gilthead seabream (*Sparus aurata*) (initial body weight: 4.1 ± 0.1g) that were fed to satiation three times a day for 8 weeks in a pulse feeding regimen. Fish were first fed the different experimental diets intercalated with the CTRL diet every 2 weeks. After 2 and 8 weeks of feeding, 3 fish per tank were sampled. Blood was collected for haematological procedures according to Machado et al., (2015) whereas liver, gut and head-kidney samples were collected for further analyses. Immune parameters were analysed on blood plasma to evaluate fish immune status

Results

All groups showed equal feed conversion ratio (FCR) and relative growth rate (RGR) values (1.2 and 3.8%/day, respectively) and attained similar final body weight (FBW) (CTRL: 41.4 ± 1.6g;C+: 42.5 ± 0.9g; PH21: 42.1 ± 1.1g and PH37: 41.9 ± 1.9g). Regarding immune parameters, no changes were observed in plasma bactericidal and anti-protease activities and IgM levels among different dietary groups.

Discussion and conclusions

β-glucan supplementation independently from the source did not affect fish growth performance. Absence of clear effects on plasma innate immune parameters suggests that the dietary treatments did not elicit a systemic response. However, from the nature of β-glucan stimulus it is reasonable to expect a local immune activation in the gut. Further analysis on liver, gut and head-kidney samples are currently underway to obtain an overall picture of fish health status

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MARINE MICROALGAE AS A SOURCE FOR LIPID, EPA AND DHA FOR USE IN AQUAFEED

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Introduction

Cultivated microorganisms can be a sustainable aquafeed resource that can meet the future needs for lipids and the essential fatty acids EPA and DHA (Chauton et al., 2011). The production of lipids and EPA/DHA varies between photosynthetic microalgae species and cultivation conditions (Reitan et al., 1994; Brown and Robert, 2002, Wang et al., 2019). Increased nutrient stress in the cultivation process is a strategy to increase lipid content of the microalgae biomass (Chen et al., 2011; Vu et al., 2016; Wang et al., 2019). The lipid and fatty acid profiles of the microalgae are believed to be important for the utilization by the fish of these biomasses in aquafeed (Sevgili et al., 2019), as the distribution of fatty acids in the lipid classes will determine the nutritionally availability in fish feed in general (Guedes et al., 2010)

In this study, typical microalgae species used in aquaculture were grown under different cultivation conditions and analyzed for total lipid and fatty acid contents. Based on the lipid content and fatty acid profile the species could be considered as a source for use in aquafeed.

Materials and Method

Screening experiments for obtaining optimal conditions for lipid production of four selected microalgae species have been conducted: (1) *Phaeodactylum tricornutum* CCMP 2561, (2) *Isochrysis* aff. *galbana* clone T-Iso CCAP 927/14, (3) *Rhodomonas baltica* NIVA-5/91, and (4) *Nannochloropsis oceanica* CCMP1779. The microalgae were grown at different temperatures (18 °C, 20 °C, 23 °C, 25 °C and 30 °C) at light intensities (50-130 µmol photons/m²sec). As a basis F/2 medium was used. For characterizing the effect of nitrogen limitation on the lipid production, the concentration of nitrogen was reduced to 10% and phosphorous increased with 25% of the original F/2 media. On the other hand, for production of high-density biomass of *N. oceanica*. the nitrate content was increased 3.3-fold and for *P. tricornutum* the phosphate content was increased by a factor of 2.4. Lipids and fatty acids were analyzed as described in Wang et al. (2019)

Results and Discussion

The percent content of EPA and DHA varied between the microalgae species when cultured with increasing nitrogen limitation, showing decreased contents in *P. tricornutum* and *N. oceanica* with increasing nitrogen limitation, wheras the contents increased in T-ISO (Table 1). Highest EPA content was found in *P. tricornutum* while T-ISO had highest DHA content.

Table 1 - Summary of Pacific white shrimp response to different feed management protocols

Treatment	IndW (g)	Survival	Yied (kg/ha)	Total Feed Input (kg/ha)	FCR	Feed Cost ($/ha)	Shrimp Value ($/ha)	Partial Income ($/ha)
SFP 130%	26.29^{a}	77.6	5226^{a}	4933^{a}	0.99	5592^{a}	43,490^{a}	37,898^{a}
SFP 145%	26.87^{a}	75.2	5115^{a}	5332^{b}	1.11	6026^{b}	42,468^{a}	36,442^{a}
SFP 160%	29.04^{a}	80.7	6128^{ab}	5844^{c}	0.96	6585^{c}	52,62^{ab}	46,03^{ab}
AQ1	32.53^{b}	81.4	6869^{b}	6984^{d}	1.02	7828^{d}	60,723^{b}	52,90^{b}
P-value	0.0096	0.9083	0.0274	<0.0001	0.7313	<0.0001	0.0073	0.0164
PSE[1]	1.18	6.52	39.62	5.07	0.097	55.3	3,362	3,380

[1]PSE – Pooled Standard Error

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All four species accumulated lipids mainly in the form of TAG when cultured under nitrogen limitation (Figure 1). *N. oceanica*, accumulated 51% of the dry weight as lipid in moderate nitrogen limitation and up to 87% of the fatty acid was in TAG.

The lipid content in the microalgae produce for high biomass was higher in *N. oceanica* than in *P. tricornutum* (Table 2).On the other hand somewhat higher contents of EPA + DHA was found in the latter one. In conclusion, broth *N. oceanica* than *P. tricornutum* are regarded as potential microalgae biomass for us in aquafeed (Sevgili et al., 2019).

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ENHANCING EUROPE-ASIA COOPERATION IN AQUACULTURE EDUCATION – ACHIEVEMENTS OF THE EU HORIZON2020 PROJECT - EURASTIP

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The European Union (EU) funded EURASTiP project is an initiative to reinforce international cooperation on aquaculture between Europe and countries from Southeast Asia. The project fosters the development of stakeholder platforms in Thailand, Vietnam and Bangladesh to link with the European Technology and Innovation Platform (EATiP) and its regional and national Mirror Platforms. Amongst other activities, brokerage events, industrial and research exchange visits support business and trade whilst education activities support educational links across the regions. Consolidating existing EU-Southeast Asian collaborations in the field of education and training as well as enhancing new collaboration through numerous activities is a key objective of EURASTiP.

One of the first activities carried out in relation to aquaculture training was a training survey to identify existing aquaculture education networks and their activities. The two most frequent type of collaborations were identified as receiving students and collaboration with research stakeholders. Important benefits of EU-Asia collaborations were considered as knowledge and skills sharing, along with the development of best practice and expertise across both regions. The major challenges were identified as lack of funding to support collaborations and cultural differences between actors from Europe and Asia. Further to the training survey, an alignment workshop was held to bring together the champions of major aquaculture education networks to exchange best practice in relation to aquaculture training provision between Europe and Asia; and to identify the key topics of common interest to both regions for the future of aquaculture education. Based on the selected key topics, three capacity building workshops were planned, two of which have taken place, covering the topics: 1) Promoting Innovative Teaching through Collaboration in the International Aquaculture Sector; 2) Ensuring Education is Responding to Industry Needs. The third and final capacity building workshop will take place in November 2019 alongside the International Fisheries Symposium in Kuala Lumpur (Malaysia) focusing on Enhancing Opportunities for Mobility.

In addition to the capacity building workshops, the EURASTiP project has supported an exchange programme for aquaculture educators to maximise collaboration and strategic partnerships. As part of a broader Asia-Europe exchange programme under EURASTiP, a series of thirteen Educator exchange visits have been supported. These are intended to help build collaborative partnerships between Europe and Asia and participants have shared their experiences through blogs, reports and videos. Additional education and support resources are being developed from these to further support understanding and cooperation between Asia and Europe.

Other educational and training materials for aquaculture educators have been developed as part of the project. These include example educational material, based partly on the exchange programme, being developed to support understanding of European and Asian aquaculture sectors. These are being made available as Open Educational Resources (Pounds & Bostock 2019a&b) which can be adapted and re-used for different purposes via the AQUACASE website (www.aquacase.org). The testing of these materials is also underway to help provide guidance for future development. Also, a Best Practice Case Study Report has been developed to help with overall capacity building. Examples of collaborative education and training between Asia and Europe have been written up and collated into a downloadable publication (Bostock 2019). This also includes a summary of issues that need to be considered when developing educational collaborations and student mobility, especially with respect to recognition of academic qualifications and credit

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EVALUATING THE ENVIRONMENTAL CONDITIONS REQUIRED FOR THE DEVELOPMENT OF OFFSHORE AQUACULTURE: IMPACT ON FARMED ATLANTIC SALMON HEALTH AND WELFARE

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Introduction

Scottish salmon is worth over £2 billion to the country's economy. It is Scotland's top food export and a particularly valuable product, at about 10% above the world price. Recently, the Scottish Government developed a plan that aims at doubling this production of salmon by 2030. However, a number of factors limit the progress of the industry in its current form, which is based primarily on sea cages in sheltered, fjordic sea lochs where water exchange is restricted. These limitations include the impact of sea lice infestations on fish health (Black 2001), the cost and environmental impact of chemical treatments against the parasite (Costello 2009, SARF 2016, Van Geest 2014), their development of resistance to these treatments (Aaen et al. 2015), planning issues (James & Davies 2010), the (regulatory) Scottish Environment Protection Agency (SEPA) biomass limit of 2500t/site, and the increasing impact of harmful algal blooms (HABs), which can be particularly acute in restricted water exchange environments (Gowen et al. 2012). Moreover, the industry also faces opposition from environmental campaigners who fear that farming in sea lochs might damage wild stocks and the ecosystem. Development of aquaculture in more dispersive "offshore" environments offers a potential solution to some of these problems. Improved, science-based evidence with direct relevance to the complex environment of the west coast of Scotland will permit planning and informed regulation of this offshore transition.

The project will address a number of the issues, specifically offshore developments, algal blooms, [sea lice/AGD] host-pathogen interactions and fish health and welfare. These will be addressed through four interconnected work packages: 1) Physical Oceanography, 2) Wave modelling and risk, 3) Hydrodynamic and sea lice/HAB modelling, and 4) Fish health and welfare implications. The latter addresses the particular issue of moving cages offshore and the impact on Atlantic Salmon health and welfare (Kirchhoff et al 2011, Ashley, 2007).

Materials and methods

Health historical data will be supplied by the partner salmon company regarding mortalities, condition, growth, health blood parameters, sea lice counts, and gross gill scores (e.g. amoebic gill disease (AGD) and proliferative gill disease (PGD)). Data on detailed morphometrics and welfare scoring indices (external appearance and fin and tissue damage) will be supplemented by 'in situ' sampling data in the same periods and at the same sites where the physical oceanography samplings take place. Environmental and physical data will also be provided by the company to complete the database from the sampling sites.

There will be 3 main sampling points per treatment (offshore vs inshore farms), at which a total of 45 fish will be sampled per site (15 fish per cage, 3 cages per site, and 2 sites). Historical and newly-collected fish heath data will be analysed, through both univariate and multivariate statistics, in relation to physical oceanographic parameters, waves, sea lice, and HAB data, as well as by elaborating model predictions.

Initial sampling was already performed in winter 2019. Fish were weighted and measured, sea lice, AGD and PGD scored and skin mucous, blood and tissue sampled and stored for posterior analysis.

The next sampling will take place in spring 2019 and at harvest (August-September 2019). By the time of the conference, more data will be available.

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Results and Discussion

Because this is an ongoing project and the current results are only preliminary, more data to support the claim of an offshore site's potential in improving welfare in Atlantic salmon is needed. However, current results indicate that offshore environments presented significantly lower incidence of parasites (AGD, *Caligus* sp. and adult male sea lice) and had better welfare indicator scores, such as fin damage and cataracts. The decreased incidence of parasites could be the result of increased water velocities, improved aeration and net cleanliness in offshore sites. This increased water velocity could also explain the lower number of sea lice due to the increased difficulty in attachment to the fish (Revie *et al.,* 2004). However, results indicate that fish were smaller in the offshore site. Later samplings will show whether this weight and length difference is maintained.

Additional work and analysis of historical data is required to confidently validate the hypothesis that high energy offshore sites improve welfare and overall health of Atlantic salmon. However, current results are promising.

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USING MROSATELLITE MARKERS TO ASSESS POST-STOCKING SURVIVAL OF HATCHERY-REARED ATLANTIC TROUT (*Salmo trutta* L.) IN TRIBUTARIES OF A PREALPINE LAKE

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Introduction

As countermeasures against ongoing declines and habitat alteration, many fish populations in Switzerland are supplemented through stocking of fish raised in captive environments. However, the success of this stock enhancement often remains unclear and hatchery-reared fish possibly are inferior to remnant wild individuals (Araki et al. 2008). Thus, the verification of the stocking success becomes more and more common and is often evaluated using genetic methodology. We conducted such a genetic success control of the stocking practice of lacustrine Atlantic trout (*Salmo trutta* L.) for the cantonal fishing office of Canton Zürich (FJV) in Switzerland

Materials and Methods

A predefined number of individuals from a broodstock were hand stripped in spring 2018 and their hatchery-reared offspring was stocked as fry in early spring 2018 in three experimental tributaries of Lake Zürich in Switzerland by the FJV. Parr in these three tributaries were caught in autumn 2018 by electrofishing and a small fin clip of each individual was taken for subsequent genetic analyses. In total we genotyped 207 parr and 48 broodstock fish (22 males & 26 females) using a set of 19 highly polymorphic microsatellite markers to assess survival of these stocked fry over the first summer past stocking using parental analysis. To further test for possible genetic differentiation between the broodstock and remnant trout populations in the three tributaries, we additionally genotyped adult fish from each of the three tributaries.

Results

Preliminary results showed that the proportion of stocked parr among the three experimental tributaries on average varied between 49% and 62%, depending on whether parentage was determined using a strict exclusion or categorical allocation approach. Analysis of genetic diversity parameters revealed poor genetic diversity and signs of bottleneck effects in the broodstock. We further observed significant genetic differentiation between the broodstock and all three groups of adult fish among the three sampling locations. Thus, although about half of all parr were positively identified as stocked fish, their long-term survival and supplementary effect on remnant populations is questionable and needs further research.

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ZEBRAFISH AND LPS, MODEL TOOLS FOR DECIPHERING EPIGENETIC CHANGES DURING SEX DIFFERENTIATION

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Introduction

Fish farmed for human consumption are reared in artificial environments that are very different from conditions that species experience in the wild. Consequently, the environment influences many aspects of the biology of cultured animals, including sexual phenotype through epigenetic mechanisms (Feeney et al., 2014). Disease outbreaks occur eventually in fish farms, causing economic loses. However, the epigenetic consequences that these reiterating infections can potentially cause in fish during their development and whether these can alter the reproductive system, remain unknown. Here we present a study for which two model research tools have been selected; the zebrafish as a suitable model for aquaculture research (Ribas and Piferrer, 2014) and the lipopolysaccharide (LPS) from Gram negative bacterial wall as a model to stimulate the immune system in fish (Forn-Cuní et al., 2017). The aim of this work is to develop a suitable *in vivo* system to study whether infections occurring in fish during sex differentiation are able to alter the final gonadal phenotype throughout epigenetic changes.

Materials and methods

AB zebrafish were housed following standard conditions (Ribas et al., 2014;) (Bioethical Committee code: 9977). Zebrafish juvenile at 15 days post fertilization (dpf) were stimulated with three different strains of LPS; *Escherichia coli*, *Pseudomonas aeruginosa* or *Aeromonas hydrophila*, during sex differentiation. First, and in order to find the median lethal dose (LD50), animals were exposed to different LPS concentrations and survival were recorded. Samples were taken at 3, 6, 24, 48 and 72 hours after LPS stimulations for studying the expression levels of three immune genes (*IL-1β*, *TNF-α*, *Casp9*) involved in the inflammatory response. Methylation patterns of these immune genes were studied by Methylation Bisulfite Sequencing (MBS), a candidate gene approach (Anastasiadi et al., 2018), by high throughput sequencing (Illumina, paired-end). Surviving individuals were transferred into tanks and kept until adulthood to check final sex ratios

Results

Fish survival was dependent to the dose and the LPS strain, being the *A. hydrophila* strain with the highest toxicity. Gene expression of the studied immune genes were downregulated after *P. aeruginosa* exposition but a significant upregulation was found when zebrafish were exposed to *A. hydrophila*. Methylation levels in the juvenile larvae showed significant changes in the methylation levels of specific CpGs of the three studied genes (Fig.1). Short exposition of LPS was not able to change sex ratios but a feminization tendency was observed after longer exposition to *A. hydrophila* in a dose-response and family-dependent manner.

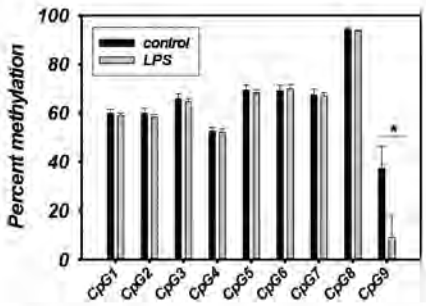

Figure 1. Methylation of immune genes (IL1β) of 15 dpf zebrafish expose during 3h to LPS of *P. aeruginosa*. Data showing as mean ± SEM. Sample size n = 6-7 zebrafish per treatment. Significant differences *($P < 0.05$) were analyzed by Student's t-test.

(Continued on next page)

Discussion and conclusions

Here we have developed an *in vivo* model to study the effects of immune stimulation during gonad differentiation that results in long-term influences in the sexual phenotype and it is able to alter methylation and gene expression patterns. We have identified the LD50 that is able to induce an activation of the immune system by reducing fish survival proving that LPS from *A. hydrophila* has a higher toxicity than the LPS of *P. aeruginosa* but less than *E. coli*, as partly shown in other studies in zebrafish (Novoa *et al.,* 2009). Zebrafish is a gonochoristic species in which all individuals starts developing as female and later, during sex differentiation, male differentiation processes appear throughout apoptotic and p53 signaling pathways that are responsible of the testes development (reviewed in Liew and Orban, 2014). Here, by stimulating zebrafish fish LPS from *A. hydrophila* we have been able to alter sex ratios by decreasing the number of males in a dose and family dependent manner. Intra-family variation of the other environmental factors (i.e., temperature and density) have been observed in zebrafish (Ribas et al., 2017a, 2017b). Previous studies in zebrafish treated with heat-killed bacteria from *E. coli* during sex differentiation showed a female bias due to the activation of NF-κB pathway that induced anti-apoptotic effects in the differentiating gonad (Pradhan *et al*., 2012). We observed that methylation and expression mechanisms were involved in the LPS stimulation in the juvenile larva and so interfered in the final sexual phenotypic fate. However, the epigenetic and transcriptomic mechanisms involved in the gonadal changes need to be further elucidated. In conclusion, our results showed interactions between immune–reproduction systems and we identified methylation differences for particular CpGs of the studied immune genes that can be worth for developing markers that can improve breeding programs (e.g., selection of high quality broodstocks).

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Enteromyxum leei TREATMENT TRIAL IN SHARPSNOUT SEA BREAM (*Diplodus puntazzo*) USING PHYTOBIOTICS

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Introduction

The myxozoan parasite *Enteromyxum leei* is a serious pathogen of sharpsnout sea bream, *Diplodus puntazzo* causing epizootic outbreaks with severe catarrhal enteritis. There are currently no prescription medicines effective against this parasite and most attempts to control it in the field and/or in experimental trials have failed. However, empirical and experimental evidence have shown the benefits of certain feed additives against this infection in Gilthead seabream (Palenzuela et al., 2017). Therefore, the aim of the present work was to evaluate in controlled conditions a commercial blend of phytobiotics (Sanacore), and its potential to control enteromyxosis. This was accomplished during a lab-controlled challenge in which feed intake and growth, survival, immunological and parasitic measurements, were studied.

Materials and methods

Sharpsnout sea bream juveniles (~2g) were given a commercial diet until they reached a size of approximately 12g, and they were then transferred to the challenge environment and distributed in 6 identical net cages (1m^3) (300 fish/cage) arranged within a large (50m^3) inland concrete tank. In this environment, experimental fish were delivered either medicated (Sanacore 0.3% included) or control extruded diets prepared in the facilities. Four weeks after the start of the challenge with donors all surviving fish were individually weighed and the following growth parameters were calculated: FCR, SGR and FE. Samples were taken for the measurement of immunological parameters (hemoglobin concentration, respiratory burst activity, lysozyme, anti-protease activity and ceruloplasmin) and for the diagnosis and quantification of *E. leei* rDNA by qPCR.

Results & Discussion

Eventually, the cumulative mortality after challenge reached values as high as 22 & 27% in the control and the treated groups respectively, indicating insignificant differences between Sanacore-fed and control groups. Growth was greatly affected by *E. leei* infection since the calculated growth parameters seemed to be considerably impaired in comparison with normal sharpsnout sea bream growth curves. Weight increase (~10g) was low in both groups. Minor differences between the two groups were observed in (impaired) measured growth parameters (FCR: 3 vs 2.86; FE: 33.6 vs 35.6; SGR: 0.55 vs 0.58 for control- and Sanacore- fed fish, respectively). Out of the 90 experimental fish examined, 69 samples were positive for *E. leei* by qPCR (76.67% prevalence). Of these, 35 belonged to the control group (77% prevalence) and 34 to the Sanacore group (75.56%). No differences were found in the mean intensity of infection between groups or among replicate cages within each group (Figure 1). Prevalence and intensity of infection among challenged mortalities trial were 63.2%, and 13.9 ± 1.5, respectively. While these values are similar to those of donor fish, they are significantly lower than in experimental fish at the end of the trial. Concerning the immunological measurements, the parasitological challenge significantly affected fis fed both the control and the Sanacore diets as observed through the reduced hemoglobin concentration and the increased respiratory burst activity of the fresh blood, observed at the end of the experiment relative to the initial levels. A decrease in the values of anti-protease and anti-bacterial activities of the challenged fish was also observed, which was significan in the case of ceruloplasmin activity in control fish. However, this infection-induced immunosuppression observed in both groups was reduced or even totally reverted (depending on the immunological parameter concerned) in fish fed Sanacore for one month before the challenge.

In a different study, Sanacore reduced the prevalence and intensity of *E. leei* infection and improved the condition of gilthead sea bream in an experimental challenge with this parasite, although it did not clear off the infection (Palenzuela et al., 2017). Given the high pathogenicity of *E. leei* in *D. puntazzo*, it is not surprising that nutraceutical strategies could not prevent clinical enteromyxosis in this trial, in which a very high infection pressure was applied. However, the drop observed in some immune-related parameters in challenged control fish appeared reduced in Sanacore-treated fish, indicating a biological effect of the supplement.

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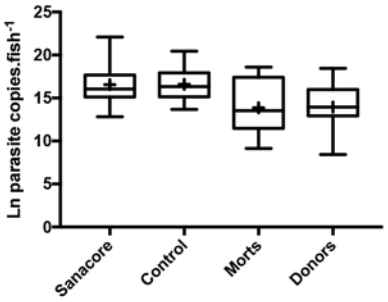

Fig 1 *E.leei* infection intensity (parasite rDNA copies.fish^{-1}) in the experimental groups.

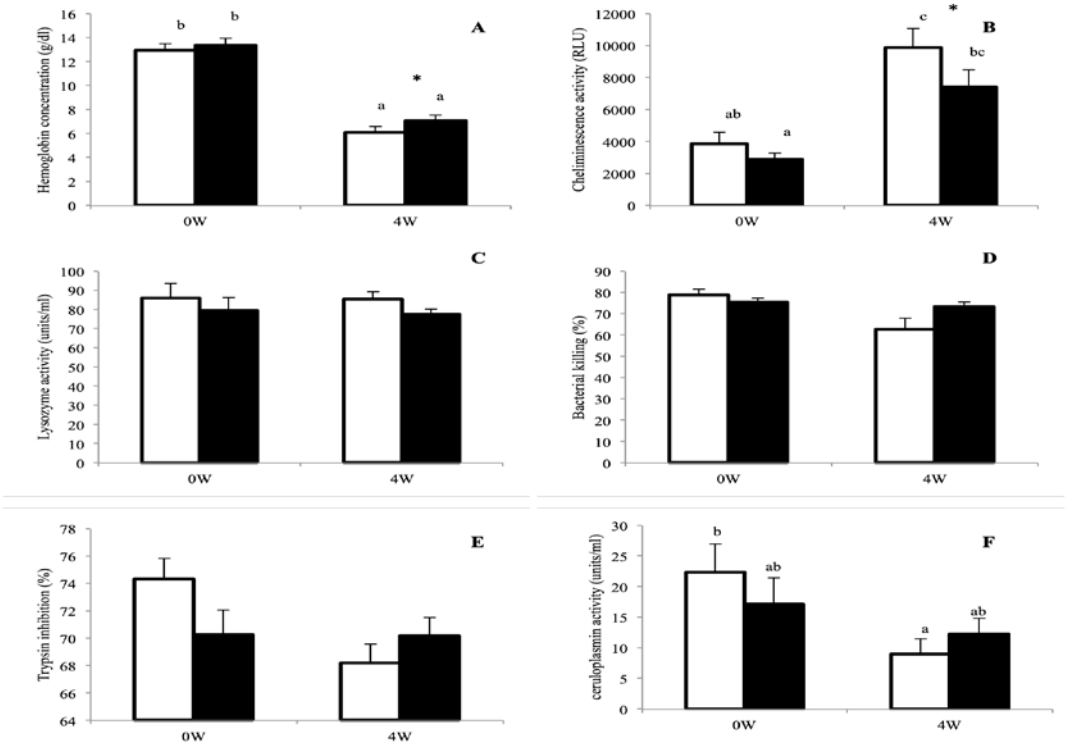

Fig 2 (A) Hemoglobin concentration (g/dl blood), (B) Chemiluminescence activity (Relative Luminescent Units), (C) anti-Gram positive activity, (D) anti-Gram negative activity, (E) Anti-protease activity, (F) Ceruloplasmin activity in the fresh blood or sera of sharpsnout seabream, *Diploduspuntazzo*, fed control (□) or Sanacore (■) diets for 4 weeks before (0W) and after 4 weeks (4W) of a parasitical challenge by cohabitation

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IMPACT OF WATER PURIFICATION AND CONDITIONING ON *IN VITRO* PRODUCED EMBRYOS AND LARVAE OF EUROPEAN EEL (*Anguilla anguilla*)

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Introduction

Aquaculture fish production first exceeded fisheries production in 2012, and is currently the fastest growing food-producing sector in the world. However, such intensified fish production is prone to disease outbreaks, of which opportunistic pathogenic bacteria are a major cause. During their early life stages, marine fish are particularly vulnerable to infections in intensified aquaculture (Vadstein et al., 2018)we explore the microbial community of fish larvae from an ecological and eco-physiological perspective, with the aim to develop the knowledge basis for microbial management. The larvae are exposed to a huge number of microbes from external and internal sources in intensive aquaculture, but their relative importance depend on the rearing technology used (especially flow-through vs. recirculating systems due to their yet undeveloped immune system and the inherent bacterial load caused by high stocking densities. This is particularly relevant for the early life stages of the European eel *Anguilla anguilla*. Bactericidal treatments may prove useful in the short term (Sørensen et al., 2014), though they also destroy benefici l bacteria and in the long term may lead to a less stable microbial community (Vadstein et al., 2018)we explore the microbial community of fish larvae from an ecological and eco-physiological perspective, with the aim to develop the knowledge basis for microbial management. The larvae are exposed to a huge number of microbes from external and internal sources in intensive aquaculture, but their relative importance depend on the rearing technology used (especially flow-through vs. recirculating systems. By optimising operational conditions such as substratum, nutrition, filtering, and water retention time, this may help to support more favorable microbial communities and hence improve the stability and rearing success in recirculating aquaculture systems (RAS). This study examines the vital processes for eel embryo survival during egg incubation and subsequent hatch and survival of larvae, coupling egg-associated bacterial colonization in different sea water environments, with pelagic bacteria levels and organic matter character.

Materials and methods

Eggs harvested from captive bred European eel were fertilized using pre-diluted milt, and activated using artificial seawater based on reverse osmosis water and reef salt (Sørensen et al., 2015). Buoyant eggs were distributed into three 60 L conical incubators connected to three different types of seawater, applied as flow-through with the water inlet at the bottom. Incubators were held at 18ºC, at low light and a constant supply of 0.2 µm filtered air. The three water masses used for incubation were treated as follows: 1) MSW - preconditioned microbial matured seawater, selected for increased microbial stability by aid of RAS retention time, and steady nutrition levels; 2) UFSW - MSW that was further treated by passing through cross flow Ultrafilter ~50kDa, removing particles smaller than bacteria and vira; and 3) UVSW - MSW which was then passed through 1 µm 20” cartridge filter and UV-treated (UVSW). Each incubator was sampled daily to estimate egg density, bacterial load, fertilization, survival, hatch, and subsequently larvae survival to day 12. Egg-associated bacteria were quantified at 0, 2, 4, 24, and 48 h post-fertilization, using the Bactiqant method (Mycometer, Hørsholm, Denmark) - a novel technique measuring enzymatic metabolic processes of bacteria. In addition to egg-associated bacteria, the bacteria in the incubation water were quantified in regards to both the in- and out-flowing water, to assess the impact of the eggs and their bacteria on water quality. The inlet and outlet water were also analysed for fluorescent dissolved organic matter (FDOM), which may have an impact on heterotrophic bacteria in the rearing medium. Furthermore, microbial growth potential in the three different water treatments was assessed to quantify microbial stability or shelter effect (Blancheton et al., 2013)”container-title”:”Aquacultural Engineering”,”page”:”30-39”,”volume”:”53”,”source”:”ScienceDirect”,”abstrac t”:”The current onshore aquaculture trend is to develop large scale production of diversified fingerlings and very large units for fish ongrowing. This requires an industrial type of approach including quality assurance and minimization of failures in addition to management of bio-technical and economic aspects. Therefore, all the key biological mechanisms involved in Recirculating Aquaculture Systems (RAS. Hatch was assessed by sampling ~100 buoyant embryos at 50 HPF from each water type, inserted into triplicate antibiotic flask containing 200ml 50 ppm rifampicin and 50 ppm ampicillin (Sørensen et al., 2014), and incubated in temperature controlled environment (18ºC) until 60 HPF, when the ratio of hatched larvae was determined. Larval survival was estimated by random selection of 3 × 20 larvae from each water type and inserting upon hatch into 200ml flasks of antibiotic similar to above. Flasks with larvae were cultured in a temperature controlled environment, and survival was quantified daily throughout the yolk sac period until day 12 DPH (Sørensen et al., 2016)

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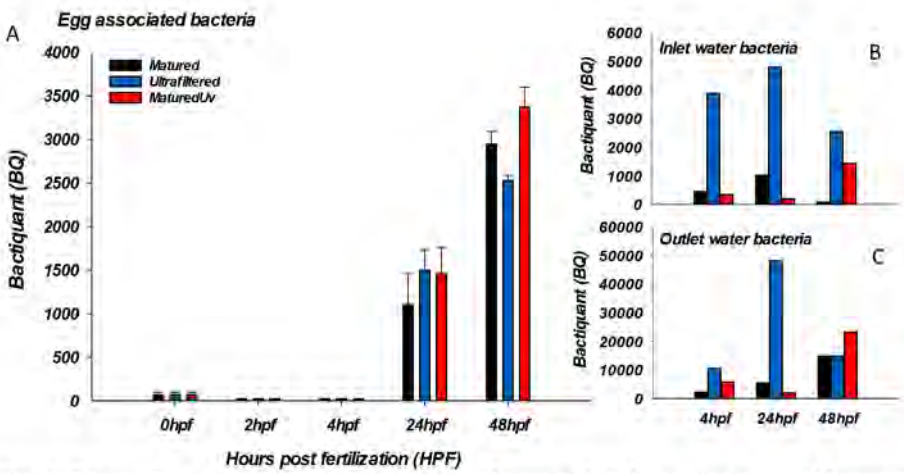

Fig. 1: Bacterial activity (Bactiquant) on incubated eggs and in the rearing water of European eel eggs reared using Matured (MSW), Ultra filtered (UFSW), and Matured UV treated water (UVSW). A: Egg-associated bacteria colonizing the egg of European eel between 0 h post fertilization (HPF) to 48 HPF. B: Bacteria levels of water inlet to the egg incubator. C: Bacteria levels of water and outlet of the egg incubator.

Results and discussion

The study showed that conditioning RAS water by microbial maturation, selecting for K-strategist, and reducing growth potential in the water effectively improved stability in the rearing environment and increasing embryonic rearing success in European eel (Fig. 1).

Bacterial colonization of eggs occurs rapidly, and various filtration techniques can improve survival, but also tend to induce instability and increase variability forming a "virgin" water mass ready for colonization by opportunistic pathogens (Vestrum et al., 2018)and that recirculating aquaculture systems (RAS. By analyzing water content and characteristics regarding fluorescent dissolved organic matter, we could differentiate between the carbon footprint of 'good' and less 'good' aquatic environments and their effect on colonization level/speed on incubated eggs.

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NEW FUNCTIONAL FEED SUPPLEMENT: EFFECTS OF DIETARY YEAST AUTOLYSATE ON INTESTINAL MICROBIAL COMMUNITIES OF SEA BREAM (*Sparus aurata*)

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Introduction

Fish protein hydrolysate is rich in free amino acids, bioactive compounds and water-soluble proteins that improve feed digestibility and palatability, thus promoting fish growth and feed utilization. Protein hydrolysate from yeast (*Saccharomyces cerevisiae*), is assumed to have similar beneficial effects of fish protein hydrolysate but, in addition, it contains several immune-stimulating compounds, such as β-glucans and mannan-oligosaccharides, which have positive influence on immune responses and stress tolerance of fish. However, the introduction of any new ingredient in the diet needs to be carefully evaluated since diet is one of the main factors shaping the intestinal microbiota. The gut microbial communities of fish is indicated being correlated with host metabolism, nutrition, growth, immunity, and disease resistance, in a similar way as reported for mammals.

The present study aimed to investigate the effects of a diet rich in plant proteins, supplemented with 5% of either fish protein hydrolysate or autolysed dry yeast (HiCell, Biorigin) on intestinal microbiota of gilthead sea bream.

Materials and methods

Seven hundred twenty S.aurata with an initial mean body weight of 122.18 ± 6.226 g (mean ± s.d.) were randomly distributed into nine fibreglass tanks (80 fish/tank) connected to a flow-through fish rearing system with three complete daily water changing in each tank. Experimental tanks were supplied with water with an approximately constant temperature of 24.5 ± 2.26 °C (Fronte et al. 2019).

Three dietary formulations (A, B and C) were produced by Naturalleva VMR S.r.l. (Italy). The diet based on commercial fishmeal (FM)/vegetable meal containing 46% crude protein and 16% fat) was used as the control diet (A). The other two diets were characterized by FM replacement with fish hydrolysate (diet B) and with the commercial additive HiCell (diet C). Fish were fed with the experimental diets in triplicate (3 tanks/diet) for 90 days.

At the end of the trial 15 fish/dietary group five fish/tank were sampled. The intestine was aseptically removed from each fish and squeezed to collect the fecal matter. For microbiome analysis, bacterial DNA was extracted from each faecal sample. The high-throughput sequencing of 16S rRNA gene was applied to analyse and characterize the gut microbial communities of rainbow trout as described in Rimoldi et al. (2019). The obtained sequencing raw data were analyzed using the QIIME software. A Two-way ANOVA was applied to test for differences in mean values of bacterial taxa between the experimental groups using STATISTICA software (StatSoft, Inc.). A $p\text{-}value<0.05$ was considered significant.

Results and Discussion

Totally 102 operational taxonomic units (OTUs) of bacteria were identified from the 2,327,049 sequences obtained by Illumina MiSeq 16S rRNA gene sequencing. Interestingly, our data revealed that inclusion of autolysed dry yeast was associated with an increased bacterial diversity compared to fish protein hydrolysed supplemented diet. Indeed, although most of the bacterial taxa were common between dietary groups, fish receiving autolysed yeast showed higher abundance of *Bacillus* and *Shewanella* genera, besides to be characterized by specific bacterial genera, such as *Megasphera*, potentially beneficial for host (able 1).

In conclusion, autolysed dry yeast could be a valid alternative protein source for aquafeed production, since it positively affects the intestinal microbial communities of sea bream by increasing the number of indigestible carbohydrate degrading and short chain fatty acid producing bacteria.

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Table 1. Percentage of most abundant genera (mean ± SEM) found in different dietary groups. "n.d." means not detected. Statistical significance: (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

Genus	A	B	C	
Prevotella	n.d.	n.d.	0.8 ± 0.3^{a}	***
Bacillus	0.9 ± 0.2^{b}	1.5 ± 0.4^{b}	2.3 ± 0.6^{a}	**
Staphylococcus	< 0.5	0.8 ± 0.1	0.8 ± 0.2	
Lactobacillus	70.9 ± 3.9	63.6 ± 6.1	67.8 ± 3.7	
Clostridium	< 0.5	1.3 ± 0.6	0.8 ± 0.5	
Megasphaera	n.d.	n.d.	1.2 ± 0.3^{a}	***
Comamonas	< 0.5	< 0.5	0.6 ± 0.2	
Shewanella	2.0 ± 0.5^{a}	$< 0.5^{b}$	1.1 ± 0.3^{a}	***
Erwinia	$< 0.5^{ab}$	$< 0.5^{b}$	0.6 ± 0.2^{a}	**
Pseudomonas	0.7 ± 0.1	0.9 ± 0.3	1.0 ± 0.3	
Pseudoalteromonas	0.9 ± 0.2^{a}	$< 0.5^{b}$	$< 0.5^{b}$	***
Photobacterium	12.4 ± 4.2	20.2 ± 5.8	10.9 ± 4.4	
Vibrio	4.7 ± 1.3^{a}	0.5 ± 0.2^{b}	2.5 ± 0.7^{a}	***

Acknowledgements

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MYTILUS LARVAE IN THE SOUTH WESTERN BALTIC SEA – A BASIS FOR MUSSEL FARMING

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Introduction

In the history of the Baltic Sea, mussel farming was a negligible element of fisher , but recently it has become a topic of great interest. Results already indicate that commercial farming of mussels can be viable and has the potential to offer environmental as well as socio-economic benefits (Lindahl et al. 2005). Especially the cultivation of *Mytilus* spp. seems to be a sustainable method to produce a high valuable feed stock and simultaneously function as a promising nutrient retention measure to relieve coastal waters of surplus nutrients.

Regardless of the cultivation candidate and strategy, a successful production of bivalve shellfish always starts with collecting mussel seeds or the direct settlement of planktonic mussel larvae originating from permanent mussel beds. Even tough, *Mytilus* spp. dominates the animal biomass on hard substrate habitats within the range of its salinity tolerance (Kautsky 1981) some areas in the Baltic Sea are characterized by sandy or muddy grounds providing only a limited amount of hard substrate as well as lacking a steady blue mussel population. Nonetheless, affected areas can still fulfill other key criteria important for site selection (e.g. sheltered positions, good logistical connections, sufficient phytoplankton concentrations). The Bay of Greifswald (GWB), for example, offers promising mussel farm sites, but due to its little amount of hard substrate, only a limited permanent mussel population as well as high fluctuations of larvae supply are presumed. Before establishing cultivation sites at GWB, it is necessary to proof if external larval transport into the target area is sufficient enough to provide an adequate mussel larvae concentration for cultivation purposes. In order to evaluate the larvae supply in GWB and the south western Baltic Sea, a mussel larvae distribution model approach is tested as well as settlement success at two test sites.

Material and methods

To trace larvae drift ways, the hydrodynamic 3d General Estuarian Transport Model (GETM) is combined with an Lagrangian particle tracking approach. Study area is GWB as well as the coastal waters of the south western Baltic Sea. The hydrodynamic model describes the years from 2016 until 2018 and has a spatial resolution of 50m (GWB) and 200m (south western Baltic Sea). Release points of particles (1 particle resembling 1000 larvae) are known mussel beds inside and outside of GWB as well as areas with a high likelihood of mussel population based on seabed habitat documentation. Particles are released on a daily basis and as soon as water temperature at mussel bed sites reach 15 °C and on a daily basis. The spawning period is set to 90 days. A drifting period of 450 degree days (temperature in °C * time in days), and a mortality were applied according to existing literature data (Stuckas et al. 2017, Kautsky, 1982). To verify the model approach and evaluate settlement success, two strategies are applied, consisting of direct larvae sampling in the water column and via observation of settlement. Settlement was recorded at two small scale test farms located in GWB and in the Bay of Wiek as well as on different sea signs in the target area.

Results

A preliminary first model approach with particles released within GWB already shows high inter-annual variations in spatial distribution. Similar results concerning spatial distribution are shown by the navigation sign monitoring. A comparison of modelled water temperature at different spawning sites, indicates that temperature induced spawning in GWB could start earlier compared to external spawning grounds. Supporting those results, bivalve larvae abundance in the central part of GWB shows concentration peaks at the end of May while sampling stations outside record highest peaks later in June or even July.

Discussion

First results of this study already indicate, that areas within in GWB most probably depend on larvae entering from the outside GWB to ensure reliable amount of larvae for production purpose. Ongoing studies will show if a sufficient amount of larvae is entering GWB and how they will complement the larvae distribution within. Modelling the larvae drift paths over the time span of three years will also give an inside look of how different environmental factors (salinity, temperature) effect temporal and spatial distribution. Therefore, final results could provide information about farm sites most suitable for mussel production in GWB as well as similar areas with a limited amount of hard substrate.

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QTL AND EQTL FOR SALMONID ALPHAVIRUS VIRAL LOAD IN ATLANTIC SALMON-IMPLICATIONS FOR PANCREAS DISEASE RESISTANCE AND TOLERANCE

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Introduction

Pancreas disease (PD) caused by salmonid alphaviruses (SAV) leads to severe economic losses in Atlantic salmon aquaculture. Outbreaks of PD lasting 3-4 months regularly occur in Norway, but as there are often no obvious external signs of infection [1, 2], and as an estimated one-third of SAV infected populations in Norway do not develop clinical PD [3], monitoring and control is difficult. Heritability of survival to PD challenge is high (~0.4 in post-smolts, ~0.5 in fry) [4, 5], and quantitative trait loci affecting resistance have been detected [6], suggesting that resistance to this disease could be increased with selective breeding. Viral load may be a more appropriate phenotype than survival for assessing resistance and tolerance to PD as mortalities to PD in the field can often be negligible even though populations of animals are carrying and shedding the virus into the environment.

Our analysis of the gene expression response to infection comparing fish ranked with high- or low-genomic estimated breeding values for PD resistance suggest that better protection of salmon against PD might be associated with earlier mobilization of acquired immunity, which accelerates clearance of the pathogen and resolution of the disease (unpublished results). Expression quantitative trait loci (eQTL) might explain the differential expression observed for some genes in response to PD challenge in fish with high- versus low-genomic breeding values. The aim of this study was to characterize QTL and eQTL associated with SAV3 viral load post-challenge in a Norwegian Atlantic salmon population.

Materials and Methods

Survival data and samples from a PD experimental disease challenge test (10 full-sib families, ~110 unvaccinated salmon parr per family, challenged by intraperitoneal injection or cohabitant infection) were obtained from SalmoBreed (Benchmark Holdings PLC UK). All challenge testing procedures were performed by VESO Vikan in a secure containment facility in Namsos Norway using standard operating procedures as approved by the Norwegian Animal Research Authority. Half of the 10 families chosen had high estimated genomic breeding values (h-gEBV), and half had low estimated genomic breeding values (l-gEBV), for PD days survival after intraperitoneal injection (Fig. 1).

Relative viral load was assessed using real-time reverse transcription PCR threshold cycle (*Ct*) value analysis on samples taken ~3.5 and 7 weeks (days 25 and 49) post-infection. Survival status (dead/alive, P_{DA}) was assessed 7 weeks post-infection. DNA was extracted from tail fin samples and genotyped using a ~55K Axiom Affymetrix SNP Genotyping Array (NOFSAL03). Heritability and genetic correlations for P_{DA} and $C_{t.}$ were assessed using a genomic relationship matrix (GRM), and a genome-wide association study (GWAS) fitting the GRM was performed to detect putative QTL. Differential gene expression between h-gEBV and l-gEBV familes was assessed using mRNAseq on heart samples taken at 0-, 4- and 10-weeks post-infection to search for eQTL.

Results

Genomic heritability for P_{DA} and C_t ranged from 0.40 to 0.74 and from 0.15 to 0.21 respectively (dependent on challenge test type, cohabitant challenge having the highest heritability). The genetic correlation between P_{DA} and C_t was high (0.91 to 0.98). The correlation of Ct values between IP and CH challenge tests was 0.73. A single genome wide viral load (*Ct*) QTL was detected on Ssa03 (Fig. 2), in the same region of the genome as a previously identified QTL for PD survival [6]. Several genes mapping to the region of the QTL were differentially expressed, and candidate eQTL were detected, including one trans-eQTL associated with several differentially expressed genes with putative immune function.

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Discussion and Conclusion

SAV3 viral load (C_t) is highly correlated with challenge survival P_{DA} and could provide a more efficient means for assessing resistance to PD under some circumstances in the field. But the C_t trait has lower heritability than P_{DA}, so that genetic gain in resistance from genomic selection would be expected to be lower for C_t. Clues about possible genes and causative mutations affecting PD resistance on Ssa03 from QTL and eQTL analyses will be discussed.

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TRACKING THE MICROBIOME IN INVERTEBRATES AS A TOOL FOR OPTIMIZING CULTURE CONDITIONS

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As the demand for seafood increases in the future due to rising human population growth, production of seafood products will have to increase to meet new and existing consumption. While fisheries will continue to provide seafood for the markets, an increasingly larger percentage will be coming from farming. To meet this demand, more sophisticated approaches to culture will be required that will involve new species, integrated culture practices like IMTA, lower trophic levels, intensification of production and more sophisticated monitoring. Regarding monitoring, recent advances in genetic technology, primarily in human health, has shown the microbiome of an organism (the microbes that live on and in an organism) is directly related to the overall health of the animal. Our team has begun to extend this genetic approach to invertebrate species in aquaculture using next-generation, high throughput eDNA sequencing technology (Illumina MiSeq). Samples were taken from 3 different species in culture, the giant scallop *Placopecten magellanicus*, the green sea urchin *Strongylocentrotus droebachiensis* and the northern sea cucumber *Cucumaria frondosa* from different body parts of the animals. In total, 1559 species of identified bacteria were found associated with these invertebrates. There were significant differences found between the 3 invertebrate species in taxonomic groups ranging from species to class. There were significant differences between body parts. Some of the dominant classes of bacteria found were Flavobacteriia, Gammaproteobacteria, Chlamydiia, Deltaproteobacteria and Epsilonproteobacteria. It is anticipated the results of this work will begin to develop a baseline for the microbiome of cultured marine invertebrates and develop into a tool that can be used to study organisms in relation to culture stress and environmental changes in marine ecosystems.

COCKLES HARVESTING, TRANSPORT AND PACKAGING: HOW TO IMPROVE THE QUALITY OF PRODUCT IN CONSUMERS TABLE

Rocha R.J.M.[1*], Pires S.F.S.[1], Martins D.[1], Rodrigues A.C.M.[1], Ofoegbu P.U.[1], Pereira V.[1], Pacheco M.[1], Costa A. P. L.[1], Soares A.M.V.M.[1]

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Introduction

In Portugal, harvesting of cockles (*Cerastoderma edule*) is still carried out using artisanal techniques, which consist in the use of hand-raking or dredges operated by small vessels. In most cases, this process starts at ebb tide and cockles remain in net bags outside water or in the boat until the harvesting is concluded, which can take a few hours. Therefore, these techniques can induce stress in bivalves, especially during summer time, as they are exposed to air and temperature fluctuations, from harvesting to arrival at depuration and expedition centers (Alfaro et al. 2019)"ISSN":"00448486","abstract":"Temperature fluctuations during the live storage and transport of bivalves are known to be one of the most important stressors for these markets. However, the biological mechanisms that induce these stresses and the immunological responses to stressors are not currently understood. In this study, a gas chromatography–mass spectrometry (GC–MS based. Temperature fluctuations can induce physiological and metabolic alterations, thermal and oxidative stress and, ultimately, oxidative degradation of lipids (lipid peroxidation – LPO) and cellular damage. The level of stress and damage in bivalves can influence the energy budget and induce mortality during the depuration and commercialization processes, reducing the quality and shelf life of the product. Besides that, lipid peroxidation is usually associated with quality losses and organoleptic alterations (Ferreira et al. 2010), which may depreciate the product and cause consumer reluctance in future acquisitions. However, if care is taken during the harvesting process, in particular by using isothermal containers to maintain the temperature, some damage can be avoided. This study was developed to compare two methodologies: *i*) bivalve harvesting by conventional methods with exposure to air and temperature variations, and *ii*) bivalve harvesting to refrigerated containers, to evaluate its effect on the quality and shelf life of the product. Additionally, we also aimed to evaluate the packaging methods after depuration, comparing the conventional net plastic bags with burlap bags, which can maintain a humid atmosphere during more time.

Material and Methods

Cockles were harvested in Ria Formosa, Algarve, South Portugal, with bivalve dredges and divided in two groups: group 1 – cockles were maintained in the boat exposed to air and temperature alterations during approximately 3 hours, group 2 – cockles were harvested and immediately conditioned in isothermal boxes with cold packs (6±1 ºC). Animals from the two groups were sampled after arriving at the expedition center in Ria Formosa (3h after harvesting). Posteriorly, the two groups of bivalves were transported in a cooled environment (6±1 ºC) to the experimental depuration center in Aveiro University (7h) and sampled at arrival. After, bivalves were depurated during 24h in recirculated 250L tanks at 15 ºC and posteriorly sampled. Finally, bivalves of the two groups were packaged into two different types of bags (n=5): traditional net bags and burlap bags. After packaging, cockles were stored for 6 days at 5±1 ºC, to simulate the conditions during commercialization period. In each sampling, edible tissue samples were immediately frozen in liquid nitrogen and kept at -80 ºC until biochemical determinations. Lipid peroxidation and cellular energy allocation (ratio between energy reserves and energy consumption) were measured. Comet assay was performed in fresh samples to evaluate the DNA damage.

Results

Cockles transported in a cooler environment presented lower levels of DNA damage after both transport and shelf life period (Fig. 1 a). Results of sampling after arriving experimental depuration center revealed that energy consumption in bivalves transported in a cooler environment after harvesting was lower when compared to the values obtained in bivalves transported at ambient temperature. Higher values of energy reserves were measured in the cooled group comparing to ambient temperature group after the transport (Fig. 1 c). Regarding the energy budget, it is possible to observe a significant increase during the ice cooled transport comparing to the ambient temperature. Additionally, the transport in cooler conditions seemed to increase the quality of cockles until 6 days of shelf life, as lipid peroxidation (LPO) was lower in these group of *C. edule*, especially for animals stored in traditional net bags (Fig. 1 b).

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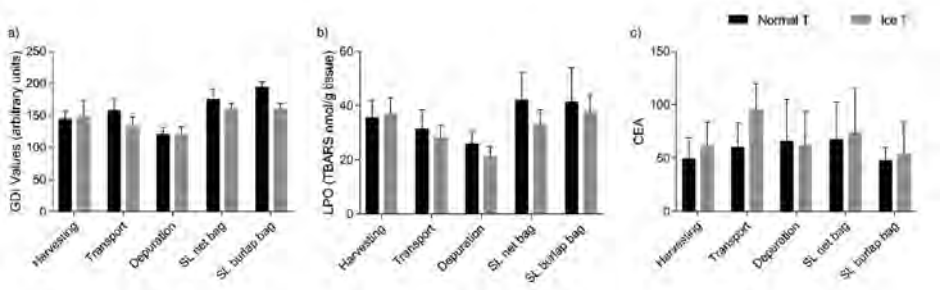

Fig. 1 Values of *Cerastoderma edule* harvested and transport in normal conditions (Normal T) *vs* transport in isothermal boxes with cold packs (Ice T) at the different points of handling until 6 days of shelf life period (SL): a) DNA damage (GDI values, as arbitrary units); b) lipid peroxidation (LPO – nmol TBARS/ g tissue); and c) cellular energy allocation (CEA). Values are presented as mean ± SD.

Discussion and conclusions

The transport of harvested *C. edule* in a cooled environment seems to be an effective measure to minimize all the stress associated with handling procedures and assure the better quality of the final product. Traditional net bags seemed to be more appropriate for the storage of cockles during the shelf life period, probably due to their bigger pore when compared to burlap bags, that allows for better rates of gas exchanges. So, our study emphasizes the importance that simple and low-cost changes in the harvesting procedure can have to improve food quality. Further work testing different times of shelf life and considering other materials for the bags may be performed and give new insights to bivalve market.

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HIGH PERFORMANCE OF *Venerupis corrugata* LARVAL CULTURE UNDER PROJECTED ACIDIFICATION AND WARMING SCENARIOS UNVEILS THE SPECIES' RESILIENCE TO FUTURE CLIMATE CHANGE

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Introduction

The pullet carpet shell *Venerupis corrugata* (Gmelin, 1791) is a clam species traditionally harvested at the Southwestern and Mediterranean Europe, mainly in Portugal, Spain, France and Italy. Poor management and pathogen-induced mortality determined natural stocks' reduction, forcing aquaculture production in order to respond to a high market demand. With an actual high market value (wholesale price over $20USD per kg) when compared to most shellfish species, intensification of *V. corrugata* aquaculture production is very likely. It may also support stocks' recovery under high consumption and increased mortality due to disease outbreaks and extreme climatic events, predictably more frequent under future environmental reality in a changing climate. This includes several undergoing global phenomena such as ocean acidification (OA) and warming (W), known to alter seawater chemistry impacting marine calcifiers such as clams. In this work we assess the performance of *V. corrugata* larval culture, from the first planktonic larval phase to settlement, under OA-W projected scenarios, aiming at foreseeing the resilience of the earliest, most fragile, life stages of the species to the expected future climate conditions.

Material and methods

Broodstock from the Ria de Aveiro (NW Portugal) was induced to spawn by thermal stimulation. Fertilized eggs were incubated to hatch in 1-µm filtered, UV-sterilized artificial saltwater under control conditions: unmanipulated pH (~8.2) and 18ºC. The D-larvae retained in a 60µm-sieve 48 hours post fertilization (48h pf) were exposed in triplicate to nine OA-W scenarios (Figure 1) resultant from a factorial experimental design of three pH levels at three different temperatures. Mortality and development stage were assessed every 3 to 4 days until settlement was complete in all experimental treatments (after 28 days of exposure, at T28). Shell length (SL) was measured at T0, T14 and T28, and growth rates calculated per treatment. The effects of temperature, pH and the potential interaction between these factors on the assessed endpoints were tested through permutational multivariate analysis of variance (PERMANOVA, *Primer v7 software*).

Results

Apart from an initial increased survival under acidity, potentially due to changes in the microbiome, there was an apparent lack of effect of the experimental treatments on mortality after 28 days of exposure (Figure 2). Still, the factors under study interact significantly (T14: *Pseudo-F*=7.5939, *P*=0.0001; T28: *Pseudo-F*=6.7797, *P* =0.0001) affecting larval development, which was notably accelerated by warming, despite the relatively delayed growth under acidity, an effect attenuated by the simultaneous increase in temperature (Figure 3).

Discussion and conclusion

Our results reveal the improved performance of *V. corrugata* larval culture under some of the tested OA-W scenarios, specifically the warmer (22ºC) at control (8.2) and intermediate (7.9) pH levels, suggesting that warming is likely to favor larval development and, to some extent, counteract growth impairment under acidity. As early life stages are generally more prone to the tested stressors (Przeslawski et al., 2015), this work points to the resilience of this bivalve to the expected OA-W and to its high productive potential under the future climate.

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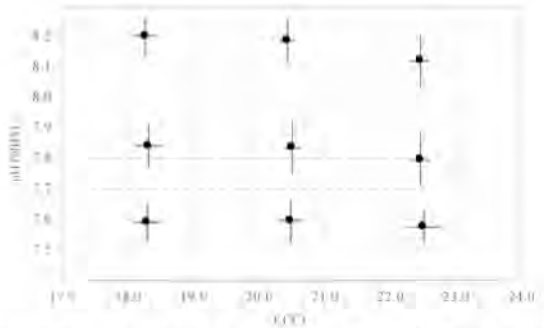

Fig. 1. Mean temperature (T, expressed in ºC) and pH (NBS scale) calculated from probe measurements performed twice a day, and respective standard deviation.

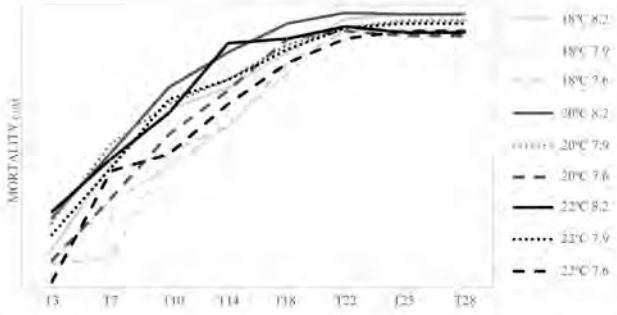

Fig. 2. Evolution of the cumulative mortality ($MORTALITY_{CUM}$) calculated from the mean survival per experimental treatment determined every 3 to 4 days throughout the 28 days of exposure (at T3, T7, T10, T14, T18, T22, T25 and T28).

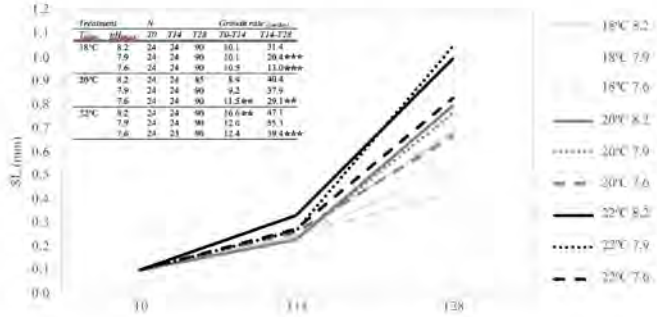

Fig. 3. Evolution of the mean shell length (SL, expressed in mm) per treatment, calculated from measurements taken at T0, T14 and T28. The insert on the upper left corner compiles information on the number of specimens (N) measured per treatment and time point, and the growth rate (expressed in µm/day) calculated for the first (T0-T14) and the next 14 days (T14-T28) of exposure. Significant differences in SL at T14 and at T28, between control pH and the two acidified treatments within the same temperature, are shown next to the growth rate value in the respective "*Growth rate*" column, at the respective "pH_{target}" line.

INDIVIDUAL FEED INTAKE AND BODY WEIGHT GAIN RELATIONSHIP ACCORDING TO GENETIC ORIGIN AND REARING TEMPERATURE IN EUROPEAN SEA BASS *Dicentrarchus labrax*

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Introduction

Fish feed accounts for around 50% of intensive fish farming costs, and fish feed production and consumption have a strong environmental impact. Using breeding programs to improve feed efficiency could highly increase aquaculture sustainability. Still, it is likely that individual feed intake (FI), body weight gain (BWG) and their interrelation could change substantially depending on the genetic background and on the rearing temperature. Genetics by environment interactions were already demonstrated on the growth of European sea bass (Vandeputte et al, 2014). Here, we studied three European sea bass populations: Atlantic Ocean (AT), West Mediterranean sea (WM) and East Mediterranean sea (EM) at two temperatures (18 and 24°C) in order to 1) assess the differences in the relationship between FI and BWG for each population, temperature and population by temperature interaction and 2) determine the variations of such traits at individual level.

Material and methods

We used 200 European sea bass from the three sea bass populations (62 AT, 66 WM, 72 EM). At the beginning of the experiment, fish were seven-months old and weighing 21.4 ± 8.3 g. These fish were reared in individual aquaria in order to permit individual FI measurement, as described by Besson et al (2019). Among these 200 fish, 100 were reared at 18°C and 100 were reared at 24°C. We firstly identified the ad libitum (ADL) feeding rate of each fish (100% ADL) by adjusting rations weekly over a four-weeks period. Each fish was then fed to 100 % ADL for 22 days, and then 80%, 60%, 40%, 20% and 0% ADL for 10-11 days at each level. Individual FI and BWG were measured at each level and FI and BWG relative to metabolic body weight were calculated ($MBW=(Wi \times Wf)^{0.4}$, with Wi and Wf initial and final weights, Saravanan et al, 2012).

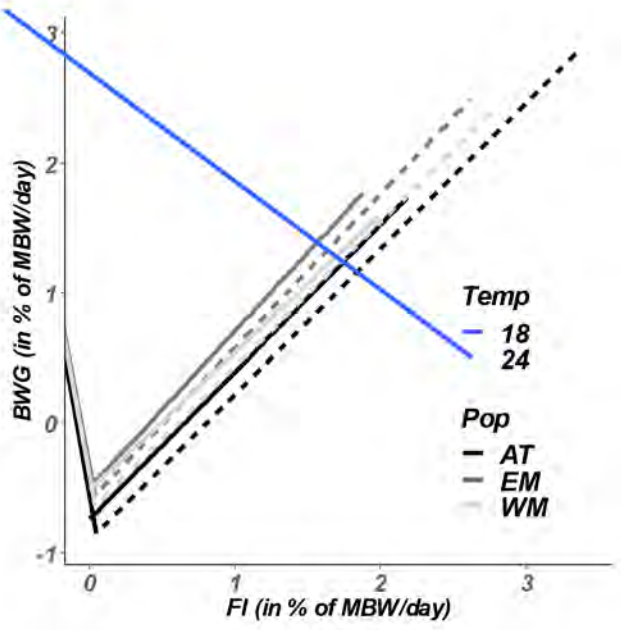

Figure 1 BWG evolution in function of FI

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We first studied the intercept and slope of the linear relationship between BWG and FI with an analysis of covariance. To assess individual variations within each interaction, parameters of the linear relationship between BWG and FI were then calculated for each individual and compared. Finally, residuals of the linear relationship between BWG and FI were calculated using the whole dataset and corrected for population and temperature effects. The Pearson's correlation between residuals ad libitum (res100%ADL) and during fasting (res0%ADL) was estimated. All the analyses were performed using R software.

Results

There was no significant difference in slope in the relationship between BWG and FI (Figure 1) between populations, temperatures, and population by temperature interactions. A significant difference in intercept was found between populations ($P < 0.001$) and between temperatures ($P < 0.001$), but interaction was not significant (> 0.05)

EM fish had a significantly higher intercept than WM ($P < 0.01$) which had a significantly higher intercept than AT ($P < 0.001$). Fish reared at 18°C had a significantly higher intercept (< 0.001) than those reared at 24°C.

At individual level, within each population by temperature interaction, coefficients of variation of intercept and slope were ranged between 15% and 25% and between 15% and 29% respectively.

Finally, Pearson's correlation between res100%ADL and res0%ADL was not significantly di ferent from 0 ($r = -0.08$).

Discussion and conclusions

Given the observed homogeneity of slope between populations, temperatures and interactions, it can be considered that a given increase in FI will lead to a similar increase in BWG, whatever the population or the rearing temperature. Moreover, intercept was higher at 18°C than at 24°C: we can make the hypothesis that maintenance costs are lower at 18°C. Similarly, intercept differences between populations may be explained by different maintenance costs. Actually, several phenotypic traits were already demonstrated as significantly different between these populations (Vandeputte et al, 2014). No genetics by environment interaction seemed to be involved in the relationship.

Furthermore, as measuring FI is complex in fish, we tried to find an easier trait (loss of weight during fasting, expressed by res0%ADL) to select for in a breeding program. As the correlation between res100%ADL and res0%ADL was not significant, weight loss during fasting cannot be used to predict which individual fish would have the best growth performance at its ad libitum FI. However, the present experiment was done using really specific rearing conditions: fish were juveniles and isolated (which induces stress). Correlation estimates may be biased by the time lapse existing between measurements done at different feeding levels.

Nevertheless, this study generated key information for breeding programs. A broad individual variability was existing in the relationship between BWG and FI, suggesting opportunities for selective breeding, if this variability is of genetic origin.

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OPTIMUM WATER TEMPERATURE BOOSTS DEPURATION EFFICIENCY CHANGING OXIDATIVE STRESS STATUS OF DIFFERENT BIVALVE SPECIES

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Introduction

Food security relies on depuration of mollusc bivalves and these good practices are regulated among the European countries (EU 2004). So far, physiological requirements of the diverse commercialized species, as optimum temperature range, are not considered in the purificati n process, which can compromise animal metabolism, welfare and quality of the final product. Further, bivalves harvested in class C areas are usually avoided by stakeholders due to economic constraints, as they must be transposed to relaying areas for a minimum of two months prior to depuration, before being commercialized for human consumption. Thus, our objectives were to evaluate: 1) if bivalves harvested from class C areas are able to depurate to class A values in 24 h; 2) if there are differences in purification capability due to water temperature; and 3) if water temperature will affect the biochemical responses of depurated bivalves.

Material and Methods

Clams (*Ruditapes decussatus*) and razor clams (*Solen marginatus*) were selected as the model bivalve species, as they have a broad distribution and high economic value in Europe. Bivalves were harvested in class C areas in Algarve, south Portugal, and transported to Aveiro University facilities in less than 24 h. Animals were depurated during 24 h in 250 L modular depuration systems equipped with a filtration system and a UV-c unit (25W UV-c, 6.000 μW.s/cm^2), testing 4 temperatures (10, 15, 20 and 25 ºC). Bivalves were sampled for microbiological and biochemical analyses at 0 (arrival) and 24 h (depuration period). Microbiological load (most probable number – MPN – of *E. coli per* 100g of tissue and intravalvular liquid), antioxidant defences (catalase, glutathione S- transferases, and total glutathione), oxidative damage (lipid peroxidation and DNA damage) and cellular energy allocation were evaluated.

Results

R. decussatus and *S. marginatus* individuals were able to depurate their microbiological load to class A values in 24 h at all tested temperatures. However, *R. decussatus* presented the lower microbiological load at 20 ºC (<18 *E. coli*/100g) and *S. marginatus* between 10 and 15 ºC (mean values of 69 and 62 *E. coli*/100g, respectively). No changes were observed on clams antioxidant defenses, but for razor clams tGSH levels were impaired with increasing temperatures (Fig. 1 a). Clams seemed to have a higher range of temperature tolerance, with DNA damage increasing only at 25 ºC, whereas for razor clams temperatures above 20 ºC caused an increase in DNA strain breaks. Regarding energy consumption, clams presented a higher activity of electron transport system than razor clams, although no significant changes in energy consumption were observed due to water temperature (Fig. 1 d).

Discussion and conclusions

This study shows that bivalves from class C areas can depurate their microbiological load to values of class A areas in 24h if optimized condition of depuration systems are available. Further, we observed that the efficacy of purification of each species was influenced by water temperature with *R. decussatus* individuals more tolerant to water temperature changes than *S. marginatus*. These differences are in accordance with the ecological niche of each species, since clams usually stay at the surface of the sediments, thus more exposed to environmental changes (thermal/desiccation fluctuations) than razor clams that live buried in the sediments (Monaco et al. 2019). Thus, it is highlighted the relevance of considering species-specific requirements to improve purification and welfare of live bivalves during the commercialization chain. In sum, strength might be given towards a rethinking of the possibility for direct depuration of animals from class C areas and the adjustment of the depuration process to fulfill species-specific requirements of cultured bivalves

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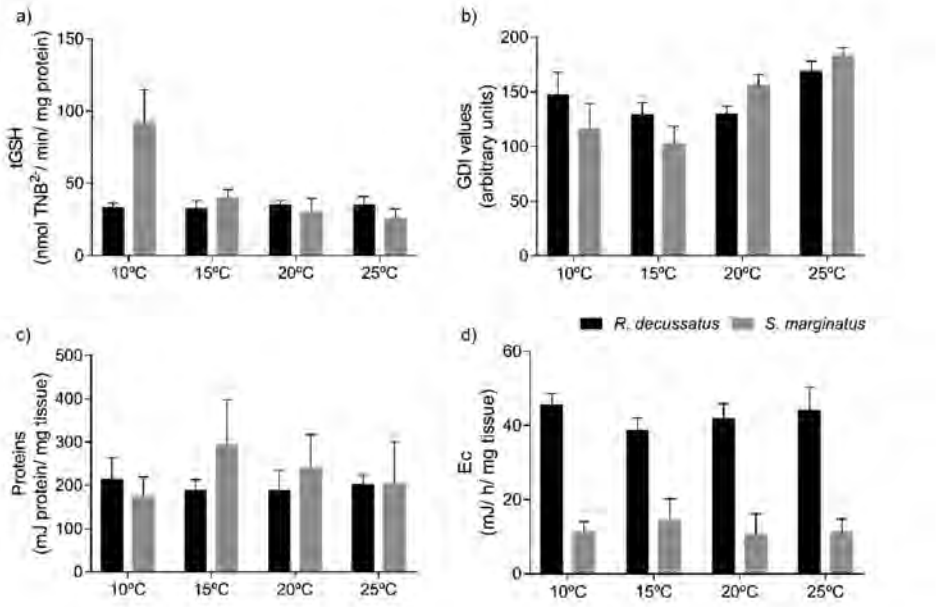

Fig 1. Temperature effects on *Ruditapes decussatus* and *Solen marginatus* a) total glutathione levels (tGSH, nnmol TNB^{2-}/ min/ mg protein); b) DNA damage (GDI – genetic damage indicator, expressed as arbitrary units values); c) protein content (mJ protein/ mg tissue); and d) energy consumption (Ec – mJ/ h/ mg tissue). Value are presented as mean ± SD.

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CORAL AQUACULTURE: THE INFLUENCE OF COLONY ORIGIN IN THE PERFORMANCE OF CULTURED FRAGMENTS IN DIFFERENT ARTIFICIAL LIGHT CONDITIONS

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Introduction

Coral reefs represent one of the most diverse ecosystems in the world, with critical importance for various organisms. People also depend heavily on this marine ecosystem in ecological, economic and cultural terms (Laurans et al., 2013; Spalding et al., 2017). However, coral reefs are declining due to various natural and anthropogenic factors such as global warming, pollution or illegal fishing with destructive gears (McClanahan et al., 2014). In addition, the capture of wild scleractinian corals is an activity practiced in several tropical and subtropical regions, mainly for the aquarium industry (Wijgerde et al., 2012). Coral aquaculture (*in situ* or *ex situ*) is, therefore, a possible solution to minimize the capture of wild organisms, allowing the production of these organisms for various purposes, such as reef restoration, artificial reef building, or commercialization for educational and ornamental purposes. However, the need to optimize zootechnical procedures persists in order to maximize the rate of survival and growth. The main objective of this work was to study the effect of light (intensity and spectra) on survival, growth, photobiology, energy allocation and energy consumption of *Montipora digitata* fragments obtained from mother colonies acclimated to different light scenarios (mimetizing different sea depths).

Material and methods

Eight colonies of *M. digitata* (with the same genetic origin) were acclimated for two months in experimental systems with artificial light (full spectrum) under two photosynthetic active radiation (PAR) intensities: 4 colonies in high light (HL, 130 ± 20 µmol quanta.m^{-2} .s^{-1}) and 4 colonies in low light (LL, 70 ± 10 µmol quanta.m^{-2}.s^{-1}) conditions. Afterward, colonies were fragmented in ~ 2 cm length fragments, originating a total of 56 fragments (28 from HL and 28 from LL acclimated colonies), which were cultured in two 235L glass tanks connected to the same filtration system, as described by (Rocha et al., 2015). Four light treatments were tested, using two light spectra (red and blue) and two photosynthetic active radiation (PAR) intensities (130 ± 20 and 70 ± 10 µmol quanta.m^{-2}.s^{-1}). Fragments originated from HL and LL acclimated colonies were identified and cultured during four months in the described light treatments (n=7). Survival, growth rate, maximum photosynthetic efficiency of dark-adapted PSII (fv/fm, *in vivo*), and energy allocation were evaluated.

Results

No mortality occurred during the experiment. An effect of mother colony origin was observed, with fragments from LL acclimated colonies presenting a higher growth rate when compared to fragments from HL acclimated colonies. Light spectra also significantly affected the growth rate of corals, with fragments reared under red light low intensity (PAR 70 ± 10 µmol quanta.m^{-2}.s^{-1}) showing a higher growth rate (Fig. 1). In terms of fv/fm, it was observed that fragments from HL acclimated colonies, reared in red light low intensity, increases the photosynthetic efficiency from T0 to T1. The mother colonies also influenced the energy reserves in the fragments exposed to different light conditions. It was observed a higher protein content in fragments from LL acclimated colonies in all treatments. Regarding lipid content, an interaction was observed with fragments from HL acclimated colonies having higher lipid levels at low light treatments and fragments from LL acclimated colonies having higher lipid content when reared in high light conditions.

Discussion and conclusions

The results suggest that mother colonies acclimated to low light conditions will originate fragments that can present better growth performance. Additionally, red light spectra with low intensity can be more appropriate to culture *M. digitata ex situ*, with organisms presenting better growth rate and photosynthetic activity. In this study, it was observed that the high light intensity provokes photo-inhibition and consequently low photosynthetic efficienc , as previously reported by Barton et al. (2017). Interestingly, the high light conditions seem to promote higher content of proteins, probably fluorescent

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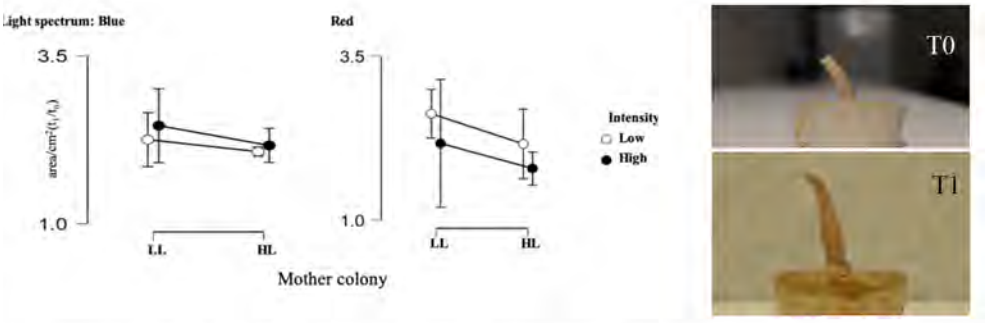

Figure 1. Growth rate of *M. digitata* fragments originated from colonies acclimated to low and high light conditions (LL and HL, respectively), cultured during 4 months in different light spectra (red and blue) and photosynthetic active radiation (PAR) intensities (130 ± 20 and 70 ± 10 µmol quanta.$m^{-2}.s^{-1}$).

proteins, that can be used for protection to high intensity exposure (Roth, 2014)creating habitat for these diverse and productive ecosystems. Light is thus a key regulating factor shaping the productivity, physiology, and ecology of the coral holobiont. Similar to all oxygenic photoautotrophs, Symbiodinium must safely harvest sunlight for photosynthesis and dissipate excess energy to prevent oxidative stress. Oxidative stress is caused by environmental stressors such as those associated with global climate change, and ultimately leads to breakdown of the coral-algal symbiosis known as coral bleaching. Recently, large-scale coral bleaching events have become pervasive and frequent threatening and endangering coral reefs. Because the coral-algal symbiosis is the biological engine producing the reef, the future of coral reef ecosystems depends on the ecophysiology of the symbiosis. This review examines the photobiology of the coral-algal symbiosis with particular focus on the photophysiological responses and timescales of corals and Symbiodinium. Additionally, this review summarizes the light environment and its dynamics, the vulnerability of the symbiosis to oxidative stress, the abiotic and biotic factors influencing photosynthesis, the diversity of the coral-algal symbiosis, and recent advances in the field. Studies integrating physiology with the developing \"omics\" fields will provide new insights into the coral-algal symbiosis. Greater physiological and ecological understanding of the coral-algal symbiosis is needed for protection and conservation of coral reefs.","author":[{"dropping-particle":"","family":"Roth","given":"Melissa S.","non-dropping-particle":"","parse-names":false,"suffix":""}],"containe -title":"Frontiers in Microbiology","id":"ITEM-1","issue":"AUG","issued":{"date-parts":[["2014","8","22"]]},"publisher":"Frontiers Media S.A.","title":"The engine of the reef: photobiology of the coral-algal symbiosis","type":"article-journal","volume":"5"},"uris":["http://www.mendeley.com/documents/?uuid=894fd674-5cb7-3a57-beb9-00ad15f16d62"]}],"mendeley":{"formattedCitation":"(Roth, 2014. This study emphasizes that several factors must be considered to a successful coral aquaculture, including the type of light (intensity and spectrum). However, the origin of mother colonies, namely PAR acclimation conditions (e.g., different depths or shadow conditions), can have a preponderant role in the optimization of coral aquaculture to maximize biomass production.

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EXPERIMENTS OF WRECKFISH (*Polyprion americanus*) LARVAL CULTURE IN GALICIA (SPAIN)

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Introduction

The wreckfish, *Polyprion americanus*, is a very interesting species for diversification in aquaculture in the European Atlantic zone because of its rapid growth, good adaptation to captivity (Machias *et al.*,2003, Papandrolakis *et al*,.2004; Rodriguez *et al*., 2014), late maturation (Sedberry *et al*., 1999), onset, high market price and very limited catches. In recent years, the Diversify project (UE) has made important progress in reproduction,, broodstock nutrition and incubation. However, larval culture is still the main bottleneck in wreckfish aquaculture. In this work, some experiments of larval culture that led to the first juveniles of wreckfish are show

Materials and Methods

The rearing trials were carried out at the Galician Institute of Aquaculture (IGAFA) in Galicia, using two batches of 5,000 larvae each, from the broodstocks of the IEO (Vigo) and the Aquarium Finisterrae (A Coruña). The larvae were released in two 400 L concave cylindrical tanks at a density of 12.5 larvae/L. The culture was carried out using a recirculation system (RAS), operating at 16-17 ºC, salinity 36 %, oxygen 7.2-8.4 mg/L, with natural photoperiod. Levels of ammonium, nitrites, nitrates and pH were monitored and recorded daily. The opening of the mouth of the larvae occurred at 6 dph (days post hatching) and feeding was initiated at 8 dph. From 8 dph to 19 dph, larvae were fed with rotifers enriched with products specifically developed for this species by the University of Las Palmas de Gran Canaria (FCPCT). From 15 to 23 dph, *Artemia* nauplii (A_0) was also given. Between days 18-48 dph, the larvae were fed with metanupliu (A_1) enriched with special products developed by the FCPCT. From day 40 to 48 dph, commercial dry food was supplied; and weaning finished at 48 dph. Samples of larvae were regularly taken for their measurement, taking photos and observing the stomach content.

Results

The newly hatched larvae had a size of 4.70 ± 0.27 mm. Before opening their mouth (8 dph) the growth was very slow, but then the growth rate increased progressively. After 65 dph, the larvae were on average 12.77 mm (Fig.1). The survival rate of the larvae was: 0.18 % (9 individuals) for the batch from IEO and 0.32% (16 individuals) for the batch from Aquarium Finisterrae. Some individuals exhibited swimming difficulties, aprobably due to dysfunction of the swim bladde

Discussion and Conclusion

Despite the low survival rates, these results represent an important first step towards the development of a larval protocol for wreckfish aquaculture. It is noteworthy that the behaviour of wreckfish larvae is quite different from the other marine species that are currently being cultivated; and therefore, a special zootechnics is needed for this species. More research is needed to identify the factors influencing the quality of the spawning as many larvae had jaw joint problems during the first days of life, which caused difficulties to capture the food and they ended up dying when the nutrients from endogenous reserves were finished

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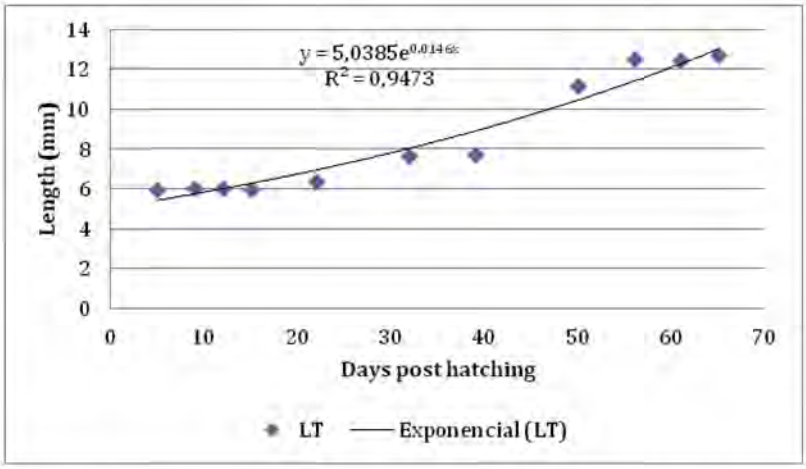

Figure 1. Wreckfish larvae length increasing (mm) since 5 to 65 dph.

THE EFFECT OF DIFFERENT WATER SOURCES ON THE POTENTIAL H_2S-FORMATION WITHIN RAS

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Introduction

In the last years, several of the serious incidents involving acute fish mortalities in recirculating aquaculture systems (RAS) for Atlantic salmon (*Salmo salar*), have been caused by hydrogen sulphide (H_2S). These incidents have mainly occurred in seawater systems, e.g. post-smolt production. H_2S is formed by sulphate-reducing bacteria which uses sulphate (SO_4^{2-}) and organic material under anaerobic conditions (Muyzer and Stam, 2008). Seawater contains 1000 times more SO_4^{2-} than freshwater (Gerardi, 2006), increasing the potential risk for H_2S production. However, using seawater is pivotal to avoid desmoltification and preparing salmon for seawater transfer (Mortensen and Damsgård, 1998). Therefore, the idea of removing sulphate from seawater through membrane filtration have been proposed as a measure for reducing fish mortalities caused by H_2S. The aim of the following study was to: 1) understand what microbial environments in RAS have the highest potential risk for H_2S-formation, 2) test the hypothesis that sulphate-removal in seawater reduces the risk of H_2S formation, and 3) to gain a better understanding of the dynamic between organic material and sulphate concentration for H_2S formation in RAS-water.

Materials and Methods

Three main environmental sources where H_2S could potentially form in a commercial RAS were selected: sludge, biofilter elements and RAS-water. A small-scale batch experiment was conducted where each of these three potential sources were exposed to seawater, freshwater, brackish water and treated water (freshwater + filtered seawater). The filtered seawater was provided by a membrane filtration technology developed during the same project that this study is a part of. The H_2S kinetics and production rate was measured for each test. The organic material was also measured in form of COD (chemical oxygen demand). To gain insight on the microbial environment, bacterial activity, number and community was also investigated.

Results

The results of this experiment are still under development and analysis and will therefore be presented at the European Aquaculture Society conference.

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MICROALGAE CULTIVATION FOR BIOREMEDIATION OF NUTRIENTS UNDER AQUAPONIC CONDITIONS

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Introduction

Microalgae are considered an important group of organisms, ecologically and for numerous applications – these include production of pharmaceutical ingredients and chemical raw materials, food and dietary supplements, (aqua)feed, biofertilizers and bioactive compounds, biofuels and bioplastics, biosensors as well as biomass production and bioengineering, e.g. CO_2-fixation and waste water treatment (Chapman et al., 2013; Harun et al., 2010)

Modern RAS production facilities create considerable amounts of nutrient rich waste water. To prevent eutrophication and recycle valuable nutrients terrestrial plants can be grown in soilless cultivation systems, a principle widely known as aquaponics.

Another approach doing so is the cultivation of microalgae, with waste waters serving as the culture medium. Nutrients contained in waste water are hereby recovered while the water is biologically remediated (Chiu et al., 2015; Queiroz et al., 2013; Sriram et Seenivasan, 2012).

In this study we aimed to assess the potential of green microalgae to develop biomass suitable for possible further applications grown in waste water deriving from a production site of African catfish (*Clarias gariepinus*) under controlled and pilot scale conditions.

Materials and methods

For this study, stocks of a limnic green algae polyculture dominated by *Scenedesmus* sp. were maintained in cell culture flasks with ventilation caps utilizing F/2 medium. To create pre-cultures used for actual cultivation stock cultures were transferred to aerated 2L glass chemostats and added to fish aquaculture waste water which had been filtered threefol

Pre-cultures were kept in a fed-batch cultivation process with artificial illumination set at a 16/8h light/dark rhythm until reaching an OD665-value (optical density obtained at a wavelength of 665nm as proxy for concentration of algal cells) of approximately 1.0.

Actual cultivation took place in a greenhouse aided by artificial illumination set at a 16/8h light/dark rhythm. To run a cultivation cycle, 7L (i.e. 5% of total volume) of pre-culture were transferred and inoculated in a 140L tubular photobioreactor (PBR) containing double filtered fish aquaculture waste water from the same source

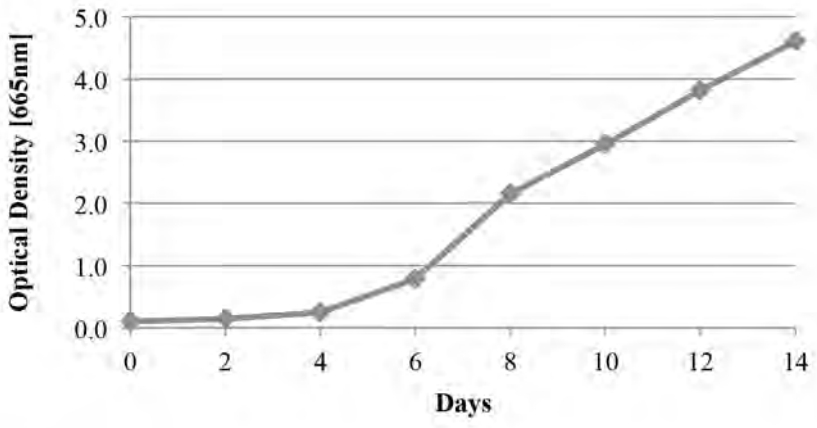

Fig. 1: Development of optical density over time measured at a wavelength of 665nm of a green algae polyculture in waste water deriving from a production site of African catfish (*Clarias gariepinus*).

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Several growth and nutrient parameters including OD665, cell densities, chlorophyll contents, dissolved organic and inorganic as well as particulate organic nutrient contents (nitrogen=N, phosphorus=P) were assessed over the course of 14 days. Additionally, the fatty acid profile of the obtained algal biomass was analyzed. To verify results a total of four cultivation cycles had been conducted.

Results

OD665-values as a preliminary result had increased from approximately 0.1 at day zero (D0) to more than 4.6 at the final D14 and hence, multiplied numerous times which indicated that under the given conditions the green algae polyculture had grown strongly, (figure 1). Further results from this study will be presented

Discussion and Conclusion

Members of the genus *Scenedesmus* have shown to efficiently remove nutrients including N and P from artificial and real waster waters alike (Voltolina et al., 1999; Martinez et al., 2000; Ruiz-Marin et al., 2010; Ji et al., 2013).

Growth curves of these algae had an appearance similar to the ones in our study indicating that the utilized polyculture was able to thrive and develop quantities of biomass suitable for further applications (Xin et al., 2010).

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RAINBOW TROUT (*Oncorhynchus mykiss*) GROWING TRIAL USING DIETS WITH DIFFERENT DOSAGES OF THE SAME ADDITIVE

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Introduction

In animal nutrition, the phospholipids are considered to play an important role in the digestion of fat. In larvae and early juveniles, dietary phospholipids improved growth and increased survival rates and decreased incidence of malformation in larvae (Kanazawa, 1993; Fontagnè et al., 1998). A specific class of phospholipids, the lysophospholipids, induces a better absorption of nutrients than other phospholipids due to their hydrophilic nature; in particular, they help in fatty acid absorption by the formation of micelle structures as shown in different animal species (Gheisar et al., 2015; Kim et al, 2018). Limited data are reported for the lysophospholipids in fish during growing

In order to investigate the effects of an emulsifie , based on lysophospholipids (LEX), on rainbow trout during the growing phase, a trial was performed evaluating the growth performances and feed conversion rate. For the aim, the (LEX) was supplemented to diet at two different doses.

Materials and Methods

For the trial, we employed 6 000 rainbow trout, averagely weighing 47.8±3g, which came from the pre-growing sector where they were reared. The fish were stocked in 12 tanks (3 tanks per diet group) at 4.93kg/m^3. The test additive (LEX) was supplied by the company in a ready-to-use powder form. For 90 days, 4 groups, with 3 replicates each, were considered: 1) Positive Control (normal energetic level); 2) Negative Control (Positive Control without a fraction of energy added); 3) Negative Control plus additive at 500ppm; 4) Negative Control plus additive at 750ppm. The Diet 1) was formulated in accordance with the person in charge of the nutrition line of the commercial feed employed for rainbow trout juveniles submitted to pre-growing phase. The diets were administered by hand twice a day, 6 days per week, and the quantity of the daily ratio was recorded.

At the end of the trial, all the fish were weighed whereas the mean final length was sampled in 20 fish. The main zootechnical performances were determined and submitted to statistical analysis. To investigate potential differences in the intestinal structure among the groups, standard histological techniques were performed by means of light microscopy (Nikon Phase Contrast 0.90 Dry, Japan). Tissue morphology was evaluated according to a grading system (absent, light and high damage) in order to assess possible changes in cell morphology. Quality traits of the fillet were examined in six fish/group at the end of the trial; a portion of dorsal muscle, deprived of skin and subcutaneous adipose panicle, was collected, homogenised and submitted to the following analysis: dry matter (determined at 105°C for 24h); total nitrogen (Kjeldahl, conversion factor of N to protein 6.25); total lipids extracted with chloroform/methanol (2:1 v/v) (Folch et al., 1957) and converted to fatty acid methyl esters following Sukhija and Palmquist (1988).
Data collected every sampling were submitted to analysis of variance using General Linear Model (PROC GLM) of SPSS 25 (IBM Corp., 2017). The differences among the means were verified by Test of Pairwise. Effect was considered significant when $P<0.05$

Results and discussion

The final mean body weight showed significant differences among the four groups: the fish fed on Diet 3 were the heaviest (166g), followed by those of Diet 4 (154g), and finally those fed on Diet 1 (133g) and Diet 2 (127g) respectively, which weighed far less. On the other hand, no significant differences were observed with respect to either the final mean body length (21-22.5cm) or the survival rate among the groups.

The two diets containing the emulsifier allowed for a significant y greater weight gain (106-118g) and specific growth rate (1.30-1.38%day^{-1}) with respect to the Positive (85g; 1.14%day^{-1}) and Negative (80g; 1.09%day^{-1}) Control diets. The Diet 3 and Diet 4 groups showed a significantly lower and, therefore, more favorable feed conversion ratio (1.14-1.15) than the other two Control groups (1.23-1.29). As far as the somatic indices are concerned, no significant differences were noted among the four groups in the VSI, PFI, and HIS, while the KI was significantly higher in the fish fed on two diets containing the emulsifie . A good general morphological condition was noted in the fish fed on Diet 3 and 4: the architecture of the intestinal wall was generally normal in terms of thickness and development of the villi whereas the Diet 1 and Diet 2 groups frequently presented an alteration in the structure of the intestinal wall.

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Concerning quality traits, protein content ranged between 19.5% in fillet of trout fed Diet 3 and 20.1% in group receiving Diet 4. Lipid fraction varied from 0.7% (Diet 4) to 1.1 in Diet 1. The highest n-3 PUFAs was detected in fish fed Diet 4 (0.227g $100g^{-1}$ fillet) compared to the others (Diet 3: 0.193g 100^{-1}; Diet 1: 0.119 g $100g^{-1}$; Diet 2: 0.069g $100g^{-1}$ fillet)

Acknowledgements
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MYCOTOXINS – A WORLDWIDE THREAT FOR THE BLUE REVOLUTION?

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Introduction

Mycotoxins are toxic secondary metabolites produced by various species of filamentous fungi, contaminating a wide variety of mostly plant origin agricultural commodities, animal feeds and human foods.

Reports of mycotoxins-related pathologies in aquatic species have been the driving force for research in this field since the 1960's.

As their chemical structures vary considerably, mycotoxins cannot be classified as one group according to their mode of action, toxicology or metabolism. (Gonçalves, et al., 2019); Additionally, specific biological classification is limited due to the species variability in the arbitrary group of aquatic organisms cultivated for aquaculture. Nevertheless, a common characteristic of mycotoxicoses in all species is the early pathogenesis and route of intoxication. Toxic molecules carried by food or water are internalized or topically come in contact with the organism's mucosal tissue, where first pathological processes can usually be detected, this is followed in most cases by the host circulation carrying the toxins and toxic metabolites, further altering the homeostasis of cells, tissues organs and systems. These changes are often clinically manifested in productivity, pathogen susceptibility, genetic and immune changes and more.

In recent years a shift towards increase use of plant origin materials and reduction of the animal component in aquatic feeds created a 'more-than-ever' risk of mycotoxicoses in aquaculture systems, which is now a globally growing and fast developing industry.

In the near future, Human societies will face an enormous challenge of feeding and caring for a global population of over 9 billion people, in what seem like, a determinative period of time for addressing impacts of climate change and environmental degradation of the resources base. (FAO, 2018).

Global sustainable aquaculture, as agreed by many, could be one of the major factors comprising the answer for this challenge, creating an equal, clean and available source of food and income for populations around the world.

Modern aquaculture needs to address many complex issues, from preservation of fisheries, water resources and the aquatic environment, through animal and human welfare, to reduction in the use of antibiotic substances in an effort to fight the problem of antibiotic resistant pathogens, which are all interconnected to the threats posed by mycotoxins. Research efforts and increased awareness for prevention, together with development of novel mycotoxins mitigation strategies, could play a major role in future aquaculture as the solution for feeding a healthy and sustainable world in upcoming years.

While currently over 350 compounds are considered to be mycotoxins, the most influential ones are Aflatoxins (AF), Ochratoxins (OTA), *Fusarium* spp. toxins such as Fumonisins (FUM) and Zearalenone (ZEN), Trichothecenes such as Deoxynivalenol (DON) ,T-2 toxin and their derivates and a growing group of emerging mycotoxins that includes sub-groups such as the Enniatins and Beauvercins (Pietsch, 2019).

These toxins are currently in the spot light of research as their impacts in aquaculture and other feed, food and health are complex and manifest themselves often with reduced, productivity, increase in disease and economical losses estimated to billions of US dollars every year (Anater, et al., 2016)

(Continued on next page)

Materials and methods

Over a period of five years (2014-2019) 114 samples of globally distributed finished aquatic feeds were analysed for mycotoxin contamination within the scope of the BIOMIN Mycotoxin Survey Program. These samples were analyzed using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS, Spectrum 380®) screening for more than 700 mycotoxins and other secondary metabolites.

Results

All samples showed co-contamination by > 1 compound with a maximum count of 78 different toxins contaminating single aquatic feed sample. From the group of major mycotoxins, ZEN was the most commonly present, detected in 73% of all samples at a mean concentration of 59ppb and maximum concentration of 615ppb for positive samples. DON and FUM B1 were detected both in 48% of samples (means of 249, 100ppb and maximums of 2486 and 2087ppb both respectively). AF B_1 and OTA occurrence is often related to storage conditions but also grain damage in the field. Both were present in 8% of all samples. Global toxin distribution was observed with foci of higher levels in Mediterranean, north European and south east Asian countries.

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FRESH-WATER-PRODUCING SEAWATER-AQUAPONICS AS A SOLUTION FOR THE DRAUGHT NOT ONLY IN THE NORTHEAST OF BRAZIL

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Introduction

The Northeast of Brazil was the countries' biggest Tilapia producer five years ago. Due to climate change today about 95 % of the productive water bodies have disappeared, as well as the Tilapia industry did.

As sustainable response, seawater aquaculture with a combined desalination process is to be established.

Project Description

The consortium of the Brazilian Piscis Industry Trade Ltda and the German Gloasis GmbH have developed an Aquaponics System for the annual production of: 175 tons of Tilapia, 63 tons of Shrimp, 250 tons of tomato and 238,000 heads of lettuce on 1.4 hectares of barren land.

The novelty of this project is that also up to 244 m³ of condensed water are produced daily as shown in Figure 1 below:

Process Description Example Lettuce Module

In November, the hottest month of the year, the Lettuce Module faces a cooling demand of 484.9 kW, already regarding the shading installations.

The plants itself contribute a part of the needed cooling energy by evapo-transpiration of 2.5 $kg_{H2O}m^{-2}$ per day. Further evaporation-cooling energy is supplied by Evaporation Cooling Units (No. 1 in Figure 1). In one of these, 25.52 g of seawater from the aquaculture is evaporated for the cooling of the greenhouse modules per second.

One advantage of this cooling is that the cold air also contains high CO_2-levels from the aquaculture and therefore realizes a CO_2-Fertilization of the plants.

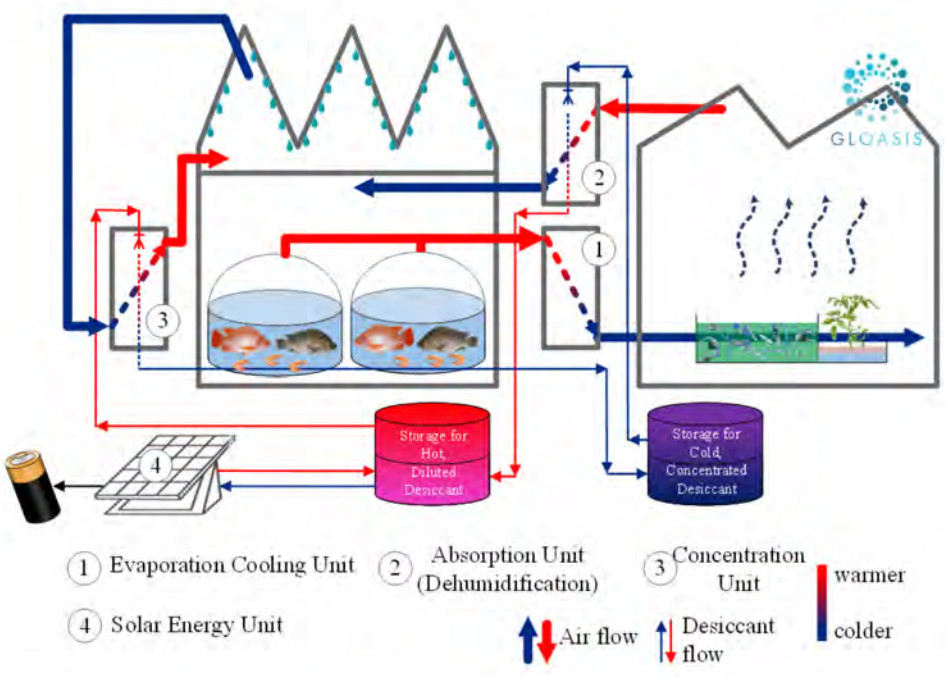

Figure 1: Cooling, Desalination and CO_2-Fertilization based on Seawater Aquaculture

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A further advantage is that the water from the aquaculture is also saturated with oxygen in this process. The peak demand of 1,060.0 $g_{O2}h^{-1}$ per tank requires a water flow rate of 135.5 ls^{-1} with an 80 % saturation efficiency in the cooling towers and a minimum level of 4 $g_{O2}m^{-3}$ water in the tanks. Thus, mayor saving in energy costs for aeration are supposed to be proven.

In the Absorption Unit (No. 2 in Figure 1), 216.7 g of water (humidity) will be absorbed by a liquid desiccant per second.

In the Concentration Unit (No. 3 in Figure 1), these 216.7 g of water will be desorbed from the liquid desiccant to the air and condensed in two adequately designed condensers.

The concentration of the desiccant requires thermal energy, which is taken from a cooling cycle of PV panels. The cooling of the PV panels automatically increases their efficienc .

Conclusion

The seawater aquaponics system with desalination is regarded to be an answer to the fresh water scarcity, lack of fertile land and climate change. In controlled greenhouse environments it is possible to maintain the perfect growing conditions for plants and animals. A key element for the success is the additional to the water treatment implemented, the use of the evaporation-condensation cycle.

The project creates closed greenhouse environments, which should therefore even be applicable in space, where all the transpired and evaporated water can be cleaned and used for aquaculture and horticulture and thus for the survival of humanity.

ANTIOXIDANT EFFECT OF THYMOL AND SORBIC ACID IN FISH HEPATOCYTES *IN VITRO*

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Introduction

Aquaculture has grown consistently over the last decade and aqua species production will continue to increase, as a result of growing demand for seafood (Huntington and Hasan, 2009). However, aquaculture has to face important challenges in terms of health management and environmental impact like intensive fish farming, increase in water temperature due to global warming, world sea metal pollution, and pathogen infections (Cochrane et al., 2009). All these challenges induce oxidative stress in fish that could depress growth performance and impact the anti-oxidative ability (Slaninova et al., 2009). There is a growing intertest in use bioactive compounds of plant extracts and organic acids as feed additives, because of their antimicrobial power as well as their potential positive effect on immune defense mechanisms, but little is known about their potential against oxidative stress. The aim of this study was to assess the effect of thymol and sorbic acid on PHLC-1 hepatocytes cell line during a H_2O_2-induced oxidative stress. The PLHC-1 (*Poeciliopsis lucida* hepatocellular carcinoma) cell line was used in this study since it is a recognized *in vitro* model to study the hepatic metabolism in fish

Materials & methods

PLHC-1 were seeded at a density of 400 000 cells/ml in Eagle's Minimum Essential Medium (EMEM). Once adhered, PLHC-1 cells were incubated with 3 different concentrations of each active principle tested: thymol (0 M, CTR-T; 10 M, T10; 25 M, T25; 50 M, T50) or sorbic acid (0 M, CTR-S; 100 M, S100; 250 M, S250; 500 M, S500). PLHC-1 were cultured for 48 hours and viability was measured by MTT test, by incubating cells with MTT 1mg/ml for 4 h, and subsequently adding DMSO to dissolve formazan, before analyzing samples in a plate reader. In parallel, PLHC-1 cells were incubated with the same concentrations of thymol and sorbic acid listed before and after 24h or 48h, 0.01% H_2O_2 was added in order to induce an oxidative stress. At both time points, it was measured reactive oxygen species ROS production, by adding dihydrorhodamine123 (DHR123) 10μM for 30min at 30ºC then reading fluorescence by flow cytometry, and mRNA expression of peroxisome proliferator-activated receptor alpha (PPARα). Data were analyzed with one-way ANOVA.

Results

Cell viability was not affected by thymol at any dosage after 48h. Sorbic acid significantly increased viability of PLHC-1 cells at all the dosages tested, compared to control *($P<0.01$)*. Sorbic acid did not show any effect on ROS production. T25 showed a significant decrease in ROS production after 48h and T50 significantly decreased ROS both at 24h and at 48h (*$P<0.05$*). PPARα mRNA expression was significantly decr ased by T25 and T50 at 24h, and after 48h all the thymol dosages tested significantly decreased PPARα expression (*$P<0.01$*). On the opposite, sorbic acid significantly increased PPARα, in a dose-response manner, after 48h of incubation (*$P<0.05$*).

Discussion

Our findings indicate that sorbic acid was able to increase viability of PLHC-1 hepatocytes, as well as increase PPARα mRNA expression. PPARα has a central role in inflammat ry responses and oxidative stress, and its activation stimulates antioxidant enzymes (Toyama et al., 2004). The positive modulation on cell viability, mediated by sorbic acid during the oxidative challenge, could be related to its ability to increase PPARα expression by modulating ROS scavenging, even if it was not possible to detect significant results about ROS production. On the opposite, thymol showed a significant decrease in ROS production, along with a reduction in PPARα expression. Considering that PPARα agonists are able to decrease ROS formation, the decrease in ROS production mediated by thymol could be related to an increased activation of PPARα receptors. This would indicate a negative feedback mechanism of regulation of gene expression: the lower the level of ROS, the higher the PPARα protein amount and the less transcription of new PPARα mRNA was required.

Conclusions

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The present data represent a preliminary study where an oxidative challenge protocol was optimized and where the effect of thymol and sorbic acid on fish hepatocytes during this challenge was studied. The research was aimed to evaluate their positive modulation on oxidative responses with the aim to prevent oxidative stress, since during stressful condition the balance of ROS production is disturbed. Both thymol and sorbic acid seem to exert a positive modulation by acting on different ways, scavenging ROS production or increasing viability and PPARα expression. Further analysis will be aimed to increase the panel of biomarkers aimed to define this mode of action, like oxidative enzymes and other markers involved in the inflammatory response

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ENVIRONMENTAL SUSTAINABILITY INDICATORS APPLIED IN SMALL SCALE FAMILY FISH FARM AT THE ATLANTIC FOREST IN SOUTH AMERICA

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Fish farming practiced on small farms is an important economic and social alternative for local populations. However, the peculiarities of each production system adopted, the lack of standardization of the zootechnical management and the potential environmental impact of the fish production compromise the permanence of the producer in this activity. In this context, the present study aims to evaluate environmental sustainability through the application of indicators in family fish farms producing Nile tilapia (*Oreochromis niloticus*) in semi-intensive ponds in the Ribeira Valley region of Atlantic Forest, Sao Paulo state, Brazil.

The family farms were selected because they met the following criteria: i) produce Nile tilapia (*Oreochromis niloticus*) in earth ponds in a monoculture system; (ii) production for commercial purposes; iii) use of extruded feeds; iv) only use family labor. During a production cycle (8 months), three ponds (sample units) monitored in each fish farm, totaling six sample units evaluated. In fish culture A, production was carried out in a single-phase system: Phase I - fish weighing 1 g distributed in three ponds until reaching a minimum slaughter weight of 600g. In fish culture B, production was carried out in a two-phase system: Phase I - fish weighing 1g to 80g distributed in three ponds; Phase II - all fish (> 80g) were transferred to a single pond until reaching a minimum slaughter weight of 600g. The zootechnical, environmental variables and environmental sustainability indicators (ESI) based on four aspects: use of natural resources, efficiency in the use of resources, release and accumulation of pollutants and conservation of genetic diversity, according to the proposal by Valenti et al. (2018).

These data then used for the determination of the ESI of aquaculture enterprises and elaboration of performance scale diagrams (Figure 1). The fish farm A presented sub-index of 1.00 in relation to efficiency in the use of energy (E), nitrogen (N), phosphorus (P) and carbon (C), showing to be greater in 46%, 2%, 27% e 18% in relation to fish farm B, for E, N, P and C, respectively.

The environmental sustainability index for fish farms A and B were 0.95 and 0.75, respectively and influenced by the high generation of pollutants in fish farms. Thus, it is evident the importance of the use of tools that help in the correct definitions and limits to help the planning and direction of fish production in tropical waters, in order to minimize the impacts of aquaculture on the aquatic ecosystem. The results shown that this methodology is an efficient tool to help aquaculture managers and producers in the planning and organization of the activity in a sustainable way.

(FAPESP, CNPq, Capes)

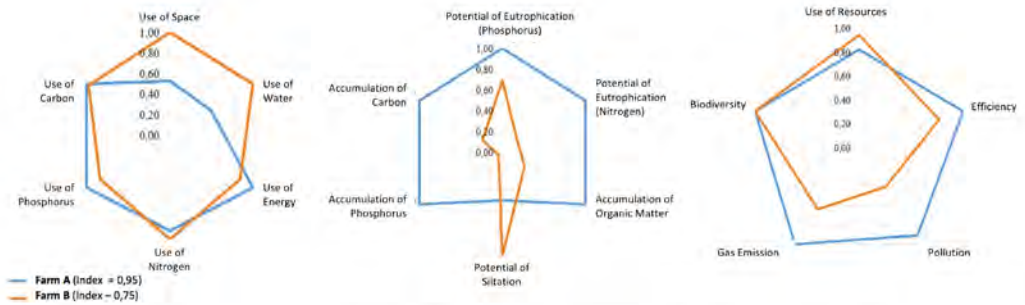

Figure 1. Environmental sustainability indicators (ESI) applied in small fish farms.

REVIEW ON SEX DETERMINATION PROCESS, ALL-FEMALE PRODUCTION AND GROWTH PERFORMANCES IN EURASIAN PERCH, *Perca fluviatilis*

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Introduction

The sex determination process in Eurasian perch (*Perca fluviatilis*), that display a sexual growth dimorphism towards females, is mainly under the control of genetic factors, govern by the sex chromosomes system XX/XY, with a probable autosomal influence as proven by sex reversal experiment, gynogenesis and hybridization. Beside this chromosomal control, sex steroids, mainly E2/11KT ratio, are strongly involved in the control of the development of the phenotypic sex, as important sex steroids levels are measured during all the embryonic period, from fertilization (maternal transmission) until the onset of histological differentiation of the gonad. Although the lability of sexual development was clearly established, no effect of temperature on sex differentiation process was described up to now for this species. This lability of the sexual development could be used to control the sex ratio and produce all-female population, that allow the improvement of the productivity under controlled conditions. This paper will review the bases of the sex determination process in E. perch, as well as the different methods of sex control, from hormonally sex reversal treatment to chromosomes set manipulation (triploïdisation and gynogenesis).

Sex determination process

Sex determination process in E. Perch is mainly under the control of sex chromosomes, with female XX and male XY chromosomic system with a probable implication of autosomes. The 11KT to E2 ratio plays a major role in the sex differentiation process in E. perch. No effect of temperature was reported under experimental conditions (Rougeot, 2019).

Sex control and all-female production

In E.perch, hormonal sex control and all-female production could be achieved within 2 generations by naturally or artificially crossing hormonally sex-reversed XX males breeders with normal XX females. The optimal treatment to obtain functional XX male is 5 to 10 mg MT per kg of food applied at 70 mg mean body weight during 30 days. The production of all-female population will allow to improve the productivity about 30% (fig.1)

Chromosomes set manipulation

The induction of 90 to 100% of triploid E. perch is obtained using a heat shock of 30°C, applied 5-7 minutes post-fertilization for 10 to 25 minutes with 45% of survival rate.

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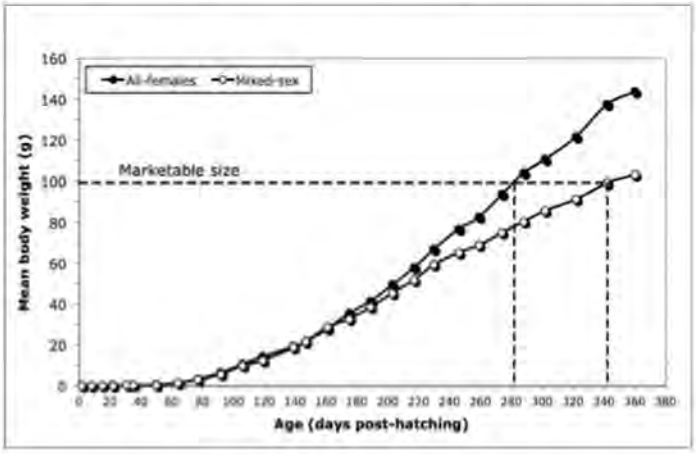
Figure 1: Comparative growth curve of all-female and mixed-sex juvenile Eurasian perch reared under intensive rearing conditions in a 0.5 m^3 tank in a recirculating system at 23°C at an initial stocking density of 2000 fish.m^3 (Rougeot and Mélard, 2008).



COMPACT PHOTOBIOREACTORS FOR LIVE MICROALGAE PRODUCTION IN AQUACULTURE HATCHERIES AND NURSERIES

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Photobioreactors in Aquaculture

Availability of high quality microalgae is a bottleneck in aquaculture hatcheries and nurseries. Numerous species of nutritious microalgae are used to feed bivalve and crustacean larvae. In marine finfis hatcheries algae is used to tint water to enhance larval survival, or to nutritionally enhance zooplankton fed to larvae. In any facility, algae contaminated with pathogens (i.e. *Vibrio* spp.) can result in high larval mortality and lower growth rates. Traditional methods of microalgal production are unreliable and labour intensive, while substitutes such as concentrates and dried algae can degrade water quality as they decompose, increasing the organic load in the system and creating a substrate for pathogenic bacteria. Automated photobioreactors offer a compact and easier method of producing live algae onsite compared to traditional methods (e.g. figure 1). They are often more cost effective than substitutes and contribute less to the organic load in the water. Closed photobioreactors provide a biosecure culture environment and reduce the chance of culture contamination. This control over the microbial environment is of paramount importance since pathogenic or probiotic organisms introduced into algae cultures will be transferred downstream to livestock, impacting survivability and ultimately profits

Fig. 1. Equivalent Algal production from PBR 1250L and 75,000L of traditional fiberglass batch tanks growing *Thalassiosira pseudonana*

IS THERE A EUTROPHICATION CONCERN IN SUPPLEMENTARY FEED BASED COMMON CARP FARMING?

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Introduction

The land-locked central European countries rely heavily on common carp aquaculture in fishponds (e.g. Czech Republic, Poland, Hungary). There has been some debate between environmentalists and carp farmers concerning eutrophication of water bodies; often clandestine (Roy *et al.* 2019, unpublished). Over the years, share of fish meal in carp feeds has come down and use of alternative plant protein sources have intensified, generally rich in anti-tryptic factors or phytate-bound phosphorus. We undertook a systematic analysis of information from six-decades of literature data, focussing on common carp's dietary nitrogen (N) and phosphorus (P) supply and their footprints (faecal, metabolic losses) from artificial feedstuffs and natural prey or combined.

Materials and Methods

Data from 70 research articles on *Cyprinus carpio* digestibility published between 1973–2017 were analysed with additional data from 34 *C. carpio* growth trials to ascertain ecologically relevant dietary N, P levels under semi-intensive rearing conditions. Statistical analyses of numerical data (inter-quartile range (IR)), tests (ANCOVA, multiple regression) and modelling (GAM, LOESS) were done in R. All ranges are expressed in IR, except thermal growth coefficient (TGC; expressed in semi-upper IR to demarcate 'good' growth). All data are presented on dry matter (DM) basis.

Estimated N, P digestibility of cereals from metadata were validated through digestibility trials in our laboratory (Guelph system; 30% diet replacement method; 1% yttrium oxide as marker; 150-475 g carps). Apparent digestibility of N and P in commonly used cereals in Czech fishponds (wheat, corn, triticale) and carp's natural prey (daphnia, chironomid larvae, cyclops) were determined. Both literature data and our experimental results were applied to budget the faecal and metabolic footprints of dietary N, P of common carp in Czech fishponds, at national level

Results

Global metadata

Inter-quartile range (IR) of nitrogen (N) and phosphorus (P) content in feedstuffs were 5–8% and 0.7–1.2% of dry matter respectively with digestible N:P 7.2:1–44.1:1. N-digestibility IR (79–99%) was high, whereas IR of P-digestibility (27–47%) was rather poor. Dietary energy digestibility (gross energy and non-protein energy) was >76%. Higher P in feedstuffs caused signific nt negative interferences for N-digestibility. IR of nutrient content in carp faeces were estimated at 0.5–1.7% N and 0.4–0.9% P, with a 'eutrophic' N:P ratio (0.6:1 to 4.7:1). Considering the metabolic losses (16.9–30.7% of N intake), the N:P ratio of carp excreta seems 1.53:1–6.83:1, which is still 'eutrophic'.

Table 1: Nutrient footprint (faecal and metabolic losses) from supplementary feeding

Cereals	Natural food
Avg. N: 2.62% and P: 0.58%	Avg. N: 9.57% and P: 1.19% (DM basis)
24.1–44.9 kg N ton carp produced^{-1}	9.9–18.2 kg N ton carp produced^{-1}
8.7–11.6 kg P ton carp produced^{-1}	0.7–0.9 kg P ton carp produced^{-1}
N:P ~3:1–4:1	N:P ~14:1–20:1
Diffused footprint	
17–31.5 kg N and 4.7–6.2 kg P ton carp produced^{-1}	
Diffused N:P stoichiometry: ~ 9:1–12:1 (non-eutrophic)	

(Continued on next page)

Under semi-intensive system, digestible 'supplementary' nutrients (N: 3.3–4.9%, P: 0.2–0.5%; even lower) can support at least 0.6–1.2 thermal growth coefficient (reasonable growth) and be ecologically relevant. Eutrophication potential of faeces of carps seems linked to P-digestibility. While brewery wastes, microbial protein and natural prey offer high P digestibility (75–90%), knowledge gaps still exist in P digestibility of ingredients.

Application of global metadata and digestibility trial results

Production of 18460 tons common carp year^{-1} (CZ-Ryby 2019) by the Czech Republic (largest producer in EU) is supported by cereals (~2–2.5 FCR; 36920–46150 tons) and carp's natural prey (~0.3–0.4 FCR; 5538–7384 tons DM basis). Carp's excretory N, P footprints from both compartments (cereals and natural prey) and the diffused footprint is given in Table 1. Collating these values with 41080 ha of Czech fishponds (CZ-Ryby 2019), a spatial footprint of 7.6–14.2 kg N and 2.1–2.8 kg P ha^{-1} was apparent. Land-based agriculture in the Czech Republic has approximately 2–3 times the N (up to 32 kg ha^{-1}; Rosendore *et al.* 2016) or P (up to 5-6 kg ha^{-1}; Csatho *et al.* 2007, Kronvang *et al.* 2007) footprints. The N and P retentions in carp were ~28–36% and 39–50%, respectively.

Considering the complex dilution, assimilation and re-generation processes of nutrients occurring within the system (fishponds), we have adopted the principle of diffused footprint rather than cumulative footprint (Csatho *et al.* 2007). Afterall, it is not practical to assume that all excreted nutrients by carp are the actual residues; furthermore, digestive and metabolic losses with varying nutrient concentrations does not 'simply' add-up. Keeping the solution chemistry in mind, can explain the contradictions from studies accusing semi-intensive carp farming for eutrophication vs. studies not corroborating such allegations (Roy *et al.* 2019). Our preliminary estimates show that it is naïve to blame supplementary feeding (or present culture practices *per se*) in Czech fishponds for causing eutrophication. Rather, ways to improve N and P retention in carps should be the priority.

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EPIGENETIC INHERITANCE AND LONG-LASTING PROTECTION AGAINST VIBRIOS INDUCED BY A PLANT-DERIVED COMPOUND IN BRINE SHRIMP MODEL

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Disease outbreaks have been considered as a major problem for sustainable shrimp aquaculture. Amongst all, Vibriosis, caused by bacteria from Vibrionaceae family, is of major concern in farmed shrimps. In recent years, acute hepatopancreatic necrosis disease (AHPND), caused by toxic strains of *Vibrio parahaemolyticus* has severely damaged the global shrimp industry causing mortality up to 100% resulting in huge economic losses. In addition, other *Vibrio* species like *V. harveyi*, continue to cause significant mortality, especially at larval and post-larvae stages of farmed shrimps. Previously, antibiotics were used to prevent or cure bacterial diseases. However, due to the emergence of concern for antibiotic resistance, their uses have been restricted. Therefore, the development of alternative (preventive) strategies against disease in these commercially important animals is of major importance. Shrimp has only innate immune system lacking both specificity and memory. But, in contrast recently there are increasing number of evidences in invertebrates which suggests that phenotypic responses to environmental factors or any insults related to fitness which exposed to the animals persist across generations. In the present study, using brine shrimp as a model organism, we verified the possibility or impossibility for development of a disease resistance phenotype after application of the immunostimulatory plant-derived phenolic compound. Exposure of phenolic compound to the brine shrimp parental population induced and transmitted the resistance phenotype in 3 subsequent unexposed generations against pathogenic *Vibrios* and epigenetic reprogramming is most likely to play a pivotal role in the underlie mechanism.

IMPLEMENTING PRECISION FISH FARMING ON A RAINBOW TROUT FARM: A DATA ASSIMILATION APPROACH

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Introduction

Innovations and decrease in costs of sensors are making it possible to apply to aquaculture the concept of "Precision Livestock Farming", introduced in the agrifood sector in early 2000. The implementation of the "Precision Fish Farming" (PFF) framework (Fore et. al., 2018) is likely to revolutionize the aquaculture industry, leading to a new generation of softwares and decision support tools, based on dynamic data and process driven models. At present, however, a few examples of PFF implementation can be found, concerning only the more mature industry, i.e. salmon farming. In this paper, a process based dynamic model of a "virtual" rainbow trout farm is presented, which includes a data assimilation algorithm, thus representing a first attempt of applying PFF to this aquaculture typology. This study was developed in the framework of the EU H2020 project GAIN Green Aquaculture INtensification in Europe (Grant Agreement 77330).

Methodology

Data Assimilation (DA) algorithms are being currently used in many scientific fields, e.g. mechanics, oceanography, meteorology, as they allow one to combine model output and field data as long as they are collected. The purpose of DA is two-fold: i) to correct the prediction of output variables, based on the information provided by any new datum; ii) to improve the estimation of the remaining state variables, which may include also some key model parameters. Key to that is the assumption that the state variables are regarded as stochastic ones: the dynamic of their expected values is driven by a set of governing differential equations but their fluctuation are driven by the differences between model output and observations. The core of the virtual farm model is a dynamic individual bioenergetic model of rainbow trout, which was identified on the basis of the literature and calibrated using a set of data collected at a trout farm in Preore, Northern Italy. The model equations (Brigolin et al., 2014) allows one to predict fish weight/length, on the basis of feed quantity and composition, as well as fish oxygen demand and ammonia excretion. This module was coupled with one simulating the dynamic of dissolved oxygen and water temperature, in relation to the oxygen level and temperature of the input. Two key parameters, i.e. the feed assimilation efficiency and the respiration rate, were included in the state vector and, therefore, treated as stochastic variables. The model was forced with hourly values of dissolved oxygen and temperature of the input water. DO and Oxygen data in the raceway system were collected every 15 minutes and processed in real time. Fish average size was determined weekly using the Vaki Biomass Daily System, purposely calibrated.

Results and discussion

Preliminary results show that the model allows one to accurately predict short term variation of DO, thus providing relevant information for managing the oxygenation system, in order to avoid hypoxic conditions and maximizing fish appetite. Furthermore, the model provides an accurate estimation of the feed ration, based on reliable short term predictions of fish average weight and of the feed assimilation efficienc , which is recursively estimated using the DA algorithm. Those findings will be further tested across a range of fish size and temperature/oxygen conditions. Subsequently, a population model will be developed, based on MonteCarlo methodology, in order to simulate the evolution of the whole weight distribution. Furthermore, the model will be used for assessing the local impact of the fish farm, in terms of release of Dissolved Inorganic Nitrogen in wastewater.

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ISOLATION AND CHARACTERIZATION OF NEW *Flavobacterium columnare* AND ITS BACTERIOPHAGES FROM FISH FARMS

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Introduction

In the past 20 years, aquaculture has been the fastest growing food production sector (FAO. 2014). The intensification of fish farming expose fish to stressful conditions and bacterial infections (Pulkkinen et al., 2009). One of the challenging diseases in the fresh water fish farms is columnaris disease, caused by *Flavobacterium columnare*, which can cause rapidly spreading epidemics and high in fish (Suomalainen et al., 2005). Antibiotic treatment is necessary and it is common that the farms are using several treatments in one season, increasing the risk of antibiotic resistance strains spread (Pulkkinen et al., 2009). A potential alternative for antibiotic treatments are bacteriophages (i.e. phages), viruses that infect bacteria. *F. columnare* phages are host specific and possible to isolate from the environment (Laanto et al., 2011). In this study, we screened the diversity of *F. columnare* and its phages in 10 different fish farms from Finland and Sweden. We were able to isolate phages that can infect virulent *F. columnare* strains. The large collection of well characterized phages and hosts allow us to study the potential for using phage therapy against columnaris disease.

Materials and Methods

Bacterial strains and phages were isolated from 10 different fish farms from Finland and Sweden using culture-based methods. Bacteria were genetically characterized using previously established methods (Suomalainen et al., 2006, Laanto et al., 2011, Kunttu et al., 2012, Almeida et al., 2019). The virulence of selected 34 strains was tested with rainbow trout fry. Host range of all new phage isolates was tested with 229 bacterial strains, and a subset of 15 phages isolates, infecting different host genotypes, were further characterized for their morphology using transmission electron microscopy (TEM).

Results

The 126 new *F. columnare* strains were clustered into genotype groups C (73), E (24), A (16) or G (8). Genotype could not be confirmed for some of the strains. Based on 16S rDNA, all strains from Finland belong to genomovar group I. Strains from Sweden belong to genomovar group I or I/II. In virulence experiment, the most virulent genotype groups were E and C, which caused 100% mortality within 15 hours post infection. 64 new *F. columnare* phages were isolated of which most infected the virulent C genotype host, the rest infecting genotype G and A hosts. Only from one fish farm were phages isolated that infected all three different kind of host genotypes. Based on the host range, most of the phages were host specific infecting same genotype group as the isolating hosts. Some phages had broader host range and were able to infect strains from two or three different genotype groups (including genotype E). TEM analysis indicated all phages belong to the *Myoviridae* family, and have icosahedral head and a stiff, unbending tail, (Figure 1).

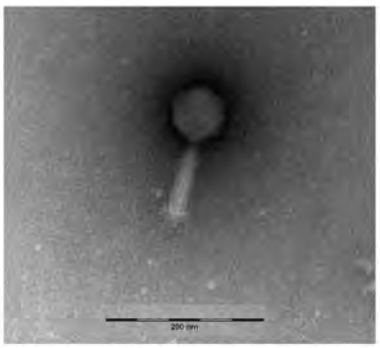

Figure 1. Negatively stained *Myoviridae* FCOV-F2, *F. columnare* bacteriophage. Icosahedral head and stiff, unbending tail. Imaged with TEM, 80 kV. Scale bar 200 nm.

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Discussion & conclusion

We isolated new *F. columnare* strains and their phages from Finnish and Swedish fish farms, and characterized the isolates. Our results support the previous findings of high variability in virulence among *F. columnare* genotypes (Suomalainen et al., 2006). Phages infecting both, highly virulent and less virulent *F. columnare* strains, can be isolated from the fish farms during columnaris outbreaks like mentioned before (Laanto et al., 2011). Our host range study indicate the isolated phages are able to infect and inhibit more than 90% of 229 bacterial strains in our collection. With these collections, we can start to plan and implement phage therapy against *F. columnare*. Combination of phages infecting different hosts could be used as part of phage cocktail to prevent or treat columnaris infections in fish

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APPLICATION OF HYPERSPECTRAL IMAGERY TO DISCRIMINATE DIFFERENT DIETS OF LIVE RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Diet, among other factors, has substantial effects on stress tolerance and health, and therefore, fish must be fed an adequate diet that meets all their nutrient requirements for proper growth and resistance to stress and disease problems. In other words, feeding fish with an inadequate nutritional diet not only affects growth and feed efficiency but also increase susceptibility to disease and induces the appearance of deficiency signs, including altered behavioral and pathological changes (Oliva-Teles, 2012). Currently, some considerable effort is addressing the replacement fish meal and fish oil with other protein and lipid sources such as the plant-based (Lazzarotto et al., 2015).

Some researchers have shown that skin colour is highly dependent on the carotenoids present in the diet (Ho et al., 2013); therefore, skin colour can provide useful information for regulating proper feeding. Despite the importance of fish skin colour, very few studies have investigated the effects of different diets on fish skin.

Hyperspectral imagery (HSI) is an emerging technology that integrates both spectroscopy and imaging in a single system; is enabling simultaneous acquisition of spatial and spectral information from an object. Hyperspectral Imaging (HSI) has been used to quantify many fish and fish products features (Saberioon et al., 2017), However, to the best of our knowledge, the feasibility of HSI to determine the impact of diet changes in live aquatic organisms has not been studied. Therefore, the main aim of this study was to investigate the suitability of using the HSI system in Vis and NIR range (400-1000 nm) to evaluate the influence of two di ferent diets on live fish skin

Materials and method

The rainbow trouts were produced at INRA-PEIMA (Sizun, France). Experiments were designed in a split-block design with three replications for each diet; therefore, 80 fish were fed a commercial based diet (3 tanks) and 80 were fed a 100% plant-based diet (3 tanks). After three weeks, all fish were mildly anaesthetised and used for hyperspectral image acquisition in the wavelength range of 393-1009 nm. Diets were manufactured at the INRA NUMEA facility of Donzacq (France). The acquired hyperspectral images were corrected. Afterwards, the reflectance spectrum from the region of interest (ROI) was computed by averaging the spectral value of all pixels in the ROI for each sample. Finally, After spectra pre-processed, Support vector machine (SVM) as a classifier was employed to develop the classification models for discriminating two different diets.

Results

The Vis/NIR hyperspectral imaging for the two diets were similar but had differences in reflectance intensity in the range from 450- 750 nm and 900-1000 nm (Figure 1). It can be seen that the mean reflectance values of PBD are higher than CBD in most of the visible bands (450 – 750 nm), but the mean reflectance values of CBD are higher than PBD in NIR bands (900 -1000 nm). This implied that different diets had induced significant alterations to fish skin in a way that can be detected by spectral information. The differences between spectra might be mainly associated with how the different types of lipids, influence the absorption and deposition of carotenoids. In short, oils contain varying amounts of stanol and sterols which interfere with the uptake of carotenoids (Kalinowski et al., 2007).

The SVM classifier showed significant accuracy for classifying fish based on their diets (CCR = 0.83, Kappa = 0.6

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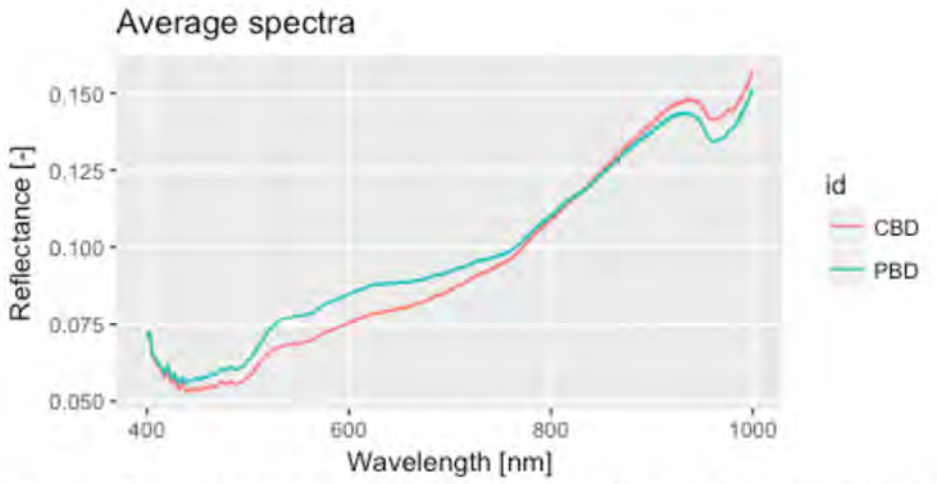

Figure1: The mean Vis/NIR spectral reflectance of rainbow trout skin for commercial based diet (CBD) and plant-based diet (PBD)

Discussion and Conclusion

The overall results indicate that Vis/NIR hyperspectral imaging combined with machine learning algorithms has the potential to be used as a rapid, accurate and non-destructive method to discriminate of fish based on their diet received during the cultivation. The skin pigmentation in farmed rainbow trout has commercial value because the high visual impact on the consumers and affects marketing prospects; therefore, the presented system in this study can provide more objective, accurate, fast tools in determining the effects of different diets on the external appearance of rainbow trout. Additionally, it will pave the way for a better understanding of the precision fish farming concept in aquaculture by controlling and monitoring of feeding impacts on fish skin during cultivation. Furthermore, it can be used in the better implementation of European Union regulation (EC 178/2002) on traceability of food and feed, food and feed producing animals, and substances intended to be incorporated into food and feed by providing diet information based on fish skin

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ASSESSMENT OF THE EFFECTS OF *Nannochloropsis gaditana* ENZYME HYDROLYSATES ADDED INTO AQUAFEEDS ON GROWTH, MUSCLE COMPOSITION, PIGMENTATION AND OXIDATIVE CONDITION OF SEA BREAM (*Sparus aurata*) JUVENILES

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Introduction

The existence of a recalcitrant, cellulose-rich cell wall in *Nannochloropsis* sp. could limit the *in vivo* bioavailability of the intracellular components, so that the theoretical feeding potential of the microalgae might not be reflected on the animals, neither in terms of fish growth or physiological condition parameters (Cerezuela *et al.,* 2013). In order to cope with such limitations, some strategies aimed at disrupting cell walls prior to the inclusion of microalgae in aquafeeds have been proposed in the literature (Tibbetts *et al.,* 2017). Unfortunately, none of them can be considered as feasible in terms of practical application, due to a numerous list of constraints. Consequently, simple, economical, and cost-effective cell-wall disruption protocols are still needed, aimed at easing industrial up-scaling. In this regard, this study evaluate the use of hydrolytic enzymes capable of weakening *N. gaditana* cell walls, namely cellulases, prior to its incorporation into feeds. Cellulase enzymes have a wide range of industrial applications, so that they are available for feed-processing industry at affordable price, and consequently, any bioprocess that includes them could be easily scalable at industrial level. The overall objective of this *in vivo* study was the assessment of possible improvement in protein bioavailability, as well as on the occurrence of potential effects of microalgae as functional additive on growth, muscle composition, oxidative status, and pigmentation of seabream juveniles.

Materials and methods

N. gaditana biomass was obtained from Estación Experimental "Las Palmerillas" (Fundación Cajamar, Almería). Two formats of algal biomass were used: raw wet algal biomass (**RAB-C**) (about 20% DM); and freeze-dried algal biomass (**FAB-C**). A batch of both formats was subjected to cellulase enzyme hydrolysis (**RAB-H** and **FAB-H**, respectively). The enzymatic treatment was standardized in order to obtain a hydrolysate directly utilizable in feed processing at low inclusion rate (5% DM). Four isonitrogenous and isolipid (47% and 17% DM, respectively) experimental diets were elaborated at the CEIA3-University of Almería facilities (Service of Experimental Diets). A microalgae-free diet was used as control (**CT**). Gilthead sea bream juveniles were randomly distributed in 15 tanks (15 fish per tank) of 400 L tank. Animals were fed at 2% of the biomass .. Fish were sampled after 90 days, and biometric and morphometric analysis were carried out. Muscle proximate analysis (AOAC, 2000) and lipid oxidation (Buege and Aust, 1978) by thiobarbituric acid reactive substances (TBARS), were determined. Instrumental colour was measured on skin side of fillets by CIE (1986) L*, a*, and b* system. The parameters lightness (L*), redness (a*), and yellowness (b*) were recorded.

Table 1. Growth performance, nutrient utilization, skin colour (L*, a*, b*) parameters and TBARS muscle content of *S. aurata* juveniles.

	CT	RAB-C	RAB-H	FAB-C	FAB-H
Initial body weight (g)	10.22 ± 0.66	10.18 ± 0.74	10.12 ± 0.78	10.21 ± 0.71	10.19 ± 0.63
Final body weight (g)	45.77 ± 8.67	44.84 ± 7.61	43.10 ± 8.24	43.52 ± 7.21	44.45 ± 8.65
Daily gain (DG, g day $^{-1}$)	0.41 ± 0.02	0.40 ± 0.01	0.38 ± 0.01	0.39 ± 0.01	0.40 ± 0.01
Specific Growth Rate (SGR)	1.74 ± 0.03	1.72 ± 0.02	1.68 ± 0.03	1.68 ± 0.02	1.71 ± 0.02
Feed efficiency ratio (FER)	0.51 ± 0.03^{b}	0.49 ± 0.01^{ab}	0.48 ± 0.00^{ab}	0.45 ± 0.03^{a}	0.49 ± 0.01^{ab}
Feed Conversion Ratio (FCR)	1.96 ± 0.11^{a}	2.04 ± 0.03^{ab}	2.07 ± 0.01^{ab}	2.22 ± 0.17^{b}	2.03 ± 0.04^{ab}
L*	89.75 ± 0.41	88.03 ± 0.58	88.07 ± 2.06	86.91 ± 1.78	87.37 ± 1.45
a*	-1.48 ± 0.16^{b}	-1.89 ± 0.12^{a}	-2.01 ± 0.38^{a}	-2.16 ± 0.11^{a}	-1.94 ± 0.08^{a}
b*	5.53 ± 0.13^{a}	8.28 ± 0.46^{bc}	9.02 ± 0.82^{c}	8.16 ± 1.46^{bc}	7.25 ± 1.07^{b}
TBARS (mg MDA Kg^{-1})	4.66 ± 0.07^{d}	3.36 ± 0.04^{b}	2.78 ± 0.02^{a}	3.62 ± 0.04^{c}	2.85 ± 0.02^{a}

Dietary codes: **CT**: control; **RAB-C**: 5% crude raw algal biomass; **RAB-H**: 5% hydrolysed raw algal biomass; **FAB-C**: 5% freeze-dried algal biomass; **FAB-H**: 5% freeze-dried algal hydrolysate. Values are expressed as mean ± SD, (n=3). Values in the same column with different lowercase letter indicate significant difference ($p<0.05$).

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Results and discussion

Overall growth performance and nutrient utilization data are shown in Table 1. Fish fed with 5% of *N.gaditana* biomass diets showed figures similar to control group, although control fish showed significantly higher protein efficiency ratio than **FAB-C** specimens. Besides higher protein content (75.68 and 75.26 % DM respectively, p= 0.0030), in fish fed with diets including microalgae hydrolysates (**RAB-H** and **FAB-H**,), compared to control fish, no differences were found in proximate analysis.

Microalgae have the potential to synthesize low concentrations of biomolecules such as astaxanthin, lutein, carotene, among other nutraceutical compounds. Many of these microalgae-derived compounds can influence tissue pigmentation, and are recognized as health-promoting substances, mostly due to antioxidant activities (Nakagawa *et al.,* 2012). In this sense, taken as a whole, *N. gaditana* inclusion in diets affected a* and b* parameters (p=0.020 and 0.009, respectively). In general, the skin of microalgae supplemented fish, compared to control batch, were greenish (a*) and yellowish (b*), mostly due to differences b* observed for raw biomass hydrolysate. In addition, *N. gaditana* at low inclusion rate (5%) in diets caused a greater muscle antioxidant response (p<0.001). Similarly, this effect is more evident when microalgae biomass in diets was pre-treated with cellulase enzymes (**RAB-H** and **FAB-H**, p< 0.001).

Conclusions

The results obtained indicated that *N. gaditana* biomass at low inclusion rates (5%) in aquafeeds could have an impact on different aspects related to protein utilization, antioxidant activity and skin pigmentation of sea bream. In addition, a prior enzymatic treatment by celullases can increase the bioavailability of metabolites with potentially bioactive and functional effects. However, further research assessing the potential effects on digestive physiology, metabolism and fish immune response are needed.

Acknowledgements

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FEED PELLETS CONTAINING pDNA AS ON-FARM STRATEGY FOR ORAL IMMUNIZATION OF AQUACULTURED FISH

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Introduction

The need to control emerging fish diseases, mostly linked to intensive rearing, that constraint the aquaculture sector has driven different promising initiatives aimed at designing effective immunization strategies. In this context, research efforts have been focussed on the development of innovative vaccines, such as DNA-based, established on gene transfer for transient gene expression of immunogenic proteins. Compared to direct administration of antigenic proteins, which stimulate almost exclusively humoral immunity, DNA vaccines are able to stimulate both humoral and cellular responses, this fact ending up in efficient immune response (Tonheim *et al*., 2008). Nevertheless, commercial plasmid DNA (pDNA) vaccines are intended exclusively for parenteral administration, owing to the degradation of pDNA taking place during its transit through the digestive tract. On the other hand, oral administration is considered the ideal route for mass administration of bioactive substances in aquacultured fish, and besides being a simple delivery strategy, research has revealed growing evidence of the key role of the intestinal mucosa as part of the immune system of fish (Xue *et al*., 2013). However, stomach acidity and digestive nucleases cause together quick, extensive hydrolysis of orally delivered pDNA that ends up in loss of biological activity. In this sense, the protection provided by encapsulation in inert biopolymers might enable substances with interest in fish immunization to resist the gastrointestinal transit (Kim *et al*., 2014). In this regard, research aimed at carrying pDNA-nanoparticles at precise dosage in practical commercial feeds particles for farmed fish still remains a major challenge. The aim of this study was the assessment of preloaded feed pellets as a delivery system for pDNA, with the purpose of evaluating the potential administration of DNA vaccines orally in aquacultured fish. Pellets were made up by usual feed ingredients, which were mixed with chitosan nanoparticles entrapping a model plasmid (pCMVβ) expressible in eukaryotic cells, before being elaborated.

Materials and methods

The plasmid pCMVβ, integrating lacZ reporter gene encoding for *E. coli* β-galactosidase was chosen for this study as eukaryotic expression vector. The preparation of chitosan-nanocapsules was based on the protocol described in Sáez *et al*. (2017). An experimental feed was elaborated (**NANO-FEED**) adding chitosan-pDNA nanoparticle suspensions to the feed ingredients during the mixing step, prior to the granulation process. The diet was elaborated by LifeBioencapsulation S.L. (Almería, Spain). Two hundred and forty juveniles (16 ± 2 g) of gilthead sea bream were used in the assays. Oral administration (**NANO-FEED)** was conducted by pellets loaded with pDNA-chitosan nanoparticles in order to guarantee the ingestion of a total amount of 40 μg pDNA per fish during 1 day. Intramuscular (i.m.) injection of equal amounts of plasmid (**IM-pDNA**) and commercial β-galactosidase antigen (**IM-β-gal**, 10 μg) were also carried out with the purpose of comparing oral and i.m. administration. A **CONTROL** group with animals that did not receive any substance completed the experimental design. All the experimental groups were fed on control diet (without pDNA) throughout the experimental

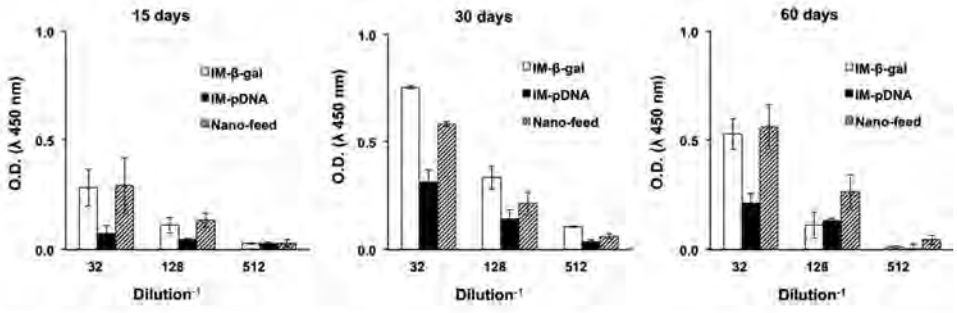

Figure 1. ELISA titration of anti-β-galactosidase IgM in fish sera. Animals were immunized by three different procedures: i) intramuscular administration of β-galactosidase enzyme (IM-β-gal); ii) intramuscular administration of pCMVβ plasmid (IM-pDNA), and iii) oral administration of chitosan-pCMVβ nanoparticles in feed pellets (nano-feed).

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period, except for the one-day oral administration of pDNA in NANO-FEED group. The possible *in vivo* expression of the exogenous gene was measured in different fish tissues during 60-d by two different procedures. On the one hand, the activity of the enzyme β-gal was detected and quantified in muscle, liver and intestine (An *et al*., 1982 and S-gal staining) on the other, specific IgM against β-gal antigen was titrated in blood samples by indirect ELISA..

Results and discussion

The expression of the reporter gene was detected in fish tissues following both oral and i.m. administration of pDNA up to 60 days. This period of sustained expression is, roughly, within the range described in other studies broaching oral delivery of pDNA (Rivas-Aravena *et al.,* 2013). However, organ distribution of the gene expression was more evident after oral (β-gal activity measured in gut, liver and muscle) than after parenteral administration (restricted to adjacent muscle tissues). In agreement, specific IgM titration indicated that humoral immune response was more intense and sustained throughout the experimental period after oral than after i.m. delivery of equal amounts of pDNA (Fig.1).

Conclusions

These results suggest that feed pellets containing chitosan nanoparticles might enable efficient oral delivery of pDNA, a fact that might imply valuable applications in terms of on-farm mass immunization purposes, especially with regard to DNA-based vaccines and small size fish, in which i.m. administration remains unfeasible. Moreover, it is also important to remark that the feed proposed here can be formulated according to the specific size of the target fish. In this regard, by adapting the feed particle size, fry could be orally vaccinated through feed loaded with pDNA entrapped in suitable nanoparticles.

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EVALUATION OF ENZYMATICALLY-HYDROLYSED *Nannochloropsis gaditana* AS FEED ADDITIVE FOR FEEDING JUVENILE GILTHEAD SEABREAM: CELL WALL DISRUPTION

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Introduction

The interest in microalgae has come up strongly in the last years, given that they stand valuable, and yet mostly untapped, potential for reducing the dependence on unsustainable conventional raw ingredients used in aquafeeds. However, the existence of a cellulosic-rich cell wall in genus as *Nannochloropsis* joined to the unappreciable digestive cellulose activity in the gut of carnivorous/omnivorous marine fish (including gilthead seabream) could limit their digestibility and the further bioavailability of the intracellular nutrients. In this sense, a pre-treatment of the algal biomass seems to be needed in order to breaking these walls for improving the nutrient bioavailability of microalgae. Some strategies using mechanical pre-treatment have been proposed (Tibbetts *et al*., 2017) however, these processes can significantly increase the price of microalgae, which is already very high, which would probably make it impossible to scale up industrially. A cost-effective alternative can be the breakage by hydrolysis using cellulolytic enzymes. The use of these industrial enzymes could solve the problem and try to increase the bioavailability of the nutrients of the microalgae biomass. With this purpose, this work aims to assess the effectiveness of using a commercial cellulase from *Aspergillus niger* for cell wall disruption in the microalgae *Nannochloropsis gaditana.*

Materials and methods

Raw wet (RAB-H) (about 15% DM) and freeze-dried biomass (FAB-H) of *N. gaditana* provided by Estación Experimental "Las Palmerillas" (Fundación Cajamar, Almería, Spain) were used. Enzymatic hydrolysis was carried out using an *in vitro* model. For each assay, a known amount of each microalgal biomass, providing a final concentration of 15mg dry weight mL^{-1}, was suspended in 100mM sodium citrate buffer solution (pH 5.0) and incubated at 50ºC under continuous agitation for 4h in presence of a commercial cellulase. Samples were withdrawn at different times and immediately immersed in a hot water bath (100ºC) for 5 min for stopping the enzymatic reaction. To understand the degree of the hydrolysis process, the evolution of total reducing sugars released from microalgae were evaluated using the dinitrosalicylic acid (DNS) method according to Miller (1959). In addition, total amino acid released was also quantified (Church *et al*., 1983).

Results and discussion.

The results obtained after the glucose equivalent and free amino acids quantification revealed the efficacy of the enzymatic pre-treatment. Quantification of reducing sugars showed a significant increase in glucose concentration over the enzymatic reaction in raw wet and freeze-dried biomass of *N. gaditana*, reaching the highest values at the end of the treatment (Figure 1). Results evidenced a significantly higher breakdown of the microalgae cell wall in RAB-H compared with FAB-H, reaching values above 15g free glucose equivalent per 100 g of microalgae biomass. Similarly, the quantification of the free α-amino groups at the end of the enzymatic procedures revealed a remarkable increase of these when the algal biomass was enzymatically treated. The final concentration of free amino acids was significantly higher in RAB-H, reaching values greater than 18g free amino acids 100g of $protein^{-1}$, compared to those obtained in FAB-H (9.6g free amino acids 100g of $protein^{-1}$)(Figure 2).

Conclusions

Results obtained evidenced effectiveness of an enzymatic pre-treatment with cellulase represents for weakening microalgae cell wall as a promising tool for increasing the nutritional value by improving the bioavailability of intracellular components, especially in raw wet algal biomass. In this regard, a previous step for cell wall disruption/hydrolysis is recommended before including microalgae biomass in aquafeeds.

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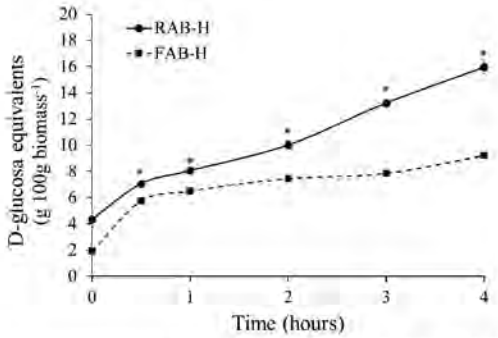

Figure 1. Evolution of D-glucose concentration during raw wet (RAB-H) and freeze-dried biomass (FAB-H) of *N. gaditana*. Values are expressed as mean ± SD. Asterisk indicates significant differences (P<0.05) among raw wet and freeze-dried biomass in each sampling point.

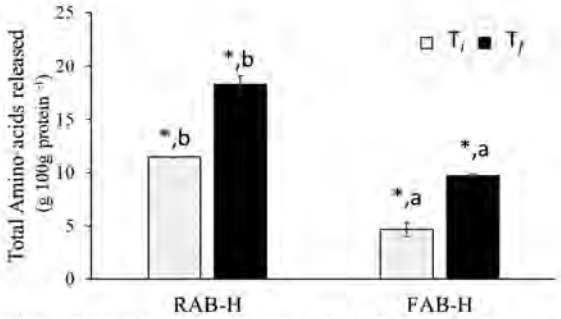

Figure 2. Total amino acids released. Data represent mean ± SD. Asterisk indicates significant differences (P<0.05) between initial and final time of enzymatic reaction (T_i and T_f, respectively). Different letters indicate significant differences (P<0.05) among raw wet and freeze-dried biomass

Acknowledgements

This research was founded by H2020-SABANA (727874), TRFE-I-2018/001-UAL and CEIJ-C05.1-CEIMAR projects. A Galafat received a grant from TRANSFIERE 2018-UAL.

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EVALUATION OF ENZYMATICALLY-HYDROLYSED *Nannochloropsis gaditana* AS FEED ADDITIVE FOR FEEDING JUVENILE GILTHEAD SEABREAM: EFFECT ON INTESTINAL FUNCTIONALITY

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Introduction

The use of microalgae as functional additive at low dietary inclusion level has been considered as a promising strategy for improving the growth and the condition status of fish. Howeve , there are studies reporting that theoretical potential of microalgae is not necessarily reflected in a significant improvement on growth rates, or in other parameters indicative of general status of fish (Cardinaletti *et a*l., 2018). Indeed, the existence of the cell wall limits the digestion of microalgae and the further bioavailability of the intracellular nutrients (Wu *et al*., 2017). Therefore, pre-treatment performed on crude algal biomass may represent a useful tool for weakening the recalcitrant microalgae cell wall before inclusion in aquafeeds in order to facilitate the action of fish digestive enzymes for increasing nutrient bioavailability. In this regard, we hypothesize that low dietary inclusion level of enzymatically-hydrolysed microalgae might improve the digestive functionality of fish. With this purpose, the overall objective of this study is focused specifically on the assessment of potential effect of using microalgae hydrolysates as functional additive in practical diets for gilthead seabream juveniles.

Materials and methods

A feeding trial was carried out at the "Planta de Cultivos Marinos" of University of Cádiz (Puerto Real, Cádiz, Spain). 225 gilthead seabream juveniles were randomly distributed in 15 tanks of 400 L capacity at a density of 15 individuals per tank and fed at 2% of the biomass with five isonitrogenous and isolipidic (47% and 17% DM, respectively) experimental diets supplemented with 5% of *Nannochloropsis gaditana* in four different formats: i) wet crude biomass (**RAB-C**), ii) freeze-dried crude biomass (**FAB-C**), iii) enzymatically-hydrolysed wet biomass (**RAB-H**) and iv) enzymatically-hydrolysed freeze-dried biomass (**FAB-H**). In addition, a microalgae-free diet was used as control (**CT**). All experimental diets were tested in triplicate. At the end of the feeding trial (90 days), fish were euthanized by spine severing, and biological samples were obtained for determination of digestive enzymatic activities and study the intestinal mucosa by scanning electron microscopy (SEM). For enzymatic activity analysis, intestines from eight fish per tank were randomly pooled to obtain four enzymatic extracts from each experimental tank in order to determine leucine aminopeptidase (Pfleidere , 1970), alkaline phosphatase (Bergmeyer, 1974), trypsin (Erlanger et al., 1961) chymotrypsin (Del Mar *et al*., 1979) and total alkaline protease (Alarcón *et al*., 1998) activities. For ultrastructural analysis, SEM visualization fields were recorded, and digital images were analysed using UTHSCA ImageTool software to obtain several measurements of enterocyte apical area.

Results and discussion

The use of *N. gaditana* at 5% inclusion level did not affect negatively none of the digestive enzyme activities measured (Table 1). Fish fed on RAB-H, FAB-C, FAB-H showed significantly higher trypsin, leucine aminopeptidase and alkaline phosphatase activity levels ($p<0.05$) than fish fed on control diet. This fact might be related to an improvement in the efficiency of the digestive processes and the intestinal absorptive capacity. Brush border enzymes such as alkaline phosphatase and leucine aminopeptidase play a fundamental role in the final stages of digestion, allowing the absorption and transport of nutrients through the enterocytes, and they are used as indicators of the intestinal integrity or as a general marker of nutrient absorption. In addition, intestine mucosa plays a key role in digestion and absorption processes, and as protective barrier. The analysis of intestinal mucosa using SEM images confirmed the absence of damage or inflammation on the intestinal microvilli. Fish fed on microalgae hydrolysate-supplemented diets (RAB-H and FAB-H) showed a significant increase in the enterocyte apical area compared with control groups (Figure 2). The results obtained indicate that the use of microalgae induced a significant e fect on the morphology of the intestinal mucosa.

Conclusions

The results obtained showed that the use of *Nannochloropsis* as feed additive for feeding *S. aurata* juveniles increases the level of activity in several digestive enzymes. In the case of fish fed on microalgae hydrolysates it was found as additional effect a significant increase in the apical area of enterocytes which might reflect a higher mucosal absorptive surface in those specimens. More studies are now in progress for determining the effects of microalgae hydrolysates on fish growth and metabolism, fish immune response, antioxidant activity and skin pigmentation

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Table 1. Digestive enzyme activities (U g tissue^{-1}) measured in intestinal extracts of juvenile gilthead seabream at the end of the feeding trial.

	CT		RAB-C		RAB-H		FAB-C		FAB-H	
Leucine aminopeptidase	0.13 ± 0.03	a	0.14 ± 0.02	a	0.23 ± 0.05	c	0.21 ± 0.04	c	0.20 ± 0.07	bc
Alkaline phosphatase	3.70 ± 0.62	a	3.42 ± 0.42	a	5.07 ± 0.97	b	5.14 ± 0.77	b	4.69 ± 0.94	b
Trypsin	0.08 ± 0.02	a	0.10 ± 0.02	ab	0.12 ± 0.03	b	0.15 ± 0.03	c	0.11 ± 0.02	b
Chymotrypsin	3.05 ± 0.69	b	2.53 ± 0.43	ab	2.91 ± 0.59	b	2.43 ± 0.53	ab	1.90 ± 0.54	a
Total alkaline protease	720.47 ± 98.61	ab	610.71 ± 154.76	a	819.31 ± 106.86	b	722.95 ± 122.88	ab	679.28 ± 75.83	ab

CT: control, RAB-C: 5% wet crude microalgae, RAB-H: 5% wet microalgae hydrolysate, FAB-C: 5% freeze-dried crude microalgae, FAB-H: 5% freeze-dried microalgae hydrolysate. Values are expressed as mean ± SD, (n=12). Values in the same row with different lowercase letter indicate significant difference ($p<0.05$).

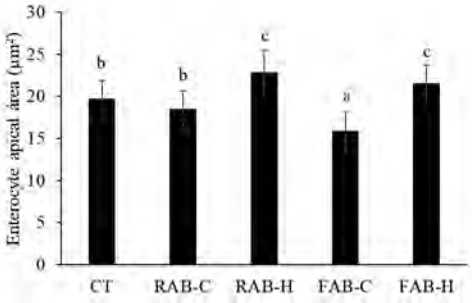

Figure 1. Morphometrical analysis of intestinal mucosa from SEM images. CT: control, RAB-C: 5% wet crude microalgae, RAB-H: 5% wet microalgae hydrolysate, FAB-C: 5% freeze-dried crude microalgae, FAB-H: 5% freeze-dried microalgae hydrolysate. Values are expressed as mean ± SD, (n=50). Different lowercase letter indicates significant difference ($p<0.05$).

Acknowledgements

This research was founded by H2020-SABANA (727874), TRFE-I-2018/001-UAL and CEIJ-C05.1-CEIMAR projects. A Galafat received a grant from TRANSFIERE 2018-UAL. The experimental diets were produced by Lifebioencapsulation SL (Almería, Spain).

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ENDOCRINE AND METABOLIC EFFECTS OF PHE-ENRICHMENT DIETS FOR ATTENUATING STRESS IN FISH

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Introduction

Stress is the most studied physiological process to assess the welfare state in farmed fish. For this reason some experiments have aimed at reducing the stress response through the use of food additives, such as essential amino acids (Herrera et al., 2017). It seems that phenylalanine (Phe) has mitigating effects on the stress system, probably due to its participation in the formation of the catecholamine hormones (adrenaline and noradrenaline) which are involved in the stress response (Saavedra et al., 2010; Herrera et al., 2017). Therefore the objective of this work is to study the physiological effects of Phe-enriched diets on two fish species, focusing on the stress system at both endocrine and metabolic levels

Material and methods

Seabreams with an average weight of 65.00±1.38g (mean±SE) and meagres with an average weight of 138.67±27.07g (mean±SE) were stocked at 20 fish/tank in 250 and 500 tanks, respectively.

Each fish species was fed two experimental fish feeds: control and control+5% Phe for seven days. At the end of the experiment, blood and brain samples were taken from 10 basal specimens and 10 specimens previously submitted to air exposure stress (3 min). The samples were analyzed using commercial kits and GC-MS; and plasma adrenaline, noradrenaline, triiodothyronine (T3) and thyroxine (T4), and brain Phe concentration were measured.

The concentration of brain Phe in meagre was higher in those specimens fed the enriched diet, however, in seabream there was only an increase in brain Phe in those fed the enriched diet and subjected to stress (Figure 2 A).

Phe-enrichment diet did not affect the T4 values in meagre, however, the T4 (Figure 2 C) concentration increased significantly in stressed seabreams. In meagre, the enriched diet led to an increase in the concentration of T3 in those specimens subjected to stress although no similar effect was observed in seabream (Figure 2 B).

Discussion

In gilthead seabream, the lack of plasma hormonal variations may be due to the fact that Phe concentration was too low for detecting effects, according to Herrera et al. (2017), who reported that only high concentrations of Phe produced endocrine effects in cod (*Gadus morhua*).

The accumulation of Phe in brain has not been previously described in fish and this first approach could indicate a quick response (accumulation) of this amino acid in seabreams after stress, although the effects on meagres could be more dependent on the feed type.

According to Peter (2011) an increase in adrenaline produces an increase in plasma T4 levels, this occurs in our results since for both species an increase in plasma adrenaline is associated with an increase in T4. The levels of T3 are lower since, as adrenaline precursor, favors the synthesis of this against the cortisol and therefore does not produce the conversion of T4 to T3.

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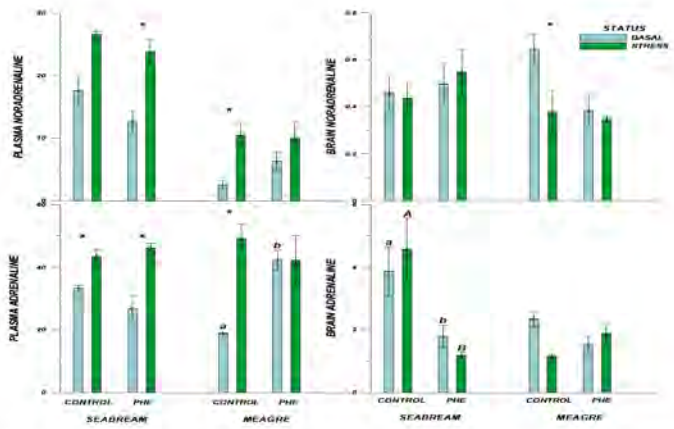

Fig. 1. Plasma an brain catecholamines concentration for each treatment (mean ± SE). Asterisks indicate significant differences between basal and stress conditions. Different letters indicate significant differences among feed groups within each sampling.

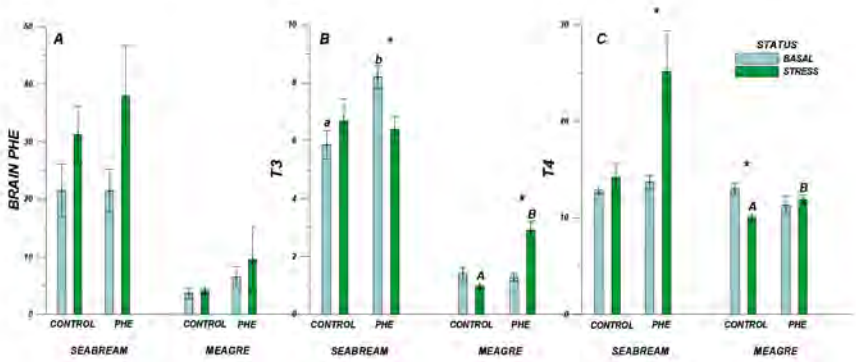

Fig. 2. Brain Phe concentration (A) and T3 and T4 plasma concentration (B) for each treatment (mean ± SE). Asterisks indicate significant differences between basal a stress conditions. Different letters indicate significant differences among groups within each sampling.

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MARINE SYNBIOTIC DECREASED THE PHYSIOLOGICAL IMPACTS OF ESTRADIOL TREATMENT IN EARLY WEANED EUROPEAN SEABASS LARVAE

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Introduction

Egypt considered the 11th world inland capture fisheries, produced 231 959 tons and the 6th world aquaculture producer, produced 1. 371 million tons (1.7% of the world aquaculture production) (**FAO, 2018**). Egypt is also the 1st Mediterranean Sea, Arab and African aquaculture producer (**FAO, 2018**). Egyptian aquaculture development production has increased from 61.6 to 1370.66 thousand tons representing 20.5 and 80.3% of the total production in the years 1991 and 2016, respectively (**FAO, 2002, 2004; GAFRD, 2010; 2016**). Egypt aquaculture production divided to 68.60% tilapia, 11.21% mullets, 14.65% carps, 0.56% catfishes, 1.78% European seabass, 1.94% sea bream and 1.17% meager (**GAFRD, 2016**). Egypt European seabass total production in 2016 was 25681 tons divided to 1183 tons (4.61%) from capture fisheries and 24498 tons (95.39%) from aquaculture (**GAFRD, 2016**). Although Egypt has 9 legal marine hatcheries (3 governmental and 6 privet sector marine hatcheries) produced 44.224 million fry, only 5.686 million were European seabass fry production (**GAFRD, 2016**), Egyptian marine aquaculture still depending on fry collected from natural resources. Egypt total wild fry collection in 2016 were 51 million fry, most of the fry were mullets, shrimp and eels., while at the same time 0.900 million fry were European seabass and sea bream fry (**GAFRD, 2016**). European seabass are a gonochoristic species and gonads are undifferentiated during the first year of life (**Gorshkov** *et al*. **2004**). **Carvalho** *et al*. **(2014)** reported that sex control in fish is one of the most promising strategies in aquaculture. The European seabass females, which show 30 to 50% higher growth rates than males (**Gorshkov** *et al*. **2004**). The estradiol-17β (E2) hormone may change the morphology of gonads, negatively affects survival and impairs growth in addition to changing the sex ratio (**Piferrer, 2001**). **Wang** *et al*. **(2008)** studied effects of E2 on growth performance of bluegill sunfish and found that the fish in the E2 treated groups grew significantly slower than the control fish. This research work aims to study impacts of Estradiol (E2) sex reversing hormone and Marine Synbiotic enriched microdiets on the European seabass larval early weaning and physiology.

Materials and Methods

European seabass larvae four treatments were control greenwater (Inve O.Range® microdiet without treatment) (G), marine synbiotic (*Bacillus subtilis* HS1 probiotic bacteria 1x 10^7 CFU + 1 mg chitosan gm-1) treated Inve O.Range® microdiet (MS), estradiol sex reversing hormone treated Inve O.Range® microdiet (150 mg g-1) (E2) and marine synbiotic and estradiol sex reversing hormone treated Inve O.Range® microdiet (MSE2) treated Inve O.Range® microdiet (E2) stocked in 30 L^3 glass aquariums triplicates in the Fish Reproduction and Spawning lab. Marine hatchery. The marine synbiotic used in this experiment developed through by Dr. Hassan A. Ibrahim and Dr. Ahmed Md. Salem according to (**Salem, 2016; Salem and Ibrahim, 2015; 2017**). Larvae samples were randomly collected from treatments tanks for body length and some fish physiology parameters to statistically determine the length growth and physiology performances

Results

The larvae weaned in marine synbiotic treated microdiet achieved the best significant ($P < 0.05$) final total length, final standard length, total length gain, total length average daily gain, total length specific growth rate and total length gain% between 35dph and 50dph. The marine synbiotic treated achieved the best significant ($P < 0.05$) final total length condition factor, final standard length condition factor and survival rate %. The larval weaning using greenwater control non treated, achieved the highest significant ($P < 0.05$) aspartate aminotransferase U/l and alanine aminotransferase U/l. The larval weaning using synbiotic treated achieved the highest significant ($P < 0.05$) alkaline phosphatase U/l. The highest significant ($P < 0.05$) in acid phosphatase U/l achieved by marine synbiotic and estradiol E2 treatements. The larval weaning tanks using greenwater control nontreated, achieved the highest significant ($P < 0.05$) Albumin g/l. The marine synbiotic treated microdiets larvae achieved the highest significant ($P < 0.05$) body supernates total protein in g/l and globulin in g/l. The highest significant ($P < 0.05$) Glucose in g/l achieved by marine synbiotic and estradiol E2 treatment. The larvae fed estradiol E2 treated microdiet showed the highest significant ($P < 0.05$) body supernates triglycerides in mmol/l. The European seabass (*D. labrax*) larvae weaned in marine synbiotic and Estradiol treated microdiet decreased the negative impacts of the Estradiol on the larval length, weight growth, condition factors, survival rate % and immunity and liver function parameters.

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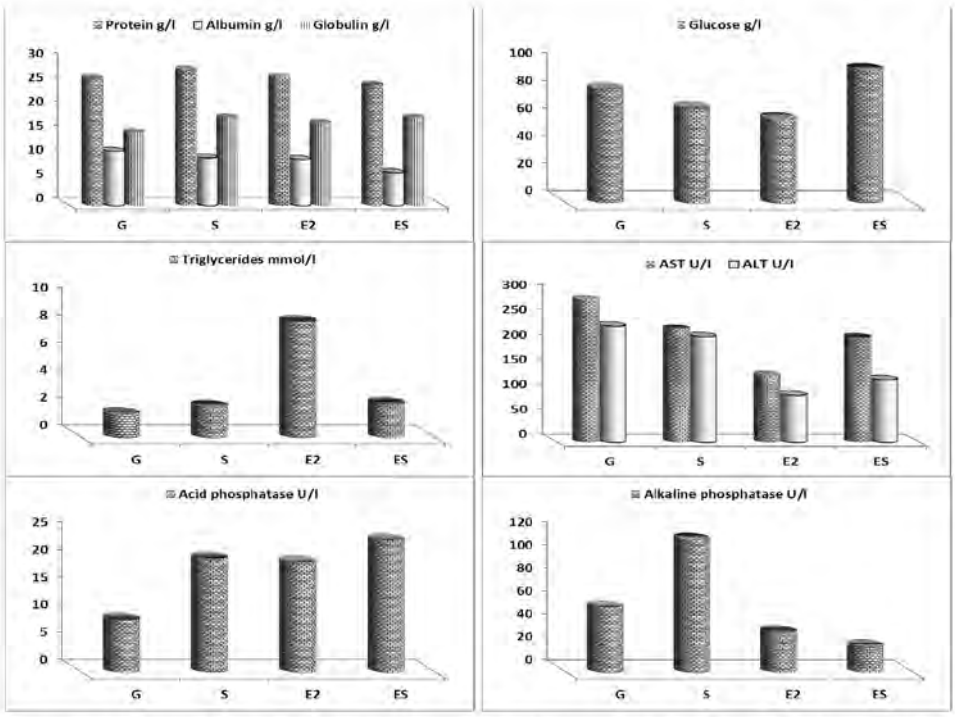

Figure 1. Effect of Estradiol (E2) sex reversing hormone and Marine Synbiotic enriched microdiets on European seabass (*D. labrax*) larval liver function enzymes.

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A DIRECT AND STRAIGHTFORWARD METHOD FOR MEASUREMENT REAL MAXIMUM FISH STOMACH VOLUME TO IMPROVE AQUACULTURE FEEDING RESEARCH

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Introduction

A common method for investigating the diet of fish is the analysis of stomach measurement (Adeyemi & Akombo, 2012; Abdul et al., 2016). Various methods have been used to measure the maximum stomach volume in fish (Kariya et al., 1968; Kimball & Helm, 1971; Jobling et al., 1977; Burley & Vigg, 1989), but the most commonly used method has been the measurement of volume under 50 cm pressure head, described by Jobling et al. (1977). The main aim of the present study is to validate a simple and direct measurement method based on Archimedes' principle, which provides real value for maximum stomach volume in fish. Likewise, Archimedes' principle is commonly used in fisheries and fishing biology since several years ago (Yañez-Arancibia, 1975). The purpose of this study results is to apply this method to aquaculture fish feeding

Materials and methods

The experiment was carried out with thirty rainbow trout Oncorhynchus mykiss (Walbaum, 1792) juveniles. Before sampling, fish were starved for four days and then fed until satiation with commercial food, once per day. Fish weight and standard length were measured. Body cavity was opened, and the stomach was removed for the measurement of stomach volume under Jobling and Archimedes' principle methods. Also, the stomach and stomach contents were weighed to determine ratio stomach volume/stomach weight.

Results

Constants, potential models and determination coefficients according to relationships and methods are presented (Fig. 1). A good positive correlation between stomach volume and fish weight was observed in both the Archimedes' principle and Jobling methods, with the R^2 values of 0.68 and 0.56, respectively (Fig. 1a). The same tendency was found in the correlation between stomach volume and stomach content, with the Archimedes' principle method being higher than the Jobling's method (R^2 values of 0.95 and 0.68, respectively; Fig. 1b). Likewise, a good correlation was obtained between stomach volume and stomach weight, where the R^2 values in the Jobling's method were slightly higher than those of the Archimedes' principle method (Fig. 1c). The behaviour of the curves created by plotting the points (X, Y) of the various relationships is as follows: with the Jobling's method, the intersection of the curve with the Y-axis (0.0499, 4.3092 and 3.7867 in Figures 1a-1c, respectively) is higher in each case than the Archimedes' principle method (0.0007, 1.4467 and 1.6138 in Figures 1a-1c, respectively). The slope values obtained with the Jobling's method (1.059, 0.6439 and 0.7575 in Figures 1a-1c, respectively) were lower than with the Archimedes' principle method (1.829, 1.827 and 1.1143 in Figures 1a-1c, respectively), in each case producing a lower curve position within the coordinate planes.

Discussion and conclusion

According to the present experiment, the Archimedes' principle method is more effective and provides greater data precision in the measurement of real maximum stomach volume in fish, based on higher R^2 (means best curve adjustment) compared to results obtained by Jobling method. The following aspects demonstrate the method's superiority: 1) it yields the maximum real value of stomach expansion in a situation where a fish wants to ingest as much as it can, 2) the measurement process is simple and quick, and 3) relationships with stomach volume observed when using the Archimedes' principle method present values that are optimally adjusted and have greater certainty than values obtained using the Jobling' method. The higher stomach volume values obtained using the Jobling's method can be attributed to the forced expansion of the stomach, owing to the constant hydraulic pressure head of a 50 cm column of water applied by liquid entering the stomach and resulting in a higher curve position corresponding higher values of stomach volume (depending variable) for each independent variables (fish weight, stomach content, stomach weight) which is not the case with the Archimedes' principle method.

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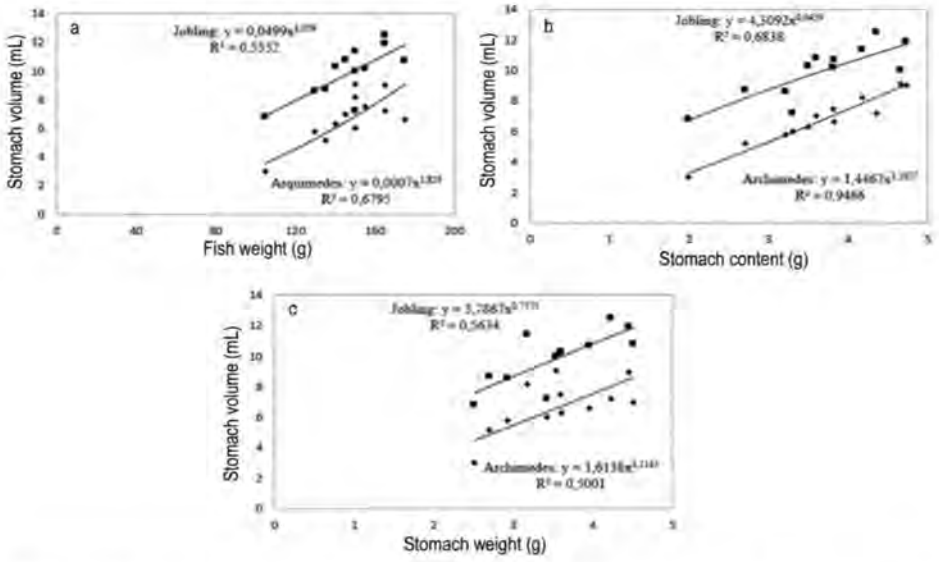

Figure 1. Relationship between stomach volume and a) fish weight, b) stomach content and c) stomach weight rainbow trout (*Oncorhynchus mykiss*) fed until satiation and following two assay methods. Each point represents an individual fish. Jobling method (▪, n = 12), and Archimedes method (♦, n = 12).

Thus, the same explanation can be applied to the ratio of volume to weight: if the same weight of the stomach divides both measured volumes, the Jobling's method will yield a higher value. According to the relationship between stomach volume and stomach content, the Archimedes' principle method presented a higher coefficient of determination than the Jobling's method; this could be because the first method considers the real maximum volume of the stomach, while the second considers the maximum forced expanded stomach volume. Because of this study, Archimedes' principle method is recommended to be applied in Aquaculture fish feeding research, due to its simplicity nature and accurately, which make more accessible the labor and time.

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DIVERGENT CORTISOL RESPONSIVENESS IN EUROPEAN SEA BASS *Dicentrarchus labrax*: INTEGRATION OF RESULTS AND FUTURE PERSPECTIVES

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Introduction

Understanding the stress responses of organisms is of importance in the performance and welfare of farmed animals, including fish. Especially fish in aquaculture commonly face stressors, and better knowledge of their responses may assist in better husbandry and selection of breeding stocks. In this respect, European sea bass (*Dicentrarchus labrax*), a species with high cortisol concentrations in blood, is of major importance.

The main objectives of the present study were to integrate results from various experiments and different populations on individual divergent cortisol responsiveness in European sea bass. Special attention was given to the repeatability and consistency of cortisol stress response, the identification of individuals showing a consistent low (LR) or high (HR) cortisol response, and the physiological mechanisms regulating these responses.

Materials and Methods

European sea bass samples from a selective breeding program were studied. The analysis used both families (full- and half-sib) and individuals as factors. Three experiments were performed using 72 fish in Experiment 1 (6 families of 12 individuals), 960 fish in Experiment 2 (96 families of 10 individuals), and 400 fish in Experiment 3 (20 families of 20 individuals).

For the identification of consistent divergent cortisol responses a standardized acute stress protocol was performed. Specificall , fish in each tank were stressed once per month for 4 (Exp. 1) and 3 (Exp. 2 & 3) consecutive months by lowering the water of the tank (confinement) and then chasing them with a net for 5 minutes. Fish were left confined for 30 minutes, and then carefully netted, immediately anaesthetized in ethylene glycol monophenyl ether (300 ppm; Merck; 807291; USA) and blood was immediately collected from the caudal vessel via heparanized syringes and centrifuged (2,000 g; 10 min). The resulting plasma was stored at -20°C until analyzed for cortisol, corticosterone and adrenocorticotropic hormone (ACTH) using commercially available ELISA assays.

For the calculation of repeatability and the characterization of LR and HR individuals the Z-score of cortisol values was used instead of the raw data. This method was employed in order to control for variations in the cortisol data between the samplings and avoid the "noise" of such variation in the analysis to obtain repeatability estimates.

Table 1. Cortisol repeatability in the three experiments.

	Exp. 1	Exp. 2	Exp. 3
Repeatability	0.389	0.293	0.441

LR and HR fish were identified in all experiments. These fish were shown to differ in their free cortisol concentration, as well as in unstressed cortisol levels either before any stress sampling or one month after the last stress sampling. Moreover, these differences were stable 1.5 years after their characterization. These fish showed no difference in their post-stress and unstressed levels of ACTH. However, there were differences in the circulating levels of cortisone, and especially the ratio between cortisone to cortisol.

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The repeatability of the post-stress cortisol levels was assessed using the repeatability statistic, *r*. The consistency of the response within an individual across all samplings was tested by a nested ANOVA, with the factor Individual being nested in the factor Family. The null hypothesis was that individual Z-scores were inconsistent within individuals and hence the variable was not repeatable.

For the identific tion of LR and HR individuals, the sum of the Z-scores of all samplings was calculated for each individual fish and ranked. Fish belonging to the upper quartile of the distribution of Z-scores were identified as HR, and those belonging to the lower quartile as LR fish

Head kidney tissues of selected LR and HR individuals were superfused through an *in- vitro* superfusion system and stimulated with the same amount of ACTH to assess their cortisol biosynthetic capacity. Moreover, the expression of important genes in cortisol regulation was assessed.

Results

In all experiments a moderate to high repeatability coefficient was estimated, showing that cortisol responsiveness is an individually consistent and repeatable trait (Table 1).

LR and HR fish were identified in all experiments. These fish were shown to differ in their free cortisol concentration, as well as in unstressed cortisol levels either before any stress sampling or one month after the last stress sampling. Moreover, these differences were stable 1.5 years after their characterization. These fish showed no difference in their post-stress and unstressed levels of ACTH. However, there were differences in the circulating levels of cortisone, and especially the ratio between cortisone to cortisol.

Analysis of head kidney capacity to produce cortisol using the superfusion technique showed that HR fish were capable to produce more cortisol and for longer time when simulated with the same amount of ACTH. Furthermore, significant differences in the expression of genes involved in the signaling of ACTH in the head kidney (*mc-2r*) and the catabolism of cortisol (*hsd11b2*) were observed between these phenotypes.

Discussion

All the above underline the genetic component of the trait, which has been estimated at 0.34-0.36 (Chatziplis et al., submitted; Vandeputte et al., 2016), while quantitative trait loci (QTL) linked to this trait have been found (Chatziplis et al., submitted; Massault et al., 2009). Therefore, cortisol responsiveness could become a suitable candidate for use in selective breeding programs as a new selection criterion. Research regarding the genetic basis of this trait using SNP based GWAS is in progress aiming additionally at providing an in-depth study of the relation between cortisol responsiveness and resistance in three common diseases that infect European sea bass in aquaculture.

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MOLECULAR IDENTIFICATION, BIOCHEMICAL CHARACTERIZATION AND ITS INNATE IMMUNE RESPONSES OF PEROXIREDOXIN 4 (HaPrx4) IN BIG BELLY SEAHORSE (*Hippocampus abdominalis*)

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Introduction

Peroxiredoxins (Prxs) belong to the family of cysteine dependent peroxidases which is able to detoxify hydrogen peroxide, peroxynitrite and organic hydroperoxides (Abbas et al., 2005)cytokines, integrins and many other gene families playing key roles in the immune response are highly represented. IRIS also includes proteins of unknown function and expressed sequence tags that may not represent genes. The predicted cellular localization of IRIS proteins is evenly distributed between cell surface and intracellular compartments, indicating that immune specificity is important at many points in the signaling pathways of the immune response. IRIS provides a resource for further investigation into the function of the immune system and immune diseases.","author":[{"dropping-particle":"","family":"Abbas","given":"A R","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Baldwin","given":"D","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Ma","given":"Y","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Ouyang","given":"W","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Gurney","given":"A","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Martin","given":"F","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Fong","given":"S","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Lookeren Campagne","given":"M","non-dropping-particle":"van","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Godowski","given":"P","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Williams","given":"P M","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Chan","given":"A C","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Clark","given":"H F","non-dropping-particle":"","parse-names":false,"suffix":""}],"container-title":"Genes and immunity","id":"ITEM-1","issue":"4","issued":{"date-parts":[["2005","6"]]},"language":"eng","page":"319-331","publisher-place":"England","title":"Immune response in silico (IRIS. Moreover, Prxs involve in cellular homeostasis, apoptosis, immune response, signaling pathways and cell growth. Peroxiredoxin 4 (Prx4) classified under the typical 2-Cys Prx class, based on their catalytic mechanism and position of cysteine (Cys) residues (Wood et al., 2003). Prx4 considered as effective H2O2 scavenger for Endoplasmic reticulum generated H2O2 (Tavender and Bulleid, 2010) and as a consequence, generates hydrogen peroxide. The production of hydrogen peroxide is thought to lead to oxidative stress that ultimately results in apoptosis. Here, we show that mammalian peroxiredoxin IV (PrxIV. Here we identified the HaPrx4 gene from the big belly seahorse transcriptome library, characterize biochemical properties and determine its innate immune responses following challenge experiment.

Materials and methods

Big belly seahorses were acclimatized at laboratory conditions (18 ± 2°C temperature and 34 ± 0.6‰ salinity) and spatial distribution of *HaPrx4* was analyzed in six healthy seahorse using qPCR. The immune challenge experiment was done for the four immune stimulants such as lipopolysaccharide (LPS), polyinosinic:polycytidylic acid, *Edwardsiella tarda*, and *Streptococcus iniae*. The mRNA expression level of *HaPrx4* in liver was explored by qPCR. The recombinant protein (rHaPrx4) was expressed and purified using the pMAL Protein Fusion and Purification System and insulin disulfide reduction assay was performed to determine the antioxidant activity.

Results

The open reading frame of *HaPrx4* is 777 bp which encodes for 258 amino-acids long protein with 28.81 kDa molecular weight. The theoretical pI is 6.36. Pairwise sequence alignment showed the highest similarity (93.5%) and identity (88.1%) with the *Miichthys miiuy* (Chinese drum) and multiple sequence alignment contained conserved 2-cys Prx motif 1(FYPLDFTFVCPTEI) and motif 2 (GEVCPA), including peroxidatic and resolving cysteines. Highest expression for the *HaPrx4* was observed in ovaries among fourteen tissues. The mRNA level of *HaPrx4* was significantly upregulated after the immune challenge experiment in the seahorse liver tissues. Insulin reduction assay showed the precipitation of insulin increased with the incubation time when increasing the rHaPrx4 concentration.

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Discussion and conclusion

Prx are thiol specific antioxidants which plays crucial role in homeostasis. The Prx motif 1 and motif 2 were highly conserved among other organisms Prx sequences, suggesting that catalytic function of HaPrx4 is conserved among different species. Also, phylogenetic analysis revealed HaPrx4 was evolutionary related to the teleost. The highest mRNA expression of *HaPrx4* showed in ovaries as a result of high ROS level in the ovaries when producing high energy for the reproduction activities (Faron et al., 2015). The prominent transcript level elevation exhibited in liver tissue to mediate the redox status during pathogenic infection. The results of insulin reduction assay showed the oxidoreductase activity of HaPrx4. Altogether, we characterized the molecular and functional levels of *HaPrx4* of big belly seahorse. Transcription levels of *Haprx4* were studied in wide range of tissues and prominently in ovaries. Moreover, immune challenge experiment revealed that HaPrx4 plays an important role in the innate immunity of seahorse against bacterial and viral pathogenic attack.

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BREWERS' SPENT YEAST AND GRAIN AS ALTERNATIVE INGREDIENTS FOR AQUACULTURE FEED

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Introduction

Aqua-feeds are formulated to contain all the essential nutrients that farmed fishes need to keep healthy and to maintain the benefits of seafood consumption. Currently, they are highly dependent on fish meal (FM) and fish oils (FO). Approximately, the 65 % and 83 % of FM and FO goes to aquaculture production, Tacon and Metian (2008). Samuel-Fitwi et al. (2013) have demonstrated that replacing FM by alternative ingredients, such as soybean or rapeseed, involves less environmental impact. The FM standard trout feed has an impact of 1,797 kg CO_2 equivalent per ton while the soybean and rapeseed meal based aqua-feeds has 1,019.65 and 1,037.13 kg CO_2, respectively.

Within this framework, alternative ingredients which successfully replace these marine components are required to result in sustainable and economical feeds. However, the suitability of these new ingredients will depend on improving feed efficiencies

Brewing industry produces large quantities of by-products in processing raw materials to beer: about 6 and 1 million tons of brewers´ spent grain (BSG) and yeast (BY). BY is often mixed with wastewater, while 70 % of BSG is used in fresh for feed, 10 % for biogas and 20 % landfilled, which implies the loss of a valuable product. However, the use of BSG as a direct supply for feed without any treatment can limit their feasibility. BSG and BY are characterized by their high protein content: about 20 % in BSG and 40 % in BY Therefore, brewers´ by-products stand as a potential alternative for replacing fish meal in aquaculture feed, due to their availability in Europe and their nutritional characteristics

Material and methods

The Brewery project aims to demonstrate the feasibility of the utilisation of Brewers' Yeast and Spent Grains in Senegalese sole, Sea bream and Trout feeding in the north-east region of Spain by carrying out five actions

1. Pre-Industrial optimization of processes for obtaining brewers´ by-products-based meal & aquafeed prototypes: An optimized drying process at semi-industrial scale will be developed obtaining 2 meal prototypes. Experimental aqua-feed diets including developed 2 meals will be formulated till optimum level of inclusion.

2. Design of a valorisation scheme for brewers' by-products including all stages of the value chain: The valorisation scheme will address the technical and administrative actions for each stage. It will be contrasted with Stakeholders.

3. Demonstration trial of the valorisation scheme applied to the case study: north-east region of Spain. A demonstration trial at a semi-industrial scale and in real operational conditions will be carried out. By-products will be taken from the most important breweries of Spain. They will be adapted and stabilized by an optimized drying process. Fish growth trial with developed aqua-feed will be carried out. Finally, the sensory quality of produced fish will be assessed

4. Feasibility assessment of the implementation of the valorisation scheme in an industrial reference scenario: The valorisation scheme will be assessed from the technical, economic, social and environmental points of view, as well as the eco-design of the valorisation plant at full scale.

5. Replicability and transferability of the valorisation scheme at European Level: The valorisation scheme will be replicated and transferred to EU. A comprehensive analysis of the replicability to 2 European areas will be carried out. A replicability and transferability plan will be performed in each of them.

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Results

The production of alternative ingredients for aquaculture feed based on brewers´ by-products has been validated from the technical, environmental and economic point of view. Four ingredient prototypes have been obtained through the combination of hydrolysis and drying process. The enzymatic hydrolysis as pre-processing prior to dehydration has been studied with the aim of assessing the increasing of the digestibility of ingredients. Then, an innovative and efficient drying process has been validated to stabilize by-products over time and to ensure their applicability in aqua feeds. Drying process consists of two steps: a low energy demanding dewatering process and, once the moisture has been reduced till 55 %, a highly efficient thermal drying process to achieve a moisture content of less than 10 %

The obtained four ingredient prototypes have been formulated and tested in digestibility tests with two fish species in RAS aquaculture systems: Sea bream (*Sparus aurata*), as a model of a Mediterranean aquaculture specie, and Rainbow trout (*Oncorhynchus mykiss*), as a model of a freshwater specie. Obtained results have shown acceptable digestibility results between 53,97 % to 93, 81 %. Hydrolysed prototypes have shown higher digestibility than non-hydrolysed.

Discussion and conclusion

Brewers´ by-products stand as a potential alternative for replacing FM in aquaculture feed, due to their availability in Europe, their nutritional characteristics and the obtained results in the digestibility trials with fishes.

All this involves an increase of the sustainability of aquaculture by providing new sustainable and economically advantageous protein sources that could replace fish meal. Their availability will contribute to reduce the environmental impact related to fish meal based aqua-feed. In addition, the reduction of aquaculture production costs will contribute to achieve the objectives established by the new European Common Fisheries Policy and the replacement of marine origin ingredients (fishmeal) will contribute to reduce significantly wild catches, contributing to achieve the goals defined in the Marine Strategy Framework Directive

Acknowledge

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EFFECTS OF NEUROACTIVE COMPOUNDS ON THE METAMORPHOSIS OF THE GROOVED CARPET SHELL CLAM LARVAE (*Ruditapes decussatus*, L.)

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Introduction

Production of bivalve molluscs spat in the hatchery encounters difficulties mainly associated with the conditioning of broodstock, and the settlement and metamorphosis of larvae. To settle and metamorphose larvae must be competent to respond to morphogenetic cues (Fitt et al. 1990; Morse, 1992). However little is known about the molecular mechanisms that take the larvae to metamorphose except for various chemical inducers that have been identified in different molluscan larvae. The γ-aminobutyric acid (GABA) has been shown to be an effective inducer in *Haliotis* sp., several clam species, oysters and mussels (Morse, 1992; García-Lavandeira et al. 2005;, Alfaro et al. 2011; Mesías-Gansbiller et al. 2008, 2013). IBMX was active inducing metamorphosis in gastropods but had not effect on the black scallop *Chamys varia* (Degnan and Morse 1995; Mesías-Gansbiller et al. 2008). The catecholamine epinephrine was effective in mussels and *Crassostrea* sp. (Coon et al. 1985; García-Lavandeira et al. 2005; Alfaro et al. 2011). L-DOPA potentiated metamorphosis in oysters and the mussel *Mytilus edulis* (Cooper 1982; Coon et al. 1985, 1986; Dobretsov and Qian, 2003; Mesías-Gansbiller et al. 2013). However, no inducers have been identified in the carpet shell clam *Ruditapes decussatus* (Linnaeus, 1758) so far. The aim of this work is the identification of inducers of the metamorphosis of *R. decussatus* larvae.

Materials and methods

One experiment of induction of the metamorphosis has been developed following the specifications described in García-Lavandeira et al. (2005) and Mesías-Gansbiller et al. (2013). Competent larvae of the clam *Ruditapes decussatus*, were supplied by the CIMA of Ribadeo and subsequently treated with several neuroactive compounds: GABA, epinephrine and norepinephrine, L-DOPA, serotonin, acetylcholine and IBMX. Three different concentrations of the possible inductor were used: 10^{-4}M, 10^{-5}M and 10^{-6}M and the exposure time to the inducers was 48 hours. Experiments were performed in triplicate in Petri dishes of 90 mm, for each one of the inducer concentrations and at a density of 4 larvae/ml. Each experiment included a control without potential inductor. Metamorphosis has been monitored with a Nikon SMZ-2T microscope. The percentage of metamorphosis was calculated as 100 (total number of larvae metamorphosed/total number larvae). A larva undergoes metamorphosis when has lost its velum and was using its foot to crawl.

Results were analysed by the SPSS 20.0 program. Percentages of metamorphosis and mortality were analysed by ANOVA. The results were considered to be significantly di ferent when $p<0.05$.

Results and discussion

GABA, epinephrine, norepinephrine, L-DOPA and IBMX did not increase the percentages of metamorphosis in *R. decussatus* larvae after a 48-hour exposure comparing to the control larvae. Maximum percentages of settlement were induced by acetylcholine and serotonin. Exposure to 10^{-4}M and 10^{-5}M acetylcholine induced significant levels of metamorphosis in *R. decussatus* with percentages of 76% and 75%, respectively, comparing to the control larvae ($p<0.05$). Exposure to 10^{-4}M and 10^{-5} M serotonin induced similar levels of metamorphosis, with percentages of 74% and 75% ($p<0.05$), respectively. The presence of acetylcholine, serotonin and other inducers did not affect the mortality of *R. decussatus* larvae comparing to the control larvae. However, it is important to point out that larval mortality ranged between 30-40%.

To our knowledge there are not previous studies of the induction of metamorphosis in *R. decussatus*. However, several authors have reported the role of GABA, epinephrine, serotonin and acetylcholine as active inducers of metamorphosis in the clam species clams *V. pullastra* and *R. philippinarum* (Urrutia et al. 2004; García-Lavandeira et al. 2005).

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THE INVOLVEMENT OF C- LECTIN A AND F- LECTIN IN THE IMMUNE SYSTEM OF REDLIP MULLET (*Liza haematochelia*); GENOMIC, MOLECULAR AND TRANSCRIPTIONAL FEATURES UPON IMMUNE STIMULANTS

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Introduction

Lectins are a class of proteins with special carbohydrate recognition domains. These proteins are able to identify carbohydrates and their conjugates by non-reversible covalent binding, forming cell aggregations (Yang et al., 2016).On the basis of their primary structure of their carbohydrate recognition domains (CRDs), structural folding, and cation requirements, animal lectins can be classified into several families, including C-, F-, P-, and I-type lectins, galectin, pentraxin, and others (Chen et al., 2011). C-type lectins are carbohydrate-binding proteins that depend on Ca^{2+} participation to recognize carbohydrates and glycoproteins and contain one or more carbohydrate-recognition domains. F-type lectin contain unique fucose- and calcium-binding sequence motifs and have been proposed to mediate molecular recognition in innate immunity (Anju et al., 2013)superoxide dismutase (SOD.

Materials and methods

Red lip mullets with an average body weight of 100 g were selected and acclimatized to the laboratory conditions. Five healthy fish were selected and blood and 11 other types of tissues were collected including the head kidney, spleen, liver, muscle, gills, intestine, kidneys, brain, skin, heart, and stomach by dissection. Another set of mullets was divided into four groups with 100 g average body weight and was subjected to an immune challenge experiment using Lipopolysaccharide (LPS), *Escherichia coli*, poly I:C and *Lactococcus garvieae*. After the challenge, tissues from the spleen & head kidney were collected from five individuals at 0, 6, 24, 48, or 72 h post injection (p.i.). After that the isolated tissues were subjected to RNA extraction and CDNA synthesis process. Finally, their molecular and transcriptional features were analyzed using bio informatics analysis and qRT-PCR.

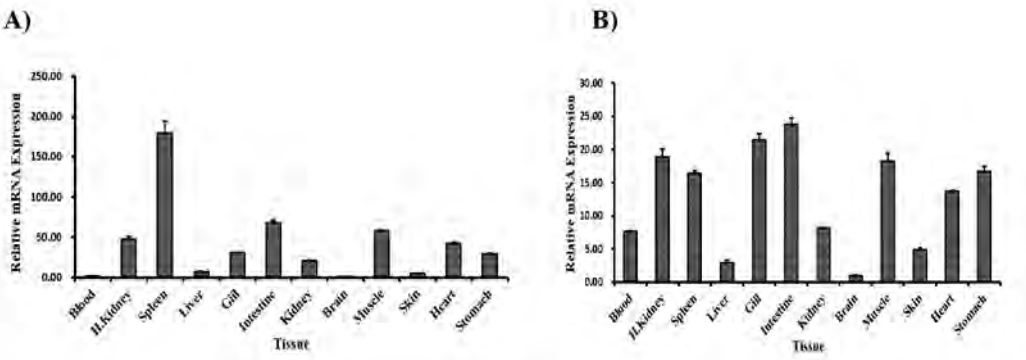

Fig. 1 A) & B) Tissue-specific transcriptional profiles of C-lectin A and F-type lectin in red lip mullets. The calculations were performed by the Livak method. Data are presented as mean ± standard deviation (n = 3).

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Results

C-lectin A consists with 462 amino acids with 52.76 kDa of predicted molecular mass. The theoretical pI calculated as 7.5. F-type lectin consists with 310 amino acids and 34.3 kDa of predicted molecular mass with 5.32 theoretical pI. In analysis of tissue-specific expression, the highest expression of C-lectin A was observed in the spleen, whereas F-type lectin was highly expressed in the intestine. Of note, we found that the transcription of C-lectin A and F-type lectin were significantl upregulated when the fish were stimulated with Lipopolysaccharide (LPS), *Escherichia coli*, poly I:C and *Lactococcus garvieae* suggesting that such immune responses.

Discussion and conclusion

This study provides an experimental insight into the molecular and transcriptional characteristics of C-lectin A and F-type lectin genes in the red lip mullet. Transcriptional analysis of C-lectin A and F-type lectin revealed their different distribution patterns in different tissues of red lip mullets. The mRNA expression levels of C-lectin A and F-type lectin were determined at different time points after the fish were challenged with bacterial or viral components. The significant changes in their expression allow us to propose possible functions of C-lectin A and F-type lectin in the innate immune system of red lip mullets. Overall, this study provides the experimental insight into the characteristics and immune-system relevance of C-lectin A and F-type lectin genes in red lip mullets.

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STUNNING OF ARCTIC CHAR (*Salvelinus alpinus*) WITH CARBON DIOXIDE AND ELECTRIC FIELD EXPOSURE: PHYSIOLOGICAL MECHANISMS OF ACTION AND WELFARE IMPLICATIONS

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Introduction

Carbon dioxide (CO_2) is still widely used to stun fish prior to slaughter in aquaculture, but the method has been criticized for resulting in poor welfare. Alternative methods such as electrical stunning, or means of alleviating the negative effects of CO_2, have therefore also been developed. Yet, knowledge gaps exist with regards to physiological stress responses and details on the mechanism of action of the various methods. This constrains further development of ethically acceptable stunning techniques for fish.

Materials and methods

In a series of lab studies on Arctic char (*Salvelinus alpinus*), an important cold-water aquaculture species in Sweden, cardioventilatory responses, blood physiological indicators of stress and behaviours were recorded during electrical field exposure (Sandblom et al. 2012), as well as during stunning with CO_2 exposure alone and in combination with excessive supply of oxygen (hyperoxia; Sandblom et al. 2013) or chilling (hypothermia; Seth et al. 2013), which have been suggested to alleviate the adverse effects of CO_2. The lab-based studies were further complemented with an on-site study where behaviours and blood samples for cortisol analysis were assessed in Arctic char prior to slaughter in a commercial facility using both CO_2 and electrical stunning in parallel (Gräns et al. 2016).

Results

Ten minute exposure to CO_2-saturated water at 10°C triggered aversive escape responses. These were accompanied by gradually reduced heart and ventilation rates, reduced blood pressure and moderately increased plasma cortisol levels before equilibrium was irrecoverably lost after ~3 minutes. Chilling to 0.25°C did not markedly affect behavior, cardioventilatory responses or the time until loss of equilibrium during CO_2 exposure, but the increase in plasma cortisol was significantly exacerbated. Ten minute exposure to 10°C water saturated with combinations of CO_2 and pure oxygen resulted in similar behavioral responses and plasma cortisol increase compared with exposure to pure CO_2. However, the hyperoxic CO_2 exposure resulted in a more variable cardioventilatory response, and fish recovered quickly when returned to water with normal oxygen levels (normoxia), which fish exposed to pure C_2 did not.

Electrical stunning (4V/cm, 125 Hz) for 5 and 30 s rendered the fish immediately motionless and was associated with a brief (~5 s) four-fold arterial blood pressure spike. Cardioventilatory arrest followed the 5 s exposure, but in ten out of eleven fish the cardiac activity recovered within 18-97s and ventilatory activity recovered within 38-733s after stunning. Nevertheless, signs of a systemic stress response were evident after the electrical exposure, which included elevated blood pressure, ventilation amplitude and plasma cortisol. After the 30 s exposure, cardiac activity initially recovered, but subsequently declined as ventilation never recovered.

In the on-site study, CO_2 exposure triggered aversive struggling and escape responses for 5-10 min before the fish became motionless. Fish exposed to an electric current became instantly motionless. On average, fish recovered from the electrical stunning within 5 min, whereas fish stunned with CO_2 did not recover. Electrically stunned fish had more than twice as high levels of plasma cortisol compared to fish stunned with CO_2. This result was surprising considering that the behavioural reactions were much more pronounced with CO_2 exposure.

Discussion and conclusion

Collectively, these data show that neither hypothermia nor additional oxygen reduce behavioral or physiological stress

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responses during CO_2 exposure in Arctic char. Moreover, additional oxygen results in quicker recovery if the fish is transferred to normoxic water following CO_2 stunning. While electrical field exposure rendered the fish instantly motionless, it appears that circulatory failure due to cardiac ischemia resulting from ventilatory failure is a principal cause of subsequent death. Thus, the relationship and timing of ventilatory failure and loss of consciousness following electrical stunning needs further study. For example, studies combining measurements of cardiac and brain electrical activities (*i.e.,* electrocardiogram and electroencephalogram, respectively) could be instrumental to evaluate consciousness and the welfare implications of this important finding. Moreover, the dramatic arterial blood pressure increase observed in char may partly explain hemorrhages and ruptured blood vessels that are frequently observed in electrically stunned fish of some species.

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UTILIZATION OF AFRICAN CATFISH (*Clarias gariepinus*) SEDIMENTS FOR THE CULTIVATION OF THE POLYCHAETE *Hediste diversicolor* (O.F. MÜLLER, 1776) UNDER DIFFERENT TEMPERATURE REGIMES

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Introduction

Aquaculture is still growing. In order to keep this process going there is the need to find additional feed ingredients to increase the amount of available feed for aquaculture. Incorporating plant material in feeds for carnivorous fish imposes certain problems, such as anti-nutritional components, which are limiting the widespread and complete substitution of fishmeal and fish oil by plant materials. In general, aquaculture organisms only convert a fraction of the supplied feed into harvestable biomass. Simultaneously, dissolved and particulate nutrients are excreted into the waters. Applying the Integrated Multitrophic Aquaculture concept and thereby converting the excessive excreted nutrients with organisms of low trophic levels, such as polychaetes, is an opportunity to produce the required valuable biomass for the additionally required feed.

This study was performed to evaluate the potential of particulate matter originating from the culture of African catfish (*Clarias gariepinus*) to cultivate the polychaete *Hediste diversicolor* as a secondary organism. Furthermore, we investigated the effect of temperature on growth and fatty acid composition of *H. diversicolor*.

Materials and Methods

Three separated culture systems were applied. These systems were subdivided into three replicate tanks and a reservoir, which combined the aerobic biofilter and the pump sump for the re-distribution of the culture water. Each reservoir was equipped with an air pump and a submerged water pump and for each tank the same water exchange rate was adjusted.

Two of the systems were additionally equipped with cooling units, to set up the three systems with different culture temperatures (12°C, 16°C and 20°C).

Hediste diversicolor were stocked in a 10 cm sediment layer (average grain size 0.5 – 2.0mm), fed with particulate matter from a Recirculated Aquaculture System (RAS) used for the commercial production of African catfish. The intended daily energy content per individual worm was set to 1 kJ. The stocking density of the worms was approximately 830 individuals per m², which resulted in about 200 individual worms stocked in an average space of 0.24m² per tank.

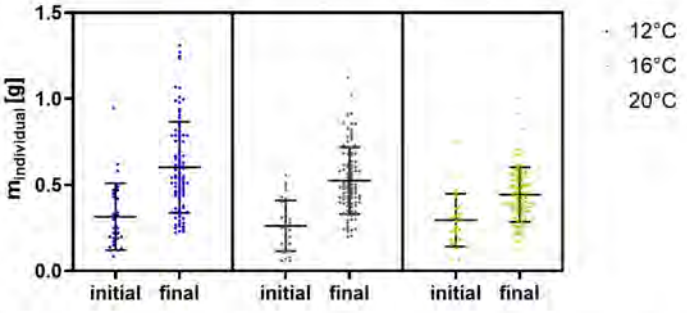

Fig. 1: Individual worm biomass recorded for the three different temperature treatments (left: 12°C; middle: 16°C; right: 20°C).

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The feeding experiment lasted for 28 days and during the feeding experiment temperature, salinity, oxygen saturation and oxygen content as well as pH were daily measured by a handheld measuring device. Furthermore, the dissolved nutrient concentrations (ammonium, nitrite, nitrate and orthophosphate) were analyzed to assure the required water quality for the worms. After stocking the worms to the tanks and an acclimatization of about 14 days, sub-samples of worms were collected from each tank and the average bodyweight at the start of the feeding experiment was calculated. This procedure was repeated at the end of the feeding experiment and the weight difference was used for calculating the Specific Growth Rate (SGR) of the differently treated worms. Further sub-samples were analyzed for their fatty acid composition.

Results

Water parameters were in the expected range and appropriate for the successful cultivation of *H. diversicolor*. Salinity, oxygen saturation and oxygen content as well as pH showed no differences, but the temperature differed significantly ($p<0.001$), resulting at 12.2 ± 0.2°C for system I, 15.8 ± 0.8°C for system II and 19.9 ± 0.8°C for system III. The dissolved concentrations of the analyzed nutrients were below the levels recommended for the culture of *H. diversicolor* (Bischoff 2007).

All groups showed a positive biomass gain but growth varied with the different temperatures (Fig. 1). The SGR decreased with increasing temperatures and was 2.47 ± 0.22%*d^{-1} for system I, 2.37 ± 0.24%*d^{-1} for system II and 1.45 ± 0.16%*d^{-1} for system III.

The analyzes of the fatty acid composition of the worms revealed significant spearman correlation between temperature and changes in fatty acid proportion for Oleic Acid (18:1cis-Δ9; ρ=-0.839), Linoleic acid (18:2cis,cis-Δ9,Δ12; ρ=-0.373) Arachidic acid (20:0; ρ=-0.588) Myristic Acid 14:0 (ρ=0.302), Palmitic Acid (16:0; ρ=0.291).

Discussion and Conclusions

Applying the particulate matter originating from the culture of African catfish under freshwater conditions to the polychaete *Hediste diversicolor* under brackish or seawater conditions is possible and leads to positive growth. These growth rates were comparable to literature under similar conditions (Bischoff 2007, Nesto et al. 2012, Wang et al. 2018) but lower compared to Santos et al. (2016). Nevertheless, the worms by Santos et al. (2018) where fed either commercial fish feed or non-proceeded makerell's filets, which were higher in protein, fat and ene gy content.

The correlation analyses revealed that the fatty acid proportions of *Hediste diversicolor* only correlated between the changes of ω−6 fatty acids and the temperature.

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MOLECULAR CHARACTERIZATION OF THE NKIRAS PROTEIN FAMILY IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

RAS proteins are a family of GTP-binding proteins, which are involved in regulating the immune system, majorly NF-kappa-B-dependent pathways. The first evidence of that was found by Julian Downward who detected an RAS-dependent activation of T-lymphocytes (Wuebbles et al., 1990).

Mutation of these RAS proteins have disastrous consequences for the respective signal transduction pathways, including a misdirected signal transfer or cancer (GOODSELL, 1999). Therefore, RAS proteins were first identified as oncogene

KB-RAS is a small but potent RAS protein subfamily, whose members act as inhibitors of NF-kappa-B is (Chen, Wu, & Ghosh, 2003). KB-RAS proteins are encoded by the NKIRAS genes. The full length sequences of both KB-RAS orthologs (NKIRAS1 and NKIRAS2) were identified in rainbow trout, showing an incredible level of sequence conservation among different phyla. To date, little is known about the NKIRAS gene family in rainbow trout *Oncorhynchus mykiss*.

Here, we characterized the gene and protein structures of the NKIRAS gene family in *O. mykiss* and functional aspects of the members NKIRAS1 and -2. We also suggest a novel function for the KB-RAS2 isoform X4, which is absent in mammals.

Material and methods

Healthy rainbow trout (*Oncorhynchus mykiss*) weighing 80–100 g was purchased from a local rainbow trout breeding farm (LFA-MV Born [Germany]) for gene isolation.

RNA was isolated from the gills, head kidney, liver, and spleen of rainbow trout using TRIzol (Invitrogen/Life Technologies) and subsequently purified with the RNeasy Mini Kit (Qiagen). Total RNA was reverse-transcribed with SuperScript II Reverse Transcriptase (Life Technologies) after priming with oligo-d(T) and random hexonucleotides. We amplified and subcloned the open read frame (ORF) and inserted it in frame to v280- Mammalian Expression Vector previously double-digested with the same restriction enzymes. The final clones were utilized for functional analyses.

CHSE-214 cells were transfected, respectively, with vectors expressing pPLUM tagged with NKIRAS1 and GFP tagged with NKIRAS2 to monitor transfection efficacy and localize the proteins in the cell

CHSE-214 cells were co-transfected with increasing quantities (100-1000ng) of the plasmids expressing either NKIRAS1 or -2 and 50 ng of the ELAM- driven NF-kB reporter vector (ELAM-1-luc (Schindler & Baichwal, 1994)). Cells were stimulated either with $3x10^5$ CFU/ml heat-killed *Pseudomonas aeruginosa* or with 100 ng/ml, FSL-ST (Invivogen) for 24 h.

Luciferase activity of cell lysates was measured with the Dual-Luciferase Reporter Assay System (Promega).

Results

Preliminary results show that NKIRAS1 protein is acting as inhibitor of activated NF-kappa-B, but not NKIRAS2.

In contrast to mammals, NKIRAS2 seems to enhance the expression of immune-related genes. Fig. 1

Confocal microscopy confirmed the same sub-localization of the protein as seen in mammals previously. Both NKIRAS1 and NKIRAS2 are expressed in nuclei and cytoplasm (Fig. 2).

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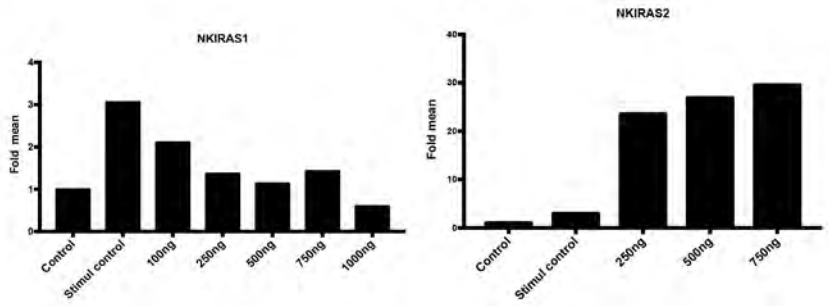

Fig.1: CHSE-214 cells were transfected with increasing quantity of plasmid containing, respectively, NKIRAS1 and NKIRAS2. Cells are stimulated for 24h with Flagellin or with heat killed *Pseudomonas aeruginosa*.

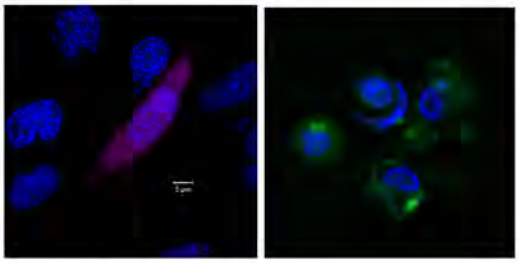

Fig. 2: CHSE-cells were transfected with 1000ng of vector expressing pmPLUM (NKIRAS1) and GFP (NKIRAS2). Cells nuclei were stained with Hoechst 33343dye and fixed with 4 Roti-Histofix 4%.

Discussion and Conclusions

NKIRAS1 and NKIRAS2 are described in literature as two inhibitors of NF-kappa-B (Fenwick, 2000). However, our results suggest that NKIRAS2 might have an additional function in rainbow trout. We observed that NKIRAS2 acts in a different direction than in a mammalian system. Our alignment of the amino acid sequence of NKIRAS2 isoforms highlighted a different N-terminus that could be responsible of the immune enhancing effect on trout. NKIRAS1 conserved its inhibiting function, as we found that it might silence the NF-kappa-B-dependent immune response with increasing quantity. The conserved structure of NKIRAS1 and NKIRAS2 suggests a well-preserved function during the evolution. Due to the whole-genome duplication in teleost fishes (Glasauer & Neuhauss, 2014), the presence of several and structurally different isoforms of NKIRAS2 must be taken in consideration for further studies.

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POLYCHAETE WORMS - SUPERIOR LIVE FOOD FOR FISH AND CRUSTACEANS

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Introduction

Feed industry contributes vital role in aquaculture. In that, fish meal is a major ingredient used to develop the fish and shrimp feed. Protein content of the feed has been decided a quality and price of the feed. Generally the fish unable to synthesizes fatty acids directly like linolenic, linoleic and arachidonic acid and whilst, decrease the production of fish meal we can move to the unconventional feed ingredients to replace fish meal. A disease free healthy stock can be achieved by feeding appropriate live feeds. Artificial feeds are not match to live feed organisms in terms of acceptance and nutritional factors. Live feeds have rich protein and essential fatty acid content (unsaturated fatty acids, HUFA) for better growth, efficient breeding, survival, sexual maturation and reproductive performance of cultivable organisms. Apart, live feed has organic acids, carbohydrates, vitamins, and minerals and hence they are commonly known as “living capsules of nutrition”. Polychaete worms have superior qualities than other live foods, so we can use polychaete worms as a live food for aquatic organisms.

Importance of Polychaetes in Aqua Feed industry:

Polychaete worms live in every type of habitat in the sea they can be found in the sands of any beach, all the way down to the deepest depths of the oceans. However, a few species live in freshwater and also abundant in seagrass beds and mangrove areas. Polychaetes worms could decay most of the organic matter present in the sediment or mud. Majority of them are filter feeders. Polychaetes are recently used as an ingredient in formulating feeds for fish, crustaceans and other organisms. It is used as feed in aquaculture either live, in blast frozen form, or as a constituent of formulated feeds. They are used as a maturation diet for shrimp broodstock (Olive, 1999). In commercial shrimp hatcheries, broodstock fed with live polychaetes, which promotes higher fecundity due to the high polyunsaturated fatty acids, especially arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid in the polychaetes. Polychaetes used for improvement of diets in hatcheries and larval rearing units for both finfis and Crustacean. Whilst, polychaete worms are rich in carotenoids so incorporation of polychaetes in the diet of ornamental fishes may increase the colour pattern

The use of polychaete worms could ensures adequate nutrition for brood stock of crustaceans & fishes. They are rich in essential amino acids like Cystine, Proline, Leucine, Lysine, Aspartic acid, Threonine, Glutamic acid, Asparagine, Phenyl alanine, Histidine, Valine, Isoleucine, Glycine, Serine, Tryptophan, Alannine, Arginine, Tyrosine and Essential Fatty acids like Alphalinolenic acid, Linolenic acid, Oleic acid, Stearic acid, Palmitic acid, Moroctic acid.

Polychaete worms have a wide applications in aquafeed industry, live bait industry, toxicity testing and prevent coral bleeching because of its nutrient profile. Development of easy and economically viable culture techniques recommended to fulfil the polychaetes demand

Save polychaetes!!!
Save ecosystem!!!

GENERATION OF THEMATIC MAP OF MACROPHYTE AND INTERPRETING ITS EFFECT ON ICHTYOFAUNA IN BHIMTAL AND NAUKUCHIATAL LAKE

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Naukuchiatal is the deepest lake with an area of around 44.5 ha in Nainital, Uttarakhand. It houses a unique biodiversity. Along with Sattal and Bhimtal, this lake is being used traditionally as primary source of drinking and irrigation water. The thematic map was created showing the quality wise distribution of macrophyte in both the Lakes. Icthyofauna regarding species distribution in the lake was collected. Macrophyte analysis was done on site and the result were interpolated to see the effect of macrophyte on icthyofauna and result obtained were Naukuchiatal Lake has obtained more transparency then Bhimtal Lake which shows that there is a low concentration of suspended sediments in the Lake indicating that there is medium to dense population of macrophyte this will help to increase prey-predator interaction. Major species avalaible in both the Lakes are omnivores but *Puntius spp, Rainbow trout, Botia spp, Garra gotyla, Glyptothorx Cyprinus carpio, Tor spp, Ctenopharyngodon idella*, *Barilius bendelisis* are macrophyte eaters.

Introduction

India has many impoundments such as tanks, ponds, reservoirs, and lakes. Lakes and reservoirs are larger in size as compared to ponds and tanks. Lakes are large inland water bodies which are surrounded by land. They are also connected to streams or rivers. In India, there is no specific definition of the lake has been given. They are loosely described as different types such as natural, man-made and ephemeral consisting wetlands **(Reddy and Char, 2006).** Bhimtal and Naukuchiatal Lake are situated in the lesser Himalayan region of Uttarakhand in India. Naukuchiatal is mesotrophic with intermediate levels of nutrients **(Sharma *et al.*, 1985; Pant *et al.*, 1985; Raina and Petre, 1999; Chauhan *et al.*, 2009)**. But in winter it has found that the lake turns into eutrophic and causing fish kills (**Pant and Sharma, 1985).** Macrophytes are directly related to the ageing of the Lakes. The colonization of macrophytes especially rooted plants enrich the water with nutrients above normal resuspension of nutrients. This colonization stimulates production in pelagic water and sedimentation to produce more area for macrophyte colonization. Littoral vegetation with the basin morphometric determines the rate of Lake Succession **(Carpenter, 1981).** Fluctuations in submerged macrophytes are experienced not only in natural but also in managed ecosystems which as there plants affects the physical and chemical environment and thereby biota. The effect on aquatic biota including epiphytes, grazers, detritivores and fishes is visible due to the variation in macrophytes abundance and diversity **(Carpenter and Lodge, 1986).** Lakes such as Loktak Lake, Bhopal Lakes, Pong Dam Lake, Kanji Lake are infested by the Water hyacinth (*Eichhornia spp*.) causing endemic diseases by providing the optimum conditions for the breeding of aquatic vectors **(Reddy and Char, 2006)**. The fisheries resources are left untapped in the majority of the upland water resources. As they are the inland water resources, there is a lack of documentation and databases of water and fisheries resources for fisheries management of these areas. As compared to other inland resources, the manual data collection and analysis of upland resources including Lakes is more tedious and they are considered in inland fisheries. Upland Lakes and aquatic organism are getting disturbed by various anthropogenic activities, tourism, nutrient runoff, and macrophyte infestation, immediate mitigation and research should be carried out to analyze some factors affecting the Lake. As the upland Lakes such as Bhimtal and Naukuchiatal selected as study area of interest are less studied as compared to other inland water bodies. Remote sensing data has become a common and essential tool in GIS analysis. Many studies have demonstrated the effectiveness of using remotely sensed data as a powerful technology tool which has considerable potential in the monitoring of coastal resources such as mangroves, estuaries and other landforms. Remote sensing can give various indices by ecosystem status which includes a compact system of the surface ecosystem at a particular time and place **(Platt and Sathyendrath, 2008)**.

Material and method

Lake was divided into five grids. In each grid variety of macrophyte were noted. Location of macrophyte was note down with the help of GPS. Data was added in the excel sheet. X and Y data was feed in software. Shape file of the area was added in ArcGIS software. X and Y data was feed in software by mentioning X as longitude and Y as latitude. Project coordinate system was set as WGS_1984_UTM_Zone_44N. In Arctool Box, Geostatistical analytical was clicked in which IDW (Inverse Distance Weightage) method was used to create a layer. The thematic map was created showing the quality wise distribution of macrophyte. Icthyofauna regarding species distribution in the lake was collected and identified morphologically (Days 1889). Macrophyte analysis was done on site and the result were interpolated to see the effect of macrophyte on icthyofauna.

(Continued on next page)

Result

In Bhimtal Lake macrophyte were more concentrated in 79↑ 33' 15 " E longitude and 29↑ 20' 58" N latitude and in Naukuchiatal Lake macrophyte were more concentrated in 79↑ 35' 10 " E longitude and 29↑ 19' 14" N latitude.

Literatures were cited and sampling survey was done on Bhimtal and Naukuchiatal Lake shows that the lake has the moderate influence of macrophyte. Table 4.8 shows the name of macrophyte in both Lakes.

Some literature were cited and some of the parameters was taken into consideration for obtaining the effect of macrophyte on fishes and the result shows that macrophyte may have positive as well as a negative effect which can be directly or indirectly on fishes. Aquatic macrophyte are needed to keep the healthy fish stock in natural water but there over dominance may harm the aquatic icthyofauna present in water. Naukuchiatal Lake has obtained more transparency then Bhimtal Lake which shows that there is a low concentration of suspended sediments in the Lake indicating that there is medium to the dense population of macrophyte this will help to increase prey-predator interaction. Major species avalaible in both the Lakes are omnivores but *Puntius, Cyprinus carpio, Tor spp, Ctenopharyngodon idella* are macrophyte eaters. Macrophyte helps as food, spawning and shelter for fishes *Cyprinus carpio* needs Hydrilla for laying their eggs which acts as a substrate for attachment of egg and their survival and also eat some macrophyte, both the Lake have Hydrilla which will have a positive effect on fish. Grass carp are considered to eat 170 different species of macrophyte which shows it has feeding relation with macrophyte showing a positive effect**. Schuytema (1977)** suggested grass carp can be used as biological control of aquatic plants but pointed out at a number of negative impacts. As *Tor spp* prefereable food is *Myriophyllum, Chara* etc which shows that macrophyte is a part of the food chain showing a positive effect on icthyofauna. **Pathani (1980)** observed that *Tor tor* and *Tor putitora* feed on the submerged aquatic plants such as *Ceratophyllum spp, Hydrilla spp, Myriophyllum spp* and *Vallisnaria spp.* Both the Lakes are abundant with submerging macrophyte which will not affect the *Tor spp*. For *Puntius sp* macrophyte helps in shelter and feeding. Puntius favourite food is Hydrilla and Vallisneria. **Devaraj *et al.* (1977)** have tried to control aquatic vegetation such as by using the ornamental fishes such as *Puntius spp* and found that the fingerlings can feed on *Lemna spp* and *Hydrilla spp* at a rate of more than fifty percentage of their bodyweight. For all other species, macrophyte acts as sheltering ground during higher temperatures. A large amount of floating macrophyte may eventually decrease the light penetration leading to a decrease in dissolved oxygen level which may hamper the fishes, But both the Lakes have the least abundance of floating macrophyte and also the average dissolved oxygen in Bhimtal Lake was 9.0 mg/l and Naukuchiatal Lake was found to be 8.65 mg/l which shows that floating macrophyte is not affecting the icthyofauna. There is a negative relationship between abundance of submersed macrophyte and planktonic algae biomass **Canfield *et al*. (1984)**. Both the lake have more infestation of submerged macrophyte as they eventually uptake more nutrients which will lead to a decline in nutrient cycling that may lower the productivity of plankton. Fish that feed directly on plankton could be negatively affected. Biodiversity in Bhimtal Lake was found to be more than Naukuchiatal Lake. Macrophyte were found to be more in Bhimtal Lake like *Lemna, Azolla, Eichhornia, Polygonum.*

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Table 4.8: Macrophytes in Bhimtal and Naukuchiatal Lake.

Floating	***Lemna, Azolla, Eichhornia***
Emergent	***Polygonum, Nelumbo***
Submerged	***Vallisneria, Potamogeton, Hydrilla, Chara, Myriophyllum***

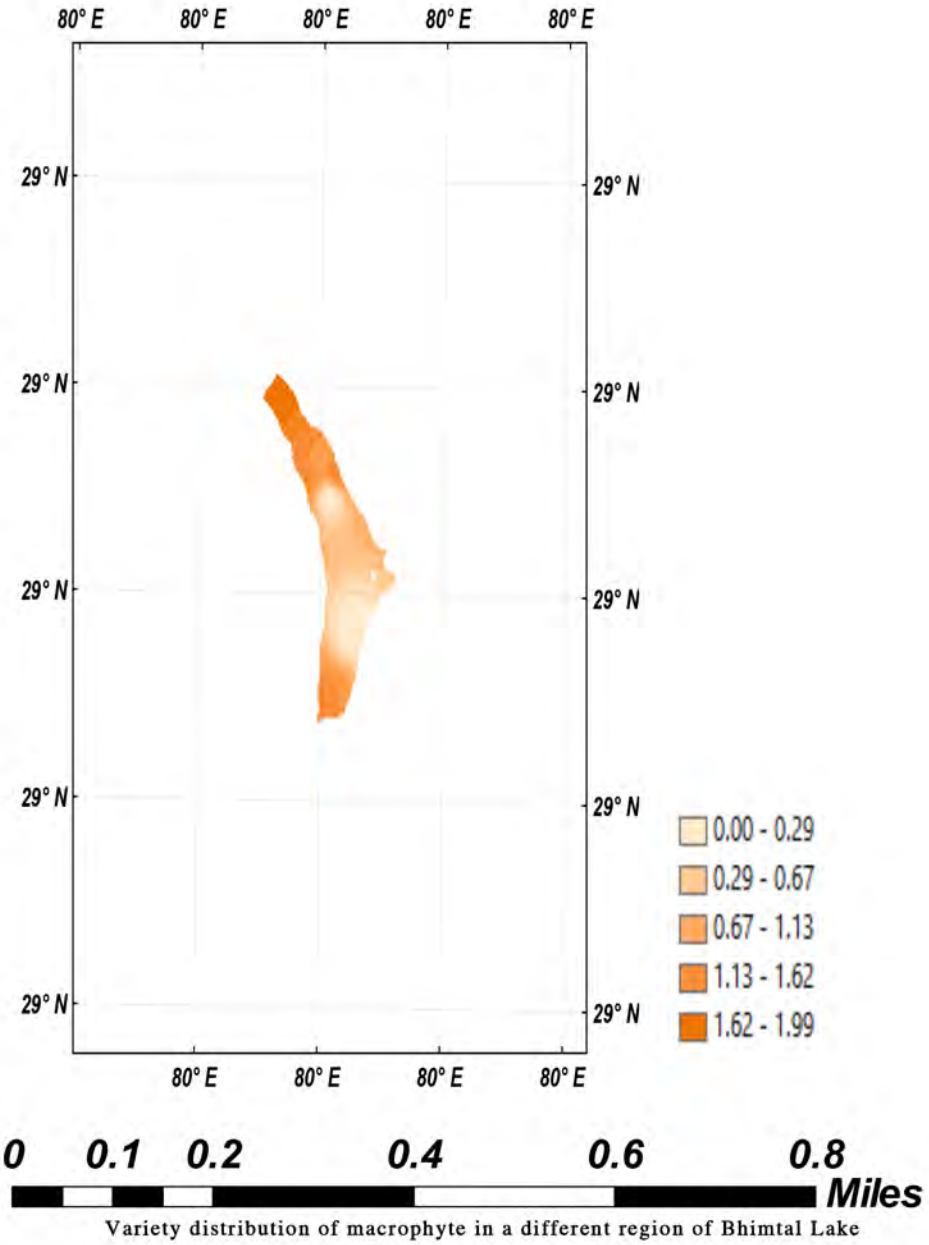
Table 4.9: Feeding habit of Bhimtal species

Herbivore	***Puntius ticto, Puntius conchonius, Labeo rohita, Schizothorax richardsonii, Catla catla, Ctenopharyngodon idella, Garra gotyla***
Omnivores	***Tor tor, Nemachilus montanus, Tor putitora, Barilius bendelisis, Schizothorax progastus, Chanda spp, Cirrhinus mrigala, Cyprinus carpio, Onchorynchus spp Hypopthalmichtys spp , Botia lohachata***
Carnivores	***Channa spp, Mystus spp, Wallago spp, Notopterus spp, Rita rita, Glyptothorax***

(Continued on next page)

Table 4.10: Feeding habit of Naukuchiatal species

Herbivore	***Puntius conchonius, Labeo rohita, Schizothorax richardsonii, Catla catla, Ctenopharyngodon idella***
Omnivores	***Tor tor, Nemachilus montanus, Tor putitora, Barilius bendelisis, Schizothorax progastus, Chanda spp, Cirrhinus mrigala, Cyprinus carpio***
Carnivores	***Channa spp, Mystus spp, Wallago spp, Notopterus sp, Onchorynchus mykiss***



Variety distribution of macrophyte in a different region of Bhimtal Lake

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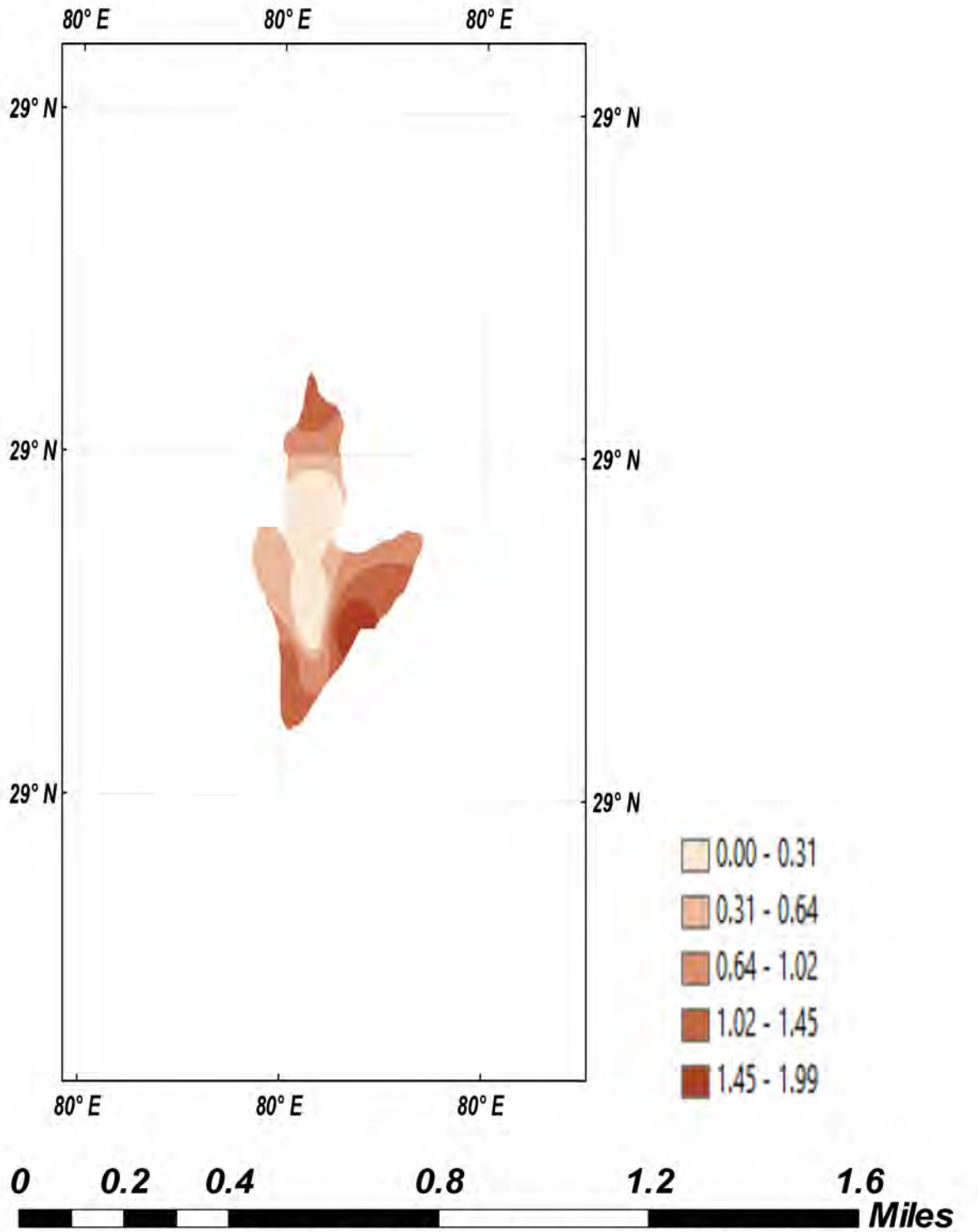

Variety distribution of macrophyte in a different region of Naukuchiatal Lake

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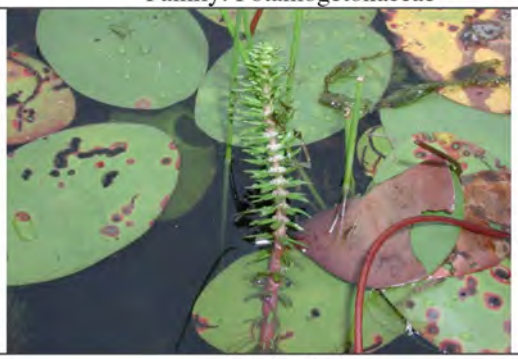
Vallisneria
Family: Hydrocharitaceae

Lemna
Family: Araceae

Potomagaton
Family: Potamogetonaceae

Chara
Family: Characeae



Myriophyllum
Family: Haloragaceae

Pistia
Family: Araceae

Polygonum
Family: Polygonaceae

Hydrilla
Family: Hydrocharitaceae

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Nelumbo Family: Nelumbonaceae	*Azolla* Family: Salvinicea

Macrophyte observed in Bhimtal and Naukuchiatal Lake

	
Puntius ticto	*Puntius conchonius*
	
Labeo rohita	*Cirrhinus mrigala*
	
Schizothorax richardsonii	*Ctenopharyngodon idella*
	
Tor tor	*Cyprinus carpio*
	
Catla catla	*Glyptothorax spp*

(Continued on next page)

Tor putitora

Chanda nama

Nemacheilus montanus

Barilius bendelisis

Channa spp

Myustus spp

Wallago attu

Notopterus spp

Rita rita

Onchorynchus mykiss

Garra spp

Species observed in Bhimtal and Naukuchiatal Lake

MONOCULTURE OF PIKEPERCH (*Sander lucioperca*) IN INTENSIVE SYSTEM OF IRAN

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Sander lucioperca (Pikeperch) is one of the economies known for its high quality meat, reduced natural resources, high prices and popularity among consumers as an ideal choice for commercial production. Intensive culture for Sander production are in the early stages of development in Europe and Asia. The habit of artificial feeding fish is gradual (the best temperature for rearing fish that has the best conversion factor, growth rate, protein efficienc , and instantaneous growth rate is at 22-23 ° C. The appropriate light period for breeding was 16 hours of darkness and 8 hours of light. 1718 intensively reared juveniles and stocked into four ponds for 240-day long culture in pond culture.

SGR was 0.762±0.441%.d-1, FCR = 1.8±1.2, FC = 0.72±0.09 and survival rate was 76.8% at the end of the 270-day rearing period. The fish density was observed about 15 kg per cubic meter. No difficulties were observed in adaptation of intensively cultured juveniles to pond conditions. During the study, it was obtained a high survival rate and excellent ability of pikeperch juveniles to consume dry feed after their re-adaptation to aquaculture system.

IMPACT OF HYPOXIA STRESS ON THE IMMUNE STATUS OF FARMED PIKEPERCH (*Sander lucioperca* L., 1758)

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Introduction

Pikeperch (*Sander lucioperca* L., 1758) is native to the northern hemisphere and increasingly important for the European aquaculture. It is susceptible to typical stress parameters in aquaculture like husbandry procedures and changing water conditions (Baekelandt et al. 2018). Low dissolved oxygen (DO) levels can have severe effects on the health of aquatic animals (Eissa and Wang 2016). Oxygen saturation is therefore an important parameter for the welfare status of farmed fish. Saturations below 40% are regarded as unfavourable for aquaculture facilities (Bregnballe 2015). In detail, hypoxia can be caused by high water temperatures, insufficient water circulation or high stocking densities, increasing the oxygen demand. Effects on the immune response of cultured fish due to adverse husbandry have been shown, amongst others, by Korytář et al. (2016). They demonstrated that high stocking densities induce specific immune pathways in kidney and liver of maraena whitefish (*Coregonus maraena* B., 1779). Until now only few details about the stress physiology of pikeperch have been evaluated (Milla et al. 2015). A comprehensive analysis of acute and chronic hypoxia stress affecting its immune system is fundamental for successful rearing practices and welfare maintenance in aquaculture. This work of the Campus bioFISH M-V was financed by the European Maritime and Fisheries Fund (EMFF) and the Ministry of Agriculture and the Environment Mecklenburg-Western Pomerania, Germany (Grant #: MV-II.1-RM-001). Furthermore it is part of the AQUAEXCEL[2020] project (TNA Identification Code AE080004).

Material and Methods

Pikeperch (mean weight 208g) were reared at 23°C for up to 28 days in recirculating aquaculture systems. The control group (n = 40) was kept under normoxic conditions (100% DO). The experimental group (n = 40) was kept under hypoxic conditions (40% DO). At day 1, 7, 14, 21 and 28 samples of peripheral blood, liver, head kidney, HK leukocytes, gills, spleen and skin were collected (Fig 1). Following analyses included the determination of blood parameters, cell compositions and transcript levels of possible biomarker genes.

Results

Preliminary data show that blood parameters like glucose, lactate and cortisol do not significantly correlate with stressed or unstressed fish. Furthermore, cytometry analyses indicate a change of immune cell composition within peripheral blood and head kidney leukocytes in stressed fish. Transcript level analysis of possible biomarker genes in liver and head kidney tissue suggests that hypoxic conditions activate stress-relevant signaling and immune-system pathways. Network analyses confirm the interaction of the investigated factors within the hypoxia-stress response

Discussion and Conclusions

The present study investigates the influence of oxygen deficienc on the immune status of farmed pikeperch. We conclude that acute hypoxia stress induces molecular mechanisms stimulating immune responses, especially the innate immunity. Chronic hypoxia stress, on the other hand, finally suppresses the immune system impairing the response to pathogens. With this work, we contribute to the knowledge on the stress-immune crosstalk in the novel aquaculture species *Sander lucioperca* and support regional farmers with identification of specific markers for a diagnostic tool assessing the health status in farmed pikeperch.

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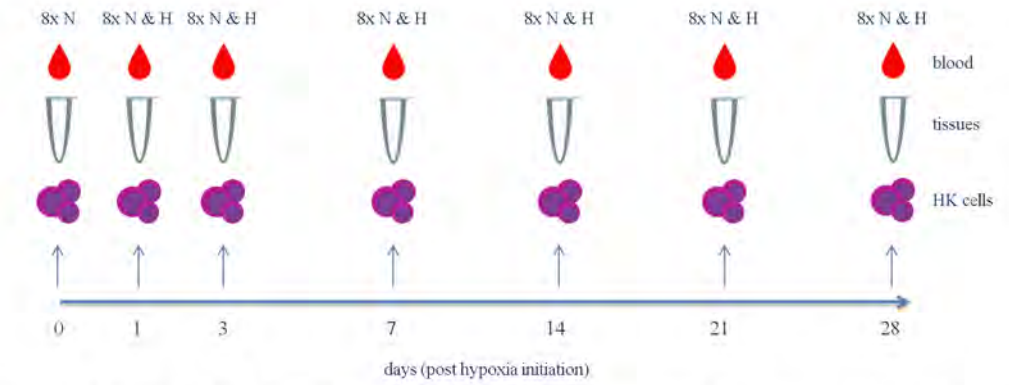

Fig. 1. **Timeline of the hypoxia stress experiment.** Day 0 = start of the experiment. N = fish kept under normoxic conditions. H = fish kept under hypoxic conditions. HK = head kidney.

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ASSESSING THE POTENTIAL OF THE COLD WATER SEA CUCUMBER SPECIES *Parastichopus Tremulus* FOR USE IN CIRCULAR AQUACULTURE

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Aquaculture in Sweden and Norway, although existing for several decades, have mainly focused on salmonids in monoculture. The recently formed research collaboration centre SWEMARC at Gothenburg University focuses on implementing new species and technology into local aquaculture, such as RAS and IMTA.

The CIRCULAR project evaluates the use of the aspidochirotide red sea cucumber *Parastichopus tremulus* as detritivorous component, eating the waste from e.g. fish, in an integrated marine aquaculture system consisting of cold-water species. *Parastichopus tremulus* is a species on which relatively few scientific studies have been made (e.g. Jespersen & Lützen 1971, Hauksson 1979, Holland 1981). It is mainly found on deep soft bottoms (35- >100m) in Scandinavia, and is occasionally caught as by-catch in trawl nets and lobster pots. They spawn during the summer months; reports show gonad ripeness between May-July (. Age of reproduction is unknown, as is growth rate.

We aim to develop a breeding protocol for *P. tremulus,* with studies on e.g. spawning induction, development time, effects of feed quantity and -quality for the auricularia and pentactula larvae on growth and survival. In order to determine the potential of this species in an IMTA, we have already performed studies on adult feeding efficiency and stress tolerances. Once the juveniles have developed, they will undergo these same studies in order for us to further assess the suitability of *P. tremulus* for farming in an IMTA.

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TOWARDS AN INDIVIDUAL CHARACTERISATION OF FARMED SALMON

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Introduction

Due to the growth of the human population and the growing demand for seafood aquaculture is an increasingly important and fast-growing industry. In order to perform salmon fish farming in a sustainable, non-harmful and environmentally friendly way it is important to gather accurate information about fis welfare along with productivity indicators as this can serve as basis for optimal operation decisions. New enabling technologies i.e. advances in machine learning and underwater camera technology allow now for an individual based characterisation of farmed salmon. In line with the recently published Precision Fish Farming (PFF) concept [1] the continuous and automatic analysis of individual salmon based on high quality video recordings is a prerequisite for measuring the current state of the fish population with highest accuracy in a cost effectively way. In this work we present recent progress of the automatic characterisation of individual salmon seen in video-streams from full scale industrial fish-cages.

Material and Methods

One objective within the INDISAL project [2] is the automatic computer vision and machine learning based characterisation of salmon in fish-cages. In a first step we find and track the best visible heads of the salmon in a underwater fish-cage video stream and analyse the relative motion of the fish mouth. We exploit a recent deep learning based approach [3] for the detection of the salmon head and/or other fish-parts. In particular we introduced the classes “fish”, “eye”, “head”, “mouth” among others like “tail” and “topfin” which are useful for an automatic analysis of typical underwater recordings in fish-cages. The deep neural network was then trained with labeled data representing a large variety of scenes in order to work robustly in many different lighting conditions. After the training-phase real time video-streams or recordings can be analysed and clearly detected fish heads (where also the mouth and eye are found) are tracked and a subsequent computer vision based analysis of the relative motion of the mouth allows us to determine the “mouth opening frequency”.

Results

The developed approach robustly finds and tracks the most suitable salmon heads that are visible in underwater video-streams from a full scale industrial fish-cage. Initial results confirm that within an additional step we are able to classify a mouth as “open” or “closed”, thereby getting information about the “mouth opening frequency”.

Discussions and Conclusion

Within the presented approach we have the focus to determinate salmon status variables that can be objectively measured. In future work we intend to relate these objective measurements also to fish welfare indicators [4]. Additional fish-status variables one may consider to extract include appearance and behaviour based variables like size, shape, physical damages/ wounds, amount of lice, swimming speed, feeding activity, stress and possibly other welfare indicators.

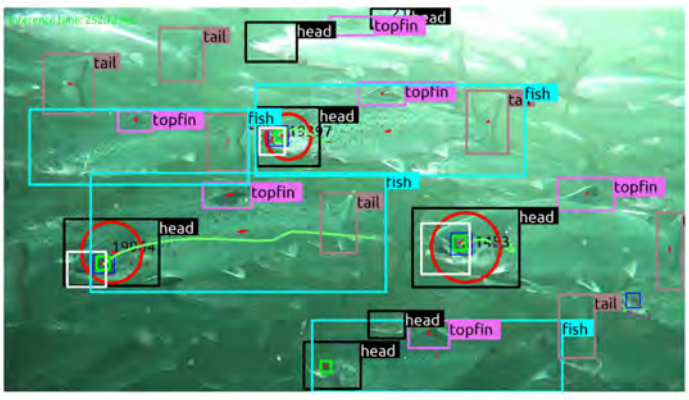

Fig. 1: The automatic recognition of salmon and fish-parts allows for example for a more detailed analysis of the mouth motion in the head region.

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CLIMATE CHANGE IMPACTS ON THE EUROPEAN ISLANDS' AQUACULTURE SECTOR

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According to the fifth Assessment Report of the Intergovernmental Panel on Climate Change, the warming of the climate system is unequivocal and continued emission of greenhouse gases will cause further warming and long-lasting changes in all components of the climate system, increasing the likelihood of severe and irreversible environmental impacts, which can induce large socio-economic damage. New policies on mitigation and adaptation are needed. In the field of Climate Change adaptation, policy makers must have detailed and accurate information about likely impact chains and about the costs and benefits of possible resilience strategies corresponding to the potential decarbonisation pathways. EU islands are particularly vulnerable to Climate Change consequences.

The SOCLIMPACT project aims at modelling and assessing downscaled Climate Change impacts and low carbon transition pathways in European islands and archipelagos for 2030-2100, complementing current available projections for Europe, and nourishing actual economic models with non-market assessment. The project is developing a thorough understanding on how Climate Change will impact the EU islands located in different regions and focuses on the Blue growth sector.

One of the fast-growing Blue Growth sectors is aquaculture. Aquaculture accounts for 20% of the fish production in Europe. Climate change impacts and risks for the sector were identified based on climate hazards, vulnerability factors and exposure.

The main risks for marine cage aquaculture recognised are:

- Change in production due to changes in seawater characteristics and temperature
- Increased fragility of the aquaculture activity due to extreme events
- Increased environmental pollution from aquaculture sites due to changes in coastal hydrodynamics

Impact chains for these risks have been developed and operationalised to identify the major risks and enable comparison between risks and islands.

Acknowledgement
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ZEBRA-MUSSEL (*Dreissena*) CULTIVATION IN BALTIC COASTAL WATERS – AN OPTION FOR BLUE GROWTH AND WATER POLICY IMPLEMENTATION?

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1. Introduction

Blue and zebra mussels (*Dreissena*) are naturally occuring the the Baltic Sea and its coastal waters. Both species are generally suitable for suspended cultivation in mussel farms. Despite that, commercial mussel farms are an exception in the Baltic Sea. Reduced salinity and the associated slower growth hardly allows a cost-covering blue mussel production for human consumption. Zebra mussels, who prefer low salinities, are too small for direct human consumption. However, both species are suitable for the production of mussel meal, which could serve as feed in fish aquaculture, replacing fish mea

The European Commission Regulation (EC 710/2009) provides detailed rules on organic aquaculture animal and seaweed production. Feeds for carnivorous aquaculture animals, for example, need to be organic and preferably of local origin. A sustainable mussel farm could provide organic feed meal and could favor organic fish aquaculture, which is hampered by limited feed availability, quality and/or high feed costs.

The Blue Growth Strategy is the European long-term attempt to support sustainable growth in the marine and maritime sectors. The European Commission intends to boost the aquaculture sector and EU countries were asked to set up multiannual plans to promote aquaculture. However, with respect to mussel farming the production trend in Europe is decreasing since the beginning of the century, whereas it is still increasing fast in other parts of the world. The same is true for the production of organic fish in German , which decreased by 35% since 2013 (EUMOFA 2017, www.eumofa.eu).

Mussel farms not only provide food and protein-rich feed. Blue and zebra mussels are efficient filter feeders, which reduce phytoplankton concentrations and bind the nutrients nitrogen and phosphorus. According to HELCOM (www.helcom.fi), eutrophication is still one of the main threats to the Baltic Sea and is caused by excessive inputs of nutrients to the marine environment. When farmed mussels are harvested, the nutrients are removed and this process counteracts eutrophication. Additionally, mussels increase water transparency. Within the EU Water Framework Directive, blue mussel farms are already discussed and tested as a measure to combat eutrophication in coastal waters.

With focus on zebra mussels (*Dreissena*) in selected Baltic coastal waters, we address the questions: Is it realistic to produce mussels as fresh feed and meal on a commercial basis and support Blue Growth? Can zebra mussel farming play a role as measure in the Water Framework Directive (WFD) by removing nutrients and combating eutrophication? Can it play a role for improving water transparency and enabling macrophyte restoration? Is it possible to use mussel cultivation to improve transparency and attractiveness of bathing waters at beaches? This presentation summarizes most recent findings by Schernewski et al. (2018) and Friedland et al. (2019a, 2019b).

2. Methods

For this study we improved an ecological model added an economic model, collected a wide range of data and carried out field and feeding experiments as well as surveys among tourists and local inhabitants. The ecological model consist of a coupled 3d-hydrographical-biogeochemical model (GETM-ERGOM) linked to a mussel module. We present the results for several spatially explicit and realistic scenarios of mussel farming.

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3. Results

Zebra mussel farming seems to be a suitable measure for removing nutrients, raising water transparency and improving ecosystem quality, but its quantitative potential in our case study site, the large, shallow Oder Lagoon, is limited. In smaller water bodies with less external nutrient loads, zebra mussel cultivation may be a suitable water quality management option.

In the Oder Lagoon, mussel cultivation could be implemented for concrete local purposes, like small scale feed production, improving water transparency at beaches or as environmental measure to enable macrophyte recovery. Zebra mussel cultivation in the lagoon would not be profitable as a business, because the market for fresh feed mussels is limited (animal parks, aquaculture) and mussel meal production would require a large production. Zebra mussel farms would produce feed of high quality that could serve as replacement for fishmeal. It would meet the standard for organic certified production and could support organic fish aquaculture (Naturland, https://www.naturland.de). The demand and price for such a product may increase in future and may make a commercial production realistic. Our model simulations suggest that as soon as a compensation for nutrient removal is considered, all mussel farm scenarios could cover the costs. Further, our experiments confirm that the conditions for an environmental friendly farming approach in the lagoon are suitable

From a practical point of view, the positive effects of mussel farms on water transparency are, at the moment, most important. Even to establish mussel farms in the surrounding of beaches to improve bathing water transparency, was perceived as a worth considering option by our local and regional stakeholders. However, the potential water transparency improvement (Secchi depth) seems limited to about 20 cm. A higher mussel biomass may cause negative impacts on the environment and increase the risk of hypoxia.

Most promising in our stakeholders' view are mobile mussel farms, which increase water transparency in shallow areas (water depth below 2 m) and initiate a recovery of submerse macrophytes, without disturbing already established submerse macrophytes. These mussel farms could potentially be moved to other places once the macrophytes are re-established. A temporary installation was perceived as a major advantage, because it limits potential local negative effects of mussel farms on sediments and the re-use in a different place would reduce costs. However, this has not been tested so far and additional field experiments are necessary to confirm the suitability of such an approac

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DEVELOPMENT OF A NOVEL, LAND-BASED AQUACULTURE PROCESS FOR THE PRODUCTION OF MARINE SPONGE *Chondrosia reniformis* AND THE PHARMACOLOGICAL USE OF SPONGE COLLAGEN

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Introduction

Marine sponges are one of the most important sources of bioactive compounds used in medicine, industry and as nutraceuticals. As sessile organisms, sponges have developed chemical defense mechanisms to avoid the covering of algae, bacteria and infectious microorganisms (Chanas et al., 1996; Thoms et al., 2006). Regarding the development of nutraceuticals *Chondrosia reniformis*, a sponge species from the Mediterranean, is of special interest due to their high amount of collagen (Swatschek et al., 2002). To gain a high yield of collagen, plenty of sponge biomass is needed. Avoiding exploitation of natural resources, special culture techniques need to be developed. Experiments showed, that mariculture is not an adequate technique for a mass reproduction of *C. reniformis* (pretrials by KliniPharm, 2004). A land-based cultivation and high mass production of *C. reniformis* and the associated collagen could not be realized yet, because the animals are very sensitive to transportation and make high demands on culture conditions (Nickel and Brümmer, 2003; own experience, 2018 and 2019).

The project AkPhaKol will address the detection of convenient recirculating aquaculture system conditions for *C. reniformis* fragments to build up a sustainable source for sponge biomass accompanied by a maximized collagen production. Reduction and loss of microorganism associated with the sponge species as well as nutrient content in the culture water shall be minimized by an optimized design of culture tank and water treatment. In addition a specific adapted food for this species shall be developed, which is not available on the market yet. The project partner KliniPharm GmbH will be provided with the extracted collagen from sponges. It will be used for the manufacturing of coatings for solid pharmaceuticals relating to the application of colon-targeting.

Materials and methods

Evaluation of adequate rearing conditions related to amount and content of collagen of *C. reniformis*, tests on culture methods, holding structures, abiotic factors and nutrition are conducted at the Alfred-Wegener-Institute. *C. reniformis* were obtained from the sampling area located in the Mediterranean Sea around the Island Kalymnos, Greece and subsequently raised in a recirculation aquaculture system at the Centre for Aquaculture Research, Bremerhaven. Despite sponge specimen collection, substrate structure, abiotic factors and composition of nutrient matter of the native marine water of *C. reniformis* are collected and identified in laboratories. Data are used for the developing of a special tank design, evaluation of the optimal rearing conditions and the detection of adequate food composition.

After cutting of sponge specimen, sponge fragments were glued to an artificial substrate and abiotic factors like temperature, light and current are tested and the effects on survival, growth and collagen content are examined. In a further experiment, feeding trials like feeding strategies and different food types are tested on sponge fragments: a self-developed food, commercial available liquid food for filter feeders and the detritus from fish or shrimp culture. Fragments growth, survival rate and collagen content are measured in specific intervals

Results

Running-in of culture tanks with water retreatment processes was successful; the values of abiotic factors adjusted to native conditions like temperature, light, current and nutrient content remain stable. Efficient transportation conditions as high water volume and cooling were essentials for survival of sponge specimen. One week after arrival, survival rate of sponge specimens was high with 90% and they attached to the bottom of the culture tank.

Started with the first experiment, sponge specimen were cut into fragments and glued to artificial substrate, they remain attached and after a week began to encrust the substrate. During this experiment, sponge fragments were fed with a mixture of commercial available liquid food and a deep-frozen floating feed consisting of different microalgae. Results on growth and collagen content will be measured in a further step. Design and development of a special adapted feed for *C. reniformis* is in progress, which will be applied in a further experiment.

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Discussion and conclusion

To avoid sudden death of sponge specimen short after arrival, the running-in of the aquaculture system have to provide stable values of abiotic factors (similar to natural habitat) and parameters in the biological water treatment.

One of the important parameters for a successful land-based aquaculture is an optimal food supply, the development of a special feed, which needs to have the correct size for filtering and meets the needs of the species *C. reniformis*. Additionally the amount and the regularity of feeding intervals is crucial; high loads of food will lead to blockage of the ostia and too low food supply in staving of sponge fragments.

Concluding, ideal transportations condition, an aquaculture system without fluctuation in abiotic factors and the development of an optimal feed adapted to this species is essential for survival, growth, amount of symbionts and of collagen content.

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INVESTIGATIONS ON NATURE AND LEVEL OF RESIDUES IN FARMED FISH AS PART OF THE EUROPEAN PESTICIDE REGULATION

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Introduction

The declining availability of pelagic forage/trash fisheries products and the increasing demand for these resources for aquafeed production is causing the price on international markets to rise as well as stimulating the search for alternatives. Plant-derived commodities are the only realistic alternative to replace fish meal and fish oil in rising demand in aquafeeds. With an increasing amount of plant material in aquaculture diets, there is an increasing risk for pesticide residues in aquaculture diets and their transfer into aquaculture products. Therefore, the EU has published data requirements for fish as part of the approval process for pesticides (EU-Commisson, 2013a). A working document on the nature of pesticide residues in fish was published to provide guidance on the performance of fish metabolism studies (EU-Commission, 2013b). Important inland aquaculture species reared for human consumption such as rainbow trout (*Oncorhynchus mykiss*) or common carp (*Cyprinus carpio*) are the recommended test species. Metabolism studies on fish are required when a pesticide of moderate to high lipophilicity (>logP 3) is used on crops and may lead to significant residues in fish feed, generally considered to be ≥0.1 mg kg^{-1} of the total diet. Fish dietary burden calculation is therefore an important prerequisite to decide on further experimental testing as part of the consumer risk assessment. Metabolism studies using radiolabelled test material are difficult to apply under aquatic conditions. Large volumes of contaminated water may result from metabolism studies and need to be treated using powerful filter technology. In addition to that fish metabolism studies reflecting oral ingestion of contaminated feed need to be carried out with animals of marketable size. This is to provide sufficient tissue for the quantitation of residues in the fish product (fillet, liver), and to enable identification of radiolabelled metabolites. The availability of an in vitro approach to provide information on metabolism of pesticides in farmed fish would be thus advantageous.

Material and Methods

The software *DietaryBurdenCalculator* was developed that allows the determination of the worst case feed composition made of plant derived feedstuffs. This is the composition leading to the maximum dietary burden of pesticide residues. Calculations are based on the simplex algorithm which allows solving large-scale linear programming problems quickly. The calculator allows estimating the specific dietary burden for common carp and rainbow trout, offers the possibility to consider maximum inclusion rates, and enables optimised residue estimation by adding non-contaminated substitutes as a source of protein, carbohydrates or fat (Klein et al. 2015).

Fish metabolism studies in both rainbow trout and common carp were carried out, following the working document on the nature of pesticide residues in fish using ^{14}C-labelled pesticide to assess the practicality of conducting metabolism studies in fish for regulatory purposes (Schlechtriem et al. 2015)

In order to evaluate the potential of in vitro fish hepatocyte assays to provide information on in vivo metabolite patterns of pesticides in farmed fish, in vitro incubations using suspensions of freshly isolated or cryopreserved primary hepatocytes obtained from common carp and rainbow trout were performed. In vitro and in vivo metabolite patterns were compared (Bischof et al. 2016).

Results & Discussion

Due to the increased amount of plant-derived materials in aquaculture diets, fish might increasingly become exposed to pesticide residues through their diets. Reasonable worst case estimates of the fish dietary burden are therefore important to decide on further testing such as fish metabolism or feeding studies, The software *DietaryBurdenCalculator* follows a realistic dietary optimisation scenario and allows the determination of the worst case feed composition made of plant derived feedstuffs leading to the maximum dietary burden of pesticide residues (Klein et al. 2015).

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Results of the metabolism studies with comon carp and rainbow trout (Schlechtriem et al. 2015) show that metabolism studies for regulatory purposes can be carried out with both fish species under laboratory conditions. The experimental design reported in the working document on the nature of pesticide residues in fish is suitable for quantifying the transfer of residues to edible tissues and enables characterisation of the chemical nature of residues. Based on the findings of the fish metabolism study and the estimated maximum residues which may occur in fish feed (maximum dietary burden), a feeding study may be required for fish, where residues at levels above 0.01mg kg^{-1} fresh matter may be reasonably expected in edible tissues. Feeding studies are required to determine the magnitude of residues in products of fish origin in order to assess possible consumer risks arising from ingestion of these products and to establish MRLs for edible fish commodities which still need to be defined. An official test protocol for feeding studies is being developed. Dietary burden calculation as outlined by the EU working document (EU-Commisson, 2013b) is currently limited to the two recommended test species trout and carp which are considered as representatives of the full range of fish species raised for human consumption. However, the dietary burden of fish species raised in aquaculture is likely to be much more diverse. Along with the development of guidance on fish feeding studies it would therefore be desirable to develop a wider range of fish feeding tables including salt water species to derive the realistic worst case dietary burden. Testing based on this dietary burden could still be conducted with the selected representative species.

The results from the in vitro/in vivo study (Bischof et al. 2016) confirm the presence of relevant differences in the metabolism of xenobiotics/pesticides between fish species. They further provide evidence that in vitro assays based on primary fish hepatocytes well reflect the in vivo metabolite patterns of xenobiotics/pesticides, in particular they reflect the in vivo species differences of metabolite patterns. Finally, the results of the in vitro study indicate that cryopreserved hepatocytes produce comparable metabolite patterns to freshly isolated cells. The in vitro hepatocyte assay could thus serve as a valuable tool to support in vivo metabolism studies on fish carried out as part of the approval process for pesticides according to the EU regulation 1107 (EU, 2009).

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COMBINATIONS OF BROWN SEAWEEDS AS FEED ADDITIVES IMPROVED THERMAL SHOCK RESISTANCE OF PACIFIC WHITE SHRIMP POST-LARVAE REARED IN BIOFLOC SYSTEM

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Introduction

Brown seaweeds have important nutritional and functional properties. These seaweeds show higher antioxidant activity than red and green seaweeds, and have a diverse content of bioactive compounds, such as polysaccharides, terpenes, phenols and polyphenols, minerals, polyunsaturated fatty acids, vitamins, and carotenoids (Balboa et al., 2013; Gómez-Gil et al., 2000). The present work aimed to assess the effect of different combinations of the brown seaweeds *Sargassum filipendula* and *Undaria pinnatifida* as feed additives on growth parameters and thermal shock resistance of Pacific white shrimp post-larvae reared in biofloc system, during nursery phase.

Materials and methods

The experiment was performed at the Marine Shrimp Laboratory (LCM, *Laboratório de Camarões Marinhos*), of the Federal University of Santa Catarina (UFSC, *Universidade Federal de Santa Catarina*, Brazil), using *Litopenaeus vannamei* post-larvae at the stage of 20 days post-hatchery (PL20, ± 0.006 g). PLs20 were stocked in 400 L tanks at 1500 PLs m^{-3}, and the rearing was managed as a superintensive biofloc system for seven weeks (until they reach 1 g), under four treatments: PLs fed diets containing different ratios 0.5%:2%, 0.5%:4%, 1%:2% and 1%:4% of the dry biomass of *S. filipendula* (S):*U. pinnatifida* (U), respectively, and control diet (without addition), all in triplicate. During the experiment, animals were fed six times day^{-1} and the water quality parameters were monitored (temperature, pH and dissolved daily, and total ammonia, nitrite and nitrate once a week). Following the feeding trial, we determined the growth parameters, and subjected the PLs to thermal shock (28.6 °C to 13.5 °C for 1h, and then back to 28 °C).

Results

The water quality parameters remained at acceptable levels for shrimp nursery, as suggested by Van Wyk and Scarpa (1999), and did not differ among the treatments. Growth parameters also did not show any significant differences among the treatments.

On the other hand, PLs fed diets containing the 1S:2U ratio showed higher survival after thermal shock (Table 1.).

Discussion and conclusion

Many studies have shown that brown seaweeds can affect the resistance of different organisms to thermal stress (Kandasamy et al., 2011, 2014; Schleder et al., 2016). In previous reports, shrimp fed *S. filipendula* did show higher survival after thermal shock (around 97% compared to 43% in the control group), while increasing levels of *U. pinnatifida* had negative effects on thermal shock resistance, with the higher level (4%) causing 100% mortality. The combination of both brown seaweeds in the diets (0.5S:1U, 0.5S:2U and 0.5S:4U) avoided the negative effect of *U. pinnatifida* on the resistance of shrimp juveniles to thermal shock, but the survival remained similar to the control group (around 30%) (Schleder et al., 2016, 2017). Interestingly, in the present work, the combination 1S:2U improved significantly the resistance of shrimp post-larvae to thermal shock.

In conclusion, the combination of *S. filipendula* and *U. pinnatifida* in the diet can improve PLs resistance to acute thermal stress without affecting their growth performance. These results are particularly important for shrimp industry, since after nursery phase shrimp are transferred to grow-out tanks in the shrimp farms, where they face several environmental stresses, such as thermal stress.

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Table 1. Survival rate (%) of *Litopenaeus vannamei* post-larvae fed diets containing 0.5%:2%, 0.5%:4%, 1%:2% and 1%:4% of the dry biomass of *S. filipendula* (S):*U. pinnatifida* (U), and the control diet with no supplementation, after 24 hours post-thermal shock .

Treatments	Survival rate (%)
Control	50.0[a*]
0.5S:2U	44.5[a]
0.5S:4U	46.7[a]
1S:2U	73.3[b]
1S:4U	55.6[a]

*ANOVA one-way, followed by Tukey test (p=0.0001)

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COMPARISON OF THE NUTRITIONAL COMPOSITION OF THREE DIFFERENT BIOFLOC SYSTEMS – CHEMOAUTOTROPH, HETEROTROPH AND MATURE BACTERIA – AND ITS EFFECT ON SHRIMP GROWTH

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Introduction

Shrimp farming has experienced strong growth in recent decades. In nature, shrimp feed mainly on plankton and microorganisms. In commercial aquaculture, shrimp are fed with pellets basically made of fish meal and fish oil (Tacon & Barg, 1998). Even when optimally prepared feed supports the best growth, extensive shrimp aquaculture has a high impact on the environment, and feeding is a cost-intensive component of shrimp breeding. Biofloc technology is an alternative system to maintain water quality and provide shrimp with an additional source of high protein and low fat nutrients (Ebeling et al, 2006). Depending on which bacteria are used, biofloc systems with different characteristics will develop. In the present study, different systems with chemoautotrophic and heterotrophic bacteria as well as a bacterial mix (mature biofloc) were compared in terms of biofloc nutritional value (assessed via proximate analysis) and their influence on shrimp growth.

Material and methods

The experiment consisted of three treatments (chemoautotrophic, heterotrophic and mature biofloc) each with four replicates (tanks) all randomly distributed. In each tank, 105 individuals of *L. vannamei* with an average weight of 3.46±0.01g were placed. The animals were kept in tanks for five weeks and fed four times a day with pelleted shrimp feed (Guabi Potimar "oti Mirim QS 1.6mm", -35% crude protein, made at Guabi Nutrição e Saúde Animal S.A. in Brazil). Consumption was controlled each 90min after feeding by weighting pellet´s rest. New feeding settings were adjusted based on previous consumption. Growth rate and survival were assessed after 35 days based on weekly weight of 30 shrimps per tank. Biofloc samples from each tank were collected lyophilized and homogenized for proximate analysis (including ash, crude lipids, carbohydrates, and crude proteins) as well as energy and fatty acids contents. For the proximate analysis the ash content, crude lipids, carbohydrates and crude proteins were determined. In addition, the energy and fatty acid content were measured in the different biofloc types

Results

There was no significant difference in crude lipid content among treatments (mature: 1.22±0.37%, heterotrophic: 0.86±0.81%, chemoautotrophic: 0.75±0.37%). In all three treatments saturated fatty acids had the highest proportion of total fat (mature: 70.70±3.91%, heterotrophic: 64.53±6.00% and chemoautotrophic: 60.25±11.27%). Ash content was higher in the heterotrophic tanks (58.22±1.01%) than in the mature (53.77±1.96%) and chemoautotrophic (47.45±1.79%). The protein content was in the heterotrophic treatments (30.03±0.47%) significant higher than in the mature (23.15±1.89%) and chemoautotrophic (22.63±1.41%). Carbohydrate content of all treatments were significantly different. The chemoautotrophic system had the highest quantity of carbohydrates with 29.2±3.11%, followed by the mature tanks (21.88±3.32%) and the heterotrophic treatments (10.89±1.55%). The chemoautotrophic (8.26±0.27%) and heterotrophic (8.33±0.62%) treatments significantly di fered on energy content from the mature (10.93±0.09%) treatment.

There was no significant difference between all three treatments in terms of shrimp growth (Fig. 1) or survival rate (mature: 93.33±2.23%, chemoautotrophic: 91.67±3.11% and heterotrophic 91.00±5.41%).

Discussion

The use of particulate organic material, such as microorganisms contained in floc-based products, has been proposed as a potential food source for aquatic animals (Emerenciano, 2011). Biofloc systems in this experiment showed low levels of crude lipids with a high proportion of saturated fatty acids and low content of unsaturated fatty acids, which is not optimal for feeding *L. vannamei*. Alday-Sanz (2010) suggested optimal lipid content between 6-8% for shrimps. Crude protein in the heterotrophic biofloc treatment was relatively high and comparable to commercial shrimp feed. The present results suggest that these different bioflocs might be partially used as feed by the shrimps. High and comparable survival rates and good growth rates indicate that the animals have grown very fast in the different biofloc systems. When shrimp is mainly fed with commercial feeds, the type of biofloc has no influence on growth and survival. Other parameters such as temperature and nutrients accumulation or even extension of the cultivation period may drive shrimps performance in systems using the tested bioflocs while the kind of bacterial groups developed within the tanks may just complement the culture conditions.

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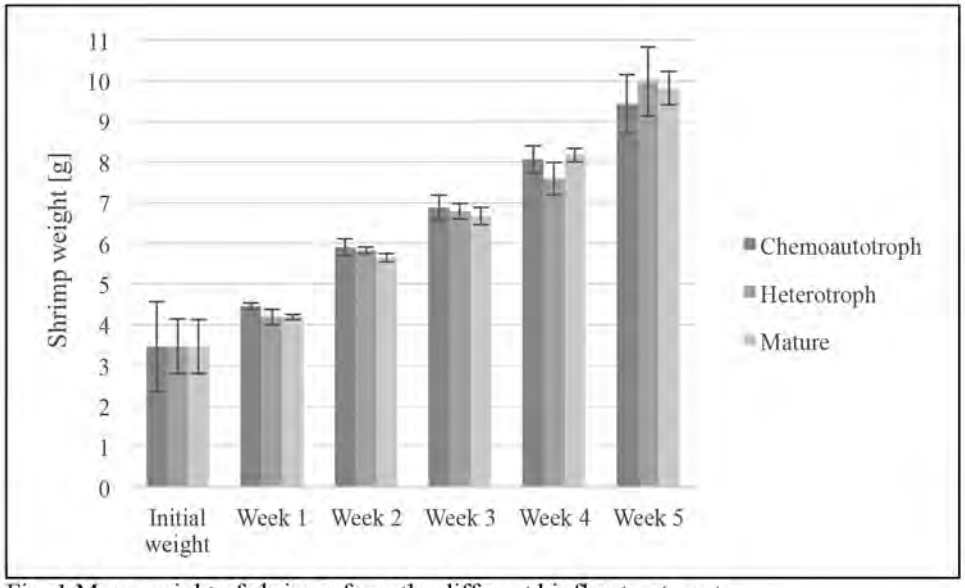

Fig. 1 Mean weight of shrimps from the different biofloc treatments.

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EVALUATION OF THE BIOFILM FORMATION ON STAINLESS STEEL SURFACES IN A MARINE RECIRCULATED AQUACULTURE SYSTEM

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Introduction

The adhesion of microorganisms on surfaces and the consequent biofilm formation is of major concern in the aquaculture sector, since biofilms are difficult to remove and may act as a reservoir for potentially pathogenic bacteria (Bourne et al., 2006). Recently the potential of using submersed sensors is increasing in aquaculture tanks or cages for water monitoring purposes. The biofilm formation can affect the function of these sensors that may lead to misinterpretation of the acquired measurements. Water monitoring sensors have parts made of stainless steel and once introduced into the water, they serve as a new niche for microbial colonization. The PCR-DGGE method is useful tool as a first approach for studying microbial communities, as it allows a quick screening of the microbial succession among temporal samples. The aim of this study was to evaluate qualitative as well as quantitative the formation of biofilms on stainless steel surfaces under seawater conditions.

Methods

Sterilized stainless steel coupons were placed in an aquarium tank, part of an experimental marine Recirculated Aquaculture System (RAS). Two fish species were reared in the RAS, *Dicentrarchus labrax* and *Sparus aurata*. Fish were fed a commercial pellet diet three times per week. Physicochemical parameters (T, salinity, pH, O_2, NO_2^- and total TAN) of the water were monitored with sensors or spectroscopic methods.

Sampling for microbiological analysis was conducted at 24h, 48h, 72h and every three days thereafter until the 30th day. Tank water and coupons (in triplicate) were sampled. Biofilm cells on coupons were sampled according to protocol by Kostaki et al. (2012).

The bacterial community composition of the tank water as well as the biofilms and their temporal changes were assessed by denaturing gradient gel electrophoresis (DGGE) based on the 16S rRNA gene. Tank water samples (100ml) and samples from the bacterial solution of the detached cells of each coupon were centrifuged at 5.000g for 5min. Then the cell pellets were lysed using a lysozyme based protocol to extract bacterial DNA. PCR amplification, using the primers 341 F (with GC clamp) and 907 R, targeted the V3-V5 region of the gene. The amplification products (~580bp) were examined by electrophoresis on 1.2% agarose gels.

DGGE was performed with the DCode Universal Mutation Detection System (Biorad). Samples were loaded on 6% (w/v) polyacrylamide gels with the denaturing gradient ranging from 20% to 60% (100% contains 7M urea and 40% formamide) and gels were run in 1x TAE electrophoresis buffer at 60^{o}C and electrophoresed at 200V for 4h. Gels were stained with ethidium bromide, visualized and photographed with a Gel-Doc imaging system (Biorad).

Results and Discussion

Sea water physicochemical parameters were stable during the experimental period, within values that ensured optimal rearing conditions. Mean values and their standard deviation are as follows: Temperature 23.5 ± 0.4 ^{o}C, salinity 35.3 ± 0.5psu, pH 7.06 ± 0.11, oxygen saturation 100.6 ± 0.6%, nitrite 0.14 ± 0.08ppm, TAN 0.07 ± 0.03ppm.
The microbiological analysis of the tank water revealed that the water microbial load ranged from 3.8 to 5.4logCFU ml^{-1} (Fig.1). The observed fluctuati n followed the feeding regime. This in line with the reports where, bacterial activity and abundance in RAS, found to be affected by the changes in feed loading (Rojas-Tirado et al., 2018).
The DGGE profiles revealed that the microbial association of the tank water remained unchanged during the experimental period. Similar results have been reported by Attramadal et al. (2012, 2014), who found that the tank water of the RAS is characterized by stable carrying capacity and microbial community composition.

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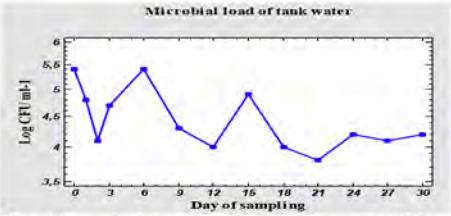

Fig. 1 Population of the marine heterotrophic bacteria of the RAS water (logCFU ml^{-1})

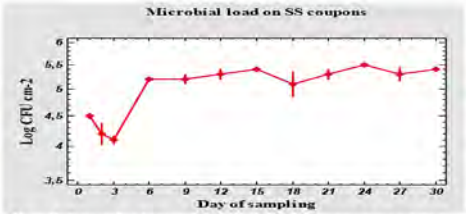

Fig. 2 Population of the marine heterotrophic bacteria forming biofilm (logCFU cm^{-2})

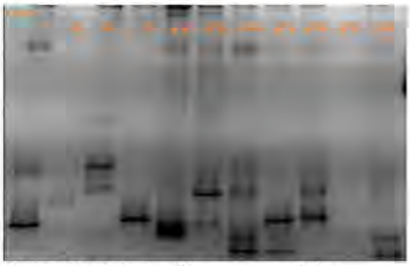

Fig. 3 DGGE profile pattern of the microbial community forming biofilm on the SS coupons during 30 days.

Within 24h the biofilm cells on the coupons reached a population of 4.5±0.05logCFU cm^{-2}. The maximum microbial load of biofilm was achieved within the 6th day, remaining stable until the 30th day (Fig.2). On the other hand, DGGE bacterial profiling revealed a constant change of microbial species during the biofilm development (Fig.3). The dynamic succession of microbial species during biofilm development on fibe glass surfaces has been also previously shown by using DGGE profiling of bacterial 16S rRN genes (Bourne et al., 2006).

Conventional microbiological analysis combined with molecular techniques demonstrated the dynamics of the biofilm development on stainless steel surfaces in a Mediterranean RAS. This work highlights the importance of conducting studies *in situ* for prolonged time periods, as an approach for better assessment of the biofilm formation on test surface materials

Acknowledgment
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RAINBOW TROUT WELFARE INDEX MODEL 1.0

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Introduction

Animal welfare is becoming an increasingly important factor in aquaculture (Ashley, 2007; Browman et al. 2018) as environmentally literate consumers pressing for more species-appropriate methods of food production worldwide. Based on the Salmon Welfare Index Model (SWIM) developed by Stien *et al.* (2013), a semantic welfare index model was designed for rainbow trout (*Oncorhynchus mykiss*) and verified for flow-through as well as recirculating aquaculture systems. The aim of this research project was to provide an applied basis for the assessment of rainbow trout welfare in aquaculture systems which can be further developed and adjusted at any time due to its modular design. The focus was set to develop a tool which can be used by fish farmers as well as governmental authorities

Materials and methods

In a first step, potential welfare indicators for rainbow trout were compiled based on literature references. These welfare indicators were then discussed and adjusted in an expert meeting of scientists, practitioners and veterinaries. Afterwards, the welfare indicators were grouped based on their application in the sections "environment", "stock" and "individual" (**Figure 1**) and weighted on their importance. Furthermore, each indicator was subdivided in practice-oriented levels to allow a gradual assessment of fish welfare. An overall index value of 1.0 represent optimal welfare conditions, whereas 0.0 stands for maximum violations of animal welfare.

Results

The applicability of the rainbow trout welfare index models for flow-through as well as recirculating aquaculture systems were finally verified in surveys of rainbow trout production systems in Germany and an experimental RAS facility. During on-site visits of the commercial fish farms, operators were informed about the background of the project and the intended procedure within half an hour. When data collection and management was in accordance with the good technical practice, information needed to evaluate the section „environment", using environmental parameter mean values from the last month of production could be obtained within about one hour. Evaluation for the section „stock" by observation of three separate rearing units and for the section "individual", examining 10 randomly selected individuals could be conducted within one hour. Final adjustments of the welfare index model were made following the on-site visits.

Overall, the example surveys proved the applicability of the welfare index model for rainbow trout reared in flow-through as well as recirculating aquaculture systems.

The study was founded by the German Federal Ministry of Food and Agriculture (support code 2817900215).

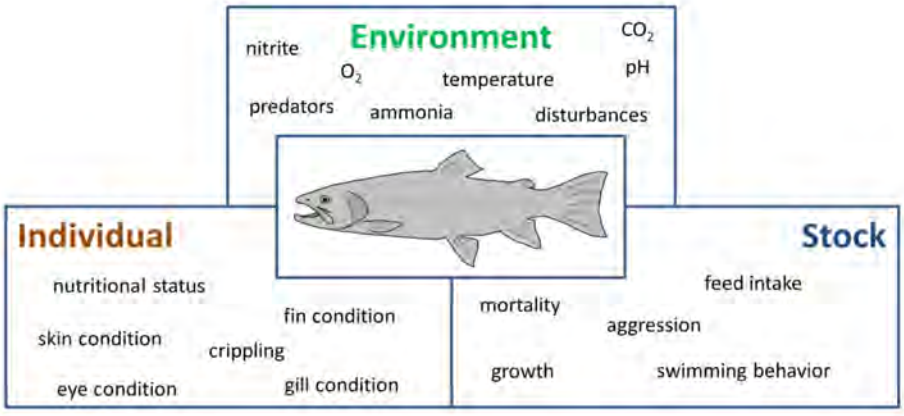

Figure 1: Welfare indicators of the rainbow trout welfare index model grouped into the three sections environment, stock and individual.

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MULTI-USE POTENTIAL OF MARINE AQUACULTURE WITHIN OFFSHORE WIND FARMS IN THE GERMAN BIGHT: A SWOT ANALYSIS

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Introduction
The European Union's Integrated Maritime Policy as well as its Framework for Maritime Spatial Planning highlight the sustainable and efficient development of ocean space as a core objective. Towards this end, users, researchers and planners alike are adopting the idea of multi-use, i.e. the combination of two or more uses in the same (ocean) space. A salient example of multi-use would be Offshore Wind Farms (OWFs) as potential locations for marine aquaculture. This combination has been widely researched and is often viewed as a sustainable and efficient way to enhance regional aquaculture production through the utilisation of synergies between sectors, however, this multi-use combination has yet to reach market readiness.

Materials & Methods
In order to better understand the external and internal factors affecting particular combinations of sectors, we applied a strategic SWOT (Strength, Weakness, Opportunities, Threat) analysis to a case study within Germany's North Sea Exclusive Economic Zone (EEZ), located within the German Bight. Case study results and factors were verified by key stakeholders from industry, research and regulatory agencies. To arrive at clear, accessible guidelines for future development, factors were analysed within the context of the following relevant dimensions: socio-economic, technological, legal and environmental.

Results
Using a SWOT approach, combined with the typology on ocean multi-use presented in Schupp et al. (2019), yields a large number of factors and exposes a large variability in the specific composition of each multi-use combination. Due to the operational differences between types of marine aquaculture, a number of possible sub-combinations can be identified, each carrying different implications for the involved actors, regulators and stakeholders.

Discussion & Conclusion
Discussions of the multi-use of OWFs and marine aquaculture have long since focused on biological and technological practicality as well as the economic feasibility and stakeholder perceptions (Buck and Langan, 2017). Nevertheless, not strictly characterizing and distinguishing the various possible types of sub-combinations could quickly lead to dismissal of the entire multi-use concept. The application of SWOT analysis, a tool more commonly used for strategic planning, to the combinations of OWFs and marine aquaculture yields a systematic overview of factors influencing their continued development. This study represents a first as it showcases all possible sub-combinations, while also taking a full account of the factors influencing their development, using the SWOT framework. It aims to serve as a fundament for pertinent marine multi-use combinations as strategic marine spatial planning continues to expand and evolve.

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THE EFFECT OF TWO DIFFERENT FEEDS ON GROWTH, SURVIVIAL AND CARAPACE COLOR IN WHITELEG SHRIMP *Penaeus vannamei*

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The whiteleg shrimp *Penaeus vannamei* Boone, 1931 is one of the most important commercial species in shrimp aquaculture and the dietary requirements of this species are a key factor during production.

The purpose of the present study was to determine the effect of two commercial feeds containing different amounts of protein on the weight gain and the survival of *P. vannamei*. Also, the pigmentation was measured with a colorimeter as the ability to market shrimps usually depends on the level of their pigmentation which is associated with freshness and quality of the product (Boonyaratpalin et al. 2001).

Two trials with commercial feeds containing 37% (Group A) and 48% protein (Group B) were undertaken with *P. vannamei* of a mean initial weight of 14.5 ± 2.1 g. In each setup, a total of 165 shrimps were held in triplicates (55 shrimps/tank) at 29°C for 100 days.

Survival in Group A was 80.6% compared to 57.6% in Group B. Also, significantly higher weight gain was recorded for Group A (F= 92.821; p= 0.0001; see Fig. 1).
Furthermore shrimps of Group A were darker compared to Group B before and after cooking (see Fig. 2).

Our findings will be complemented by further analysis of biochemistry (e.g. fatty acid-, amino acid profile) as well as astaxanthin content of *P. vannamei* and the commercial feeds.

The final results of these measurements are still in progress and not available at time of abstract submission. They will be presented at the resubmitted version of this abstract.

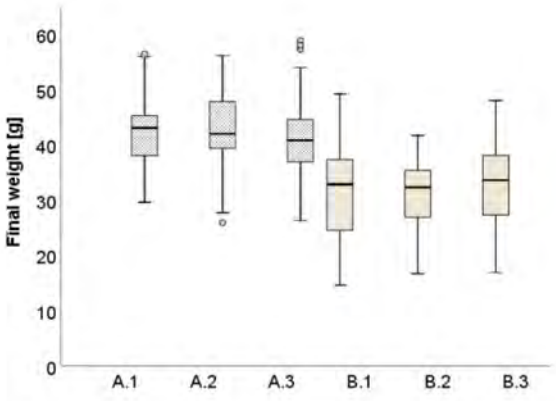

Fig. 1. Average final weight of *P. vannamei* after 100 days fed with two different commercial feeds with 37% (A) and 48% (B) protein content, respectively.

Fig. 2: Visual appearance of *P. vannamei* before (top) and after cooking (bottom), fed with two different commercial feeds with 37% (A) and 48% (B) protein content, respectively.

GROWTH PERFORMANCE AND SURVIVAL OF WHITELEG SHRIMP *Penaeus vannamei* UNDER VARYING STOCKING DENSITIES

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The whiteleg shrimp, *Penaeus vannamei* Boone, 1931 is one of the most farmed crustaceans globally (FAO 2018). The production of animals does not only occur in open ponds but also in indoor clearwater recirculating aquaculture systems (RAS). In this regard, management factors such as water quality and stocking density are crucial for an economic production of cultivated shrimp.

The production performance of whiteleg shrimp reared with different stocking densities was evaluated for postlarvae (PL 12) and juveniles (Ø weight 2.5g) for 2 and 4 weeks, respectively. Individuals were cultivated in triplicates under controlled experimental conditions in a recirculation system of 100 l aquaria with a salinity of 20 ppt. Postlarvae were held at densities of 6, 35 and 70 shrimps/l and juveniles at 300, 600, 900 and 1200 shrimps/m². Length-weight ratio and survival were compared between treatments at the end of the experiment.

Postlarvae showed higher survival of more than 50% when held at densities of 6 and 35 shrimps/l in contrast to a density of 70 shrimps/l with only 30% survival. The specific growth rates (SGR) were 3.5 %/day for 6 shrimps/l and 2.8 %/day for 35 shrimps/l and 70 shrimps/l.

In juveniles, also the two groups held at lower densities revealed higher survival of 90% compared to the two higher densities with 70% survival. The results also indicated a higher SGR for the lower stocking densities with 3.3 %/day (300 and 600 shrimps/m²) compared to 2.5% (900 and 1200 shrimps/m²).

FEEDING STRESS DUE TO SOY BEAN MEAL AS A MODEL FOR THE DEVELOPMENT OF MOLECULAR IMMUNE MARKERS IN RAINBOW TROUT

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Introduction
The increasing intensification of aquaculture practices has led to public debate about the welfare status of cultured fish

In order to verify the immune status of trout in context of husbandry stress and to investigate the relationship between chronic stress, immunosuppression, husbandry and feeding we established molecular stress markers using a feeding stress experiment with soybean meal. The welfare status of trout was verified by investigation of mRNA expression of different potential stress regulated genes in whole blood to establish a minimal invasive method.

Materials and methods
A 56-day feeding experiment was carried out. The triplicate fish groups were fed isoenergetic and isonitrogenic feed mixtures in which the fish meal (50 % of the total diet) was replaced by 0 %, 33 %, 66 % and 100 % soybean meal. EDTA blood was collected from the caudal vein of immobilized trout. In total 88 different genes were tested for their suitability in stress detection using a Fluidigm Biomark HD and a Light Cycler System. Specific primers were designed. Regulated genes belonging to the superior signal transduction pathways such as SERPIN G superfamily, intracellular PI3K/actin, Toll-like receptor, NF-κB, MAP kinase and JAK-STAT signal transduction or intracellular pathogen recognition receptors were tested. The mRNA expression of blood cells was tested for different pro- and anti-inflammatory cytokines, chemokines, substances involved in the acute phase reaction, complement cascade or inflammatory reactions, and heat shock proteins. Finally, different marker genes for specific cell populations were investigated. The housekeeping genes β-Actin, EF1 and RPS5 served as internal standards.

Results and Discussion
Different genes (e.g. SAA, MPO, NOS2, UCP2) emerged as suitable stress and immune markers and therefore as welfare indicators on a molecular level, while some genes (e.g. IL10, IFN, HSP47) revealed no correlation to feeding stress.

The results represent an important basis for a better assessment of animal welfare in trout farming. They are an important first step towards making well-founded, early assessments of chronic feeding stress of trout in the future, which are minimally invasive. So far, the parameters have often been based on observations such as behavior, color changes and such aspects that are difficult to standardize. These results provide a basis for the development of practical detection systems - comparable to a diabetes test.

EFFECTS OF FERMENTED AND UNFERMENTED DUCKWEED AS FEED ADDITIVE ON GROWTH PERFORMANCE OF COMMON CARP (*Cyprinus carpio*)

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Introduction

As a favoured ingredient, fishmeal is still a major part of most aquacultural diets (Lim and Webster, 2006). Since fishmeal and fish oil resources are decreasing, the replacement of these typical feed ingredients with more sustainable dietary components is desired. Therefore, duckweed with its high protein content and enormous growth rate is a promising substitute for the animal-based components in aquacultural diets.

Both fermented and unfermented duckweed have already been fed to fish. Best growth performance was achieved with fermented duckweed, whereas the fermentation process also improved other nutritional properties, e.g. it reduced the fibre content and the concentrations of antinutritional factors (Bairagi et al. 2002).

To develop more sustainable diets for Switzerland's aquaculture, the effects of either fermented or unfermented duckweed (*Spirodela polyrhiza*) were investigated in a feeding trial using common carp (*Cyprinus carpio*).

Materials and Methods

The best starter culture for the fermentation of duckweed was investigated in small-batch trials, whereby four different cultures of single strain or multi strain cultures of fermentation bacteria or fungi were tested. The large-scale fermentation was carried out with a combination of *Pediococcus pentosaceus* and a commercial mixture of bacteria and yeast (EM 1, EM Schweiz AG, Switzerland). The fermented duckweed and freshly harvested duckweed were dried, grinded and used for diet formulation. An animal protein-based control diet and the two duckweed-containing diets were extruded to contain 30% unfermented or fermented duckweed powder, respectively.

For assessing the growth performance of common carp fed the experimental diets, a subsequent feeding experiment was conducted at the Zurich University of Applied Sciences. Therefore, 630 fish with an initial weight of 60g were reared in three identical and independent recirculating aquaculture systems. Each diet was fed in triplicates to groups of 70 fish for 94 days. Three samplings were carried out to evaluate the effects of the experimental diets by measuring the length and weight of fish, calculating organosomatic indices and their specific growth rate. In addition, sensory tests were conducted and the filet quality was investigated

Results

The small-scale fermentation tests revealed that the fermentation of duckweed with *P. pentosaceus* and EM 1 had positive effects on the content of anti-oxidative substances, whereas the protein content was only slightly reduced. The fermentation process also affected the content of anti-nutritive substances. First estimations of the effects of all experimental diets on the growth performance of carps will be available for the second submission date in July whereas the results of the entire feeding experiment will be ready for presentation at the conference.

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GENOMIC CHARACTERIZATION AND EXPRESSION LEVEL ANALYSIS OF MALECTIN FROM BIG BELLY SEAHORSE *Hippocampus abdominalis*

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Introduction

Malectin is a novel carbohydrate-binding lectin type ER resident protein, with unique selectivity for the Glc_2-N-glycan. It was first identified in *Xenopus laevis* as an intracellular lectin involving in glycoprotein quality control. Structure determination by nuclear magnetic resonance showed the luminal part of malectin is a carbohydrate binding domain that recognizes glucose oligomers. Carbohydrate microarray analyses revealed a uniquely selective binding to a Glc2-*N*-glycan probe (Chen et al., 2011; Schallus et al., 2008)nine mannoses, and two N-acetylglucosamines (Glc(3. Most of endoplasmic reticulum (ER) synthesized proteins are glycoprotein in nature. The N-linked oligosaccharide moieties in glycoprotein is the modificatio which employed for highly diverse function. They serve as a ligand for multiple recognition process and stabilize proteins from denaturation and proteolysis, increase solubility, immune response modulation, facilitate protein orientation to relative membrane, regulation of protein turn over, fine-tune the charge and isoelectic point of protein, and pathogen interaction (Helenius and Aebi, 2004). In the present study we identified and molecularly characterized the malectin from big-belly seahorse *Hippocampus abdominalis* (*HaMLEC*). The immune response was evaluated using challenge experiment.

Methodology

Seahorse transcriptome library was constructed and the cDNA sequence of *HaMLEC* was identified. In-silico study was performed using different bioinformatic tools as follows; Unipro UGENE software, SMART, ExPASy Prosite, ExPASy ProtParam tool, EMBOSS Needle Pairwise Sequence Alignment Tool, ClustalW 2.0 program, and MEGA version 7.0.26 software. Tissue specific mRNA expression and mRNA expression at time post challenge (*Edwardasiella tarda*, *Streptococcus iniae,* polyinosinic: polycytidylic, and lipopolysaccharide) in blood tissue was analysed by qPCR using respective cDNA sample.

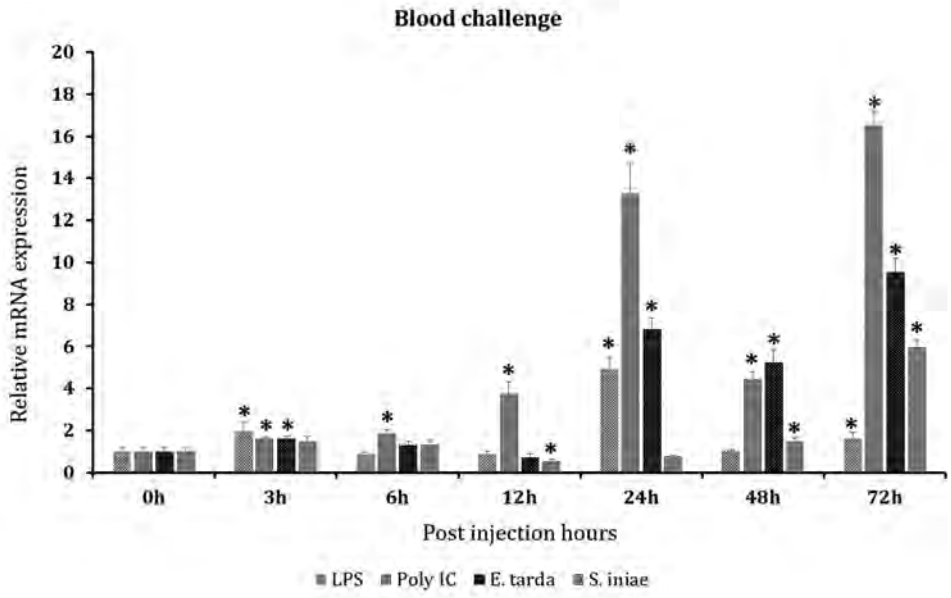

Fig. 1. Temporal mRNA expression level of *HaMELC* in blood following LPS, poly I:C, *E. tarda,* and *S. iniae* stimulation. The relative *HaMELC* transcript levels were determined using the Livak method. Each bar represents the standard error (SE) of triplicate (n=3).

(Continued on next page)

Results

The *HaMELC* cDNA sequence was identified with an ORF of 864 bp, encoding 288 amino acids with the predicted molecular weight of 31.99kDa and estimated pI of 5.17. Protein domain database "SMART" revealed Malectin super family (39 - 199), N-terminal signal peptide (1 – 26) and C-terminal transmembrane region (267 -286) from the amino acid sequence of HaMELC. The deduced amino acid sequence of HaMELC showed 88.5%, 87.2%, 74.4%, 71.9%, 61.6%, and 37.3% identity with that of *Cynoglossus semilaevis, Oryzias melastigma*, *Bos taurus*, *Homo sapiens*, and *Nanorana parkeri, and Drosophila serrata* respectively. In the phylogenetic analysis HaMELC was main clustered into vertebrate and subclustered into fishes with *Cynoglossus semilaevis* and *Oryzias melastigma*. The *HaMELC* mRNA expression was observed in all tested tissues by real-time PCR (blood, brain, gill, heart, intestine, liver, kidney, muscle, ovary, pouch, skin, spleen, stomach and testis) with highest expression in ovary followed by brain. The *HaMELC* mRNA expression in blood showed significant upregulations during the experimental period except the down regulation at 12 hour post challenge with *S. iniae*. Significant upregulation was observed at 72 hour post immune challenge with all four stimulants (Figure 1)

Discussion and conclusion

According to the in-silico analysis, the typical MELC super family was obtained with signal peptide and transmembrane region from the identified *HaMELC* cDNA sequence. The sequence homology of HaMELC with MELC sequences from other species was further confirmed by phylogenetic analysis, pairwise sequence alignment and multiple sequence alignment. The observed significant upregulations following the immune challenge suggested that HaMELC might have a significant role in host immune response

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HOW AQUAPONICS CAN HELP URBAN AGRICULTURE

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Aquaponics is an environmentally friendly method of food production benefiting from the integrated cycle of aquaculture and hydroponics. It uses a closed-loop water cycle with little run-off to the environment and minimum requirement for plant fertilizer. Considering the water scarcity around the globe and all of the problems agriculture industry is facing concerning water, more water efficient practices in the field such as aquaponics are becoming the wave of the future. Aquaponics might be a candidate solution for urban agriculture specificall . This is a study on the water efficiency of an outdoor semi-commercial aquaponics system growing lettuce and tilapia located in California State Polytechnic University at Pomona. The annual water consumption of a raft bed system with the growth capacity of 250 plants per cycle was measured. The results were compared to the published data for the conventional soil farming in California and the world. Our finding shows that the outdoor aquaponics system can produce crop with 25% water efficiency enhancement in Southern California climate.

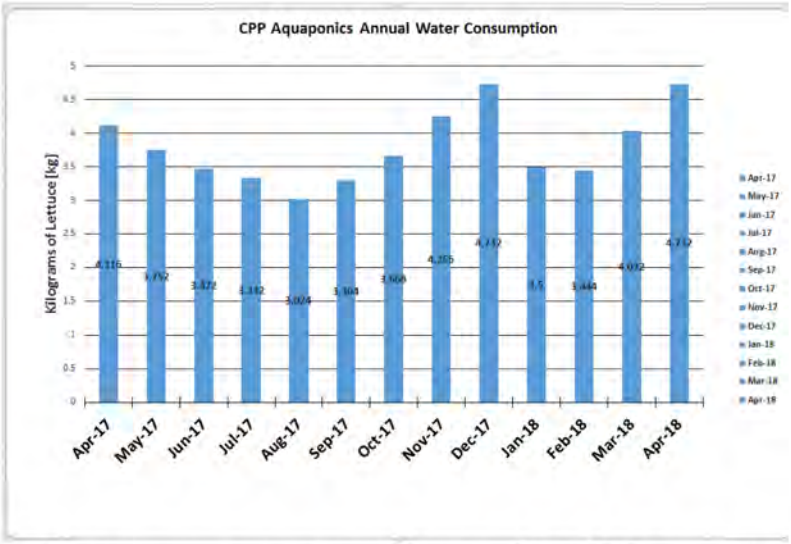

Figure 1- The annual water consumption per crop yield measured in Cal Poly aquaponics outdoor system.

CFD MODELING OF SEMI-CLOSED CONTAINMENT FLOATING SYSTEM WITH FLEXIBLE WALLS: EFFECT OF INLET ORIENTATION ANGLE ON THE FLOW FIELD HYDRODYNAMICS

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Introduction

Aquaculture industry is progressively interested in producing and utilizing larger semi-closed containment systems (SCCS) to accomplish high production goals and economy-of-scale. Consequently, the large-scale systems lie in a category of high Reynolds number, which makes the flow in SCCS fully turbulent. The overall hydrodynamic performance of the system is influenced by inflow characteristics, [4] i.e. turbulence produced by inlet orientations [5]. An actual experimental study on such a high flow condition that involve velocity, uniformity, vorticity and swirl number changes is not feasible. Therefore, computational fluid dynamics modeling (CFD) is considered as a most appropriate tool to investigate the hydrodynamics of large systems. The main object of this study was to investigate the effect of inlet orientation angle on the hydrodynamics of SCCS with flexible walls and establish the optimal inlet orientation set-up for use during Atlantic salmon production in this system.

Materials and Methods

The cone shaped floating bag with flexible walls and total volume of 10 000 m^3 received water from four inlet pipes. The water was discharged through 14 sidewall outlets located at depth up to 15.5 m and bottom central drain located at 21 m depth. In order to determine the most optimal pipe orientation set-up for the system total of six cases were developed in CFD (Table 1) and compared with the empirical data collected from floating bag. Grid convergence tests was used to verify CFD model and Acoustic Doppler velocimetry (ADV) was used for velocity measurements. The hydrodynamics of the system was evaluated using different flow field indicators, such as flow velocity, distribution of vortices, turbulence in the system and vorticity.

Naiver Stokes equation for incompressible fluids was solved with SIMPLE algorithm, which is Semi Implicit Method for Pressure Linked Equations. Where initially pressure and velocity values are estimated by algorithm and later pressure-correction equation $\nabla^2 p\uparrow = 1/\Delta t\ (\nabla \cdot V)$, is solved to obtain a corrected value of pressure and velocity field and at the solution convergence is checked [3]. A k-omega SST turbulence model with first order accuracy in space and time is used to solve Turbulence Kinetic Energy (k) and Specific Dissipation Rate (ω) [1,2]. In present study, one assumption in selection of boundary conditions is that no external force factors are included in the system (sea waves effect).

Results

In all 6 cases empirical data show relatively higher velocity by a factor of 3 (at 2 and 7.5-meter depth) and 14 (at 15.5-meter depth) as compare to CFD simulations. However, both empirical and CFD data show similar velocity pattern and mixing across the whole system. Out of 6 analyzed cases, the best hydrodynamics in the system (Figure 1a and 1b) was achieved by case 2 set-up (Table I) while the large variation in hydrodynamics was observed between cases. Observed differences in velocities between CFD models and empirical measurements were further investigated.

Discussion and conclusion

To investigate the hydrodynamics of large systems, we adopted two methods (Empirical and Computational) and compared them, respectively. The understanding of flow patterns could be performed more efficiently and relatively cheaper with the help of CFD modeling and simulations. In order to achieve a good qualitive and more reliable results, it is very important to develop a Solid CFD bench model. In this study, initially we developed a reliable CFD bench model and then examined the complete flow patterns for 6 cases with different inlet orientation angles (Table I). Large difference between the flow patterns are observed among various cases, based on their inlet orientation angle change. This factor has large impact on mixing and velocity factor across the system, which in turn effect water quality for optimal fish growth/welfare, health performance and particle removal. This study shows that further optimization of the system set-up is achievable with the help of CFD modeling.

Table (I). Different inlet orientations setup

CASE	Inlet 1 Angle	Inlet 2 Angle	Inlet 3 Angle	Inlet 4 Angle
1	36.55°	36.55°	36.55°	36.55°
2	36.55°	90°	36.55°	90°
3	5°	90°	5°	90°
4	5°	5°	5°	5°
5	36.55°	36.55°	36.55°	90°
6	45°	45°	45°	45°

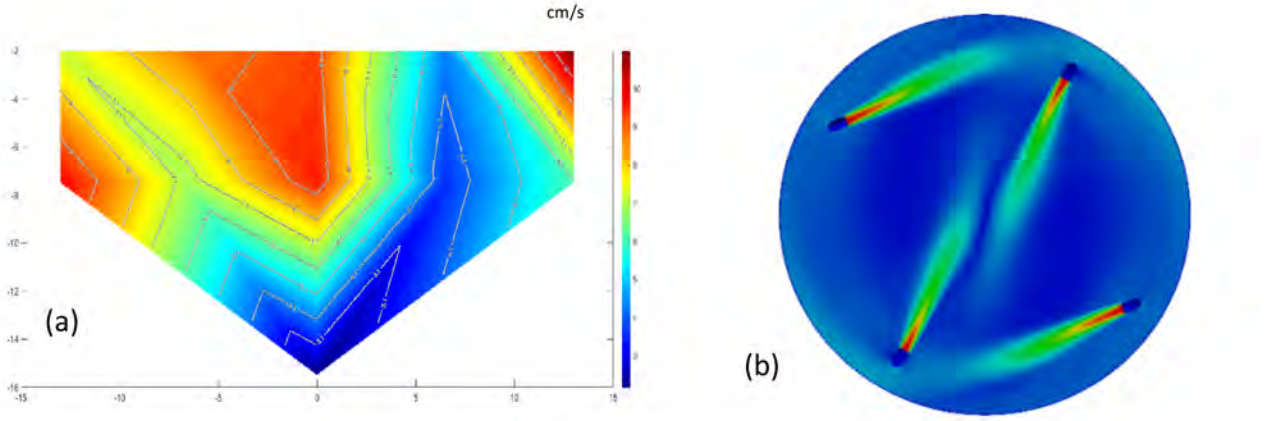

Figure 1 (a) Empirical velocity measurements slice at different depth and distance from the tank wall for case 2 and (b) Top view slice of velocity at 4 m depth. Four intake pipes orientation based on case 2 is shown.

Acknowledgements

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INFLUENCE OF GENETIC SELECTION FOR GROWTH AND DIETARY n-3 LC-PUFA LEVELS ON REPRODUCTIVE PERFORMANCE IN GILTHEAD SEA BREAM, *Sparus aurata*

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Introduction

It is well documented that dietary fatty acids such as arachidonic (ARA, 20:4n-6), eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids are important for ensuring egg and larval quality and they significantly improve fecundity, fertilization, viability, hatching and larval survival rates in marine fish (Fernandez-Palacios *et al*., 1995; Izquierdo *et al*., 2001). Gilthead sea bream is a major marine fish farmed in Europe and is also subject to selective breeding programmes to improve growth performance, disease resistance and carcass quality (Afonso *et al.,* 2012). So far, no study has dealt with the influence of genetic selection for growth trait on the improvement of reproductive performance of gilthead sea bream. Recent work has also shown that potential of broodstock selection improved the spawning quality and the subsequent nutritional programming had a positive effect on the utilisation of low fish meal (FM) and fish oil (FO) diets by the progeny (Izquierdo *et al*., 2015; Turkmen *et al*., 2017). The present study was undertaken with gilthead sea bream broodstock to determine the effects of selection for high (HG) or low (LG) growth and dietary fatty acid source, fish oil (FO) or rapeseed and linseed oil (VO) and its interaction on reproductive performance of gilthead sea bream.

Materials and methods

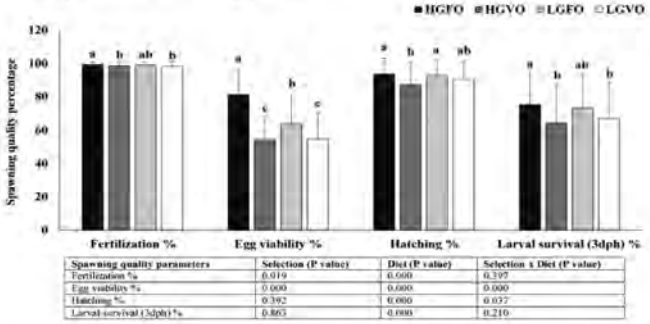
Experimental broodstock

Gilthead sea bream broodstock were used from third generation of selection under the PROGENSA (Spanish National Breeding Program) project (Afonso *et al.,* 2012). Two broodstock groups expressing either high growth (HG), or low growth (LG) were selected for the assessment of reproductive performance. HG and LG trait broodstock were kept separately for mass spawning in four tanks at the facilities of ECOAQUA institute (Marine Science & Technology Park, ULPGC, Canary Islands, Spain).

Dietary treatments

The broodstock feeds were formulated to be iso-proteic and iso-lipidic with either fish oil (FO) or rapeseed and linseed oil (VO) as the lipid source and were produced by Skretting ARC, Stavanger, Norway. Broodstock were fed with the respective diets at the rate of 1% body weight in twice a day meals for over 3 months.

Figure 1. Reproductive performance (% spawning quality) of gilthead seabream broodstock selected for high (HG) or low (LG) growth fed with either FO or VO diet during three months of experimental feeding period.



Spawning quality parameters	Selection (P value)	Diet (P value)	Selection x Diet (P value)
Fertilization %	0.019	0.000	0.395
Egg viability %	0.000	0.000	0.000
Hatching %	0.392	0.000	0.037
Larval survival (3dph) %	0.863	0.080	0.210

Means bearing different superscript letters differ significantly (P<0.05 by One-way ANOVA, Tukey Post-Hoc).

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Analysis of sperm quality

Sperm quality parameters such as sperm cell concentration (10^9 ml^{-1}), sperm viability (%) and sperm motility (%) were analyzed immediately after the semen collection.

Evaluation of broodstock spawning quality

The floating eggs were collected six days per week at 09:00 h from each mass spawning tank and egg number was counted under light microscope to estimate total fecundity, number of fertilised, unfertilised, viable and dead eggs. The viable eggs of each group were used to calculate hatching and larval survival (3 days post hatch, dph) rates.

Results

Broodstock selected for high growth fed with either FO or VO significantly (P<0.05) increased the sperm cell concentration (Table 1). In same way, HG broodstock fish significantly (P<0.05) improved the motility percentage irrespective of the dietary group. All the male broodstock exhibited same quality of sperm viability percentage (Table 1). In case of spawning quality, broodstock fish selected for high growth fed with fish oil (HGFO) based diet significantly (P<0.05) improved the egg viability percentage (Fig 1). The high or low growth selected broodstock fish fed with FO based diet shown the significantly (P<0.05) higher hatching and larval (3 dph) survival rate. Whereas, both HG or LG broodstock fed with VO based diet group exhibited significantly (P>0.05) lower level of egg and larval quality (Fig 1). The two-way analysis result revealed that both genetic selection for HG or LG and high n-3 LC PUFA (FO) diet fed broodstock group significantly improved the fertilization and egg viability percentage. Whereas, hatching and larval (3 dph) survival rate were only significantly improved by the high n-3 LC PU A (FO) diet broodstock group.

Conclusion

This study emphasizes the strong positive effect of broodstock (HG) selected for high growth improved the sperm concentration and motility percent and HG broodstock fed with high n-3 LC PUFA (FO) diet improved the egg viability percent. Further studies will address the effects of broodstock selection and dietary fatty acid level on progeny growth performance and their utilization of low FM/FO diets and assess the possible epigenetic changes in the offspring as affected by the nutritional history of broodstock.

Acknowledgements

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DIETARY UTILIZATION OF MEALWORM AND BLACK SOLDIER FLY FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Insects have received a great attention for their potentials as a protein resource in aqua-feeds. There is limited information on the insects for shrimp feeds. This study was conducted to replace partial fish meal (FM) with two insect meals for Pacific white shrimp. In a feeding trial, we used mealworm (MW) and black soldier fly (BSF) as the FM replacement. Tuna meal was used as the main protein source in a control diet and its 20, 40 and 60% was replaced with MW or BSF. Shrimp (body weight: 0.09g) were randomly stocked into twenty-eight experimental tanks with 4 replicates and fed the diets for 65 days. At the end of feeding trial, body weight was significantly higher in shrimp fed BSF 40 and 60% than that of shrimp fed the FM control diet. The lowest body weight or feed intake was observed in shrimp fed MW60%. There were no significant differences in composition of shrimp whole-body. This study indicates that BSF and MW can replace FM by 60% and 40%, respectively, in diets for Pacific white shrimp

Table 1. Growth performance of Pacific white shrimp *Litopenaeus vannamei* fed the experimental diets for 65 days (initial body weight: 0.09g)

Diets	FBW[1]	FCR[2]	PER[3]	FI[4]	Survival (%)
Con	5.28±0.22^{b}	1.42±0.05^{a}	1.90±0.06^{c}	7.38±0.13^{a}	92.0±2.00^{ab}
BSF 20%	5.54±0.29^{b}	1.36±0.03^{ab}	2.04±0.04^{b}	7.41±0.32^{a}	94.0±6.93^{ab}
BSF 40%	6.40±0.17^{a}	1.16±0.04^{c}	2.33±0.07^{a}	7.30±0.23^{a}	95.0±2.00^{ab}
BSF 60%	6.17±0.69^{a}	1.17±0.07^{c}	2.26±0.12^{a}	7.06±0.43^{ab}	93.0±6.83^{ab}
MW 20%	5.06±0.11^{bc}	1.36±0.04^{ab}	1.95±0.06^{bc}	6.75±0.27^{bc}	98.0±2.31^{a}
MW 40%	5.39±0.33^{b}	1.33±0.04^{b}	1.98±0.07^{bc}	7.04±0.31^{ab}	90.7±7.66^{b}
MW 60%	4.67±0.53^{c}	1.41±0.09^{ab}	1.88±0.11^{c}	6.40±0.36^{c}	94.0±2.31^{ab}

Values are mean of quadruplicates, and presented as mean ± SD. Values with different superscripts in the same column are significantly different ($P < 0.05$). [1]Final body weight (g); [2]Feed conversion ratio = dry feed fed / wet weight gain; [3]Protein efficiency ratio = wet weight gain (g) / total protein fed; [4]Feed intake (g) = dry feed consumed (g) / shrimp

THE GENETIC ANALYSIS OF TWO STERLET POPULATIONS AND THEIR INTER-POPULATION HYBRIDS

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Introduction

The sterlet (*Acipenser ruthenus*) is a Eurasian rheophile freshwater species which is relatively small in size among the Acipenserids. Although this species is less important for caviar production, it is threatened according to the IUCN Red List (2013). Restocking activities are being undertaken along the stretches of Danube in Romania and Hungary. Genetic variability considered as a key element for adaptability against the drastic climate change and anthropogenic pressures. Investigation of genetic patterns within and between populations is considered as a prerequisite for a successful recovery program (Reinartz et al., 2011; Friedrich, 2018). As such the question of the proper source for recovery, stocking is becoming an urgent question for the initial phase of the recovery programs (Boscari et al., 2014). One likely option for restocking could be the mixing fish from different isolated populations to increase the genetic diversity and allow future selection based upon the natural pressures in the natural waters (Friedrich, 2018). The fitness of intra-specific hybrid can be higher (heterosis) or lower (outbreeding depression) than that of their parental populations. Besides, heterosis or neutral outcomes are not considered detrimental to conservation efforts, henceforth the focus of selecting candidate populations should be on the prevention of outbreeding depression. Here, we performed the genetic analysis of hatchery stocks originated from Volga and Danube sterlets using a set of microsatellite loci and, selected the best candidate parents for production of intraspecific hybrids. Also, we compared the genetic diversity among the produced purebreds and hybrids in order to find any hybridization incurred genetic incompatibilities and the genetic fate of F1 hybrids.

Materials and Methods

The tissue sample (fin clip) from 100 fishes were collected from hatchery stocks originated from Danube (Rybnikarstvi, Pohořelice) and Volga (Genetic Fisheries Center, Vodňany) respectively and stored in 96% ethanol. Six microsatellite markers viz., AciG 35, AfuG 135, Aox 45, Spl 101, Spl 163 and Spl 173 were used for amplification. The PCR amplification was carried out according to the procedure described by Havelka et al. (2013). The mean number of effective alleles (N_A), allele diversities of each locus, the fixation index (F), pairwise population differentiation (G_{ST}), DA genetic distance, expected (H_e) and observed (H_o) heterozygosities between Danube and Volga populations were calculated using GeneAlex software. The male and female candidates which expressed more number of discriminatory alleles in both populations were chosen for production of hybrids (also reciprocal hybrids) and purebreds. The level of polymorphism among produced hybrids and purebreds was assessed as described above.

Results

The allele diversity was found to be highest in Danube population for locus Aox45 (0.882). The overall allele diversity among all the loci was high in Danube population when compared to Volga population. The observed heterozygosity followed by expected heterozygosity was found highest in Danube population. The pairwise G_{ST} and D_A matrix value between Danube and Volga populations were 0.136 and 0.549 respectively. These significant values showed that the Danube and Volga stocks are moderately genetically differentiated. Likewise, the mean number of alleles was significantly higher in Danube × Volga hybrid, whereas Volga purebred displayed least mean number of alleles. The observed (0.6962±0.0498) and expected (0.7589±0.0685) heterozygosities was high in the Danube × Volga hybrid.

Discussion

Our experiment revealed that the Danube and Volga stocks are moderately genetically differentiated. It is highly essential to track the genetic condition of the broodstock constantly. The selection of broodstock containing spawning individuals characterized by high genetic variation is done when the genetic diversity values reaches critical level. In such instances, selection of spawning pairs based on genetic profiles is highly required (Kaczmarczyk and Fopp-Bayat, 2013). The mean number of alleles and, expected and observed heterozygosities were significantly high in one of the hybrid. Inheritance of genetic material from one population to another by hybridization serves as a source of adaptive genetic variation (Grant and Grant 1994). Our results shows that these hybrids can be used to exploit the heterosis over purebreds for aquaculture purposes. We also suggest to avoid use of these hybrids for restocking purposes since our results revealed the selected Danube and Volga broodstocks has moderate genetic divergence which eventually can possibly give rise to outbreeding depression in successive generations and can be detrimental to the wild stocks.

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EFFECTS OF SALINITY ON CA^{2+} TRANSPORTERS IN THE MANTLE TISSUE OF *Crassostrea gigas*

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Introduction

The bivalve shell is composed of 95-99 % calcium carbonate ($CaCO_3$) while the rest is made of organic matrix consisting mainly of proteins and polysaccharides which are thought to control the crystallization of $CaCO_3$ (Marin et al., 2012). To build and maintain the shell, calcium needs to be taken up from the environment and transported to the area of shell growth through the mantle tissue which separates the shell from the rest of the animal body. The final layer of transport is the outer mantle epithelium (OME) facing directly the shell growth area.

In theory, calcium could be transported across in the OME either as free ionic Ca^{2+} either intra- or paracellularly, bound to organic or inorganic ligands, bound to proteins and/or within vesicles or hemolymph cells (Marin et al., 2012). Ionic Ca^{2+} transport across the OME has been shown to consist of both passive paracellular and active transcellular transfer. Ca^{2+} enters the OME through voltage-gated Ca channels across the basal cell membrane and transported further to the shell growth area via plasma-membrane Ca^{2+} ATPase (PMCA) and Na^+/Ca^{2+} exchanger (NCX) (Sillanpää et al., 2018).

Global increases in temperature will intensify the Earth's water cycle, creating increased evaporation which in turn will result in storms with increased precipitation in certain areas but also contribute to drying over some land areas. It will also result in increased melting of the sea ice and the ice sheets of both the Arctic and Antarctic areas. This increase in fresh water output to the oceans does not only create rising sea levels but will potentially also affect the salinity of the waters (IPCC 2014). Lowering seawater salinity and thus ion concentration could potentially affect calcifying organisms which dependent on the supply of Ca^{2+} and CO_3^{2-} ions from the environment.

In this study, the expression of potential Ca channels and transporters was measured in the mantle tissue of *C. gigas* exposed to different salinities. Additionally, ion transfer across the OME was assessed using the Ussing chamber methodology.

Materials and methods

Pacific oysters (*Crassosotrea gigas*) were exposed for two salinities, 16 ppt and 32 ppt for the duration of 14 days. After the exposure, tissue samples from mantles were sampled and frozen in liquid nitrogen. The gene expression of Na^+/K^+ ATPase, PMCA, sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA), NCX, L-type Ca channel and T-type Ca channel were measured using qPCR.

Additionally, to study the ion transport across the outer mantle epithelium, the OME of ten *C. gigas* from both salinities was dissected from the ventral side of the animal. Inner mantle epithelium and connective tissue were carefully removed and the remaining OME was placed on an Ussing chamber. The transepithelial resistance (TER), transepithelial potential (TEP) and short circuit current (SCC) were measured during the whole experiment (150 minutes). On the left half chamber (the inner side of the OME) radiolabelled ^{45}Ca as well as ^{3}H-Mannitol were applied. Samples were taken from the "cold" side at 10, 15, 20, 60, 80, 85 and 90 minutes from sampling. Concentration of transported calcium was calculated from the slope of appeared radioactivity against time.

Results

The expression of NKA and SERCA as well as Ca^{2+} transfer and total ion transport (measured as SCC) across the OME were higher in the salinity of 32 ppt compared to 16 ppt. The expression of T-type Ca channel as well as paracellular permeability (measured as TER) were higher in 16 ppt compared 32 ppt.

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Discussion and conclusions

The electrophysiological parameters, TER, TEP and SCC revealed that the salinity affected ion transport. The net ion transport (measured as TEP) remained the same despite the salinity. Active transport (SCC) was higher in 32 ppt compared to 16 ppt, most likely due to the higher sodium content of the oysters' environment. This could also been seen from the higher NKA expression compared to the lower salinity as NKA is directly affected by the external sodium concentration (Meng et al. 2013). On the other hand, the TER was higher in 16ppt compared to the 32 ppt, which indicated lower paracellular permeability. Together these results show that the overall ion transport, both passive and active is decreased in 16 ppt compared to 32 ppt.

Although the active Ca^{2+} output (PMCA and NCX) did not seem to be affected by salinity, the expression of Ca channels, especially the T-type Ca channel increased when salinity was decreased to 16ppt. Meng et al. (2013) found a similar increase in Ca-channel expression in low salinities (10 and 15 ppt compared to 20 – 30) in the gills of *C. gigas*. They suggested this to be due the fact that the increased Ca^{2+} current in low salinity would induce the activity of calcium-gated K^+ -channel partly responsible for maintaining the ionic balance inside the mantle cells.

From the perspective of calcifi ation, the increase in the expression of Ca channels in low salinity could aim to keep the Ca balance stable. Similarly the decrease in the expression of SERCA would indicate this, as less Ca is sequestered inside the mantle cells. However, Ca^{2+} transfer did decrease in 16 ppt as was seen from the Ussing chamber measurements. An alternative explanation is offered by the theory that OME cells produce vesicles containing amorphous $CaCO_3$ (ACC) which are then transported to the shell growth area (Li et al., 2016a). To be able to precipitate ACC, OME cells would need to take up calcium but the deposition would be executed by exocytosis instead of using active transport. This would explain the increase in ionic Ca^{2+} transfer into the cytosol without similar increase in the expression of active Ca^{2+} transporters.

Ca^{2+} uptake into the cells might also be related to Ca-dependent signaling pathways (Zhao et al. 2012; Zhang et al. 2016). The animal might try to maintan Ca homeostasis despite the low environmental salinity, ie. to keep the intracellular Ca^{2+} concentration stable

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Sinularia flexibilis AQUACULTURE: DOES LIGHT MATTER?

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Introduction

The growing demand for corals as ornamentals (Wabnitz et al., 2003)worth an estimated US$200-330 million annually, and oper- ating throughout the tropics. Ornamental marine species (corals, other invertebrates and fish, potential organisms for bioprospection (Leal et al., 2012), or good biological models (Watanabe et al., 2007) support a rather new sector for aquaculture. Coral cultures are a potentially sustainable, and constant biomass supply, with reduced impact on the ecosystem. A decisive next step is to overlap the gap of knowledge on culture protocols for key coral species that can meet the industry demands. *Sinularia flexibilis* (subclass Octocorallia, order Alcyonacea) is an exploited soft coral commonly found in Indo-Pacific reefs (Ofwegen, 2000) with suitable features for husbandry (*e.g.* bioactive compounds production). Photosynthetic corals rely on unicellular dinoflagellates (zooxanthellae) for photosynthesis. As mixotrophs, photosynthesis plays a decisive role on this organism energy budget (Falkowski et al., 1984). Therefore, understanding *S. flexibilis* response to light is crucial for a better husbandry. In this study we observed the energy allocation and photosynthetic response to light intensity and spectra variations.

Materials and methods

Coral specimens were collected in Indonesia and acclimated during two months in 100 -120 μmol.m^{-2}.s^{-}1 with total spectrum artificial light (12 hours photoperiod) in a standardized modular system (Rocha et al., 2015)biotechnological applications, and to supply the marine aquarium trade has prompted researchers to optimize coral culture protocols, with emphasis on ex situ production. However, the diversity of experimental systems employed to investigate ex situ coral production may be a bottleneck to the advance of the state of the art, as it impairs reliable comparisons between experiments, as well as the replication and optimization of culture protocols. This study presents a versatile modular culture system for experimental coral production ex situ, assembled using materials and equipments available from suppliers all over the world and thus allowing researchers worldwide to truly replicate experimental setups. The validation of the modular culture system was performed using the soft coral, Sarcophyton cf. glaucum, as a model organism. The validation experiment tested the effect of different light spectra on the photosynthetic performance, symbiont density, chlorophyll and carotenoid pigments concentration, survival, and growth of coral fragments. The validation experiment confirmed the potential of this modular culture system, which ultimately enables researchers to perform direct comparisons among experiments, and more efficientl contribute to advance ex situ coral aquaculture.”,”author”:[{“dropping-particle”:””,”family”:”Rocha”,” given”:”Rui J.M.”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family”:”Bont s”,”given”:”Bogdan”,”non-dropping-particle”:””,”parse-names”:false,”suffi ”:””},{“dropping-particle”:””,”family”:”Ca rtaxana”,”given”:”Paulo”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”family” ”Leal”,”given”:”Miguel C.”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”fami y”:”Ferreira”,”given”:”José M.”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”f mily”:”Rosa”,”given”:”Rui”,”non-dropping-particle”:””,”parse-names”:false,”suffi ”:””},{“dropping-particle”:””,”fami ly”:”Serôdio”,”given”:”João”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””},{“dropping-particle”:””,”fa ily”:”Calado”,”given”:”Ricardo”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””}],”containe -title”:”Journal of the World Aquaculture Society”,”id”:”ITEM-1”,”issue”:”3”,”issued”:{“date-parts”:[[“2015”]]},”page”:”235-251”,”title”:”Development of a Standardized Modular System for Experimental Coral Culture”,”type”:”article-journal”,”v olume”:”46”},”uris”:[“http://www.mendeley.com/documents/?uuid=685fb87c-913a-40f9-98e8-b9ed8b4c9834”]}],”mend eley”:{“formattedCitation”:”(Rocha et al., 2015. After acclimation, one mother colony was fragmented into 28 fragments and randomly assigned for the different treatments (n=7). Red and blue spectra were used along with 120 ± 10 μmol.m^{-2}.s^{-1} (high) and 70 ± 10 μmol.m^{-2}.s^{-1} (low) photosynthetic active radiation for each spectrum. After four months photosynthetic performance (Rapid Light Curves) and energy allocation were assessed. Data was analyzed in R (2018) and a two-way ANOVA was performed.

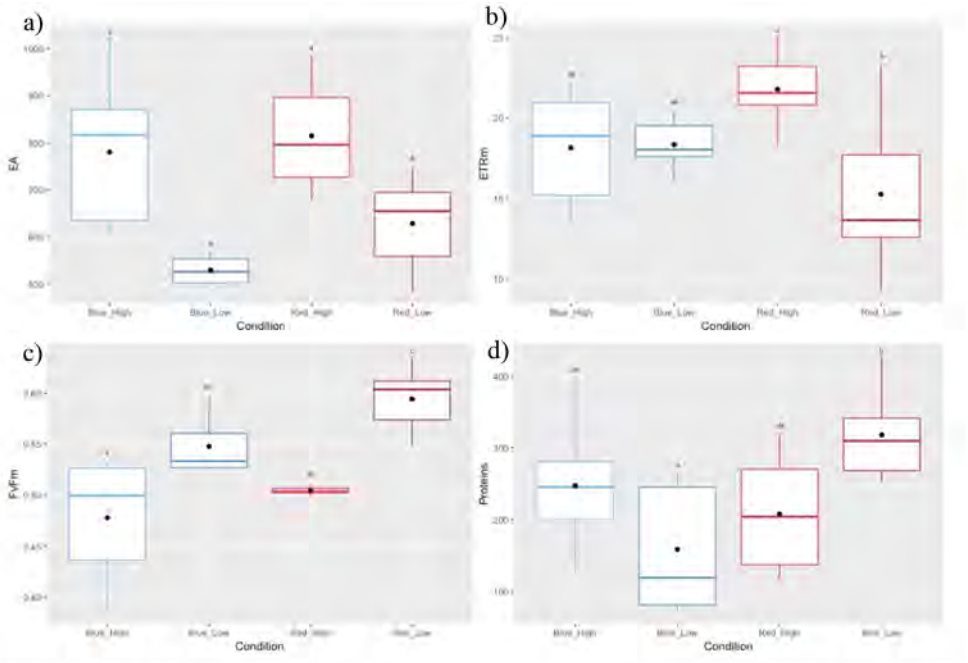

Figure 1- Boxplots for a two-way ANOVA significant differences for each condition (color and light intensity). a) Energy available significantly different for light intensity (p=0.001); b) Maximum electron transport rate significantly different for light intensity (p=0.033) and interaction between light intensity and color (p=0.024); c) Maximum photosynthetic potential significantly different for color (p=0.007) and light intensity (p<0.001); Total protein significantly different for interaction between light intensity and color (p=0.005).

Results

Red spectrum with low light intensity displayed more total protein (=309.35), better photosynthetic response (α) (=0.12), and improved maximum photochemical efficiency of photosystem II in a dark adapted state (Fv/Fm) (=0.60). On the other hand, the same condition showed lower tolerance to high light intensity, specificall , Ek (=110.61) and ETRmax (=13.63), and scarce energy available (=654.78). Blue spectrum with high light intensity showed significantly more energy available (=816.79) and better tolerance to high light intensity, namely high Ek (=211.06) and ETRmax (=18.88), but also lower total protein (=245.50), lower maximum photosynthetic potential (=0.50) and worse photosynthetic response (=0.09).

Discussion

Our data suggest that red spectrum with low light intensity incites *S. flexibilis* to allocate energy on protein assimilation (e.g. growth) while being photosynthetically efficient, which are better characteristics for husbandry under stable and controlled conditions. Alternatively, blue spectrum with high light intensity seems to be a better condition to prepare for less stable conditions (e.g. restocking), suggesting higher resistance to environmental variation, but lower fit to husbandry.

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MAIN DIFFICULTIES FACED BY AQUAPONICS PRACTITIONERS IN BRAZIL

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Introduction

Aquaponics is a novel and developing industry in which the combined production of aquatic organisms and plants is carried out. Aquaponics faces multiple challenges since it is a relatively new activity (Yep and Zheng, 2019). According to Goddek et al. (2015), more quantitative research is needed to develop the economic feasibility of aquaponics systems. Technical issues like optimum pH (Zou et al., 2016), nutrient balance (Nhan et al., 2019) and pest and disease issues (Mori and Smith, 2019) among others have been studied. The economic feasibility of aquaponics also has been the subject of research; however, there are not consensus about it (Greenfeld et al., 2018). Also, research has been carried out that aims to characterize the participants of aquaponics activities through surveys; in these works, the economic sustainability of the production system and the necessity of more training related to fish diseases and plant pest were identified as challenges (Love et al., 2015; Villarroel et al., 2016). In order to propose research for the improvement of aquaponics systems and management it is necessary to know the manner in which the production is being carried out and to understand the current problems faced by the participants. The aim of this work was to identify the difficulties that the Brazilians in aquaponics are facing, with the intention of elaborating research avenues specific to improving the industry in the countr .

Material e Methods

From July to September of 2018 a questionnaire for the identification and characterization of aquaponics participants in Brazil was made available in Google Forms. This was promoted and shared through social media, schools, research and extension institutions, professional platforms and discussion groups related to aquaponics. In addition to identifying the participants, the questionnaire covered other topics such as their production purpose and their problems. The problems considered were: System implantation (SI), control of climate conditions (CC), lack of technical knowledge (LTK), lack of specific supplies (LSS), diseases in the aquatic component (DA), diseases in the plant component (DP), aquatic production performance (APP), plant production performance (PPP), aquatic production commercialization (APC), plant production commercialization (PPC), organic certification (OC). The respondents were asked to assign to each one, in ascending order, a score from 1 to 4 according to the level of difficulty (being 4 the highest) or they had the option to inform if that problem does not apply in their enterprise, which would receive the score 0. A general average value was calculated for each problem using the obtained data. Also, the average score of each issue was calculated and a cluster analysis was performed for each one of the production purposes groups.

Results

A total of 39 aquaponics participants from all the regions of Brazil responded to the online questionnaire and were classified into 8 groups according with their production purpose. The issues with the highest scores were LSS, CC and SI with an average score of 2.26 ± 1.23, 2.13 ± 1.22 and 2.00 ± 1.21 respectively. The lowest score was for APC with a value of 1.03 ± 1.29 (Figure 1).

The average score for the production purpose group showed that the highest score is different for some groups. For example, the highest score for “Commercial” and “Graduate and undergraduate” groups was obtained in OC with a value of 2.91 ± 1.87 and 4. And, in the case of the “Hobby” group the highest score was in the CC issue. The lowest score is also variable for the different production purpose groups. For “Hobby”, “Others” and “Subsistence” groups the score of OC was 0 (Figure 2).

The production purpose groups can be clustered in four sets according to the main problems identified. From these, the “Others” and the “Graduate and undergraduate” groups are alone in the first and second clusters respectively, the “Subsistence”, “Subsistence and surplus sale” and the “Commercial” groups belong to the third one and the “Hobby”, “Highschool” and “Scientific” groups belong to the fourth.

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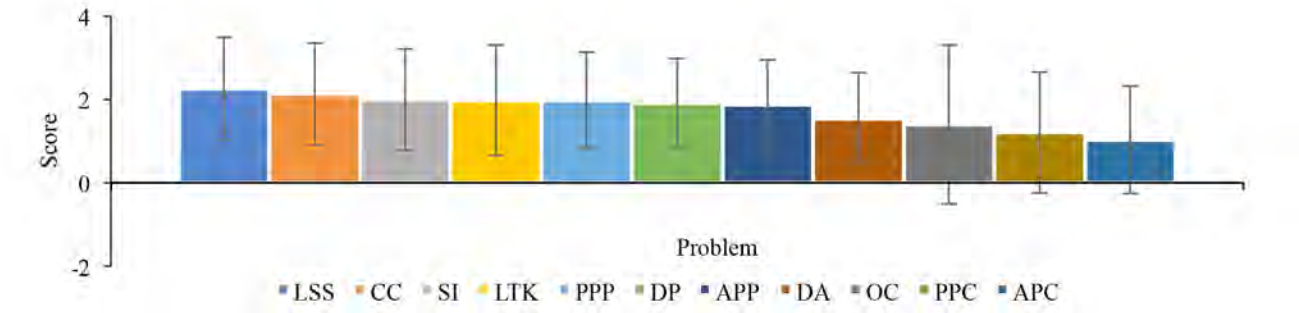

Figure 1. Average scores for each issue

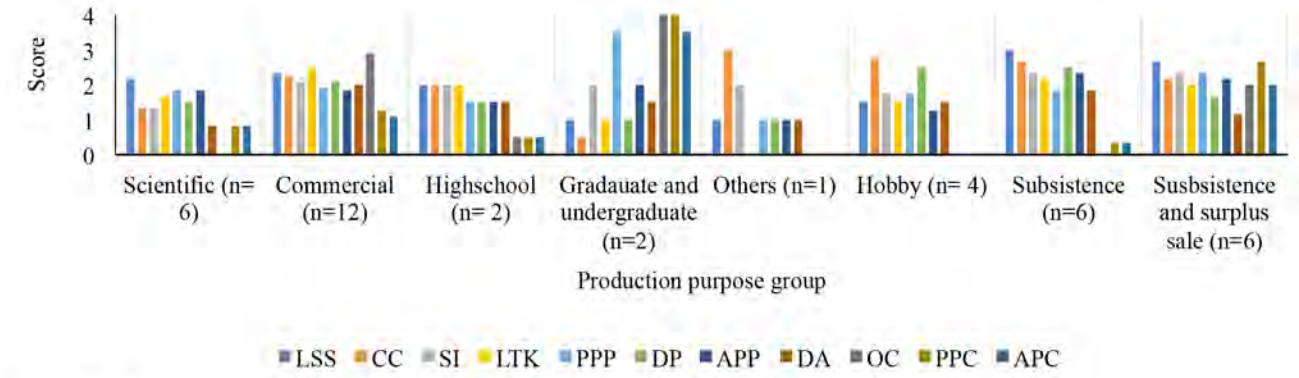

Figure 2. Average score obtained for each issue in the purpose groups.

Discussion and Conclusion

Depending on the target production group, the problems to be solved are variable. Besides the research interest of the scientists, efforts should focus on solving the problems of the target group. For the development of commercial aquaponics, research that covers the requirements of organic production should be carried out since this subject may improve the economic feasibility of the enterprise (Tokunaga et al., 2015).The research for subsistence aquaponics should be directed to systems designed with less specialized infrastructure, adapted to the climate conditions of the region and disease plant control.

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MICROALGAE BIOTECHNOLOGY AS A POTENTIAL NUTRITIONAL SOLUTION FOR FISH FEED

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Introduction

Aquaculture sector is growing faster than any other food-producing sector (FAO, 2018) and to meet this demand, different nutritional sources for the aquacultured animals have been discussed. Fishmeal is currently the preferred protein ingredient of feed in aquaculture industry; however, its complete or partial substitution by alternative sources is needed. Microalgae have been commonly used worldwide as an alternate protein source replacing fishmeal

Fishmeal substitution by microalgae is considered to be environmentally sustainable, as it reduces dependency on finite marine resources and surpasses the issue rased by the extensive use of land when using plant-based ingredients. However, the nutritional requirements of certain aquatic species may limit the fishmeal substitution due to unbalanced nutrient rates (Huntington and Hasan, 2009).

Microalgal biotechnology has emerged in recent decades based on their extensive different applications, such as nutraceutical research, renewable energy source, production of bioactive and health-beneficial biomolecules like β-carotene, astaxanthin, PUFA, bio-colorant production, wastewater treatment, bioremediation, human food and animal feed (Daphne *et al.*, 2015). Besides used since early, biotechnology focused in microalgae is a relatively recent field and these organisms´ growth conditions and nutritional information still need clarification

In this context, the aim of this work was to access the potential of microalgae to be used as aquafeed, concerning their industrial production.

Material and methods

Different microalgae were addressed during the present study, namely *Chlorella vulgaris*, *Nannochloropsis oceanica*, *Tetraselmis* sp. *Chlorococcum* sp., *Scenedesmus obliquus*, *Phaeodactylum* sp.. All experiments were performed at the industrial production facilities of CMP/AlgaFarm/Allmicroalgae, Pataias, Portugal. A five step scale-up was approached, with cultures starting at 5 L up and being scaled-up until reaching the 2.5 m^3 pilot-scale photobioreactor (PBR) in appropriate culture medium. After growing experiment, cells were filtered and spray dried. Spray dried microalgae samples were used for analytical quantifications: protein, ashes, starch and fiber content, as well as the digestibility rate.

Results

Preliminary results indicate that, among the studied microalgae, *Chlorella vulgaris* was the one with higher productivity, reaching 0.22 g/L/day which were immediately followed by *Scenedesmus obliquus* (0.19 g/L/day). *Chlorococcum* sp. presented the lower productivity rate, growing at 0.10 g/L/day.

Chlorella vulgaris presented the higher protein content (59%), followed by *Scenedesmus obliquus* (53%). *Tetraselmis* sp. was the species with the lowest protein values, however with the higher digestibility rate, 89%. In accordance, this microalga presented a low rate of indigestible fibers. *Tetraselmis* sp. had also the higher starch content, 14%, while the other strains showed values between 1 and 4%. Together with *Nannochloropsis oceanica*, *Tetraselmis* sp. showed the highest level of ashes, 28%, when compared with the other analyzed microalgae.

Scenedesmus obliquus presented the lowest ash content, 8%, when compared with the other five studied microalgae, followed by the *Chlorella vulgaris* with 9%. *Scenedesmus obliquus*, *Nannochloropsis oceanica*, *Chlorococcum* sp. and *Phaeodactylum* sp. have minimum levels of starch, which vary from 1 to 2%.

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Discussion and conclusions

Chlorella vulgaris industrially grown at the ALGAFARM facilities seem to meet the nutritional characteristics for a good fishmeal substitute. Fish feed are mostly characterized by their protein content which is primarily needed for muscle tissues (Delgado and Reyes-Jaquez, 2017). *Chlorella vulgaris* observed protein levels are within the range of other aquaculture composition formulations (Rodríguez-Miranda, 2014). The amount of carbohydrates in fish feed is usually low since fish have a low capacity to digest carbohydrates (Delgado and Reyes-Jaquez, 2017). *Chlorella vulgaris* presents relatively low starch content; however, starch makes it also more suitable for the extrusion process (Delgado and Reyes-Jaquez, 2017). Besides, *Chlorella vulgaris* shows also to be the most productive algae, when compared to the others tested, in what concerns to biomass.

The present study shows that microalgae, particularly *Chlorella vulgaris*, can represent a nutritional sustainable source for aquaculture feed. However, further complementary studies are needed in order to evaluate other nutritional parameters such as the lipid content, the amount of vitamins present in the microalgae, as well as *in vivo* feed experiments with aquacultured fish

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CHALLENGES OF CONSUMER ORIENTED COMMUNICATION ABOUT SCIENTIFICALLY BASED SUSTAINABILITY STANDARDS FOR AQUACULTURE

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Problem and research questions:
Research demonstrates that consumers' knowledge about fishery and aquaculture is low. Their ideas are not so much based on facts but more on competing schemata. Requirements for sustainable fishery and aquaculture are vague and very general. Aquaculture should be "natural", "space allowance", "no drugs". Moreover fishes should live in fish friendly environment. (Verbeke et al, 2007a; Verbeke et al. 2007b, Feucht and Zander, 2015).

According to surveys sustainability aspects as well as ethical issues are relevant for a large share of consumers. However in purchase decisions these aspects play a minor role (Verbeke et al, 2007b; Feucht and Zander, 2015). But the relevance of sustainability activities in Germany increased during the last years especially fuelled by activities of retailers. In order to be able to communicate efficiently to consumers and valorise the benefits of higher production and processing standards it is necessary to get a deeper insight into the way that consumers perceive and process information on fisher .

Method:
To better understand the discussion about fishery and the psychological relevance of fish in consumers' everyday life we conducted interviews and group discussions during which the interviewees describe their everyday life with respect to fish and aquaculture. In line with the narrative paradigm (Fisher, 1984), we acted on the assumption that the experienced reality of human beings is organized and interpreted in stories and that interviewees reveal unconscious contexts based on detailed descriptions.

The interviews were analysed in the team of psychologists together with an expert in food markets and food value chains and one in fishery and aquaculture. This reduces individual biases and integrates knowledge on markets, value chains and sustainable fishery into the research process

30 in-depth interviews and six group discussions with eight to ten participants each were carried out in two waves, between November 2017 and May 2019.

Results:
Our analysis confirms previous results regarding the low level of knowledge about fisher . Moreover, ignorance of aspects that reduce the pleasure in food is a well-established way to reduce the complexity of everyday life and to enjoy food. It turns out that participants want to know, but that they feel overstrained to learn details about the systems and to bring them into a consistent concept.

However, a lot of ideas, images observations, and reflections came up during the interviews and group discussions. The mental representation of fish in general and aquaculture in particular is characterised by competing, contrasting, and ill-matching imageries. It is like pieces of a puzzle that do not result in a uniform picture.

Wild fish can trigger imageries of holidays. As a creature living in another element wild fish is a material symbol for a transition into another world. But even though wild fish evokes romantic narratives it is susceptible regarding images and ideas about polluted fish environment, contamination of fishes, exploitation of the oceans, and fishing methods that are neither fish-friendly nor environmental sound

"Aquaculture" mainly evokes schemata comparable with mass production. These schemata are very close to mass animal husbandry and characterised by an unworthy treatment of animals (cp. Simons et al., 2017). The use of antibiotics often is regarded as necessary in an environment supposed to be far away from natural. Compared to wild fish romantic and emotionally positive aspects are of minor relevance. A few emotional narratives came up, e.g. in the context of direct marketing of trout.

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The relevance of animal welfare issues with respect to fish seems to be lower than in the context farm animals. Still it is supposed that production and processing systems which are not close to nature and require the use of antibiotics negatively impact the food quality and safety. As a consequence fish welfare and fish friendly production is indirectly an important issue for consumers linked by perceived quality.

"Naturalness" is one of the dominant categories in the context of assessing food: as natural as possible is at the same time as good as possible. As aquaculture is supposed to be not natural, it has a disadvantage compared to "natural" wild fish. But wild fish has its dark sides, too. It faces a lot of image problems that can easily be activated and reduce the pleasure in eating. Against this background, farmed fish is a good but not perfect alternative in which the environment for fishes can be controlled

Conclusion:
Given the low level of consumers' knowledge there is a need for a scientifically based standard that takes into account consumers concerns and translates them into process requirements. Moreover, credible controlling and mediating institutions are necessary so that consumers can make the right purchase decision without exactly knowing why.

As consumers will hardly understand a scientific discussion and assess management practices based on their own knowledge, communication about the standard should be embedded into the narratives of consumers in order to bridge the gap between scientific arguments and consumers' perception. An example: Transferring fishes from one basin or pond to another through tubes and by help pumps may cause immediate stress to consumers as the idea of being helpless in a water stream arouses claustrophobic fears. In order to communicate that this is a fish friendly procedure it may be necessary to highlight that fishes are in their element and therefore do not fear to suffocate. Thus uncomfortable feelings that may arise unconsciously can be prevented.

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FISH QUALITY: A PSYCHOLOGICAL PERSPECTIVE

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Problem and research questions:
In a systematic literature review on purchase behaviour for fish and seafood products in developed countries Carlucci et al. (2015) identified sensory perception, health believes, fish eating habits, convenience perception, self-efficacy in the fish preparation process, price perception, and fish availability as main factors analysed and explain the quantity of fish consumption. Product attributes as country of origin, production method, preserving method, product innovation, packaging and eco-label influence the choice with respect to different product offerings. Besides purchase behaviour a lot of papers address the willingness to pay (WTP) for sustainability attributes in general and/or of fish welfare in particular; e.g. Zander and Feucht (2018) provide a comparison between different European countries with respect to the WTP for sustainable seafood.

To the knowledge of the authors no publicly available research explicitly focusses on the opportunities of fish and fish products for satisfying specific psychological needs. A basic study on the relevance of fish in every-day life of consumers provides a deeper insight in fish consumption and in opportunities to position fish products in their competitive environment as well as a better understanding of the impact of communication on fish

Method:
The study is based on the theoretical framework of Morphological Psychology that focuses on "impacts", which are specific for a product. Pursuant to that, the method is product-oriented and identifies the ability of products to satisfy psychological needs or motives. Insights into the impact on products are collected in qualitative interviews and group discussions in which participants describe the meaning of the product in their every-day live (Melchers and Ziems 2001). As qualitative methods are interpretative and therefore susceptible for biases, research was carried out by a team of specifically trained psychologists to improve the validity of results. Moreover market data provided additional insights and help to reduce biased interpretation.

10 in-depth interviews and three group discussions with eight to ten participants each were carried out in November and December 2017 in Germany. Given that the psychological impact of products is dependent on the cultural environment, results cannot be simply transferred to other countries.

Results:
Fish has fascinating and threatening aspects at the same time. Fishes stand for agility as well as for fluent, sudden and apparently weightless movements in any directions. They live in a submarine world that is perceived as fascinating but difficult to access for humans. Moreover fish can easily be associated with memories of holidays and of adventures. Agility, imaginations of holidays, adventures and a fascinating world support the ability of fish to enact time-outs from rigid and restrictive daily routines. The specific psychological characteristic of fish and fish products is enhanced by a low level of consumption in Germany, which amounts to about 14 kg/head/year compared to about 90 kg/head/year of meat and meat products (FIZ 2019).

The "bright" side of fish is contrasted by a "dark" side. Ideas about spoiled and awfully stinking fish and the danger of poisoning can ruin the fascinating imageries and the appetite for fish. The same holds for ideas about a polluted environment in which fishes may grow and which have negative impacts on the perception of fish as valuable food. Fish bones are a further issue that may require care in preparation and caution in eating. Stories of nearly suffocated fish eaters appeared in several interviews.

The bright side of fish in combination with its dark sides provides opportunities to demonstrate knowledge about fish, specific skills in handling fish, and to present oneself as a connoisseur and gourmet. In this respect fish can be used as a mean for impression management. Some participants told that they usually eat fresh fish in restaurants so that they are not dependent on their own skills and indulge in a time-out by being served in a particular surrounding with fish

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The results especially hold for fresh fish even though it amounts for only about 10% of the overall fish consumption in Germany (FIZ 2019). Apparently fresh fish has a dominant role regarding narratives about fish. Preserves, frozen or smoked fish do not influence spontaneous perception to the same extent

The strength of fresh fish is a barrier at the same time. The high market share of prepared fish reveals a need for consuming fish without great effort. Respective products are easy to prepare and easy to eat. They fit into everyday routines and enrich the diet in a less spectacular way.

Conclusion:

The different preparations of fish with fresh fish on the one side and prepared food like fish sticks on ready to eat products on the other address the need of enriching the daily routine in specific ways. But against the background of the "dark" sides of fish, appetite for fish and its ability to address psychological needs is not stable. Fish scandals as well as negative communication about fish can easily trigger, activate and arouse fears and spoil appetite. As a consequence the image of fish can be severely harmed with an influence market performance of the products.

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DIGESTIBILITY OF FILAMENTOUS FUNGI IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Microbial proteins including filamentous fungal biomass contain noticeable levels of protein, fatty acids and other nutritious components (Karimi et al., 2018). Also fungi cell wall contains chitin and chitosan and other polysaccharides as their bioactive components (Mario et. al., 2008). These bioactive compounds possess immunomodulatory and antimicrobial properties (Kumar et. al., 2009, Esteban et. al., 2001). Thus, fungi may play a role in improving the health of farmed fish. At the same time, it is essential to understand how digestibility of fungi is affected by sustained feeding. *Neurospora intermedia* is an edible filame tous fungi with high protein content (57.3%). In this study, a 30-day feeding trial was conducted to examine the digestibility of diets supplemented with filamentous fungi *N. intermedia* in *Oncorhynchus mykiss*.

Materials and methods

Reference diet (Ref) was prepared with fish meal as the main protein source, whereas diets NPC (non-preconditioned) and PC (preconditioned; 95^{o}C for 5 min) were prepared by mixing the reference diet mash and the dried *N. intermedia* in the ratio of 70:30. Titanium dioxide (TiO2) was used as an inert marker. Twenty fish were distributed randomly in each experimental tank (mean weight: 127± 4 g) and were acclimated for 9 days on commercial diet. Fish were fed to satiation. Faecal samples for digestibility were collected daily for 30 days and were separated at 10 day intervals for assessment of ADC per period.

Results

Significant differences between diets in apparent digestibility coefficients (ADC) were observed for dry matter (77.25-80%), crude protein (91.68- 93.34%) and gross energy (81.63- 85.35%) ($p<0.05$). There were no significant differences for interaction between diets and time intervals of fecal sampling for either test diets and test ingredients ($p>005$). Diet and interval of fecal sampling had significant effect on respective ADCs but no significant effect of diets on ADC of CP was seen at end of the feeding trial. Also, no significant difference in the ADCs for test ingredients were observed after 30 days ($p>0.05$). Furthermore, there were no significant di ferences in weight gain between the dietary treatments (>0.05).

Discussion and conclusion

Based on the above results, it can be suggested that the ADC of DM, CP and GE
depends on diet and time interval. Although no positive effect on digestibility was observed due to heat processing in this experiment, positive effect has been previously documented for extrusion cooking in diet of rainbow trout (Barrow et. al., 2007). Overall, diets with *N. intermedia* had lower mean ADCs of DM, CP and GE than fish meal diet at the end of experiment. Similar results were obtained when Arctic charr was fed with another filamentous fungi, *Rhizopus oryzae* (Vidakovic et. al., 2016). In this experiment digestibility for diets did not change at 10th and 20th day of fecal sampling. Although, apparent digestibility increased with time interval of fecal sampling and since there were no significant difference in the final weight gain of fish henc prolonged feeding trial and further investigation is recommended.

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A LONG-TERM STUDY OF DOMESTICATED AND WILD ATLANTIC SALMON IN THE RIVER GUDDALSELVA

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Farmed Atlantic salmon are domesticated, and where significant interbreeding with wild conspecifics occurs, this may lead to changes in fitness traits, and ultimately, less productive wild populations (Reviewed by Glover *et al.* 2017). However, thus far, only a handful of studies have compared the fitness of domesticated and wild Atlantic salmon in the natural environment. Here, we present highlights from the most recent and extensive of these studies (Skaala *et al.* 2019).

250 000 eggs from 75 families of domesticated, F1-hybrid, and wild salmon were planted into Guddalselva, a river containing up- and downstream traps. In addition, >40 000 hatchery-produced smolts from the same pedigrees were released. Over eight years, 6669 out-migrating smolts and 356 returning adults were recaptured and identified to their families of origin with DNA.

In comparison with wild salmon, domesticated fish had substantially lower egg to smolt survival (1.8% vs 3.8% across cohorts), they migrated earlier in the year (11.8 days earlier across years), but they only displayed marginally larger smolt sizes and marginally lower smolt ages. Upon return to freshwater, domesticated salmon were substantially larger at age than wild salmon, but displayed substantially lower released-smolt to adult survival. Overall, egg to returning adult survival ratios were 1:0.76:0.30 and 1:0.44:0.21 for wild:F1-hybrid:domesticated salmon respectively (using two different types of data).

We conclude that spawning and hybridization of domesticated escapees can lead to i) reduced wild smolt output and therefore wild adult abundance, through resource competition in freshwater, ii) reduced total adult abundance due to freshwater resource competition and reduced marine survival of domesticated salmon, and iii) maladaptive changes in phenotypic traits.

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SUPLEMENTING AQUAFEEDS WITH NEW ALTERNATIVE INGREDIENTS: A PROMISING SOLUTION TO IMPROVE PLANT-BASED DIET EFFICIENCY IN RAINBOW TROUT

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Introduction

Aquaculture experienced a huge development during the last decades and is now expected to further increase. Indeed, the United Nation Food and Agriculture Organization (FAO) rely upon aquaculture production to provide sufficient animal proteins to more than 9 billion of people at the horizon 2050. However, to meet this challenge, aquaculture has to overcome bottlenecks with respect to fish nutrition. As the demand in fishmeal and fish oil for aquafeed production is going to exceed the offer soon, the sustainable development of aquaculture production implies the total replacement of fishmeal by affordable and suitable protein sources in fish feeds. Even fish feeds now include a large proportion of plant ingredients, the total replacement of fishmeal and fish oil by plant feedstuffs has not been successfully reached as it worsens growth performance and flesh quality, especially in carnivorous fish like rainbow trout[1]. At a nutrition level, much research is now being directed into novel feedstuffs including insect meal and yeast products as alternative protein sources or microalgae as n-3 long chain polyunsaturated fatty acids supplier to replace fish oil[2–4]. However, genetic selection of fish also appears as another key enabler for improving growth performance[5] when fed a plant-based diet. Therefore, in the present project, we decided to evaluate the benefits of combining selection of rainbow trout for better growth on plant-based diet and supplementation of plant-based diet with new ingredients including insect derived product, single cell protein (yeast) and processed animal protein on growth performances.

Materials and methods

In order to evaluate the impact of plant-based diets supplemented with new ingredients on growth performances of two lines of rainbow trout, we implemented a trial at the INRA fish farm facilities of PEIMA (Pisciculture Expérimentale INRA des Monts d'Arrée, France), using a rainbow trout line selected for better survival and growth on a 100% plant-based diet and trout from the initial unselected population. Rainbow trout (50g initial body weight) were fed twice a day until apparent satiation during 12 weeks, with one of the following diets: a commercial-like diet containing fishmeal and fish oil, a totally plant-based diet containing µalgae for DHA supply, the same plant-based diet supplemented with insect-derived product yeast proteins or a diet similar to the previous one but further supplemented with processed animal proteins. The total biomass of each tank and the quantity of feed distributed per tank were recorded every 3 weeks to determine the impact of the diets on feed intake, weight gain, specific growth rate (SGR) and feed efficienc . Whole fish were used for analysis of body composition (dry matter, protein, lipid and energy content). Blood, liver and muscle samples were also collected for plasma metabolite level determination (glucose, triglycerides, cholesterol and total amino acids) and gene expression RT-PCR analyses.

Results

Evolution of body weight gain and specific growth rate indicated that compared to a commercial-like diet containing fishmeal and fish oil, plant-based diet significantly reduced growth with a more important impact on the unselected line. Supplementation of plant-based diet with insect-derived and yeast protein products significantly improved growth performance but did not reach performances observed with the commercial-like diet. Daily feed intake decreased in unselected trout and in fish fed plant-based diet but it was partially restored by addition of alternative ingredients to plant-based diets. Compared to the commercial-like diet, all diets devoid of fishmeal and fish oil exhibited lower feed efficiency and this was significantly more pronounced in the selected line. Analyses of nutrient and energy gain and retention revealed that plant-based diet reduced nitrogen, lipid and energy gain. All were partially restored by dietary supplementation with insect, yeast protein and animal processed protein products. As regard retention, only lipid and energy retention were negatively affected by plant-based diets and partially restored by supplementation of these vegetable diets. Analyses of plasma metabolites indicated that plant-based diets reduced triglycerides, cholesterol and total amino acids.

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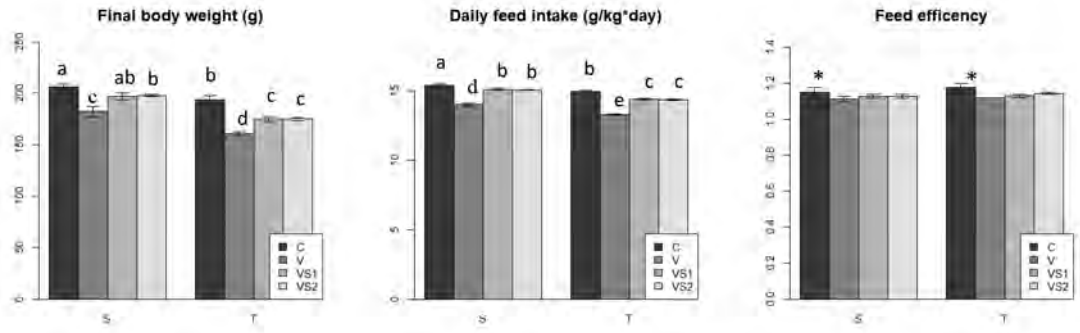

Figure: Growth parameters measured after 12 weeks of feeding trial. C: Commercial-like diet, V: Plant-based diet, VS1: Plant-based diet supplemented with insect-derived and yeast protein products, VS2: Plant-based diet supplemented with insect-derived and yeast products and processed animal protein, S: selected line, T: unselected line

Discussion and conclusion

The present study confirms the efficiency of the selection, with a global better growth of the selected line compared to the control unselected population, especially when fed a plant-based diet. When looking at the impact of the diets, the best growth occurs with the commercial-like diet that contains fishmeal and fish oil while PB diet still reduces growth performances because of lower feed intake and lower feed efficiency resulting in reduced nutrient gain. Whatever the fish line, substitution of part of the plant ingredients by a mix of insect derived product and yeast proteins increases growth without totally reaching the performance obtained with fishmeal and fish oil diets. However, interestingly, the selected line fed plant-based diet supplemented with insect-derived product and yeast proteins reaches the same growth as the control line fed the commercial-like diet, suggesting that combining genetic and new alternative diets could be a promising solution to totally suppress marine ingredients from aquafeeds dedicated to carnivorous species.

This study also highlights the leading role of feed intake in the better growth of the selected line. Enhanced feed intake also appears as a key element of the improvement of growth in trout fed supplemented plant-based diets. On the contrary, feed efficiency remains as a major bottleneck, because neither selection nor diets used in the present study were able to improve it. Therefore, in order to better understand the mechanisms underlying this negative impact of plant-based diets on feed efficienc , investigations at the level of metabolic pathways are ongoing.

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MARINE RESOURCES IN CIRCULAR CLIMATE SMART FOOD SYSTEMS: A CASE STUDY OF SEAWEED CULTIVATION IN THE NORTH SEA

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Introduction
Growth in global population will lead to an unprecedented increase in the global demand for food, and thus societies will face the challenge to increase sustainable food production. This has to be achieved in the context of a growing set of climate change-related risks, competition, scarcity of resources and the need to preserve the world's ecosystems. Hence, there is a need to make the transition from traditional towards circular and climate smart food systems. Marine resources have an important role to play in circular and climate smart food systems, for multiple reasons.

Not only can marine resources help meet future food demand – locally and abroad – they can also play an important role in stimulating food production on land and at sea, and contribute to ecosystem services. On land, marine resources can assist with improving soil quality and terrestrial production system efficienc , for both crops and livestock. At sea, marine resources can increase nursery habitats and provide valuable ecosystem services such as CO2 and mineral fixation, nutrient recycling, and long term carbon storage.

In the North Sea, development of aquaculture is becoming increasingly prominent. In particular seaweed cultivation is often considered a sustainable form of protein production. This is partly because seaweed cultivation provides ecosystem services, for example, stimulating biodiversity, improving water quality or capturing nitrogen and carbon. Seaweed cultivation can contribute to a circular economy in which nutrients that are washed away from the country by the rivers are then re-established in marine biomass. However, in large-scale cultivation, negative impacts can also occur. Large-scale seaweed cultivation demand a wide range of nutrients and the competition for these nutrients from the surrounding ecosystem can have an impact on the environment.

Objective
This study identifies the relevant ecosystem services provided by seaweed cultivation, and the positive and/or negative impacts associated with the services. It surveys the methods that can be used to estimate the economic and/or non-market values for ecosystem services from seaweed cultivation, and attempts to calculate the net benefit generated from seaweed cultivation in the North Sea.

Methodology
The study is carried out via a multidisciplinary research programme "Towards a circular and climate positive society: Marine Resources". From the economic perspective, the first phase involves a systematic review of literature on economic valuation of ecosystem services to characterise the methods that can be used to monetarise the ecosystem services, and the advantages and limitations with each method. In the second phase, primary valuation is calculated based on method(s) selected from the literature review and applied to information outputted from marine scientists regarding the types and volumes of ecosystem services produced by seaweed cultivation. Finally the results will be compared to a benchmark evaluation conducted using estimates from value-transfer of previous literature on economic valuations of ecosystem services produced by seaweed cultivation.

Intended output
The results from the study will support development of business application possibilities for low trophic species (e.g. shellfish, seaweed, worms, crustaceans) in animal production chains, carbon emission initiatives and blue growth domain. It will also be used to inform policy decisions in meeting goals set out under EU strategy to Sustainable development for climate smart food systems and circular economy.

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EFFECTS OF NUTRIENT EMISSIONS FROM AN EXPOSED FISH FARM ON THE UPPER WATER COLUMN

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Introduction

Input of inorganic nutrients to surface waters has the potential to affect primary production in the pelagic zone (Cloern, 2001; Olsen et al., 2014). Atlantic salmon (*Salmo salar*) aquaculture represents a source of nutrient loading to coastal waters of Norway (Wang et al., 2012). With the intended future increase of aquaculture in Norway, it is likely that large fish farms get located at more exposed sites than today. This study aimed to identify potential impacts following nutrient loading from Ocean Farm 1, a large-scale exposed fish farm, on the surrounding waters during its first summer season of production.

Material and methods

Integrated water samples (1 – 10 meters) were collected in Frohavet (N63.942 E009.134), Norway, every other week from June to September (n = 9) from a potential affected station 3000 meters downstream of Ocean Farm 1 (Affected) and from a nearby control station not affected (Control). The water was filtered through a 200 µm net at the stations and later analyzed for ammonium (NH_4), nitrate (NO_3), phosphate (PO_4), chlorophyll *a* (chl *a*) and particulate organic nitrogen (PON), phosphorous (POP) and carbon (POC).

Results

Results showed no statistical significant difference between the Affected and the Control station of the measured variables, except phosphate, which showed lower concentrations at the Affected station than at the Control station (Table I). The elemental C:N:P-ratio (weight) of particulate organic matter on the downstream station was 74:11:1 whereas it was 71:10:1 in the Control station. The differences between the stations was not significant ($p > 0.05$).

Discussion and conclusion

Ammonia is released from fish farms to surrounding waters whereas nitrate is supplied from deep water. The total concentrations of chlorophyll *a,* and dissolved inorganic nitrogen (ammonia plus nitrate) and phosphorous in this study were well within normal concentrations, according to the Norwegian Water Management Regulations and the OSPAR Commission (OSPAR, 2005; Vannforskriften, 2006), indicating a healthy pelagic ecosystem. The nutrients are quickly taken up by the phytoplankton and can be traced through changes in the C:N:P ratio reflecting phytoplankton biomass. Inorganic nitrogen and phosphorous emissions from aquaculture are characterized by a higher N:P ratio than natural supply from deep water and an enhanced N:P ratio of particulate suspended matter may reflect an influence of aquaculture of surface waters (Wang et al., 2013). In this study, the ratios suggested a weak phosphorous limitation of the water masses, which could be driven by emissions from aquaculture. However, no difference in particulate N:P ratios was found between the Affected and the Control station, and the results could be because of natural high N:P loading, perhaps in combination with total aquaculture emissions in the region. As aquaculture is growing, it is important to keep monitoring the effect that fish farms may have on the environment, both at well-established sites and at new, more exposed locations, in order to monitor the influence on the marine ecosystem.

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Table I. Mean background concentrations of chemical and biological variables of upper water column at an assumed affected station downstream from Ocean Farm 1 (Affected) and a control station (Control), from June – September 2018. P-values from Wilcoxon-rank-sum-test (+) or Student's t-test.

Variable, mg m^{-3}	Affected Mean ± SD (n)	Control Mean ± SD (n)	p-value
NO_3	6.7 ± 4.6 (9)	8.7 ± 3.4 (9)	> 0.05 (+)
NH_4	0.4 ± 1.2 (9)	0.5 ± 1.1 (9)	> 0.05 (+)
PO_4	1.6 ± 0.7 (9)	2.4 ± 0.7 (9)	0.04
PON	27.5 ± 8.3 (9)	24.2 ± 6.4 (9)	> 0.05
POP	2.6 ± 0.8 (9)	2.3 ± 0.6 (9)	> 0.05
POC	186 ± 45 (9)	172 ± 45 (9)	> 0.05
Chl *a*	1.12 ± 0.4 (9)	1.25 ± 0.2 (9)	> 0.05

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CULTURAL SERVICES OF MARINE BIVALVE AQUACULTURE

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Introduction

Marine bivalves have been a resource for human nutrition since pre-historic times. Their easy access and high nutritional quality have favoured their use throughout human history. Bivalve aquaculture and wild catch have shown a steady increase from 5 to 17 million tons per year over the period 1995 - 2015. Bivalve aquaculture nowadays dominates over wild catch almost 9-fold and this figure still increases. In addition to aquaculture for production, both wild and cultivated bivalves have a suite of functions in the ecosystem. These functions are defined as goods and services (Smaal et al., 2019). Examples of regulating services are water filtration that improves water quality by turbidity control and nutrient regeneration, and nutrient uptake and accumulation for eutrophication control. They also contribute to coastal defence as reef building eco-engineers and to nature quality by providing habitats that are biodiversity hotspots.

Cultural services are defined as the nonmaterial benefits people obtain from ecosystems, such as cultural diversity, spiritual and religious values, knowledge systems, educational values, inspiration, aesthetic values, social relations, sense of place, cultural heritage values, recreation and ecotourism (Millennium Assessment, 2005) . For marine bivalves many examples exist of cultural services. Shells are well-known collector items. Collecting seashore shells is worldwide spread leisure activity, and an organised profession as well, in the framework of the scientific discipline of malacology. This links to marine bivalves as a source of knowledge. Shells as fossil records have information for evolutionary studies, and their mineral content can reflect past climatological events as long-term archives. Shells are widely used for decoration and in art. Educational programs and community involvement exist in bivalve restoration programs. Sea gardening of marine bivalves is an upcoming leisure activity. Hence cultural services link directly to social structures that provide the framework for the appreciation of these services. Nonmaterial services may be more difficult to quantify than the other services, yet the benefits for people go far beyond the material benefits, as it concerns the core of human life that is able to reflect on all different services of – in this case - the marine bivalves (Harari, 2018; Daniel et al., 2012). In this presentation some examples of cultural services are reviewed

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NUTRIENT-BASED MODELS AS COMPLEMENTARY TOOLS TO ASSESS FINFISH AMMONIA EXCRETION AS THE PRIMARY NUTRIENT SOURCE IN A SEAWEED COMMERCIAL IMTA FARM

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Introduction

A critical process when planning Integrated Multi-Trophic Aquaculture (IMTA) systems, and that drives the success of their implementation, is the estimation of nutrient fluxes among the different trophic-levels to be farmed and consequent production volumes. Often-used approaches in this process are: (i) the upscaling of small-scale experiments, carried out under similar conditions of those observed at the farm site (Abreu et al., 2011)”container-title”:”Aquaculture”,”page”:”77-87”,”volume”:”312”,”issue”:”1”,”source”:”ScienceDirect”,”abstract”:”The use of ecological engineering tools for the development of a more sustainable aquaculture is crucial. In this context, seaweed based Integrated Multi-Trophic Aquaculture (IMTA; and (ii) heuristic assessments based on simple mass-balance models (Nobre et al., 2010)”container-title”:”Aquaculture”,”page”:”116-126”,”volume”:”306”,”issue”:”1”,”source”:”ScienceDirect”,”abstract”:”Integrat ed multi-trophic aquaculture (IMTA. However, such approaches can be time consuming, require historical data, cannot provide long-term predictions neither simulate changes in production conditions, for instance different feed compositions and retentions efficiencies for the IMTA fed component. This is a relevant aspect for IMTA systems that couple organisms with a long production cycle (e.g. fish) with organisms with a shorter production cycle (e.g. green seaweed). In the context of the ValorMar project (www.valormar.pt), the nutrient contribution of the fed component of a land-based commercial IMTA system, integrating finfis (*Dicentrarchus labrax*) and seaweed (*Ulva spp.*) was simulated by means of a detailed model that can simulate fish ammonia excretion with a resolution of hours for an entire production cycle (~3 years). The main objective was to provide insights for the seaweed production considering business as usual and an expansion scenario.

Methods

Total ammonia (TAN) production by the seabass culture over its production cycle (~2 years), was predicted using the dynamic nutrient-based model FEEDNETICS (Soares and Silva, 2018), calibrated for seabass (D*icentrarchus labrax*). This application was carried out by simulating the seabass production following the planned feeding regime, under the expected environmental conditions, and for two different expected mortality rates (i.e. 15% and 20%).

In order to provide insights for the seaweed production, the ammonia contribution (TAN, kg N.day^{-1}) was compared with the Ulva requirements, estimated using a static model (Nobre et al., 2017)to assess water renewal rates and nitrogen limitation in commercial seaweed farms”,”container-title”:”Journal of Applied Phycology”,”page”:”3039-3055”,”volume”:”29”,”iss ue”:”6”,”source”:”Springer Link”,”abstract”:”Key factors affecting the economic sustainability of any aquaculture industry and in particular the seaweed industry are its ecological interactions and impacts. Understanding these issues requires an extended production analysis and simulation, given the natural variability and dynamics of external factors that affect those interdependencies. As such, making sense of production data is required for suitable planning and resource optimization in a seaweed farm. The present work calculates the required water renewal rates for seaweed flow-through production units, using a novel simple user-friendly nitrogen budget model. The user interface is straightforward and the model parameter inputs and outputs are minimal, whereby the target users are commercial seaweed farmers. The model was parameterized for production kinetics of Ulva spp. based on an extensive literature survey, and was evaluated with published data on seaweed-growing experiments. Results for the estimated number of volume renewals per day are in agreement with the experimental data. The outputs indicate that this application can be used to estimate the envelope, i.e. average lower and upper ranges for water renewal rates for a given production in a given site. This model and corresponding parameterization for Ulva spp. are available to be used by farmers, managers and researchers in the form of a spreadsheet file (available as Supplementary Material, for two production scenarios: business as usual and an expansion scenario, representing the latter an increase by 6 fold in production (Nobre et al., 2018).

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Results

Figure 1 shows model results, indicating that the ammonia produced by the seabass can contribute as the nitrogen source of the current Ulva requirements. However, if considering the scenario of production expansion, the ammonia produced by the seabass culture seems to be insufficient to contribute significantly as the main source during the entire production cycle.

Discussion and conclusion

The use of a nutrient-based model for the estimation of seabass ammonia production provides reliable detailed outputs over the entire production cycle (~2 years), while the simple Ulva nutrient budget model provides threshold for the TAN requirements of a much shorter production cycle. This approach provided valuable insights about the finfis culture potential as the main nutrient source of the seaweed production, which can be used for production planning for the current production volume and for the 6-fold expansion scenario. This work is a preliminary approach to an overall assessment of nutrient sources and requirements within the IMTA system boundaries. Further work must be developed in order to account for water renewal rates, and nutrient mass fluxes among compartments (i.e. finfis earth-pond and seaweed tank) and from water inlets that supply the system.

Acknowledgments

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DOMESTICATION LEADS TO INCREASED PREDATION SUSCEPTIBILITY

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Each year, tens of thousands of domesticated Atlantic salmon, *Salmo salar* L., escape from Norwegian sea cages and into the wild. Most of the escapees disappear into the wild without ever being observed again, but some survive and return to freshwater as sexually mature adults. Escaped domesticated salmon have lower spawning success than wild salmon, but successful spawning and introgression of domesticated salmon have been documented in several Norwegian rivers. Offspring of domesticated salmon also have lower survival than offspring of wild salmon in the wild, which may reduce productivity in wild populations following introgression. Why offspring of domesticated salmon display increased mortality in the wild is not known, but one possible explanation is that domesticated salmon are more susceptible to predation. Wild fish live in an environment where early survival is low and there is a huge risk of predation. In contrast, domesticated fish live in a sheltered environment without the threat of predators. This has resulted in domesticated salmon showing a reduced anti-predator behaviour, as compared to wild salmon, when exposed to predators in controlled studies. In the project QuantEscape, a study was carried out in 2015 that suggests that a maladaptive behaviour towards predators results in increased mortality in domesticated salmon relative to wild salmon. The study was carried out in a semi-natural environment where domesticated, wild and hybrid offspring competed for natural food under the risk of predation by older trout.

PROJECT INTEGRATE. CULTIVATION OF SEA SPAGUETTI (*Himanthalia elongata*)

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INTEGRATE is an European Project funded by the ERDF through the Interreg Atlantic Area Programme, investigating the next steps in research and commercialization of Integrated Multi-Trophic aquaculture (IMTA) in the Atlantic Area (AA) in Europe. It was born from an initiative by a group of researchers and production experts aiming to foster cooperation for industrial transition towards IMTA. The project will provide tools to increase competitiveness and contribute to removing barriers for growth within the eco-aquaculture sector, while improving the quality and public image of aquatic products.

In within the project we are looking to cultivate new species in IMTA systems. In work package 4, pilot case study 1, the Irish Seaweed Consultancy (ISC) is developing the cultivation of *Himanthalia elongata* (Sea spaghetti) as a potential new species in an IMTA system. For that, eggs and sperm were released twice a month, from August to October 2018. Throughout this time scallop shells were used as a seeding substrate, with good attachment results. Shells cover on fertilise Himanthalia eggs were then submerged in tanks with air waiting to be deployed at sea. The shells were divided in two batches, and were deployed in January and March 2019. Plant growth was followed monthly until September 2019. Some results and images from this work are presented in this poster.

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GENOME-WIDE ASSOCIATION ANALYSIS OF LIPID RELATED TRAITS IN EUROPEAN SEABASS

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Background
Lipid related traits are important both for the consumers that buy fish for the health benefits of omega-3 fatty acids and for the fish that also need omega-3 fatty acids to develop healthy cell function and immunity. Other lipid related traits are related to liver health of the fish, because liver is the most important organ for producing and storing fat. Finally, deposition of fat in different body parts, e.g. viscera, liver and muscle are related to production efficienc . In A. salmon, we know that omega-3 content of the fillet and fat content of the muscle and liver are low-mid heritable traits (Leaver et al., 2011; Horn et al., 2018). We also know that genes that regulate lipid contents of liver and muscle differ (Horn et al., 2019). In European seabass, which is a leaner fish species than A. salmon, a heritability of 0.48 has been found for viscera%, and 0.28 for muscle lipid content (Saillant et al., 2009).

The aim of this study is to perform a genomic analysis of lipid-related traits using one yearclass of the Culmarex breeding program of European seabass.

Material & Methods
1000 European seabass with mean weight of 350g coming from three different cages were sampled. These fish were recorded at the slaughterline for round and cascass weights, sex and weights of viscera, heart, gonad, liver, guts and fillet fat content. Subsamples of these fish were analysed for total fat and omega3 fatty acid contents of the fillet and liver and blood contents of ASAT, ALAT, CK and ALP. The fish were also genotyped with the combined SNP array for European seabass and gilthead seabream, containing in total ~55 kSNP.

Results
This data will be analysed for phenotypic and genotypic correlations and heritability. A genome-wide association analysis will also be performed.

This work is done as part of the MEDAID project.

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WHOLE OR BROKEN CELLS OF *Phaeodactylum tricornutum* AND *Tetraselmis chuii* FED TO ATLANTIC SALMON *Salmo salar*

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Introduction

Microalgae are promising alternatives to fishmeal and fish oil (Gong et al., 2018; Kiron et al., 2012; 2016; Kousoulaki et al., 2015; Sørensen et al., 2016; 2017). However, their rigid cell walls may prevent accessibility of the valuable nutrients by fish. Nevertheless, nutrient digestibility of microalgae can be improved through disruption of their cell walls. A study was conducted to investigate the effect of bead milling on cell wall disruption and eventually on nutrient digestibility. The aim of the experiment was to investigate the digestibility of dry matter (DM), protein and lipid when Atlantic salmon was fed whole or broken cells from *Phaeodactylum tricornutum* and *Tetraselmis chuii*. Data were also collected to assess the skin and muscle pigmentation and for calculation of weight gain of the salmon.

Material and methods

The microalgae biomass [*Phaeodactylum tricornutum* B58 (PHA) and *Tetraselmis chuii* UTEX LB232 (TET)] were produced at the National Algae pilot Mongstad (NAM) (Mongstad, Norway) in a fed-batch process, in four 800L photobioreactor systems (GemTube MK2-750 from LGem B.V., Netherlands). The microalgae were harvested twice per week, concentrated to a paste by centrifugation (Evodos 50, Evodos B.V., Netherlands), vacuum packed and directly frozen at -20^{o}C before further downstream processing. The microalgae paste, containing 22.9 and 18.7 % DM, in PHA and TET, respectively, was processed by a single pass through a Dyno-Mill Multi Lab bead mill (WAB, Muttenz, Switzerland); using a 0.6 l chamber and small glass beads (0.25-0.4 mm), at 80% chamber filling, 12 m/sec agitator tip speed and 6-9 kg/h biomass flow rate. The whole and cell-wall disrupted biomasses were spray dried to obtain the fine biomass powder that was used for feed production. A reference feed (RD) was formulated to contain 660 g kg^{-1} protein and 150 g kg^{-1} lipid. Four test feeds with whole or broken microalgae were formulated by mixing 700g kg^{-1} of the reference feed and 300 g kg^{-1} of either PHA or TET. All feeds contained yttrium oxide (0.2 g kg^{-1}) as inert digestibility marker. The feeds were produced using extrusion process. For the digestibility trial, Atlantic salmon (315 g) were kept in three replicate experimental tanks (55 fish/ tank) for 44 days. Feces from individual fish were collected by stripping and then a pooled sample was made for each tank. Fish were individually weighed at start and termination of the experiment. Skin and flesh color were measured using a portable spectrophotometer (CM-700d, Konica Minolta Sensing Inc., Singapore).

Results and discussion

The preliminary results show that the average fish weight increased by 40%; growing from a start weight of 315 g to a final weight of 441 g. The corresponding average SGR and TGC values were 0.77% and 2.40, respectively. Fish fed broken TET-feed had significantly lower weight gain, SGR and TGC values compared to the RD-fed fish. However, there were no differences in the growth indices between the other study groups. As for the digestibility of whole cell TET-feeds compared to RD-feeds, the intact cells had significantly lower digestibility of DM and lipid while the corresponding broken cells-containing feeds yielded lower digestibility of protein. The lower growth of the fish fed broken cell TET-feeds may thus be explained by the lower protein digestibility. The experimental setup did not allow measurement of feed intake, however, the lower growth of fish fed broken TET may also be linked to poor palatability of the broken cells. Analyses showed that the predominant phenolic compound in TET is capsaicin, present also in hot chili, which become more available following cell wall disruption. Protein and lipid digestibility of the broken PHA was the highest, comparing the 2 algae, with both whole and broken cells. The Minolta values showed that there were some differences in pigmentation among fish fed the different diets, which can probably be explained by the content of carotenoids and vitamins and other bioactive components with antioxidant effects.

Conclusion

Disruption of cell walls employing bead milling improved apparent digestibility coefficient of protein and lipid of *Phaeodactylum tricornutum* and the procedure seems to be a promising method to improve nutrient utilization of the algae.

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Acknowledgements
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OZONATION OF SEMI-CLOSED AQUATIC SYSTEMS

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Introduction

The water quality in intense recirculating aquaculture systems (RASs) is characterised by accumulation of pollutants, potentially allowing fish pathogens to grow. Although ozonation has been applied for years in aquaculture improving the water quality (Langlais et al., 1991; Bullock et al., 1997; Krumins et al., 2001; Liltved et al., 2006; Summerfelt et al., 2009), there is a lack of knowledge regarding the reaction kinetics, the control of the dosage and side effects of excessive ozonation.

The analytical efforts to detect and monitor organic matter (DOM) in aquatic systems have included among others, absorption spectroscopy of the coloured fraction (CDOM). A part of CDOM also fluoresces (FDOM). FDOM fraction has been widely used in aquatic environments as a quantitative and qualitative measure of DOM. Recently, it was shown that RAS water consists of FDOM (Hambly et al., 2015) which was highly sensitive to ozone, suggesting that fluorescence spectroscopy could be used as an indirect method to determine ozone delivery within these systems (Spiliotopoulou et al., 2017).

This study aims to develop a method to predict the ozone demand for the specific system improving the water quality without compromising the fish welfare and to pursue a more direct approach to measure and control the delivered ozone dosage in RASs using fluorescence spectroscop .

Material and Methods

Water samples from a pilot-scale RAS were subjected to ozonation in bench scale experiments to define the ozone demand and ozone lifetime in this water matrix (Spiliotopoulou et al., 2018). All samples including the non-ozonated control sample were measured with a fluorimeter to define the ozone effect on natural fluorescence degradation (Spiliotopoulou et al., 2017, 2018). The predicted ozone dosages were applied in side-stream to pilot-RAS systems where trout were farmed to evaluate the ozone effect on a wide range of water quality parameters (Spiliotopoulou et al., 2018).

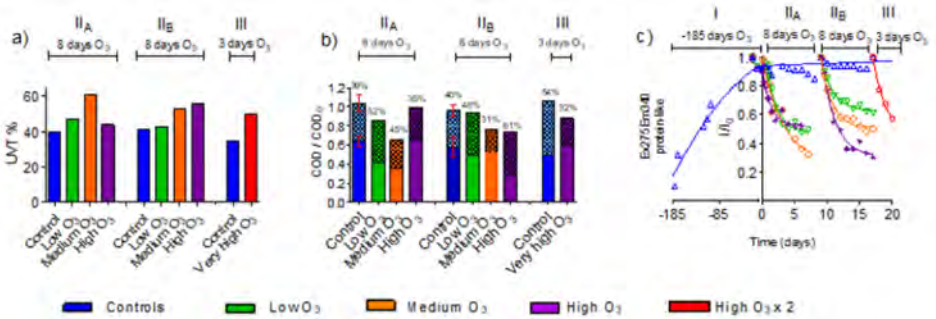

Figure 1. Ozone effect on a) UVT% after 8 days, b) COD after 8 days (the % and the dotted (upper) parts of the bars represent the particulate COD, while the lower part is dissolved COD-normalised data, standard deviation only in control, c) protein like-fluorescence degradation-normalised data.

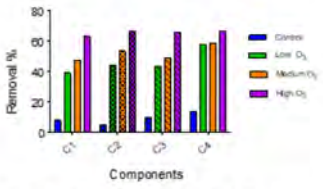

Figure 2: Effect of ozone on FDOM after 8 days of treatment.

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Results and Discussion

The analysis of a few mL of water sample in the laboratory was sufficient to determine the ozone demand and the ozone lifetime of the system but also to predict the ozone dosage that was required for the pilot and/or full-scale RAS (Spiliotopoulou et al., 2018). The four ozone dosages, including a control (non-ozonated), that were selected to be tested in pilot-RAS (ranging from 52-130 mg O_3/h, equivalent to 10-25 g O_3/kg feed) improved remarkably the water quality (Fig. 1). Residual ozone was not detected in any trial, not even after the ozone reaction chamber.

Along with the commonly studied water quality parameters in aquaculture water, the effect of continuous ozonation on the FDOM character was also investigated (Fig. 1d,e). A more detailed identification of the different fluorescent fractions was conducted using the coupled EEM-PARAFAC technique (Fig. 2) revealing the presence of four independently varying components. A UV wavelength fluorescent fraction (C4) typical of proteinaceous material was removed by 13-20% immediately after ozone initiation. The remaining fractions that exhibited visible wavelength fluorescence, at first were unaffected but during the following days, were gradually degraded, reaching a removal of 34-66%. By the end of the experiment, the fluorescence intensities of all fractions were diminished up to 60% in all applied dosages.

Conclusions

The method applied to predict the optimal ozone dosage of pilot-RAS based on laboratory studies was efficient with remarkable proximity, achieving improved water quality without affecting the fish health

The high sensitivity of FDOM to ozone and its selectivity to specific fluorescent components, suggested that fluorescence could be used as an online sensor to control the organic matter in a RAS and determine indirectly the delivered ozone dosage in the system. This study attempted to clarify misinterpretations regarding ozonation and to offer new technological concepts to make its implementation safe, convincing the aquaculture managers to integrate ozone in their water treatment processes.

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ARTIFICAL WORLD – NEW INSIGHTS ON OCTOPUS EGG REARING AND LARVAL FEEDING BEHAVIOUR

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Introduction

Global demand for octopus as seafood has been increasing but supply is constrainedby the limits of wild octopus fisheries. Establishing octopus aquaculture would help to meet the global demand and could reduce fishing pressure on wild populations (Josupeit 2008). However, rearing the larvae of various species of octopus has proven to be extremely challenging (Iglesias et al. 2007). Larval rearing and feeding behaviour of *Octopus tetricus*, an octopus species native to Australasia, was examined in this study to describe larval development and also to attempt to incubate octopus eggs without a female.

Material and methods

Thirty-six egg-strings were removed from each female three days after the first egg strings were visible. Egg strings were randomly split into groups of three, attached to a PVC-pipe and placed in one of 12 upwelling tanks for incubation. Four experimental treatments representing different culture arrangements were tested. Treatment one (T1) and two (T2) had high sea water inflow (4 l min^{-1}). Treatment three (T3) and four (T4) had low seawater inflow (2 l min^{-1}). Tanks of T1 and T3 had an additional air stone placed beneath the egg strings to increase water circulation around the developing eggs.

Newly-hatched paralarvae were kept under starving conditions in an upwelling tank. Each day a group of 20 paralarvae age 0-, 1-, 2-, 3-, 4- and 5 dph (days post hatch) were removed from the upwelling tank and used for short term feeding experiments. Two different prey items, live brine shrimp (BT) and artificial diets (AT) were used. Total amount of strikes and successful capture of food particle as well as handling time per paralarvae were measured.The experimental duration was 15min, starting when prey items were added.

Results

Hatching success of over 90% was achieved in both upwelling systems were aeration was added (i.e., T1 and T3) compared to a hatching success of 31.4% and 10.3% in the high and low water inflow treatment without aeration.

The percentage of paralarvae that captured at least one food particle increased from 0 dph and peaked at 2 dph and 3 dph in the BT and AT treatment, respectively (Fig. 1). In general, the percentage of paralarvae that had successful captures was always higher in the AT treatment compared to the BT treatment from 1 dph onwards with significant differences between the two treatments at 3 dph. The highest percentage of paralarvae that had a successful capture was 41.7% in the AT treatment at 3 dph.

Discussion

The high hatching success of over 90% which was achieved in two out of four treatments in this study, shows the possibility to rear *O. tetricus* eggs without maternal care. Earlier attempts to culture eggs of *O.* (cf) *tetricus*, *O. vulgaris* and *Enteroctopus megalocyathus* without maternal care have resulted in poor egg survival of between 30-60% (Joll 1978; De Wolf et al. 2011; Uriarte et al. 2016).

Highest feeding success was observed in paralarvae at 2-3 dph in this study. This is similar to *O. vulgaris* where the highest feeding activity was at 2 dph (Iglesias et al. 2006). In both cases paralarvae showed a decrease in success or activity after 2 dph, suggesting that a point of no return in nutritional resourcing might be reached.

These results are encouraging in the further development of octopus aquaculture. However, more research is needed to determine how much of the artificial diet was ingested and which diet features (e.g. texture, buoyancy) are most suitable for promoting consumption of artificial feed by newly-hatched larvae.

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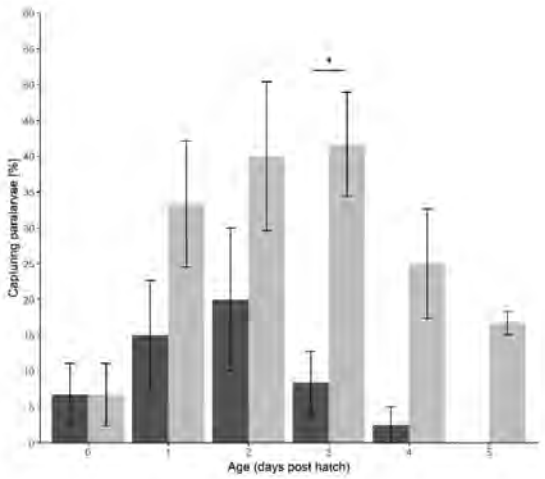

Fig. 1: Mean percentage of paralarvae that performed at least one successful capture of a food particle. Dark grey bars represent the BT treatment (live brine shrimps) and grey bars represent the AT treatment (artificial diet). Error bars indicate the standard error. Stars indicate significant differences: $p < 0.0001$ = ***, $p < 0.001$ = **, $p < 0.05$ = *.

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EFFECT OF ORGANIC ACIDS IN GROWTH, SURVIVAL AND CONTROLLING CONTINUOUS MORTALITIES OF *Litopenaeus vannamei* CULTURE AT KATTUKALAVA, WEST GODAVARI DISTRICT, ANDHRA PRADESH, INDIA

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The present study conducted to find the impact of organic acids when given through feed to the cultured penaeid shrimp *Litopenaeus vannamei*. In this study one control pond and two experimental ponds were taken at Kattukalava, West Godavari district of Andhra Pradesh, India. In the present study, periodic sampling done for estimation of growth & survival rate of shrimp in control pond as well in the experimental ponds. The results were recorded with standard measurements during the culture period.

This study was focused on the growth and survival rates in the control pond and also in the experimental ponds (pond A and pond B) where the control pond without inclusion of organic acids in the feeds was affected with unknown continuous mortalities (Generally known as Running Mortality Syndrome) in both winter and summer crops of the year 2018 when compared with the experimental ponds A & B where the organic acids are added continuously during culture. The findings of the current study concluded that the application organic acids through feed prevents the continuous mortalities in the experimental ponds as well enhance the growth and survival rates in *Litopenaeus vannamei*.

TWO DIFFERENT INSECT MEALS AS FISHMEAL REPLACEMENT FOR EUROPEAN PERCH – *Perca fluviatilis*

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Introduction

In recent years, insects have been discussed and advertised to be a sustainable alternative as feed ingredient for a variety of fish species and poultry. One of the most frequently utilized insect species, *Hermetia illucens*, could be grown on food waste and animal manure and thus use a substrate which would otherwise be burned or thrown into biogas plants. Insect meal (IM) has been utilized as fishmeal replacement in several fish species and its success depends on nutritional quality. Especially salmonids can tolerate higher levels of IM compared to other species such as turbot (*Psetta maxima*). Up to now, IM has not been tested in European perch (*Perca fluviatilis*), a commercially important fish species in Switzerland. In this trial, two different IMs (one medium de-fatted containing medium protein level and one high-defatted containing high protein level) were fed in three levels each (25, 50 and 75% fishmeal-protein replacement) to perch fingerlings and compared to a non-IM control diet.

Material and methods

Two different *H. illucens* IMs were used for this experiment. IM1 contained around 30% lipids and 48% protein while IM2 contained 5% lipids and 60% protein. Six experimental diets were formulated for a final concentration of 50% protein, 12% lipids and an estimated digestible energy content of 17.5 kJ/g. In the control diet, 47% of fishmeal served as main protein source besides 20% wheat gluten as minor protein source. Each insect meal replaced 25, 50 and 75% of fishmeal protein (diets IM1-25, IM1-50 and IM1-75 and IM2-25, IM2-50 and IM2-75) while an IM free diet served as control. 420 juvenile perch (approximately 2 g) were stocked into 28 aquaria connected to a recirculation system at 15 fish per aquarium. Each diet was allocated to four randomly chosen aquaria and fish were hand fed 2% of their body mass per day divided into 3 feeding stations during the week and two feeding stations on weekends and holidays. Once a week, the fish were group weighed and feed amount adapted accordingly. After 28 days of experimental feeding, growth (absolute and relative), feed conversion and protein efficiency ratios were evaluated

Table I: Growth, feed and protein utilization of European perch fed with control and insect meal diets.

	C	IM1-25	IM1-50	IM1-75	IM2-25	IM2-50	IM2-75
Initial BM (g)	1.93 ± 0.14	2.08 ± 0.11	1.95 ± 0.11	1.94 ± 0.16	2.02 ± 0.07	1.98 ± 0.05	1.91 ± 0.11
Final BM (g)	2.60 ± 0.23	3.10 ± 0.40	2.65 ± 0.26	2.61 ± 0.18	2.99 ± 0.23	3.12 ± 0.20	2.90 ± 0.28
BM-gain (g)	0.67 ± 0.10^{a}	1.02 ± 0.29^{ab}	0.70 ± 0.15^{ab}	0.67 ± 0.12^{a}	0.97 ± 0.22^{ab}	1.13 ± 0.20^{b}	1.00 ± 0.24^{ab}
BM-gain (%)	34.5 ± 4.23	48.8 ± 11.7	35.5 ± 5.50	34.7 ± 7.51	47.7 ± 10.9	57.2 ± 10.3	52.5 ± 12.8
SGR (%/day)	1.48 ± 0.30	2.10 ± 0.48	1.55 ± 0.35	1.50 ± 0.25	1.98 ± 0.28	2.13 ± 0.22	1.87 ± 0.34
FCR	2.36 ± 0.27	1.83 ± 0.48	2.31 ± 0.32	2.41 ± 0.48	1.78 ± 0.42	1.55 ± 0.25	1.65 ± 0.48
PER	0.88 ± 0.10	1.18 ± 0.28	0.89 ± 0.12	0.88 ± 0.17	1.19 ± 0.26	1.34 ± 0.25	1.28 ± 0.32

Values = mean (N = 4) ± SD, BM = body mass, SGR = specific growth rate, FCR = feed conversion ratio, PER = protein efficiency ratio

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Results

All diets were accepted well and feed consumed during the first few minutes after serving. All diets resulted in at least equal growth compared to control diet while the low fat and high protein insect meal (IM2) showed better growth results compared to control and to IM1 except in the lowest replacement level, where IM1-25 grew similarly to IM2-25. Although in BM-gain (%), SGR, FCR and PER relatively large numerical differences were observed between treatments, none of them were significant in a Tukey's B post-hoc test.

Statistical differences were found in absolute body mass gain, where perch fed with IM2-50 showed the highest gain. However, since the starting weights slightly differed, the relative growth would be more meaningful, where again, no significant differences were detected. In total, all insect meal diets resulted at least in a performance as good as the control diet.

Discussion and Conclusion

Due to a delayed start (fish feed had to prepared three times, since the fish did not accept the first two formulations, where, besides fishmeal the minor protein source was soy protein concentrate and had to be replaced by wheat gluten), the fish grew already differently and thus the starting BM differed stronger compared to the day of stocking. Based on the presented data, both insect meals, the high fat and medium protein (IM1) and the low fat and high protein (IM2) meal, resulted in similar performance characteristics compared to the control diet. Despite significant differences detected for relative and absolute BM-gain and SGR and trends for FCR and PER by the overall ANOVA, subsequent Tukey contrast failed to detect significant pairwise differences except for absolute gain. Perch fed with 50% fishmeal-protein replacement by IM2 gained significa tly more weight compared to control fed perch and those fed with 75% fishmeal-protein replacement by IM1. Overall, our data shows, that both insect meals are at least equally well performing compared to a high-fishmeal diet. Although non-significant, the data seems to point towards a positive trend in IM2 fed fis

EFFECT OF DIETARY NON-PROTEIN ENERGY SOURCE (STARCH VS. FAT) ON TOTAL BILE ACID POOL SIZE AND COMPOSITION OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Bile acids play a key role in digestion and absorption of dietary fat (Tocher, 2003). Synthesis of primary bile acids occurs in the liver, by oxidation of cholesterol and conjugation with either taurine or glycine. Bile acids are actively secreted into bile and stored in the gallbladder. The gallbladder awaits hormonal signals (cholecystokinin) to contract after a meal, and releases bile into the small intestine (Hagey et al., 2010). The majority of bile acids secreted into the small intestine is actively reabsorbed within the ileum, after which it returns to the liver for reuse (Cai et al., 2007). Under homeostatic conditions, the total bile acid pool is maintained relatively constant, compensating faecal bile acid loss with *de novo* synthesis (Lanzini and Lanzarotto, 2000). The process described above is known as enterohepatic circulation of bile acids (EHC).

Diet macronutrient composition can affect both the rate of EHC and bile acid synthesis. Hepner (1975) showed that the level of gallbladder contraction (level of EHC) in response to a meal regulated the bile acid pool size in humans, with a low fat diet decreasing the rate of contraction and increasing the bile acid pool size. Furthermore, less secondary bile acids were produced in response to decreased EHC, as there is less exposure of primary bile acids to bacteria in the intestine. Dietary fat level and fat saturation level have also been shown to influence bile acid synthesis. Feeding a diet high in fat stimulated bile acid synthesis, while a low fat diet decreased bile acid synthesis in rats (Botham *et al.*,1983). Furthermore, Cheema *et al.* (1997) found that the bile acid synthesis in mice was upregulated (CYP7A1) in response to a diet high in unsaturated fat. Besides dietary fat, also carbohydrates can affect bile acid synthesis. Andersén and Kjell (1980) showed an increase in bile acid synthesis in humans changing diets in which 60% of the energy was respectively supplied by fat and by carbohydrates. The non-starch polysaccharide fraction of the latter increased faecal bile acid loss, leading to increased *de novo* synthesis of bile acids. Quantitative/qualitative data on the effect of diet macronutrient composition on bile acid pool size and composition of fish are lacking. The main objective of this study was therefore to quantify and qualify the total bile acid pool in rainbow trout fed a diet differing in the main type of non-protein energy source (starch vs. fat).

Materials and methods

Two diets with a constant digestible protein to digestible energy ratio were formulated, while differing in either inclusion of corn starch or rapeseed oil as the main non-protein energy source. A vegetable oil was chosen as lipid source for the fat diet to avoid additional introduction of cholesterol, as the latter is known to increase bile acid synthesis. Each diet was tested in triplicate. Fish were housed in 6 experimental units (200 L each) connected to a common RAS-system. Water temperature was maintained between 14 ± 1 °C and water flow was set at 7 ± 0.5 L min^{-1}. At the beginning of the experiment fish were batch weighed and randomly distributed among the experimental units at a stocking density of 25 fish $unit^{-1}$. Fish were hand-fed twice daily to satiation for 44 days. Faeces were collected from week 2 till week 6, and pooled. Diets and faeces were analysed for proximate composition and bile acid content. At the end of the experiment, fish were batch weighed and sampled for proximate body composition and bile acid content.

Results

Diet did not affect growth (SGR), survival and feed intake (RFR), although, the fat diet resulted in a significant better FCR ($P<0.01$) compared to the starch diet. Diet did have significant effects on body composition. Compared to the starch diet, feeding the fat diet significantly decreased protein content, and increase fat and energy content of the fish (figure 1 A and B). Diet did not affect ash content ($p>0.05$) of the fish. Samples are currently still being analysed, and results on digestibility, bile acid pool size and composition will also be presented.

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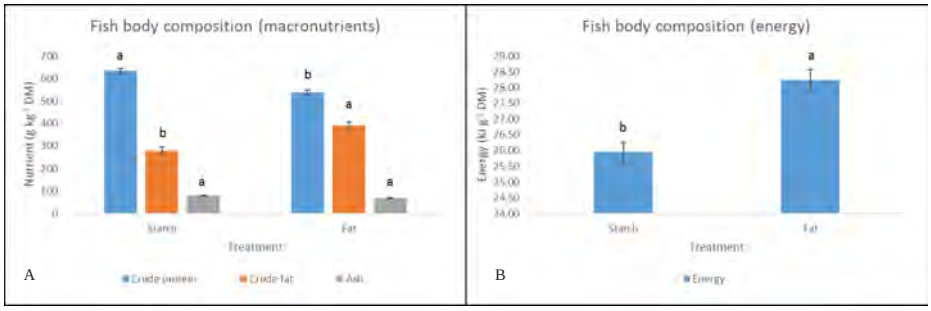

Figure 1: (A) Protein, fat and ash composition and (B) energy content of fish fed the experimental diets. For each parameter, treatments sharing a common letter are not significantly different ($p<0.05$).

Acknowledgement

Data on fish performance and body composition presented in this abstract belong to the AquaExcel2020 project 'D6.1: Effect of early life water oxygen concentration on experimental outcomes at later life in rainbow trout'. The AquaExcel2020 project has received funding from the EU's Horizon 2002 research and innovation programme under grant agreement No. 652831.

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DEVELOPMENT OF AN INDIVIDUAL-BASED GENERIC AQUAPONIC MODEL USING OBJECT ORIENTED PROGRAMMING

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Aquaponics is the association of fish and plant farming, exploiting the natural phenomenon observed in aquatic ecosystems. The microbiota transforms the fish waste into different chemical forms that are less toxic for fish and very suited for hydroponic plants culture. Due to its recent and quick development, aquaponics can now be found in a variety of areas of interest such as personal installations, education fields, private and public research projects and reached nowadays the food production at medium and large scales industries.

The diversity found in these installations makes it difficult to identify the mechanisms that rule aquaponics and to generalize the findings to other systems. Few researches established the equations that govern the behaviour of one particular system, in order to estimate the productions and to apply sensitivity analysis. These models often satisfy the goals of the developers but cannot be applied to different system setups.

In this context and as part of a research financially supported by the France-Wallonia-Flanders Interreg program, a generalist model is being developed for aquaponics. The equations are based on aquaculture, hydroponics and aquaponics studies and are implemented using an object-oriented programming in order to generate a completely generic model. The European Smart Aquaponics Project provides numerous systems with different architectures. All these installations are equipped with a full set of aquaponics sensors to generate a strong aquaponic database that will be used for model development.

The computed outputs are various and new ones are regularly added to keep pace with the continuous development of aquaponics. In this case study, the model has been applied to an experimental setup to illustrate the basic calculated parameters such as the dynamical behaviours of: the water volumes and several nutrient concentrations, the vegetal and animal biomasses, the water consumption, a few economic criteria, etc.

MEASUREMENTS OF BEHAVIOUR, STRESS RESPONSES, MONOPEPTIDE LEVELS AND CRYPTIC COLOURATION IN LUMPFISH (*Cyclopterus lumpus*) AFTER INTERACTION WITH ATLANTIC SALMON (*Salmo salar*), WITH FOCUS ON SPECIFIC SENSORY CUES

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Introduction

Salmon aquaculture has approached a trending use of facultative cleaner fish to control epidemics of ectoparasitic sea lice. The cleaner Lumpfish (*Cyclopterus lumpus*), endemic to the North Atlantic, is highly sought of due to the species ability to graze sea lice (Imsland et al., 2014a, Imsland et al., 2014b, Imsland et al., 2016) and preferences for lower temperatures (Nytrø, 2013), typically observed along coastal Norway during winter. In addition, rearing of lumpfish has been successful due to prime survivability at larval stages and adaptiveness to given artificial rearing environments (Powell et al., 2017). With annual production and distribution of more than 40 million lumpfish, in Norway only (Powell et al., 2017), an inquiry regarding animal welfare and ethics is shadowing the industry, as a large portion of deployed lumpfish in sea cages vanish during the production period of Atlantic salmon. Commonly cause of death among lumpfish has been related to disease outbreaks (Rønneseth et al., 2016), suboptimal environment (Hvas et al., 2018, Iversen, 2016, Jørgensen et al., 2017), or during handling of Atlantic salmon. In addition, the natural interspecific relationship between lumpfish and Atlantic salmon in nature is not completely understood, and due to little knowledge on how these species interact outside a controlled environment, it is uncertain how they cope together in captivity over time and how adaptive the lumpfish is to the compilation of stressors they are exposed to. The following study focused on the relationship between lumpfish and Atlantic salmon, and how separated Atlantic salmon predatory sensory cues impacted behaviour and physiology in lumpfish. These sensory cues included the introduction of salmon olfactory, perception and live salmon to lumpfish in controlled tank environments. The study hypothesised that different sensory cues could trigger different levels of stress responses, and that lumpfish might show individual variation in boldness during first interaction with the specific cues measured in stress hormones, monopeptides and/or cryptic adaptation.

Material and methods

Experiments were conducted from October to November 2018 in Mørkvedbukta research station in Bodø. A total of 144 lumpfish and 12 Atlantic salmon were used for animal experimentation after permit from the Norwegian Food Safety Authority (FDU 17231) based on the Norwegian Law on Regulation of Animal Experimentation (FOR-1996-01-15-23). The lab facilities had eight tanks (1 x 1 x 1.4 m) with 500 l/h flo , video monitoring and automatic feeders. Lumpfish were first acclimated to the tank environments for three weeks, before all fish were ID tagged with Floy Tags™. After tagging, six lumpfish with unique ID tags visible with video monitoring were put in each tank. After three days of further acclimation, lumpfish were introduced to the different sensory cue treatments. Four treatments split in duplicates, consisted of two tanks with the introduction of water flow from an adjacent tank with Atlantic salmon, two tanks where 3D model salmon were introduced, two tanks where two Atlantic salmon were introduced in each tank and two control tanks. Lumpfish behaviour was monitored 30 minutes prior to the introduction of each specific treatment and 30 minutes after. One hour after lumpfish had been introduced to the treatments, a 3 ml/L dosage of the cortisol-blocking hypnotic Metomidate was added to each tank and recordings stopped. Further, lumpfish were euthanized, and blood was sampled. Next, the telencephalon of the brain was dissected to assess measurements of monopeptides. Additional measurements of morphology included growth measurements and photography of each fish before and after the experiment. Photography was done to observe changes in cryptic blue-green colouration in the epidermis in addition to changes in black and white ratio, from melanophores. The experiment was run three times in total with new fish in each experiment, which gave six replicates for each treatment

Results

No fish died or showed signs of diseases during the experimental period. Lumpfish exposed to salmon odour showed increased vigilance in comparison with lumpfish exposed to 3D model fish, alive Atlantic salmon and control groups. Measurements of monopeptides related to stress and social behaviour showed variations in means of serotonin (5-HT) among the replicates, with lowest measurements among lumpfish exposed to odour. Lumpfish exposed to 3D model salmon were observed inspecting and attaching to the model fish

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Discussion

Results from the following study might provide useful knowledge on how lumpfish respond to Atlantic salmon with different predatory cues separated and accounted for through perception of single sensory variables. Any changes in morphology related to stress including changes in blue-green colour or black-white ratio, could be implemented as a non-invasive measurement of stress in field.

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FORECASTING CLIMATE CHANGE IMPACTS ON GREEK AQUACULTURE PRODUCTION: A CLIMEFISH CASE STUDY

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Introduction

The potential impacts of climate change on fish farming have been well documented. For the Mediterranean, several rising risks and potential opportunities have been recognized in relation to climate drivers such as temperature and extreme events (Brander et al., 2018; Rosa et al., 2012). There are, however, significant knowledge gaps regarding the effect of other drivers such as salinity, acidification, or HABs on both wild and reared fish stocks. It is therefore imperative that future changes are evaluated so that informed management incorporates climate change impacts and ensures sustainable future production. In that regard, the present study simulates mainly the effects of temperature under climate change on Greek aquaculture production for two species, the European sea bass (*Dicentrarchus labrax*) and the meagre (*Argyrosomus regius*), as part of the work conducted for a case study under the ClimeFish project.

Materials and methods

Models based on DEB (Dynamic Energy Budget) theory (Kooijman, 2010) were developed and validated for E. sea bass (Stavrakidis-Zachou et al., 2019) and meagre. These models simulate the bioenergetics of individual fish as a function of temperature and food availability following with an extrapolation to population level. Subsequently, downscaled projections of temperature under climate scenarios RCP4.5 and RCP8.5 were forced to simulate changes in nine Greek regions and for three time periods (2015-25, 2025-35, and 2045-55). In each region two theoretical farms were considered, one inshore and one offshore (>20km from the coastline) and production cycles of three stocking months (March, June, and September) were simulated for a group of fish. Furthermore, using the wind velocity projections from the models, extreme events (storms, i.e. wind velocity >50 km h^{-1} for more than 4 days and heatwaves, i.e. T>29^{o}C for more than 4 days) were defined. For these, an additional mortality was considered together with reduced feeding. The simulations report on variables such as individual growth, biomass, feed consumption and FCR (Feed Conversion Ratio).

Results

With respect to growth, simulations suggest that both species will grow faster in response to higher temperature, requiring shorter periods (up to 3 months, depending on the region) to reach various commercial sizes. The intensity of the trend is influenced by management options (stocking period, harvest size and site selection) with stocking time having the largest effect. An example of such results for RCP4.5 is given in figure 1. However, despite the benefit in growth due to rising temperatures, the effect on a population level is negative when extreme events are also considered. This is due to the extreme events influencing mortality rates and feeding, which can result in substantial losses in total biomass.

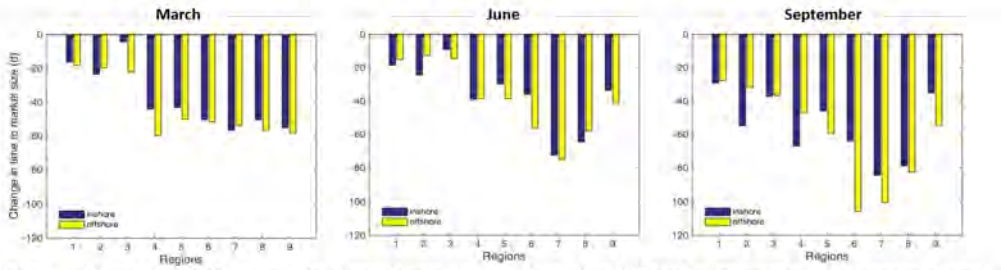

Fig. 1. Absolute differences in time (days) to market size 800g for E. seabass between short (2020)- and long (2050)- term climate projections for the climate scenario RCP 4.5. Inshore and offshore sites within the 9 regions are given in different colours.

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Conclusion

The study is a first step towards an assessment of the status of the Greek aquaculture under climate stressors. It considers the effect of temperature and wind velocity on the production of E. seabass and meagre. Simulations show that, overall, temperature will have a positive effect on the growth of both CS species, E. seabass and meagre but extreme events may also induce higher mortality rates and therefore losses in biomass. However, other impacts of climate change that may be severe for the industry have not been addressed. For instance, the positive correlation between pathogens and temperature may lead to increased frequency of disease outbreaks in the future which can be detrimental for production. Therefore, the findings of our study should be critically evaluated and potential threats from non-modelled climate drivers ought to be also taken into account for a more comprehensive assessment of the effects of climate change on aquaculture production.

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Acknowledgement

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BIOLOGICAL PERFORMANCE OF MEAGRE (*Argyrosomus regius*) UNDER HIGH TEMPERATURE

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Introduction

Meagre (*Argyrosomus regius*) is a eurythermal species that is becoming increasingly important for the Mediterranean aquaculture (Duncan et al., 2011). It was recently shown that meagre juveniles prefer temperatures between 26-30°C following acclimation in the temperature range of 18-30°C (Kir *et al.*, 2017). However, the response of the species towards the upper edge of its temperature tolerance range as well as the biological performance under prolonged exposure in those conditions remain largely unknown. The present study investigates the thermal tolerance and the biological performance of meagre in the 24-34°C temperature range.

Materials and methods

Juvenile meagre (mean weight 149g) were reared under three temperature regimes (24, 29 and 34°C) in triplicate tanks ($2m^3$) in a marine RAS for three months. Feeding was provided to satiation twice a day with commercial feeds and a 12D:12L light cycle was implemented. The fish were individually measured for length and weight on a monthly basis and 15 fish per treatment were sacrificed for collection of blood and tissues and subsequent determination of hematological, biochemical and hormonal parameters. Intermittent-flow respirometry was used (16 fish per treatment) to determine the standard (SMR) and maximum (MMR) metabolic rates and calculate the aerobic scope.

Results

Husbandry findings showed that fish performed poorly at high temperatures with growth being maximum at 24°C and ceasing completely at 34°C (figure 1) while the Feed Conversion Ratio (FCR) was the lowest at 24°C. In addition, mortality was particularly high at the highest temperature with more than half of the fish perishing by the second month. Preliminary analysis indicates that most of the considered physiological parameters were significantly different between the 34°C and the other two treatments. Finally, SMR increased linearly with temperature but this was not the case for MMR, resulting in the 34°C treatment exhibiting the lowest aerobic scope.

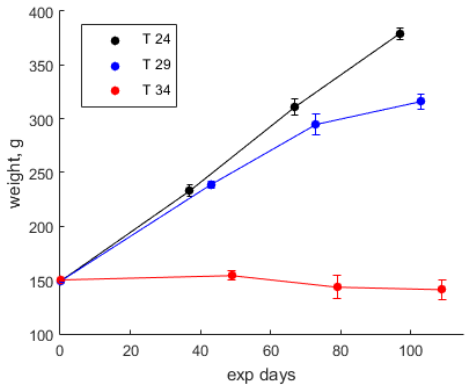

Fig. 1. Evolution of weight (g) during the trial under the three temperature treatments (24, 29 and 34°C). Points denote mean weights at sampling dates and vertical bars express the standard deviation.

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Conclusion

Overall, husbandry findings from the long term temperature tolerance experiment showed that fish perform best at 24°C while the temperature of 34°C is sharply close to the upper end of their temperature tolerance range. At 29°C the performance is appreciable, but not as good as at 24°C in terms of growth and FCR. The decreased aerobic scope exhibited at the highest temperature, suggests diminished capacity for metabolic performance while the overall poor physiological status is reflected by the pronounced mortality. The study provides insights into the performance of the species under high temperatures. This is highly relevant for the industry with respect to the exploitation of the species farming potential at a latitudinal scale, and especially in the context of climate change.

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SELECTING MICRO-ORGANISMS CAPABLE OF REMOVING THE COMMON RAS OFF-FLAVOR, GEOSMIN

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Introduction

Off-flavor of aquaculture RAS products is a major problem for the industry that affects market demand and prices (Tucker, 2000). Off flavor can originate from the animal's diet or post-harvest management strategy but is mainly caused by odorous microbial metabolites which are absorbed from the water and deposited in edible tissues (Tucker, 2000; Schrader & Rimando, 2003)»type»:»book»,»volume»:»8»},»uris»:[«http://www.mendeley.com/documents/?uuid=ebfed729-2163-41a3-bb1d-d680c9f57928»]},{«id»:»ITEM-2»,»itemData»:{«DOI»:»doi:10.1021/bk-2003-0848.ch001\r10.1021/bk-2003-0848.ch001»,»ISBN»:»0-8412-3821-9»,»abstract»:»The demand for aquaculture products continues to accelerate worldwide. One of the most economically significant problems encountered in aquaculture is \»off-flavor\» in the cultured product. Off-flavors can be related to the diet of the cultured animal, caused by inadequate post-harvest management strategies, and/or environmentally derived. The latter has received the most attention in terms of research approaches for developing methods to prevent off-flavors in aquaculture products. The production of off-flavor compounds by certain species of cyanobacteria is the greatest contributing factor to off-flavor in fishery products. In this chapter, the types and causes of off-flavor that may occur in a wide variety of aquaculture products are discussed. In addition, the most recent developments in research for the prevention of offflavor in aquaculture are discussed, and recent technological advances in off-flavor detection are presented.»,»author»:[{«dropping-particle»:»»,»family»:»Schrader»,»given»:»Kevin K»,»non-dropping-particle»:»»,»parse-names»:false,»suffix»:»»},{«dropping-particle»:»»,»family»:»Rimando»,»given»:»Agnes M»,»non-dropping-particle»:»»,»parse-names»:false,»suffix»:»»}],»containe -title»:»Off-Flavors in Aquaculture»,»id»:»ITEM-2»,»issue»:»848»,»issued»:{«date-parts»:[[«2003»]]},»page»:»1-12»,»title»:»Off-Flavors in Aquaculture: An Overview»,»type»:»article-journal»,»volume»:»848»},»uris»:[«http://www.mendeley.com/documents/?uuid=377d5050-e1de-4e7b-b197-c368a2d4a4d8»]}],»mendeley»:{«formattedCitation»:»(S. Tucker, 2000; Schrader & Rimando, 2003. The most common description of off-flavor is the musty, earthy smell which originates from geosmin that has been described by Mallevialle & Suffet (1987) and Suffet *et al*. (1999). In this study, micro-organisms, capable of degrading geosmin, were selected, identified, and evaluated on applicability in RAS systems. This research is part of a larger study that aims at developing a biological filter to reduce off-flavor problems in depuration systems or full-scale RAS.

Material and methods

Micro-organisms capable of degrading geosmin were selected from a biofilter by inducing growth on the off-flavor molecule as sole carbon source. Thirteen selected strains of bacteria and fungi were evaluated on several criteria related to applicability in RAS. Growth potential were established on LB (I). Toxicity of the strains was tested on *Artemia* (II). Biolog assays were performed on all strains (III). Strains were identified using ITS4 and 16s sequencing (IV). Cell metabolic activity on geosmin was tested using MTT assays (V). Finally, the selected strains were tested for their geosmin degradation potential using low geosmin concentrations (VI). Geosmin degradation was evaluated using SPME. Growth on goesmin as sole carbon source was evaluated using flowcytometr , formation of flocculants, OD measurement and CFU

Results

A total of 13 strains were selected during two selection experiments. These 13 strains were then identified and evaluated on applicability in RAS.

(I) After a duration of 96 hours, all the strains reached stationary phase. Indicating that all selected geosmin degrading strains are particularly slow growing micro-organisms. Strain F3 was characterized by a very slow growth and a low maximum cell density and was therefore considered unfit for application in geosmin degrading biofilter

(II) In general, mortality of *Artemia* varied widely both between different strains and between repeated experiments. Among the strains tested, strains F5, F6, F7, F11 and F12 showed significant higher mortality towards the control treatments. Since toxicity was tested towards a robust species, these strains were considered unfit for use in aquaculture facilities. Further toxicity screenings are needed before any of the strains can be used in geosmin degrading biofilters

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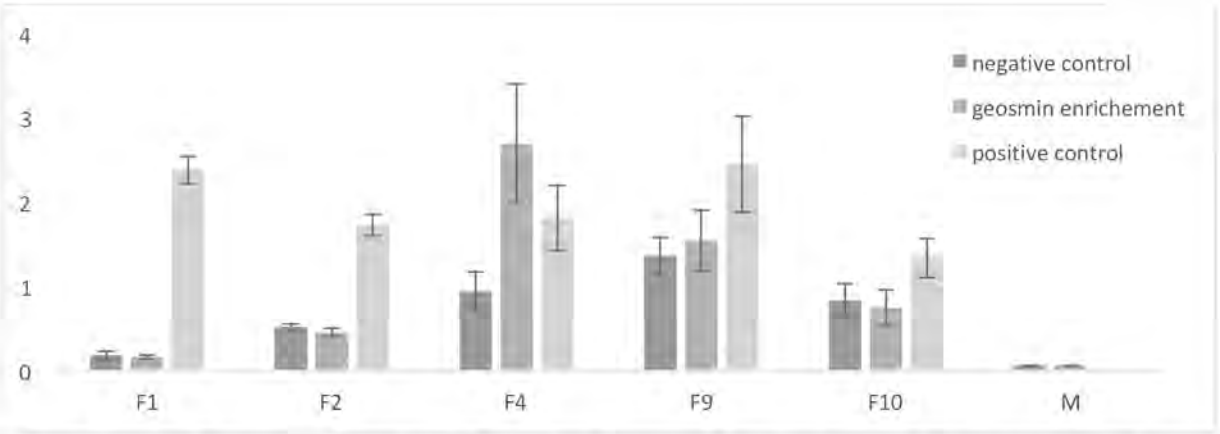

Figure 1. Optical density at 570nm (MTT assay) of treatment with cells suspended in mineral media (no geosmin), mineral media enriched with geosmin and LB.

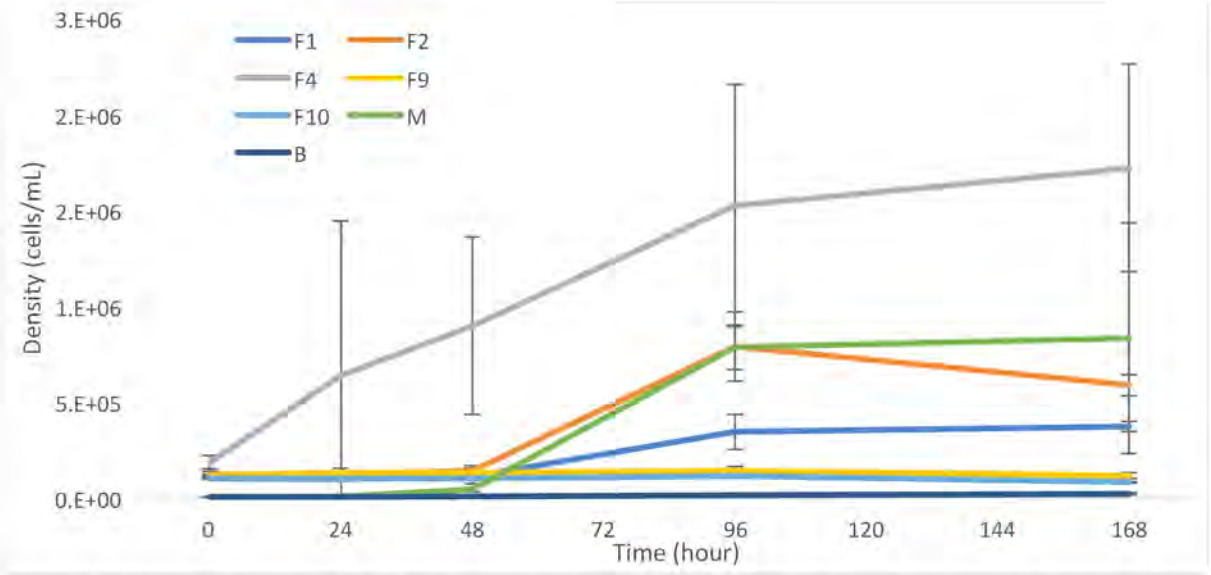

Figure 2. Microbial growth with low concentration geosmin as sole carbon source; cell densities were measured using flowcytometry

(III) Biolog assays indicated that strains varied significantly in affinity for certain C-sources, indicating that strains are metabolically different from each other.

(IV) Identification resulted in the following strains

F1: *Bosea .sp*
F2: *Bosea .sp*
F4: *Mycobacterium .sp*
F9: *Roseomonas .sp*
F10 *Brevundimonas .sp*

(V) MTT assays (figure 1) clearly show that all strains show high metabolic activity in LB (positive control) compared to mineral medium (negative control). F4 and F9 show an increased metabolic activity when grown in a with geosmin enriched environment. MTT assays may therefore suggest that F4 and F9 might have the possibility to degrade geosmin.

(VI) Growth of micro-organisms in media with only geosmin as carbon source resulted in overall slow growth (Figure 2). Strain F4 (*Mycobacterium .sp*) shown best performance compared to others. F9 and F10 showed no growth over the duration of the experiment. Analysis of geosmin concentration showed comparable results; with F4 and F10 being the only strains able to degrade geosmin.

Conclusion

Results from this study are promising, with at least 2 strains (F4: *Mycobacterium .sp* and F9: *Roseomonas .sp*) proven to be able to remove geosmin. To be able to apply these strains in biofilters for aquaculture purposes; further research on toxicity and mechanism of action is required.

DIVERSIFICATION OF GLOBAL SHRIMP FARMING THROUGH TRANSPARENCY – THE NEW ASC METRICS METHODOLOGY

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Introduction

The Aquaculture Stewardship Council (ASC) is an independent not for profit organisation founded in 2010 by World Wildlife Fund (WWF) and the Dutch Sustainable Trade Initiative (IDH) It aims to be the world's leading certification and labelling programme for responsibly farmed seafood. The ultimate goal of all ASC standards is to reduce the environmental and social impact of aquaculture worldwide. The standards are reviewed and revised every 3 – 5 years at a minimum in order to incorporate learning from stakeholders' feedback and from the Monitoring and Evaluation Programme. The revision process can also include adding new species to an existing standard.

The ASC Shrimp Standard v. 1.0 is currently under revision. The Standard currently covers species under the genius *Penaeus* (and *Litopenaeus*) and is oriented towards the production of *L. vannamei* and *P. monodon*. Other species of shrimp are eligible for certification if they can meet the specified performance thresholds. The increased interest in more diversified shrimp farming practices over the last years is not only driven by the market but also by lower susceptibility to certain diseases, easier reproduction, as well as different environmental requirements such as broader optimal temperatures and salinities (Briggs et al., 2004; Laubier and Laubier, 1993). Related to this and advancing technology a species diversification has occurred and should be reflected in the standard

An ASC desk study was facilitated in 2017 in order to identify potential new candidates for the inclusion in the standard. As a result it was decided to further evaluate the potential inclusion of *Penaeus stylirostris* (Blue Shrimp), *Penaeus merguiensis* (Banana Prawn) and *Penaeus japonicus* (Kuruma Prawn). Discussion with various stakeholder further identified *Penaeus ensis* (Greasyback or Pink Shrimp) as a species of interest.

Materials and Methods

The focus of this work was to investigate the necessity to include species specific metrics for the above mentioned species in the revised ASC Shrimp Standard. Therefore producer countries and volumes were identified using FAO data. Information about on farm production was so far collected mainly via literature research. Data from ASC certified farms was also included in order to compare literature data with actual data on ASC certified farms for the potential new species as well as for *P. mondon* and *L. vannamei*.

Results and Discussion

There is only limited data available for *Penaeus stylirostris* (Blue Shrimp). *P. stylirostris* has a similar protein requirement to *P. monodon* (~ 36 – 42%) and comparatively higher survival rates and tolerates higher stocking densities (Briggs et al., 2004). FCR of the two ASC certified farms was reported as 2.3, thus similar to literature data were the FCR was reported as 2.82 ± 0.22 (Hernandez-Llamas et al., 2004).

P. merguiensis is often a by-product of extensive shrimp aquaculture thus there is only little information available on survival rates or feed efficienc .

Penaeus japonicus is often considered the most demanding penaeid in terms of protein requirements (up to 55%) and only little data is available on feed efficiency with FCR fluctuating between 0.47 to 7.04 (Laubier and Laubier, 1993; Türkmen, 2007).

Data availability on *P. ensis* is scarce and literature research is still ongoing for this species.

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Overall it appears that both *P. merguiensis* and *P. japonicus* are similar to *P. monodon*, with slightly higher protein requirements. Protein requirements are often overestimated and are likely similar for most penaeid shrimp (Guillaume et al., 2001). It is therefore suggested, to add *P. merguiensis* and *P. japonicus* to the ASC Shrimp Standard, using the same values as for P. monodon.

P. stylirostris appears to be similar to *L. vannamei* in terms of survival rates with slightly higher protein requirements (more similar to *P. monodon*). It is therefore somewhere between the two species. There are currently two farms producing *P. stylirostris* certified to the ASC Shrimp Standard, based on the metric requirements for *P. monodon*. Based on the low production values of only 2,401t it should be considered, whether adding *P. stylirostris* specifically to the ASC Shrimp Standard is reasonable or not. It is therefore suggested to continue certifying *P. stylirostris* according to the metric requirements for

P. monodon.
However, as the obtained data is so far only based on scientific literature research. Including production data in the evaluation of metric requirements is indispensable according to the ASC Metrics Methodology. The ASC is therefore currently working on the acquisition of production data for all three species and requests producers to contact the ASC if they would be able to share data on any of these species.

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THE MULTIFUNCTIONAL GILL – THE ACHILLES'HEEL OF FISH HEALTH?

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Introduction:
Fish gills with their large surface area, thin branchial epithelium and high vascularization are a fascinating organ which is involved in oxygen and carbon dioxide exchange, in water and ion transport as well as in the excretion of ammonia. Due to this multifunctional nature and the numerous interactions between the processes taking place in gills, branchial diseases may have serious implications on fish health and can cause several complications for the organism. Fish gill can be infected by numerous pathogens, including parasites, bacteria and viruses. In common carp, *Cyprinus carpio*, the infection of gills with the poxvirus carp edema virus (CEV) is often associated with severe clinical signs including lethargic behavior of the infected fish, a characteristic lying at the bottom of the tank and with progression of the disease the activity of the fish decreases until its eventual death. Because of the characteristic lethargic behavior of clinically affected carp, the disease is assigned as "Koi sleepy disease" (KSD). Because this virus infects predominantly the gills of carp, CEV infection can serve as a unique model for studying branchial disease in carp.

Materials and Methods
Blood, gill and kidney tissue were collected from common carp experimentally infected with CEV and suffering from clinical KSD. The blood samples were subjected to gas analysis and flame photometry as well as global non-targeted metabolomics using GC-MS and LC-QTOF/MS. Tissue samples were subsequently subjected to DIGE based proteomics and RT-qPCR gene expression analyses. A salt rescue model was used for further investigating immune responses by analysing mRNA expression of genes (*cd4*, *cd8*, *igm*, *casp9*, *inos*, *tcr a2*, and *mpo*) involved in different immune responses.

Results
The analysis of blood gasses, ions concentrations and metabolite profiling indicated that observed clinical signs are most likely related to disturbed transport processes across the gill epithelium, resulting in ion dysregulation (drop of the blood sodium level from >600 µmol l^{-1} in control carp to <90 mmol l^{-1} in infected carp) and an accumulation of ammonia in circulating blood rather than effects of hypoxia or hypercapnia. Analyses of over 2,500 metabolites showed changes in the pyrimidine and urea cycle as well as the beta-alanine and amino acid metabolism in blood plasma at day 6 p.i.. The clinical signs can be abolished by the addition of salt to the tank water, which circumvents the drop of sodium levels and also rescues from ammonia accumulation in the blood of infected carp. Haematological analyses confirmed that the KSD affected carp experienced severe leucopenia and granulocytosis, with a 4-fold drop of leucocyte counts by day 6 post infection. The DIGE based proteomic studies of gill tissue from these carp indicated that 86 proteins were significantly changed during the onset of severe KSD. Besides an up-regulation of antiviral and antimicrobial innate immune responses (Mx, LyzC and ApoAI), signs of an immuno-suppression were noticed. This included a down-regulation of the antimicrobial peptide NK-lysin-like, which could indicate lower activity of NK cells, and T-cell responses in KSD affected carp. The Down-regulation of calpain and caspase indicated a pro-apoptotic effect of the infection. Increased inflammation was mediated by the down-regulation of the anti-inflammatory proteins GSN, ANXA1, SCIN. An immuno-suppression in KSD affected koi was also indicated by significantly lower mRN expression levels of the genes encoding cd4, tcr a2 and igm proteins.

The proteomic analysis of gill tissue from KSD affected carp revealed also increased concentrations of several heat shock proteins, which could indicate elevated stress response in infected gills.

Interestingly, the salt treatment eliminated all observed immunosuppressive effects of the CEV infection.

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Discussion and Conclusion

Common carp, which is moderately tolerant to hypoxia, has the ability to circumvent the impairment of oxygen availability related to proliferative changes of the branchial architecture during CEV infection. The physiology of KSD affected carp is more seriously affected by the dramatic disturbance of the ionic homoeostasis and the severe accumulation of ammonia in circulating blood. This is impressively documented in the salt rescue experiments; in which the restoration of physiological sodium levels abolished all clinical signs. Even though poxviruses encode multiple immunomodulatory proteins, the results from the salt rescue experiment suggest that the immunosuppression observed in carp suffering from KSD was also related to the increased ammonia level and the loss of the osmotic balance. The suppression of T- and B-cell responses in KSD affected carp could foster the development of secondary infections, which often accompany clinical KSD. In conclusion, the studies on KSD affected carp clearly underline that a disturbance of osmotic balance and ammonia excretion during branchial disease of carp can be decisive for the outcome of the disease and even may impact immune responses to pathogens.

A NEW CONCEPT IN MULTITROPHIC AQUACULTURE; FARM CULTURED EURASIAN PERCH (*Perca fluviatilis*), RAINBOW TROUT (*Onchorhynchus mykiis*), COMMON DUCKWEED (*Lemna minor*) AND GIBBOUS DUCKWEED (*Lemna gibba*)

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Introduction

Aquaculture remains the fastest growing food production sector in the world. However, this rapid expansion is increasingly limited by the lack of suitable water sources, availability of suitable sites and by the ecological carrying capacity of the surrounding environment. For this reason, indoor, fully controlled recirculating aquaculture systems (RAS) are attractive. However, at present these systems do not contribute much to global aquaculture production and currently the majority of aquaculture production is realised from more traditional ponds (Hargreaves 2006). Concerns about the environmental impacts of the rapid expansion of intensive aquaculture systems have recently led to increased research interest in integrated multitrophic aquaculture systems (IMTA). Because inland freshwater IMTA is a new practice, a study was conducted to assess water quality in a hybrid system comprised of combined split-pond and duckweed culture lagoons. Here, we present the first data from this unique aquaculture system. A key aspect of the farm trial is the development of a sustainable multitrophic culture system holding European perch (*Perca fluviatilis*), rainbow trout (*Oncorhynchus mykiis*), common duckweed (*Lemna minor*) and gibbous duckweed (*Lemna gibba*), with associated organic status.

Materials and methods

The farm trial is located within a cutaway bog, that has been historically harvested for peat by Bord na Móna company. The site is located in County Offaly, Ireland. The farm itself is powered by a wind turbine. The aquaculture system consists of 4 split (pill) ponds connected with through a duckweed lagoon that acts as a treatment system. The farm is designed to discharge excess water only during heavy rainfall. Water is sourced from existing peat drainage systems using sub-surface water pumps.

The fish are kept at a density that does not exceed the organic farming standard (< 20 $kg.m^{-3}$ for perch), using screens at the D-ends of each split pond (Fig. 1). Each D-end is designed to hold fish of a different size category (mesh size varied from 7 to 25 mm). The space between two D-end fish culture areas is used to treat waste with free living algae and bacteria (in suspension). Algae also produce oxygen during daytime hours which is utilised by the fish. Flow in each split pond is generated and water is circulated using an airlift (SKV-tec GmbH). Each D-end fish culture area is equipped with oxygen and temperature probes (Oxyguard Pacific) connected to paddlewheels (Aquaculture Fishtechnik GmbH) to provide extra oxygen when necessary (when saturation is below 75-85 %). Moreover, a Seneye on-line system is placed in each pond to monitor pH, free ammonia and temperature. The AQUAMONA farm is currently holding European perch and rainbow trout. Lagoons are planted with common duckweed and gibbous duckweed stabilised. These cultures are stabilised with different kinds of shelters to prevent the negative impact of wind. Lagoons are monitored for total duckweed cover every two weeks using a drone (DJI Phantom 4 Pro V2.0). Measurement of cyanobacteria, chlorophyll level and turbidity is performed on daily basis using the AlgaeTorch (bbe moldanke GmbH). Total ammonia, nitrite, nitrate and orthophosphate concentration are measured every 3 days using a HACH DR3900 spectrophotometer and appropriate LCK kits.

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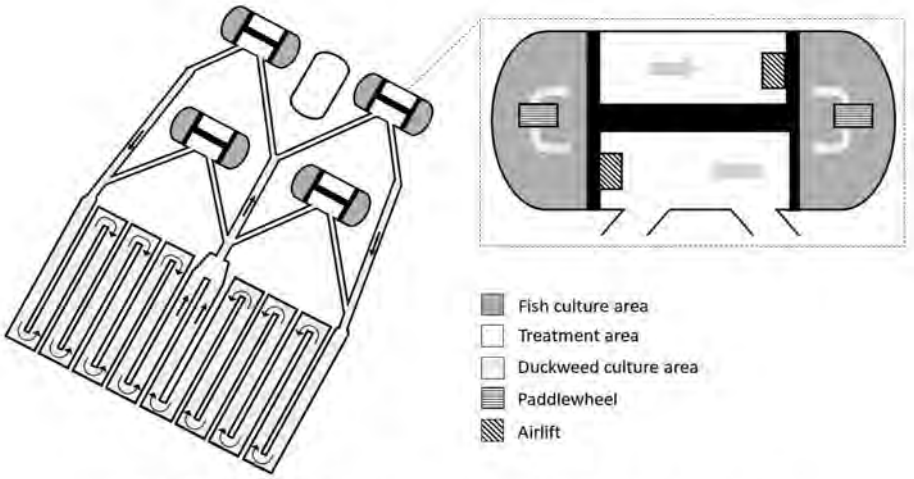

Fig. 1. AQUAMONA farm layout at Mount Lucas (Bord na Móna)

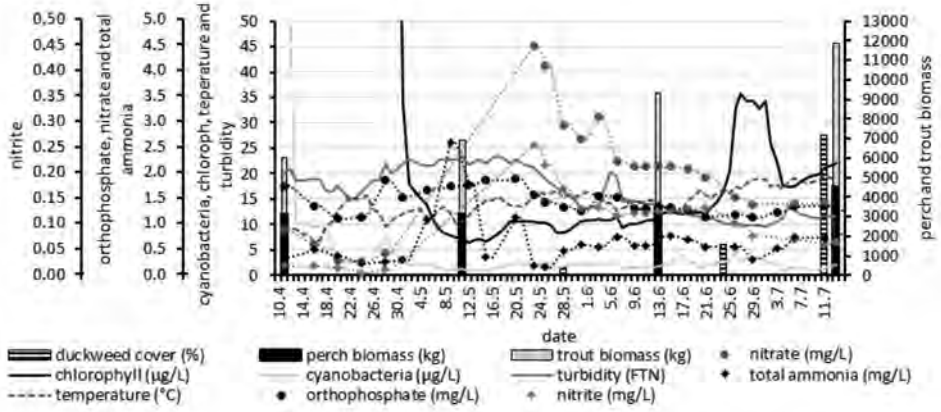

Fig. 2. Development of fish biomass (perch and rainbow trout), feed usage, total ammonia, phosphate, nitrite, nitrate, cyanobacteria, chlorophyll and turbidity level.

Results

Previous results about water quality, fish and duckweed performance are presented in Fig. 2. Values for all critical water parameter was within suitable values recommended for perch and trout. Moreover, oxygen levels (nor shown) varied from 75 to 145 % depend on chlorophyll level and day phase.

Conclusion

The semi-commercial scale system for perch, trout and duckweed production based upon IMTA principles is now successfully in operation in Ireland. Emphasis is now on fine-tuning hydrodynamics, microbial ecology, probiotic effects associated with elevated bacteria and algae densities, and duckweed effectivity.

Acknowledgements

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THE USE OF BIOLOGICAL TRAPS FOR WATER TREATMENT IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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Introduction

The high concentrations of dissolved nutrients in recirculating aquaculture systems (RAS) allow development of technologies for exploiting the waste as a valuable resource (e.g. integrated multi-trophic aquaculture approach) (Martins et al., 2010) and to reduce nutrient release into the surrounding environment. This project evaluates the use of microalgae and filter feeder cultivation in RAS effluent for efficient removal of nutrients. So far we have evaluated (1) the effectiveness of different microalgae species to remove dissolved nutrients from the RAS wastewater, (2) the use of three different LED grow lights with continuous spectra on microalgal biomass production and nutrient removal, and (3) the potential of filter feeding microcrustacean *Daphnia magna* to filter the microalgae from the water. This project produces new knowledge on nutrient recycling and thus supports the concept of circular economy, and conservational and sustainable management.

Material and methods

The wastewater originated from a laboratory scale (total volume *ca*. 1000 L) RAS at the University of Jyväskylä, Department of Biological and Environmental Science, containing whitefish (*Coregonus lavaretus*, Salmonidae) fed with Circuit Silver Opti 2.5 dry feed (Raisio aqua, Finland) with a belt feeder 24 h per day, at *ca*. 17°C.

The growth and nutrient uptake of 10 microalgae strains was assessed in unfiltered RAS wastewater in comparison with an algal growth medium. The experiments were conducted in 400 mL aerated batch cultures that enabled axenic maintenance of the cultures during sampling.

Results

Green microalgae showed a good growth potential and nutrient removal not only in reference medium but also in unfiltered RAS wastewater, while non-green microalgae performed poorly both in wastewater and in reference medium. The densities and specific growth rates of the green microalgae species did not differ between wastewater and reference medium. Microalgae *Acutodesmus* sp. and *Selenastrum* sp. had the highest density followed by *Monoraphidium griffithii*, *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, and *Haematococcus pluvialis*.

The growth and nutrient removal of *C. reinhardtii*, *M. griffithii*, and *Selenastrum* sp. in unfiltered RAS wastewater did not vary between the three light spectra, whereas the three microalgae differed from each other for growth parameters and nutrient removal.

Waterfleas (*Daphia magna*) increased their dry weights 2-4 times when fed with four different green microalgae (*C. reinhardtii*, *H. pluvialis*, *M. griffithii*, and *Selenastrum* sp.) previously cultivated in filtered RAS wastewater. After 48h, waterfleas removed 80% of *M. griffithii*, 70% of *H. pluvialis*, and 20% of *Selenastrum* sp. from the RAS wastewater.

Discussion and conclusions

The main results obtained from the project: (1) Unfiltered RAS wastewater promotes growth of green microalgae in similar extent as in reference medium at *ca*. 17° C. (2) Biomass production and nutrient removal in RAS wastewater varies among the tested species of microalgae. (3) Green microalgae can be used for RAS wastewater treatment as they improved the water quality by reducing the concentrations of nitrate and phosphate. (4) Continuous spectrum LED lights may be used efficiently to obtain high removal of dissolved nutrients and high microalgal biomass. (5) Waterfleas (Daphnia magna) are capable of filter ng and consuming green microalgae for their growth in RAS wastewater. (6) This technique has a potential to promote circular economy.

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ESTABLISHING BENTHIC ECOLOGICAL STATUS AROUND SALMON AQUACULTURE CAGES USING DNA-BASED ENVIRONMENTAL MONITORING

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Introduction

The rapid growth of coastal-based cage aquaculture has yielded in significant socio-economic benefits but is accompanied by increasing environmental impact. Through continual organic enrichment (uneaten feed and faeces), the receiving benthic environment around fish cages may experience pronounced changes in sediment geochemistry and benthic communities. The seabed eventually becomes acidified and oxygen-depleted because of microbial degradation processes. Because there is a tradeoff between acceptable environmental impact of aquaculture and socio-economic benefits, international regulatory systems for sustainable industrial development with minimal environmental impacts are in place worldwide (Borja et al., 2013). One objective of such Environmental Impact Assessment (EIA) is to determine the (spatial) scale of aquaculture impacts. Traditionally, these monitoring programs rely on the microscopic identification of benthic macroinvertebrates as bioindicators, based on which the ecological quality status is determined via index or metric calculation. However, this is very expensive, time consuming and error prone. Given the growth of the aquaculture sector, and the demand for more frequent and stricter monitoring programs by regulators, there are concerted efforts to develop alternative environmental monitoring strategies. We present a "ready-to-use" pipeline, which infers the ecological quality status of samples from traditional monitoring transects through the exploitation of eDNA metabarcodes obtained from benthic bacterial communities as bioindicators.

Material and methods

In parallel to a traditional macroinvertebrate-based environmental monitoring of salmon farms in Scotland and Norway, we from the same samples have obtained genetic markers (eDNA metabarcodes) of benthic bacterial communities. Therefore, DNA was extracted from sediment samples using Qiagen's PowerSoil DNA extraction kit. According to a protocol described in detail by Stoeck et al. (2018), we then amplified the hypervariable V3/V4 region of the bacterial SSU rDNA gene as taxonomic marker. The obtained PCR products were then subjected to high-throughput sequencing (Illumina MiSeq). Obtained sequence data were then processed and taxonomically assigned according to a standard pipeline as described in Stoeck et al. (2018). Non-metric multiple dimensional scaling (NMDS) was used to compare sample sites with each other based on bacterial community profiles and to relate community patterns to environmental variables and macrofauna-derived ecological quality status groups. Sample site patterns obtained from bacterial DNA markers were then compared to patterns obtained from macroinvertebrate communities from traditional monitoring.

In a next step, two approaches were compared and assessed to infer ecological quality. (a), indicator value (IndVal) inference (Dufrene and Legendre, 1997): indicator values were calculated for eDNA metabarcodes, based on which a variant of the widely used AZTI Marine Biotic Index (AMBI) was calculated to establish ecological quality of the samples analyzed. (b), supervised machine learning (SML) (Cordier et al., 2019): using eDNA metabarcodes and ecological quality information for each sample obtained from macroinvertebrate-based monitoring, we trained an algorithm, which identified indicator eDNA metabarcodes to predicted ecological quality status.

Results

Clustering of sample sites obtained from monitoring transects of salmon farms showed excellent pattern-matching between bacterial eDNA patterns and microscopy-derived macroinvertebrate patterns. Clustering of sites based on bacterial eDNA correlated significantly among others with distance from cage edges, macroinvertebrate-derived metrics and indices, and with ecological quality. Both approaches, SML and IndVal inference for eDNA metabarcodes with subsequent calculation of an AMBI variant performed equally well in establishing ecology quality for the investigated salmon farms. Accuracies of correct ecological status assignments to the investigated samples were ca. 80% for both methods, when compared to traditional macrofauna monitoring results of the same samples. This is a remarkable achievement considering that the number of investigated farms in this study was relatively low for SML and IndVal inference.

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Discussion and Conclusion

Our study showed a) the high potential of eDNA metabarcoding of benthic bacterial communities for environmental monitoring of salmon farms, and b) how eDNA metabarcodes can be used to establish ecological quality as known from traditional macrofauna-based monitoring. Metabarcode-based metrics are much cheaper to generate, more objective and are not dependent on a declining pool of taxonomists. Metabarcode-based metrics can be obtained within two weeks of sampling (compared with 3 – 4 months for traditional methods) enabling near-real-time management (e.g. of stock biomass). This near-real time management could be used to prevent a severe ecological impact beyond the allowable zone of effect (AZE). But also, a more frequent monitoring or a modified, more informative monitoring scheme at no additional costs compared to the current macrofauna-based monitoring would be possible through eDNA metabarcoding. Further research efforts are to make this technology ready for the implementation into official regulations. This includes for example the eDNA monitoring of more salmon farms from different geographic areas as well as in different seasons. SML and IndVal accuracies will increase with sample numbers, and, also, we will obtain a better understanding of seasonal and spatial dynamics of bacterial eDNA indicators.

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STUDY OF THE AMINO ACID AND FATTY ACID COMPOSITION AND ANTIMICROBIAL ACTIVITY AGAINST PATHOGENIC BACTERIA IN AQUACULTURE OF EIGHT MICROALGAE SPECIES

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Introduction

Microalgae account for the basis of the food chain in aquatic ecosystems and they are ubiquitously distributed throughout the biosphere (Amaro et al., 2011). This wide span of ecosystems contributes to the myriad of chemical compounds that they are able to synthesize, thus accounting for their unique potential in blue biotechnology. In aquaculture, bacterial infections are nowadays considered as the main responsible for serious mass mortalities and considerable economic losses and antibiotics have been largely used in intensive farming, however, the risk of transferring resistance to humans bacteria have led to a great concern for public health about the misuse of antibiotics. The antimicrobial activity of microalgae has been largely studied and it has been attributed to compounds belonging to several chemical classes – including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons. In this work different species of microalgae cultivated in ANFACO-CECOPESCA have been studied to check the antimicrobial activity against *Vibrio anguillarum* CECT 522 and *Aeromonas salmonicida* subsp. *salmonicida* CECT 894. Also, amino acid and fatty acid composition of these species have been analyzed to study the correlation among these two parameters and antimicrobial activity results.

Materials & methods

Amino acid composition, fatty acid composition and antimicrobial activity of eight microalgae species (*Tetraselmis chuii, Chaetoceros calcitrans, C. salsugineus, Nannochloropsis gaditana, Pavlova gyrans, Conticriba weissfloggi, Rhodomonas lens* and *Isochrysis galbana*) against two fish pathogens *V. anguillarum* and *A. salmonicida* has been determined.

Microalgae were cultured in 10-L carboys with seawater supplemented with the commercial nutritive solution Goldmedium (Aqualgae S.L., A Coruña, Spain) following manufacturer's directions. Aeration was supplemented with CO_2. When cultures reached early stationary state, cells were harvested by ultrafiltration through a 300 kDa Pall membrane and further spray-dried with a Büchi B-290 unit,

Total protein and amino acid profile were analyzed using Kjeldahl method and high-performance liquid chromatography (Vázquez-Ortiz et al., 1995) and fatty acid using the quantitative SOXHLET method based on the extraction of fat from the sample by ethyl ether and it was performed by gas chromatography based on the separation and determination of fatty acid methyl esters.

Microalgae aqueous and ethanolic extracts were obtained from lyophilized microalgae. The antimicrobial activity of these extracts was tested by agar well diffusion technique (Fajardo et al., 2014) and by spectrometry (Garrido et al., 2013). In the first case, extracts were pipetted in TSA and marine agar previously inoculated with *A. salmonicida* and *V. anguillarum* respectively, and incubated at 26°C and 30°C for 24h, before observing the presence of inhibition halos. In the second case, the growth of both pathogens, in presence and absence of the different extracts, was determined over 24h by quantifying the optical density (at 600nm) of the culture medium in a spectrophotometer; the experimental growth data obtained were fitted with a logistic equation (Falaise et al., 2016).

Results

The percentage of proteins was higher in *R. lens* and *N. gaditana*. In both cases they exceeded 30% of the total composition. Regarding lipids, *I. galbana* showed a fairly high level compared to the rest of microalgae analyzed with a percentage of total lipids close to 10%. AA profile was similar in the majority of the microalgae analised.

Fatty acid profiles showed that *C. weissflogii*, *R. lens* and *P. gyrans* have more saturated than unsaturated fatty acid. *N. gaditana* stands out with more than 70% UFA of which 75% are PUFA. Unlike, *T. pseudonana* has more than 49% of the fatty acids as MUFA.

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The test of aqueous extracts by agar diffusion techniques did not allow the formation of inhibition halos for the fish pathogens tested, while the ethanolic ones presented antimicrobial activity; however, the DMSO used for resuspending the extracts may cause part of the inhibition. More tests are needed to confirm the results. On the other hand, promising results have been obtained by spectrometry in liquid medium in 96-well plate, where ethanolic extracts (0.5 and 0.1mg/mL) of all tested microalgae produced a complete inhibition of *A. salmonicida* growth. Also, the aqueous extracts influenced significantly ($p<0.05$) the bacterial growth parameters: *K* obtained after 24h was reduced for all extracts except for those obtained by *R. lens;* some extracts reduced the μmax and increased the *b* of this pathogen. For *V. anguillarum* only concentrated ethanolic extracts gave positive results. Similar results have been obtained previously for *Tetraselmis sp.* and *Chaetoceros sp.*

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AQUAPONIC WATER SUPPRESSIVENESS ON *Pyhtium aphanidermatum* ROOT ROT IN LETTUCE AND ITS ORIGIN

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Introduction

In couple aquaponic system, hydroponic plants production, fish rearing and nitrifying bacteria are constituents of the same system and consequently share the same water. Because of this coexistence, phytosanitary treatments for plant diseases management are a delicate matter. Among plant pathogens, fungus-like Oomycetes responsible of plant diseases are among the most problematic because of their possible fast spread linked to zoospores (mobile spores) release in the irrigation water. However, it appears that aquaponic systems could be naturally armed against plant pathogens. This protective action is called suppressiveness (i.e. suppressive action) and refers to the cases where (i) the pathogen does not establish or persist; or (ii) establishes but causes little or no damage (Postma et al. 2008). In *in vitro* laboratory experiments, aquaponic water has shown a direct inhibitory effect on Pythium spp. growth (Gravel et al. 2015; Sirakov et al. 2016; Stouvenakers et al. 2018). To confirm this discovery, *in vivo* experiments using *Pythium aphanidermatum* plant pathogen have been carried out on lettuces growing in aquaponic (AP) water, hydroponic (HP) water or complemented aquaponic (CAP) water. CAP water results from the addition of mineral salts in AP water to obtain identic concentrations of mineral nutrients as the ones contained in HP water.

Materials and methods

Suppressiveness of AP, CAP and HP water were evaluated by comparing root symptoms, leaf yields and root yields of P. aphanidermatum inoculated lettuces with the corresponding negative controls. For each treatments, samples of lettuces rhizosphere, rhizoplan and endosphere have been conserved for microbiota analysis by Illumina high throughput sequencing of ITS and 16S rDNA genes.

Results and conclusions

Results show that root symptoms of inoculated lettuces are significantly lower when they grow in AP water compared to CAP and HP waters. Foliar yield, root yield and leaf turgidity also decrease significantly less when using AP water. These results also highlight that the physicochemical modifications of AP water to make the CAP water probably disturb the natural microbiota and lead to the loss of its suppressive capacity. To elucidate the origin of AP water suppressiveness, a comparative bioinformatics analysis of microbiota diversity and OTU's composition of the different samples is in progress. Moreover, this study could contribute to find novel biocontrol agents for plant pathogens control in aquaponics

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A RETROSPECTIVE OF OUR INTERNATIONAL RESEARCH COLLABORATION ON THE USE OF PERACETIC ACID IN AQUACULTURE

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Introduction

Peracetic acid (PAA) is a compound that is produced from acetic acid, hydrogen peroxide (H_2O_2) and water; it also contains a stabilizing agent. The resulting product is an equilibrium solution of these components that has long shelf stability. In addition, PAA breaks down quickly in the environment to water and vinegar. PAA has greater reactivity and lipid-penetrating properties than H_2O_2 alone and is not deactivated by catalase and peroxidase (naturally occurring on organism membranes) which happens with H_2O_2.

Thus, PAA eliminates some unwanted organisms easier and faster. Most importantly, it is eco-friendly and does not leave dangerous residues, such as trihalomethanes and haloacetic acids when it decomposes or reacts with naturally occurring organic matter as many compounds do (e.g., chlorine compounds). It is a potent disinfectant and has replaced chlorine-based disinfectants or sanitizers in many industries. Uses include: sanitation in food/beverage plants, agricultural facilities, wineries/breweries, greenhouse equipment, animal housing, preventing bio-film formation in paper/pulp industries, wastewater treatment, commercial laundries and poultry processing.

Discussion

When we started our collaboration in 2007 (Straus and Meinelt 2019), we recognized that PAA had much potential in aquaculture. But, there was very little information on the use of PAA in aquaculture, both in the U.S. and Europe, so we devised a plan to develop research and promote PAA. We understand that there are other publications on the use of PAA in aquaculture, but there has not been a concerted effort like ours. This collaboration is important because of the expertise of each scientist and the limitations on live fish experiments in the European Union (EU).

PAA has been used in Denmark and Germany for some time to treat saprolegniasis and parasites such as *Ichthyophthirius multifiliis* (Ich). This approval is as a water disinfectant "for cleaning and disinfection of equipment and facilities in the presence, as well as in the absence, of aquaculture animals". Through our research, PAA is now introduced and registered by the EPA for use in U.S. aquaculture, but currently only when fish are not present.

This presentation reviews our collaborative research that has resulted in 19 peer-reviewed publications to date, along with several magazine articles and many presentations. We began with research on Ich and have gained other scientists with expertise in a variety of areas so that our research now includes pathogen control, degradation of PAA, toxicity to fish and use of PAA in RASs.

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EFFECTS OF DIETARY MICROPLASTIC (100-400 µm) EXPOSURE ON INTESTINAL PHYSIOLOGY OF RAINBOW TROUT (*Oncorhychus mykiss*)

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Introduction

Microplastics (MPs), plastic particles between 5mm and 0.1 µm in diameter, can be found in the marine environment, seawater, lakes, rivers, estuaries, sediments and many species of biota. Reports show presence of MPs in commercial species of finfis and shellfish in the wild, and in fishery and aquaculture products. Concerns are raised that MPs, and their associated chemicals, represents a risk for fish productivity, fisheries resources with potentially negative effects on food security (Lusher et al., 2017).

The accumulation of plastics in the gastro-intestinal (GI) tract of fish has been documented and ingestion has been proposed as a prominent exposure route for MPs. The GI tract of fish is a multifunctional organ important for osmoregulation, nutrient and fluid absorption, and acts as a barrier towards the external environment (Sundh and Sundell, 2015). The luminal content contains nutrients, ions, fluid and the endogenous microbiota essential for health but also potentially harmful components, such as pathogens, antigens, pro-inflamm tory factors, biological, or synthetic toxins. Uncontrolled passage of harmful substances across the epithelium into the circulatory system can cause disease, tissue damage and/or inflammatory responses. Therefore, a prominent function of the intestinal epithelium is to maintain selective permeability, ie. allow uptake of nutrients, ions, and fluids while harmful substances are restricted from reaching the circulatory system (Sundh and Sundell, 2015).

Despite the fact that ingestion of MPs has been demonstrated in >690 aquatic species, including many fish species (Provencher et al., 2017), biological effects from ingestion of these particles are considerably less documented and potentially negative effects on GI function of fish is unknown. The aim of the current study was to assessing the impact of ingestion of MPs on GI function with emphasis on intestinal barrier and transporting functions in combination with local and systemic inflammatory responses.

Materials and methods

For this study, juvenile rainbow trout (*Oncorhynchus mykiss)* were exposed via diet to polystyrene (PS) particles (100-400 µm, 10mg of PS MPs/fish/day) for a period of 4 weeks. Fish were fed four types of diets: control (no PS MPs) and diets containing untreated PS particles (PS-virgin) or particles exposed to sewage (PS-sewage) and industrial harbor (PS-harbor) effluent. Thereafter, physiological condition and integrity of proximal and distal regions of the intestine was assessed with Ussing chamber technique. Electric parameters, including TER (Ω*cm2), TEP (mV), SCC (µA), were recorded. A time-dependent transport of radioactively-labeled molecules (C^{14}-Mannitol and H^3-Lysine) across metabolically active epithelia was investigated to assess the functional adversity of dietary PS MPs exposure. Histological analysis was performed. Gene expression analysis of immune system-related genes (TGFβ, TNFα, IL-8, IL-10, IL-17, IL4/13A) and tight junction proteins (Occludin, ZO-1, Tricellulin) was additionally performed to examine if polystyrene particles and chemical contaminants induced inflam ation in the intestinal tissue. Additionally, innate immune response (lysozyme stability and complement system) in blood plasma was evaluated to assess the presence of systemic inflammation.

Results

Overall, the findings of the study indicated no or minor functional effects on fish intestinal tissue inflicted by particle exposure. Tendencies towards inflammatory responses were detected and were accompanied by upregulation of tight junction proteins, suggesting activation of intestinal homeostasis processes in response to PS MPs exposure.

(Continued on next page)

Discussion

With present study, we could not show that PS MPs (100−400 μm) act as physical or chemical or immunotoxic hazards upon ingestion. Our results collectively suggested that 4 week dietary exposure to both virgin and environmentally deployed PS MPs had limited impacts on functional integrity of intestinal epithelium in rainbow trout with no adverse effects observed in tissue morphology, barrier integrity, active transport, and/or inflammation. Given the inherent structural and functional complexity of the intestinal epithelium as well as active presence of various agents in the gut lumen (e.g., commensal microbiota, mucus), the intestinal environment represents a complex, dynamic system, which when subjected to particle exposure (and associated chemicals) may have resilience and capacity to cope with this stressor.

Animals living in the natural environment are exposed to a great variety of mineral and organic non-digestible particulates and are likely to possess adaptive protective physiological "housekeeping" mechanisms to counteract unwanted exposure of such indigestible particulates. The rainbow trout inhabits environments with high water turbidity and this species is known to tolerate moderate levels of particulate exposure . Our findings indicating the absence of tissue-specific (functional) effects in the intestine should therefore not be extrapolated to other fish species or animals from other taxonomic groups. One must account for potential differences in anatomical structures of the digestive tract and physiology (e.g., gut retention time, digestive fluid composition, presence of enzymes degrading plastic polymers), and adaptations to different ecological niches. Also, more investigations on MPs-gut lumen interactions are required to confirm, or contradict, the lack of effects on intestinal physiology associated with exposure to other polymers (e.g., having different chemical composition, level of degradation, ability to sorb chemicals, etc.). As dietary exposure to MPs has been shown to cause false satiation and subtle changes in energy metabolism on organismal level, we need to increase current understanding about potential impacts on an organisms' survival and fitness in long-term, under more ecologically relevant scenarios

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GEOSMIN PRODUCERS AND MICROBIAL COMMUNITY COMPOSITION IN COMMERCIAL RECIRCULATING AQUACULTURE FARMS

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Introduction

Geosmin and 2-methylisoborneol (MIB) are the best known odorous metabolites produced by several different microorganisms, e.g. actinobacteria, cyanobacteria, proteobacteria and fungi. Geosmin and MIB are the most common causes of off-flavor episodes in fish industry. Geosmin and MIB cause problems already in small concentrations and humans have very low odor threshold for both metabolites. Especially in recirculating aquaculture farms (RAS), the accumulation of geosmin and MIB in fish is problematic and causing economic losses. Currently purging of the fish in clean water is the best method to remove geosmin and MIB from fish. Although purging is effective, it reduces the profitability of fish production in RAS. Objective of this study was to study the presence of different potential geosmin and MIB producers in commercial RAS farms. Additionally, the microbial community composition of farms was studied.

Materials and methods

Five commercial RAS farms were sampled to quantify the potential producers of geosmin and MIB and to study microbial community composition. Water samples were collected from the inlet, fish tank, and outlet of the farm. Biofilm samples were collected from fish tank walls and biofilters. Abundance of the currently known main geosmin producers (*Streptomyces*, *Sorangium*, cyanobacteria, *Myxococcales*) were quantified using real-time PCR (qPCR) with targeted primers. Microbial community composition was analyzed using next generation IonTorrent PGM sequencing targeting V4 region of 16S rRNA gene.

Results and discussion

The diversity of geosmin-producing bacteria and abundance in incoming water had differences between studied farms. The potential producers in incoming water had no direct effect on quantities of potential geosmin producers in fish tank indicating that the conditions and water treatment inside the farms had greater impact. Most of the studied RAS farms had one main geosmin producer and the most common producer was *Sorangium*. Also, *Streptomyces*, *Myxococcales* and cyanobacteria producers were detected from the farms. No potential MIB producers were detected on the studied samples. The microbial community composition was studied in order to identify the key microbial groups in commercial RAS farms. It is crucial to identify the conditions and potential producers of odorous metabolites in order to develop preventive methods in future

CHALLENGING SALINITY TOLERANCE LIMITS OF THE EUROPEAN EEL LARVAE (*Anguilla anguilla)*

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Introduction

Most fish species are hyper-osmotic in freshwater, where plasma osmolality is higher than the environment and hypo-osmotic in seawater, where plasma osmolality is lower than the environment (Marshall and Grosell, 2005). Thus, in freshwater, fish need to actively take up ions to counteract the diffusive ion loss and osmotic water gain, while in seawater they need to maintain osmotic balance through a desalting process to counteract osmotic water loss. Euryhaline species, such as European eels (*Anguilla anguilla*), have adapted to cope with both, hyper- and hypo-osmotic environments, likely due to regular salinity changes in their habitats (i.e. estuaries) and migrations between freshwater and marine environments to complete their life cycle (Tesch, 2003). Eel offspring naturally occur in a hypo-osmotic environment in the ocean. Interestingly though, reducing salinity during culture of early life history stages, was shown to benefit eel larval ontogeny (Politis et al., 2018). In particular, lower salinity towards a more iso-osmotic environment reduces stress and conserves energy, probably due to lower cost for osmoregulation, resulting in higher survival and growth of early life history stages (Politis et al., 2018). The main objective of this study was to identify efficient technical solutions that accommodate these physiological preferences of eel larvae from a biological and cost/benefit point of view. Thus, we investigated when drastic salinity reduction from 36 to 18 psu should be implemented and how this affected European eel larval biometry (morphology and growth), deformities and survival, in order to identify a cost-efficient rearing protocol.

Materials and Methods

The experiment used seawater, originally pumped into the EEL-HATCH facility Hirtshals, Denmark, and treated through a stepwise filtering process, before entering a conditioning system. Here, salinity was adjusted to 36 psu using artificial sea salt (Blue Treasure Reef Sea salt, Qingdao Sea-Salt Aquarium Technology Co., Ltd., China). Thereafter, water was filled into 2 identical Recirculating Aquaculture Systems (RAS), each consisting of a 22 µm membrane filte , a UV lamp (UltraAqua, Aalborg, Denmark) and a 850 L sump. One system was kept at 36 ± 0.2 psu, while the other was adjusted to 18 ± 0.4 psu. European eel larvae were reared in 12 acrylic 2 L flow through jars (drz400sm hank, JugDesk Type, Taipei, Taiwan) with custom designed bottom inflow and top outflow for 6 days. On the day of hatch (day0), all experimental units were initially connected to the 36 psu system, while 3 jars were transferred to the 18 psu system on day1 (drastic 1), another 3 jars on day2 (drastic 2) and finally another 3 jars on day 3 (drastic 3). The last 3 jars remained in the 36 psu system (control) the entire period (Fig 1). Temperature was maintained at 18.8 ± 0.4 °C (Politis et al., 2017) and light intensity was kept at a minimum (Politis et al., 2014). Water exchange rate was set at ~0.1 L per min. The experiment was repeated 3 times, every time using offspring from a different parental combination. From each batch, larval sampling occurred on day0, 1, 2, 3 and 6 for each treatment and replicate. Here, ~10 larvae were randomly sampled, anesthetized using MS-222 (Sigma-Aldrich Chemie, Steinheim, Germany) and photographed using a zoom stereomicroscope (SMZ1270i fitted DS-Fi2 Camera Head, Nikon Corporation, Tokyo, Japan) for assessment of biometric characteristics (length, body area, oil drop area and deformities). Moreover, ~20 randomly sampled larvae from each batch, treatment and replicate were euthanized by an MS-222 overdose and preserved in RNA later at -20°C for molecular analysis. Furthermore, every day, dead larvae were counted and removed from all experimental units. Additionally, all larvae at the end of the experiment, as well as of all sampled larvae from each experimental unit were enumerated and recorded. Then, larval cumulative mortality was calculated as a percentage from hatch until day6.

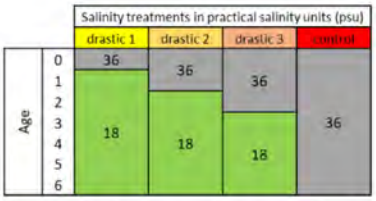

Figure 1 Experimental set-up. Larvae reared in constant 36 psu (control) were compared to larvae experiencing a drastic change in salinity from 36 to 18 psu either on day 1 (drastic 1), day 2 (drastic 2) or day 3 (drastic 3), respectively.

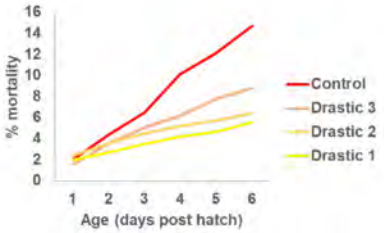

Figure 2 Larval mortality in constant 36 psu (control) vs drastic change in salinity from 36 to 18 psu either on day 1 (drastic 1), day 2 (drastic 2) or day 3 (drastic 3).

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Results

Our preliminary results show that larval mortality was highest when salinity was kept at 36 psu (control), but the earlier the drastic salinity regime was introduced, the higher the larval survival, resulting in up to 3-fold increased production (Fig 2). However, samples collected during the experiments will be further analysed leading to a deeper understanding through morphological and physiological measurements in order to define optimized rearing protocols in detail

Discussion and conclusion

The findings are quite interesting, as the control treatment (36 psu) resembles the salinity regime, larvae would naturally encounter in the oceanic spawning area in the Sargasso Sea. Thus, they would naturally never encounter any salinity changes during this period and especially not a physiologically extreme change like the one applied in this study. However, it was previously shown that salinity reduction benefits European eel larvae in terms of lower mortality and improved growth efficienc , which is likely facilitated by an energy surplus associated to lower osmoregulation demands (Politis et al., 2018). The results of this study confirm these observations while providing new insights in terms of methodology, applicability and timing from a hatchery point of view, as we have established the technically most efficient salinity reduction technique from a cost-benefit point of view. We conclude that a drastic salinity reduction can be applied resulting in increased larval survival, by only requiring two stable RAS engines.

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CLIMATIC FACTORS AND SERUM STEROID HORMONES IN NILE CATFISH (*Clarias gariepinus*).

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The study was conducted on the River Nile around Banha city and Idku Lake. Fishing trips to the studied areas were carried out every three months over a period of one year. Ten fish (*Clariasgariepinus*) samples (from each sex) were collected seasonally from each fishing site. The climatic changes in both sites were recorded. Quantitative determinations of serum steroid hormones (progesterone, P; estrogen, E and testosterone, T) were determined. Serum progesterone concentrations were significant y increased in male than female fishes in autumn and spring from Banha and in summer and spring from Idku Lake. Moreover, (E) levels were significantly increased in male than females in summer and autumn in fishes from Idku Lake. Also, the (T) levels were significantly decreased in male than females in autumn and winter from Banha and in summer and winter from Idku Lake. Climatic changes have a great effect on the reproductive hormones of male and female catfish *C. gariepinus*).

SUPPORTING THE SUSTAINABLE DEVELOPMENT OF THE IRISH FRESHWATER AQUACULTURE INDUSTRY

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Introduction

Irish freshwater aquaculture involves mainly traditional flow-through salmonids production (rainbow trout and smolt) that is generally performed without any inlet/outlet water treatment and with high flow rates to ensure oxygen replacement and good water quality. Various EU and national policies envisage a significant increase in the production of sustainably produced fish based food products. However, due to more and more stringent regulations and to path the way to an environmental practice the expected production increase must be a sustainable intensification aquaculture. EcoAqua is BIM funded project (2017-2019). The main aims of the project is to implement a water quality and energy monitoring program on each of the 4 Irish freshwater stakeholder fish farms. A graphical abstract of the project approach and objectives is presented below (Figure 1):

This particular study had a number of major objectives (i) ascertain the impacts of each site in terms of water quality, energy emissions and other water framework directive (WFD) biodiversity related impacts (ii) leverage this analysis to inform targeted decision making relating to capital investment and operation/control on each site and thus increase the efficiency on site, (iii) to reduce the levels of nutrient discharge of selected farms to meet WFD criteria by implementation of appropriate technologies to treat the wastewater and facilitate re-use of the treated water, thereby reducing both the volumes of extracted and discharged waters and (iv) help position the industry as a sustainable food producing sector with evidence based research.

Materials and methods

The facilities chosen have various configurations (i.e. pond/tank based, trout/salmon smolt/perch production) that are highly representative of the whole freshwater aquaculture industry and was be first benchmarked during year-1 of the program. This involved extensive and regular water quality monitoring – influent and effluent – of the 4 aquaculture sites to assess the performance of each and subsequent identification/benchmarking of their performance. Monitoring was performed weekly throughout a whole year using periodic 24 hour sampling campaigns augmented by remote monitoring using various sensors (e.g. pH, conductivity, dissolved oxygen, ammonium). An example of the approach undertaken for one type of farm (i.e. flow through, tank-based o ganic smolt production) is presented below (Figure 2) :

Results and discussion

Results will be presented on a farm by farm basis regarding the impact these flow through fish farms had on water quality parameters. General recommendations regarding new technologies and systems to be operated to increase aquaculture production in a environmentally sustainable way will be provided and discussed.

If accepted a presentation stating the general layout, objectives, approach and first results of the EcoAqua project will be given.

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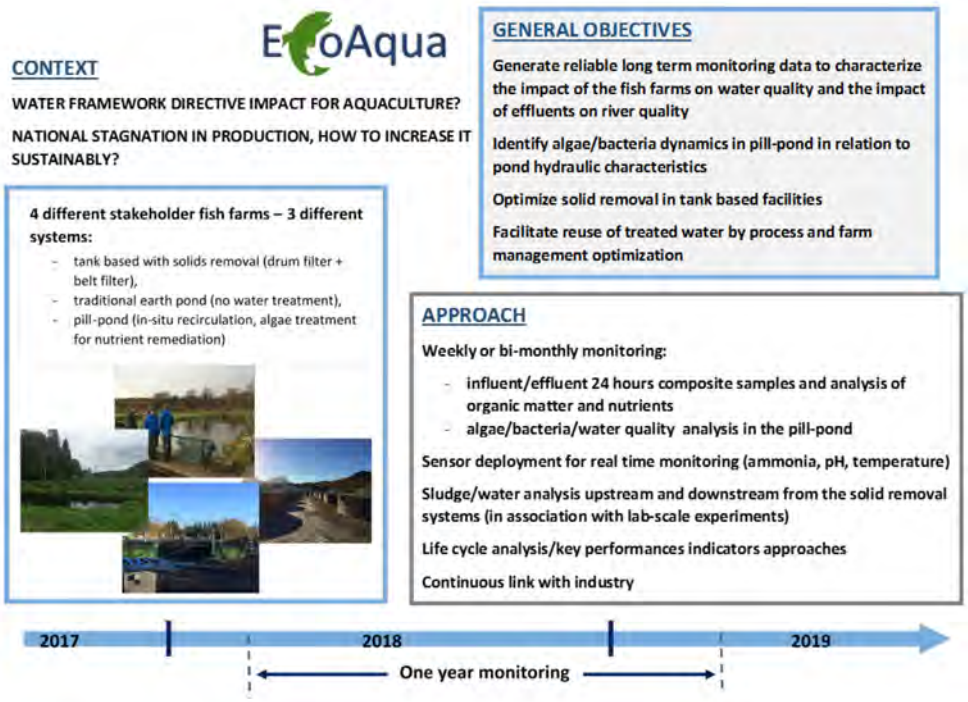

Figure 1: EcoAqua programme general approach and objectives

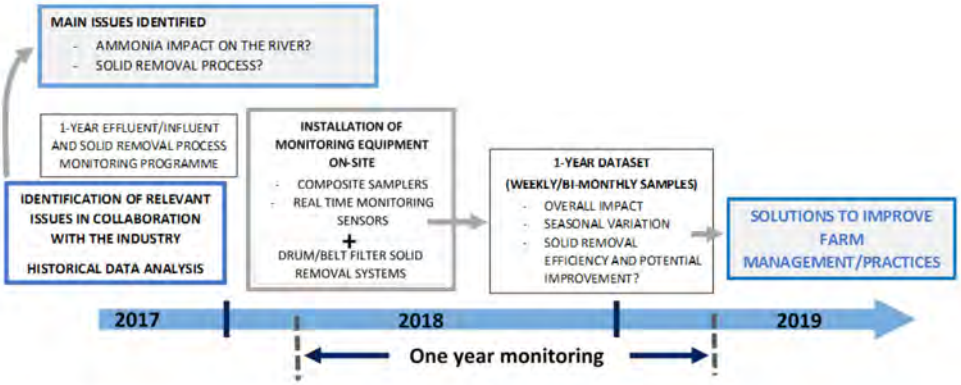

Figure 2: EcoAqua example of farm specific approach

TEMPORAL CHANGES IN GONAD QUALITY OF THE SEA URCHIN *Mesocentrotus nudus* FED ON SPOROPHYLL OF *Undaria pinnatifida* AND BASAL FROND PORTION OF *Saccharina japonica*

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Introduction

Our previous research revealed that gonads of the sea urchin *Mesocentrotus nudus* from a barren were highly improved to desirable quality, compared to those from an *Eisenia* kelp bed (fishing ground) by feeding *Saccharina japonica* during May−July. In addition, gonad quality of *M. nudus* fed the basal frond portion of *S. japonica* and sporophyll of *Undaria pinnatifida* were accepted by sushi chefs of sushi restaurants achieved three Micheline stars in Tokyo. The gonads were evaluated as the ones provided to customers in the restaurants. Present study aims to find the appropriate period when gonad quality is improved by feeding two different thallus portions of the kelps.

Materials and methods

We collected 155 sea urchins from a barren in Shizugawa Bay in northern Miyagi Prefecture, Japan on 1 May 2017. These sea urchins were reared in 31 aquaria (5 urchins/10 liter aquarium) with filtered seawater. After rearing all sea urchins without food for nine days, feeding experiment was conducted for nine weeks from 10 May to 12 July. At the start of the experiment, 20 urchins from four aquaria were dissected for measurements and analyses. Sea urchins fed basal frond portion of *S. japonica* (1/3 of frond) (BS) and sporophyll of *U. pinnatifida* (SU) *ad libitum* every 3−4 days were held in each nine of 18 aquaria. Sea urchins in other nine aquaria were reared without food (ST). Sea urchins in three aquaria of each treatment were dissected 3, 6 and 9 weeks after the start of the experiment. Test diameter, body wet weight, gonad wet weight, gonad color (C.I.E. *L*a*b**) and gonad hardness of all dissected urchins were measured, and FAA contents in the gonads were analyzed. Significant differences in these traits among treatments at each time, and among each time by treatment were analyzed by nested ANOVA and Tukey's test.

Results

Gonad indices of BS increased significantly from 8.9 (at the start of experiment) to 13.2 (after 3 weeks) and to 19.5 (after 9 weeks). Those of SU increased significantly from the start to the end of the experiment. After 6 weeks, the indices exceeded 18 which is the minimum size for commercial landing (Agatsuma, 1999), and reached to 24.1 at the end. The *L** value of gonads of BS increased significantly 3 weeks later, and that of SU increased 6 weeks later from the start. Gonad hardness of BS and SU decreased significantly 3 weeks later, and were significantly lower than that of ST until the end of the experiment. Sweet tasting alanine content in gonads of SU increased 3 weeks later. In contrast, that of BS increased significantly from 6 to 9 weeks later (Fig. 1). Although alanine content in gonads of SU was significantly higher than those of BS and ST until the 6th week, it was not significantly different from that in BS after 9 weeks. Bitter tasting FAAs in gonads decreased definitely from the start to the end. Of them, arginine, methionine and valine contents in gonads of BS and SU decreased significantly from the start to 6 weeks later. Arginine content in gonads of BS and SU did not change from 6 to 9 weeks later, in contrast, methionine and valine contents decreased significantly until the end.

(Continued on next page)

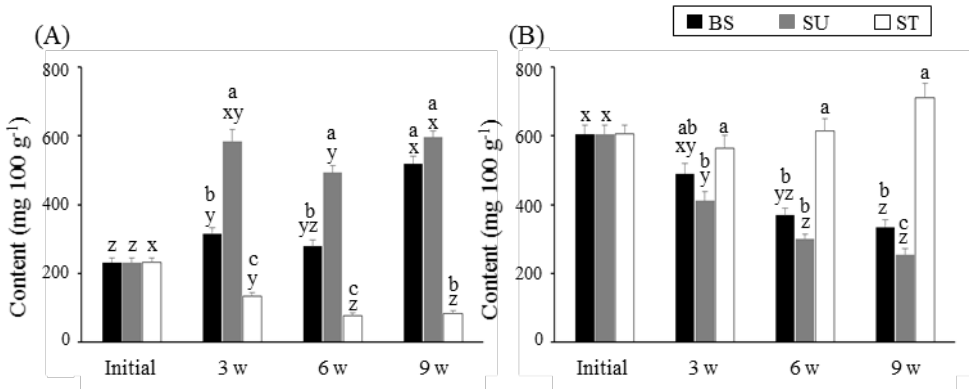

Fig. 1. Alanine (A) and arginine (B) contents in gonads of *Mesocentrotusu nudus* reared in tanks. Initial: at the start of experiment; 3 w, 6 w and 9 w: 3, 6 and 9 weeks from the start of experiment. BS: *M. nudus* fed the basal frond portion of *Saccharina japonica*; SU: *M. nudus* fed sporophyll of *Undaria pinnatifida*; ST: *M. nudus* starved. a, b and c: significant differences among treatments at each time ($P < 0.05$). x, y and z: significant differences among each time by treatment ($P < 0.05$).

Discussion and conclusion

Sweet tasting FAA contents in gonads of all treatments were lower than each threshold value (Kirimura et al. 1969) except for alanine and glycine. Increase in alanine content in gonads enhances sweetness (Takagi et al. 2017). Gonad sweetness of SU was improved 3 weeks after the start, faster than that of BS (9 weeks later). Bitter tasting arginine in sea urchin gonad makes the taste undesirable (Komata 1964). Past studies suggest that decrease in arginine content in gonads contributed to taste improvement and decrease in bitter taste (Takagi et al. 2017; 2018). Significantly lower arginine contents in gonads of BS 6 week later and those of SU 9 weeks later than that at the start would contribute to improvement of gonad taste. Low methionine and valine contents in gonads of BS and SU 9 weeks later compared to those 6 weeks later would also decrease bitterness of gonads.

Feeding basal frond portion of *S. japonica* to *M. nudus* from a barren improved gonad color and hardness 3 weeks later, and gonad size and sweetness 9 weeks later. Feeding sporophyll of *U. pinnatifida* improved gonad size, color and hardness 6 weeks later, and gonad sweetness 3 weeks later. Feeding each of kelp thallus portions could decrease undesirable bitterness of gonads 6 weeks later. Gonad bitterness would further decrease from 6 to 9 weeks later. Clarification of the components affecting the improvement of sea urchin gonad quality in basal portion of *S. japonica* frond and sporophyll of *U. pinnatifida* is needed.

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POSITIVE EFFECTS OF SUPPLEMENTING ALGAL 1,3-BETA GLUCAN ON THE SURVIVAL OF ATLANTIC SALMON *(Salmo salar)* IN CHALLENGE CONDITIONS

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Over the last decades, aquaculture practices have been intensifying and challenging sustainability and disease management. These two aspects are of great interest to the aquaculture industry that is often limited by them. The recent development of functional feeds has helped to provide balanced diets with feed additives that can improve the performance, health, and resistance to diseases. β-glucans are effective immunostimulants that have proved in several studies how they can improve the immune status and how the disease can be controlled in fish culture. *Euglena gracilis*, a freshwater microalga, used as a candidate for bioremediation and a nutrient source, is identified as a source of linear β-1,3 glucan. It accumulates up to 90% of the cell mass with linear β-1,3 glucan and equal to 50% of its cell volume when cultured under heterotrophic conditions. Genotoxicity tests were done using bacterial reverse mutagenicity and mammalian micronucleus tests to positively demonstrate that it is safe to use as a feed supplement. Safety was also proven with dermal and inhalation studies.

An experiment of a duration of ten weeks assessed the growth and disease resistance of Atlantic salmon (*Salmo salar*), as well as the immune stimulating effects resulting from dietary supplementation of algae-derived β-glucan compared with yeast-derived β-glucan when challenged with Furunculosis (*A. salmonicida*). All tests were performed at Lake Superior State University Aquatic Research Laboratory (LSSU-ARL), in a controlled environmental chamber (10-12 ˚C) with ten tanks of ~113 liters using a total of 300 fish equally distributed. Fish were fed twice daily at a rate of 3% of body weight and water was changed (half volume) every other day. Performance and growth parameters were recorded every two weeks. Two control tanks received standard feed without β-glucan supplementation, two tanks were assigned for the supplementation of yeast-derived β-glucan and the remaining six tanks received algae-derived β-glucan at increasing dosage: 200ppm, 500ppm and 1000ppm. After six weeks the fish were challenged via injection into the peritoneal cavity with *A. salmonicida* (1 x 10^5 CFU/fish; LD50). Mortality was recorded each day of this 4 weeks phase. After two weeks and at the end of the trial period, three fish from each tank were removed and processed via immunological and hematological testing procedures.

A linear correlation was observed between increasing algae-derived β-glucan dosage and survival rate with results comparable to yeast-derived β –glucan (Fig. 1). No statistically significant differences were observed in weight gain, gained length, or hematocrit or red blood cell numbers between any groups at any time points.

Data suggest that supplementation with algae-derived β-glucan can increase the survivability of salmon in a challenging environment. With additional study directed at the mechanisms and metabolic cascades involved, these results could prompt an improved understanding of the role of algae-derived β-glucan in enhancing survival, and significant advances in optimal dietary supplementation with algae-derived β-glucan in fish and shrimp diets

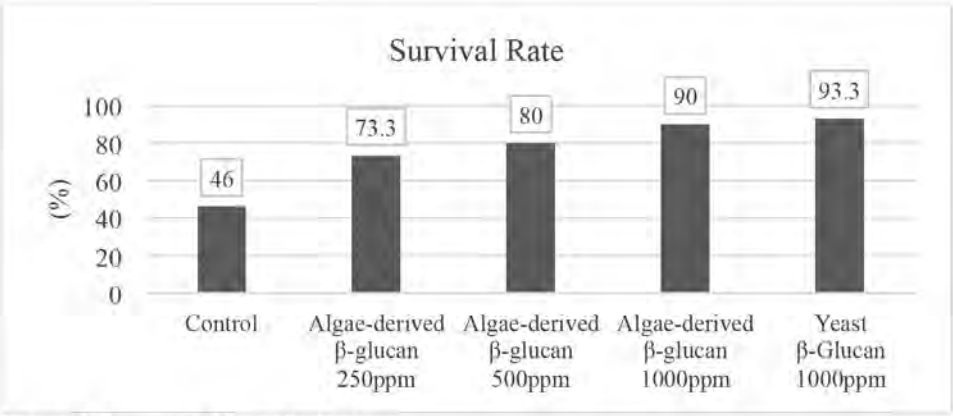

Figure 1 Summary of survival rates

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SPATIO- TEMPORAL DISTRIBUTION OF *Labeobarbus Species* IN LAKE TANA

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This study was conducted from May to December 2016 and the aim was to investigate the spatio-temporal distribution of Labeobarbus species of Lake Tana. Descriptive statistics was used to present the data. Chi-Square Test was used to compare the spatial variations of species between areas, the temporal variations between seasons. *Labeobarbus intermedius* was the most dominant specie, containing 38.8 % of catch followed by *L. tsanensis*, *L. platydorsus*, *L. brevicephalus* within a catch composition of, 18.96%, 13.1%, 10.5%, respectively. *Labeobarbus dainellii* and *L. gorguari* were rarely collected. *Labeobarbus species* of Lake Tana showed significant variation in catches at four sampling sites ($p<0.01$) and also these species showed significant variation in dry and rainy seasons ($p<0.01$). *L. intermedius*, *L. tsanensis*, *L. brevicephalus*, *L. nedgia*, *L. gorgorensis*, *L. truttiformis* were significantly more abundant in rainy season than dry season. The maximum possible diversity of four sampling site. The highest 'J' value was obtained at Gumara site with the value of 0.5. Effort control regulations, limiting the gillnet fishery in spawning seasons and/or areas, will be appropriate to prevent the Labeobarbus fish species of lake Tana.

HOW DOES TEMPERATURE AFFECT PRIMARY BARRIER FUNCTIONS IN ATLANTIC SALMON (*Salmo salar L.*) POST-SMOLTS?

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Environmental temperature has a considerate impact over near all aspects of physiology of both wild and cultured salmonids. In addition to climate change, insight regarding the effects of temperature over physiology is crucial for the aquaculture sector, as seasonal differences in temperature during early open pen rearing of post-smolt salmonids may ultimately be great and variable. For example, fish transferred during summer months will likely experience higher temperatures, whilst low temperatures will prevail during winter months. Due to these seasonal variations in temperature, in combine with the need for better predictability of fish stock robustness, it is essential to know how temperature influences tissue characteristics and modulates physiological functioning and adaptation, which processes maybe challenged during stress, thereby contributing to the stress response and overall robustness of the fish. Here we present how differences in temperature, affects Atlantic salmon (*Salmo salar* L.) post-smolt primary barrier compositions and functioning of osmoregulation in seawater. In this study we aim to elucidate how prior temperature acclimation history affects the capacity of these barrier systems to respond when confronted by an additional acute stress. Our results suggest that primary barrier characteristics change with shifting temperatures, resulting in new physiological set points to match osmoregulatory demand in those conditions. Here, we investigated key changes in gill and skin phospholipid (PL) and fatty acid (FA) compositions utilizing electrospray ionization-tandem mass spectrometry (ESI-MS/MS) and gas chromatography (GC-FID), in concert with changes in qPCR expression profiles of vital genes involved in osmoregulation and Using Chamber data on skin trans-epithelial resistance (TER). Complementation of methodologies allowed for accurate estimations to correlate the link between shifting biochemical and molecular changes at tissue sites with changing performance patterns of primary barriers manifesting at the different acclimation temperatures.

SPREAD OF A NEW DISEASE AGENT FOR RAINBOW TROUT (*oncorhynchus mykıss)* IN TURKEY: STRAWBERRY DISEASE

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Introduction

Strawberry disease (SD) is a disease of rainbow trout (*Oncorhynchus mykiss*) which is characterized by bright red, raised inflammatory lesions (Olson et al., 1985; Oman, 1990). The disease was described as a skin disorder with an unknown etiology (Lloyd et al., 2008). It was first recorded in the USA in the 1950s then noted throughout the USA and Europe (Olson et al., 1985). Similar conditions with indefinite etiology have been described as 'warm water strawberry disease' (WWSD), 'cold water strawberry disease' (CWSD) and in some conditions 'red mark syndrome' (RMS) from different researches (Fleury et al., 1985; Ferguson et al., 2006; Verner-Jeffreys et al., 2008; Lloyd et al., 2011). In some sources, WWSD was considered as the same disease with SD (Ferguson et al., 2006). Despite the temperature distinction, WWSD and CWSD are regarded as two separate conditions in order to their epidemiological, pathological and histological differences (Ferguson et al., 2006). Also, RMS was associated with the presence of rickettsia-like organism (RLO) (Lloyd et al., 2011) but the etiology is still under investigation.

Materials and methods

This study reports the diagnosis of SD from market-sized rainbow trout in Turkey. The outbreak was observed in rainbow trout farms for 2 years, especially on warm seasons in the Southwest Aegean Region. 4 different farms were monitored and the diseased fish samples of 300-350g with multiple ulcerated skin swellings were examined. To diagnose the cause of the disease, fish were dissected and from spleen, liver, kidney and also from skin lesions the isolates were streaked on Tryptic Soy Agar (TSA, Oxoid) and Tryptic Soy Agar supplemented with 5% defibrinated sheep blood (BTSA) because of their capability of growing a wide range of bacterium species. Then, the plates were incubated at 20-28°C for 24-48h. Tissue samples were taken in order to get electron microscopy views. Samples were sputtered with gold by QUORUM Q150 RES and examined in a Carl Zeiss 300 VP scanning electron microscope in the Central Research Laboratory, Izmir Katip Celebi University. DNA isolation was conducted using the GeneMATRIX Tissue & Bacterial DNA Purification Kit. For PCR amplification of the 5.8S rRNA gene, ITS1 and ITS4 primers were used. Amplified products of the template DNA were sent to the Macrogen direct sequencing service (Macrogen, Holland) for sequence determination.

Results

The outbreak occurred at about 12-13°C between April-September for two years (2016-2017). Mortality rates were not directly associated with the SD in this case. The infected fish showed no behavior changes during the condition. Single or multiple skin lesions, especially around the abdomen and fin base area were the main pathological signs of the disease. The superficial lesions were usually haemorrhagic with desquamations, central ulceration and redness (Figure 1).

Petechial haemorrhages in the stomach, swim bladder, visceral fat and intestine were detected on pathological examinations. In addition, yellowish mucous content with blood was determined in the intestines. There is no bacteriological growth was observed on TSA and BTSA. From scanning electron microscopy overviews, rod-shaped coccoid bacterium was observed in the inflammatory skin samples of infected fish (Figure 1)

Figure 1. The pathological signs of the disease and SEM overviews

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Discussion and conclusion

No pathogenic bacteria was associated with the infected fish during the study. It is known that bacteria which dominate in the aquatic environment may not culturable on conventional bacteriological media (Amann et al., 1995). Verner-Jeffreys et al (2008) indicated that the bacteria may not readily recoverable on standard bacteriological media. Also the 16s rDNA libraries from the skin lesions couldn't identify the main bacterial pathogen associated with the SD. For these reasons, a preliminary diagnosis of the causitive agent with bacteriological and molecular techniques were not effective and successful in this study. The cause of the condition couldn't be identified. Meanwhile, Athanassopoulou et al (2004) claimed that fish were more susceptible to secondary bacterial and parasite infections during the disease. However, neither bacterial nor parasitic agent was detected during the study. The SD may not have effect mortality rate or production period but the inflammatory skin lesions cause problems on the marketing and processing of the fish. If the lesions heal, it remains a remarkable patch on the surface of the skin. This situation affects the carcass quality and lowers the value of the product. In conclusion, further research is necessary to identify and understand the agent in detail. If the significant association will be asserted between the SD and the agent, treatment and vaccine procedures could be developed to prevent this economic loss.

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ASSESSMENT OF WOMEN'S PARTICIPATION IN AQUACULTURE POST-ECONOMIC STIMULUS PROGRAM IN KENYA

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Introduction

Fish farming in Kenya was introduced by colonialists in Kenya in the 1920s and in the 1960s Ngugi et al. (2007). Women are at the forefront of the rapid growth of the aquaculture sector, especially in activities such as farming, processing, and marketing. Women's participation along the aquaculture value chain is typically higher than in capture fisheries. In particular, improving women's income, access to information, technology and decision making processes have a profound effect on the quality of life at household level and by extension at the community level. It is against this background that the Kenyan Government sought to establish aquaculture as an income generating activity through a national *Economic Stimulus Program (ESP)* in the Financial Year 2009/2010. The program was geared towards commercializing aquaculture in the then 140 political constituencies. Each constituency received funds for the construction of 200 fish ponds, purchase of 15 kilograms of fertilizer and 1000 fingerlings. The second phase of the project was executed in 2011/12 financial year during which an additional 20 constituencies received funds for 300 fish ponds. The 140 constituencies that had received funds in the previous financial year received additional funding to construct another 100 ponds. In total, 48,000 ponds were constructed at a cost of 15 million US dollars (Watsuma, Bernard and Henry, 2012). Since then no assessment has been done to assess the impact of the program on the women participation in aquaculture.

Materials and Methods

A cross sectional survey design was used for a target population of 25 women in 5 counties namely; Busia, Kakamega, Vihiga, Bungoma and Uasin Gishu. Data were collected through unstructured interviews with women farmers and stakeholders in the aquaculture value chain, field visits, and desk reviews of relevant document

Results

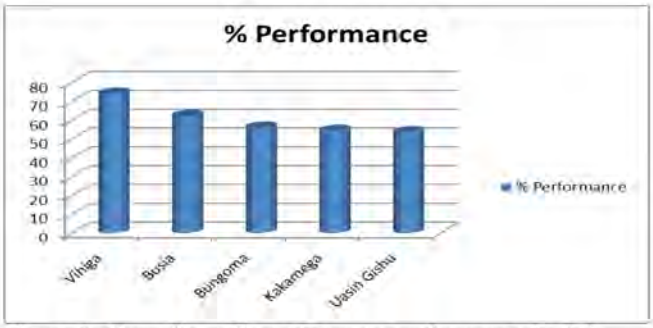

Figure1: Overall ranking of county performance in fish farming under Economic Stimulus Program

Table 1: Summary of findings from the 25 women interviewed in the 5 counties

Indicator	Percent
Ownership of ponds (by women)	100
ESP funded	81.2
Improved livelihoods as a result of ESP	78.5
Tilapia farmers	81.8
Post ESP farmers	65.6
Farmers with more than one pond	63.6
Trained farmers	85
No. of ponds that collapsed after ESP	30
Self-employed farmers	90

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Discussions and Conclusion

The study shows a positive impact of ESP on the women in the sampled counties. According to T. Nduku 2015, the overall efficiency of the counties in implementation of the Economic Stimulus Program was 65 per cent. This study shows that there is a positive correlation between ESP and women's participation in aquaculture. It is evident that the more gender is mainstreamed at higher governance levels, the more gender issues will feature in national fisheries and aquaculture policy documents. There is therefore need to carry out gender audits and best practices in successful gender integration in the work place and in the field among stakeholders in aquaculture. It is also apparent that women in aquaculture face a number of challenges that affect their quest towards commercialization of aquaculture. These challenges call for interventions which include capacity building and effective implementation of policies.

The assessment showed that most of the female farmers rely on government interventions in order to engage in aquaculture. This overreliance on government subsidies and other interventions is not sustainable. The study recommends more training and improved access to credit to enable the farmers to be self reliant in the aquaculture ventures.

Acknowledgement

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TOWARDS A SEMI-AUTOMATED NMR-BASED METABOLIC PROFILE OF FISH PLASMA: RESPONSE TO GLYCEROL-SUPPLEMENTED DIETS IN SEABASS

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Introduction

Although aquaculture production improved in the last decades some issues still persist, especially in terms of sustainability FAO (2018). Fish meal is the major cost in aquaculture and a major effort is being made to optimize feed composition and finding sustainable feed ingredients. A ^{1}H NMR metabolomics approach may provide an accurate and high-throughput data in the context of fish nutrition. Our research focused the inclusion of glycerol (0%, 2.5%, 5%) in the feed of European seabass (*Dicentrarchus labrax*). Glycerol is a cheap carbon substrate expected to enter metabolism through the glycolytic pathway, Rito et al. (2019).

Material and Methods

The fish (224 ± 35g) were kept in 3 x 350 L RAS system (22 ºC, 30 ‰ salinity) and fed 60 days. Five fish per condition were sacrificed (331 ± 59g) at 6 h and 24 h after feeding and blood was sampled. 450 µL of filtered plasma (<3 KDa) were run on Varian 600 MHz, *noesypr1d* sequence on a 5 mm triple resonance probe followed by an automated NMR spectral profiling analysis provided by Bayesil platform (http://bayesil.ca/), Ravanbakshs S et al. (2015)

Results

Spectra were successfully analysed with the automated Bayesil routine. We were able to identify and quantify 51 metabolites. The fish digested the glycerol within the first 6 h and are able to clear the circulating glycerol within 24h in the 2.5% diet. However, due to the supraphysiological concentrations of glycerol, namely in the 5% diet, the Bayesil algorithm is not able to properly integrate the signals.

Discussion and Conclusion

To our knowledge, this is the first time a high-throughput automated routine is applied to NMR data in aquaculture research framework. This approach is ideal for quantitatively assay several metabolites at once. Although supraphysiological concentrations of aqueous dietary nutrients, such as glycerol in this case, may require manual adjustments, we are able to quantify aqueous metabolites in the range of µM to mM.

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OPTIMIZING PRODUCTION OF MITIGATION MUSSELS

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Leveraging bivalve ecosystem services to counter eutrophication, and integration into water management plans, is a growing focus in many regions. Wild and cultivated bivalve assemblages assimilate nutrients bound in organic matter, can enhance nutrient cycling, and provide potential for net nutrient extraction through harvest. Intensive cultivation of mussels (*Mytilus edulis*) designed for maximizing nutrient extraction in eutrophic estuaries is developing in terms of technical and policy implementation. In Denmark, mussel mitigation culture is under consideration as a management tool within the third management cycle of the Water Framework Directive. Intensive research in optimization and further refinement in production outputs has been carried out by the Danish Shellfish Center (DTU Aqua) to document the mussel mitigation production volumes and nutrient removal for different productions technologies and environmental conditions.

Over two growing seasons (2017-2019), six test-line sites and three full-scale mitigation mussel farms were monitored for production potential in Denmark. As principle factors, linear density of settling material, vertical coverage, cultivation substrate, and relative position within the culture unit were analyzed for yield in terms of total mussel biomass and condition. In the 2017 growth cycle, differing configurations of traditional long lines were tested, yielding ~1300 t per model farm (18.8 ha) in Limfjorden. In 2018, different technologies were tested; preliminary results demonstrate capacities for exceeding 3000 t per model farm (Figure 1). High resolution biomass sampling demonstrated significant clustering of mussel condition within farm units, indicating meso-scale carrying capacity limitations, however, larger farm-scale yields were not demonstrably different between sites or by substrate quantity; indicating mitigation farms within Limfjorden conditions can be appropriately applied to reduce nutrient loads within the marine environment. In other tested estuaries, settlement and growth of mussels have been positive, suggesting mitigation culture can be applied as a nutrient management tool for many coastal waters.

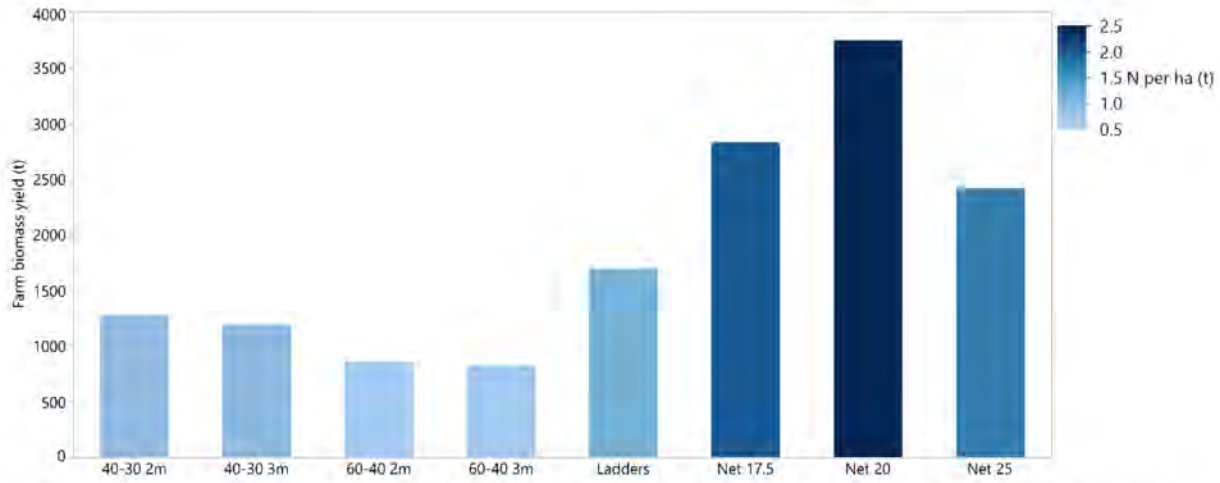

Figure 1. Potential farm (18.8 ha) mussel biomass yield and harvestable N-content per hectare by cultivation technology. 40-30 refers to 30cm spat collector spacing, 60-40 is 60cm spacing, 2 and 3m refer to spat collector depth. Ladders refer to a linear gridded collection system, and Net treatments refer to respective mesh size (cm) on 100m long, 3m deep nets.

FIGHTING THE PARASITIC CHALLENGE IN MODERN FISH FARMS: ALTERNATIVE REDUCTION STRATEGIES AGAINST *Ichthyopthirius multifiliis*

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Introduction

The control of parasitic infections is one of the major challenges for the European aquaculture. In particular *Ichthyophthirius multifiliis* poses a serious threat to farmed fish. Especially trout are affected, where aggravated by limited water supply and increasingly warm weather conditions an infection can lead to substantial losses. Since anti-parasitic treatments like the use of malachite green oxalate or other therapeutics are merely impossible in modern fish farms due to drug legislation, as of today environmental disinfection is the last resort in battling infections with *Ichthyophthirius multifiliis*. Besides the economic impact the lack of effective methods of treatment against *Ichthyophthirius multifiliis* poses also an ethical dilemma. In times where animal welfare and sustainable production are becoming more important it is not tolerable to just relay on rather ineffective environmental disinfection as a control measure. In this study filtration of parasite stages from the water and vaccination protocols against the parasite were followed to contribute to the solution of the urgent need for alternative strategies to control parasitic infections and produce healthy, ecological sustainable fish.

Material and methods

Two strategies to reduce infections with *Ichthyophthirius multifiliis* were tested. By filtering out the theronts from the water by nanofiltration the life cycle of the parasite should be interrupted. For this purpose two different filtration units were tested under controlled conditions in a lab scale keeping facility. Infected trout and carp were kept in two separated recirculating aquaculture systems (RAS) with four keeping-tanks each. On one of these RAS the filtration unit was implemented. The fish were evaluated for their parasitic burden every other day over a period of two to four weeks under a binocular. Additionally water samples were filtered and tested for parasite cells with a PCR developed and established for this purpose

As fish develop immunity following infection with *Ichthyophthirius multifiliis*, resulting in both specific and nonspecific immune responses, different preparations of *Ichthyophthirius multifiliis* were used in vaccination trials. On top of that preparations of the ciliate *Tetrahymena* sp. were tested for their ability to induce cross-immunity. In addition to living theronts, formalin and isopropanol inactivated theronts and isolated cilia of *Ichthyophthirius multifiliis* were used as well as living *Tetrahymena* sp. and also formalin and isopropanol inactivated *Tetrahymena* sp.. The living parasite stages were administered intraperitoneally while the other preparations were administered via a bath treatment. In the case of the fish vaccinated in the bath treatment, it was additionally examined whether the immunity would be stronger after enhancing the antigen intake by inducing micro-lesions in the skin with fine needles or the use of ultrasound to irritate the mucosa directly bevor vaccinating compared to a simple bath vaccination. The vaccinated fish and an untreated control group were marked by chip to be able to evaluate them individually. After a period of 30 days and a minimum of 450 day-degrees (°C) in which the immunity could build up, the fish were challenged for 8 hours with theronts harvested in a lab cycle. Afterwards they were distributed evenly to the four tanks of one RAS described above. The parasitic burden of the fish was evaluated after 7 day post infection.

Results and discussion

First results on nanofiltration indicate that the number of parasite stages in the water can be reduced by this measure. However practicability in commercial aquaculture seems to be questionable and will be investigated further.

Unfortunately a cross-immunity of *Tetrahymena* and *Ichthyophthirius multifiliis* seems to be not sufficient to protect the fish whereas preparations with *Ichthyophthirius multifiliis* seem to have a positive impact.

These approaches should now be optimized and put into testing under field conditions in fish farm

Conclusion

Parts of the methods used in this study are promising for a preventative take on *Ichthyophthirius multifiliis*. Especially vaccination might be part of the solution. However one major challenge remains partly unresolved. As tenacious *Ichthyophthirius multifiliis* is in the environment and in our aquaculture the fast, easy, cheap and controlled production of large numbers of theronts in the laboratory for example for the preparation of vaccines remains difficult and needs to be investigated further.

EFFECTS OF OXYTETRACYCLINE THERAPEUTIC TREATEMENTS ON GROWTH PERFORMANCE AND IMMUNITY IN NILE TILAPA (*Oreochromis niloticus*) JUVENILES

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Introduction

Oxytetracycline (OTC) is one of the most commonly used antibiotic in aquaculture (Reda *et al.*, 2013). Antibiotics have been associated with negative effects on the immune system of fish (Guardiola *et al*., 2012) and dysbiosis (Pindling *et al.*, 2018). The aim of this study was to evaluate the effects of OTC medicated feed on growth performance, immunity, liver and kidney functions, histopathological findings and microbiota in Nile Tilapa (*Oreochromis niloticus*).

Materials and Methods

A group of 315 Nile tilapia (average body weight 118±0.8g) was randomly distributed among nine tanks (300 L; 35 fish each tank) in a freshwater recirculating system (23ºC, 10h light /14h dark) at UTAD (University of Trás-os-Montes e Alto Douro, Portugal) facilities. All animals were acclimated to the experimental conditions and fed with a control diet (CTRL) that fulfilled the known nutritional requirements for Nile tilapia during 15 days. Then, animals from six tanks started to be fed with a medicated feed supplemented with OTC at a therapeutic dose (55mg/Kg body weight/day) whereas fish from the other three tanks continued to be fed with the CTRL diet. During OTC treatment, water from those tanks where not shared and freshwater was continuously added daily (at same temperature) to allow water renewal (50% d^{-1}). The fish was feed twice a day) at 2% of body weigth. After 10 days of medicated feed administration, Nile tilapia from three tanks were fed with the control diet (OTC-CTRL) while fish from the other three tanks were fed with an extreme diet, rich in vegetable nutrients (OTC-ED). Several samplings (n=9 per tank) were performed: i) at 15 days to determine health condition and gut microbiota prior to OTC treatment; ii) at 25, 26 and 33 days to evaluate effects of the medicated feed; iii) at 40 and 55 days to evaluate the recovery. Blood was collected to evaluate immunological and biochemical parameters, intestine tissue and faeces to evaluate microbiota, gene expression and histopathology, and liver to evaluate oxidative stress status. The methodology used was described by previous studies (Guardiola *et al*., 2013; Almeida *et al*., 2014 and Machado *et al*., 2015).

Results

This study showed a decrease in weight gain in Nile tilapia fed OTC-ED (74.1 ± 5.6 g) compared to those fed CTRL (91.8 ± 6.9 g) at the end of the experimental period (i.e. 55 days). Haematocrit and red blood cells were higher in animals fed OTC-ED than in those fed CTRL at 26 days, whereas anti-proteases activity in plasma showed the opposite pattern with higher levels in fish fed CTRL than in those fed OTC-ED also at 26 days. Moreover, circulating white blood cells (WBC) and peroxidase activity in plasma increased in fish fed CTRL compared to those fed OTC-ED and in fish fed both CTRL and OTC-CTRL compared to those fed OTC-ED at 55 days, respectively. Total proteins in plasma were higher in medicated groups than those fed CTRL at 40 days.

Discussion and Conclusions

Serezli *et al.* (2005) found an increase of total erythrocyte and leucocyte numbers after oral administration of OTC in seabream, but in the present trial, only haematocrit and erythrocyte cells were increased. The decrease in WBC observed in Nile tilapia fed OTC-ED at the end of the trial could be indicative of a possible suppression action of both OTC and the extreme diet in circulating leukocytes. While the present study showed a decrease of plasma anti-protease and peroxidase activity after oral OTC administration, other authors did not find such differences in the serum peroxidase activity in gilthead seabream Guardiola *et al*. (2012). Further analyses will be performed to evaluate the effects of oral administration of OTC in intestinal microbiota, gene expression and histology and liver oxidative stress.

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Acknowledgements

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AN 'AQUACULTURE SUSTAINABLITY TOOLBOX' FOR FLEXIBLE LICENSING AND REGULATION OF AQUACULTURE IN EUROPE

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Introduction

The EU Horizon 2020 'Tools for Assessment and Planning of Aquaculture Sustainability' (TAPAS) is a four-year project from March 2016. Its aims are to evaluate existing aquaculture regulatory practices and the methods, tools and technologies used for implementation, to identify gaps, needs and bottlenecks and propose improved approaches for licensing and regulation of aquaculture in Europe. An overall objective is to establish a comprehensive 'toolbox' to support transparent and efficient licensing that will enhance sustainability, aquatic food security and blue growth in all marine and freshwater environments for different types of aquaculture systems and species.

The target users of the Aquaculture Sustainability Toolbox are aquaculture regulators, for evaluating license applications efficientl , and producers or their consultants in preparing license applications. A further aim of the 'toolbox' is to improve access of information on aquaculture production and regulations to the public, through a communication platform, to improve understanding and perception of aquaculture practice throughout Europe.

Development of the 'toolbox' has been guided by extensive stakeholder consultation and development of recommendations for improved flexible approaches to licensing, in other parts of the TAPAS project. This ensures it is fit for purpose and fulfils the needs of the end-users

"Tools", in the context of the Toolbox, is a broad term including various types of instruments such as licensing and monitoring guidance, new and existing evaluated analysis tools (e.g. for environmental and socio-economic assessments) for impact analyses and site selection, and case studies illustrating the use of the tools.

Aquaculture Sustainability Toolbox structure

The structure of the 'toolbox' has been constructed to be intuitive and follow licensing procedures and guidelines under the EU Environmental Impact Assessment Directive (85/337/EEC and amendments). This allows the user to "enter" the toolbox from a number of points, i.e. seeing if a location is suitable for aquaculture, carrying out an EIA for the licensing process or, for more advanced users, using models and tools directly with data collected. This can help inform licensing procedures or as a guide to carrying out these procedures. Figure 1 illustrates the prototype of the front navigation page of the toolbox.

The Toolbox, comprises a HOME page and several supplementary pages addressing specific subjects and giving links to relevant tools and licensing models as well as tool pages, with the option to search for tools and a "Get meta data" link. All pages are linked so that it is possible to move around in the Toolbox as efficiently as possible for the user (see Figure 2). Users wanting to go directly to the tools can use the navigation panel which is in the header of all pages

The Aquaculture Sustainability Toolbox will be developed to a proof of concept stage (TRL6/7) at the end of the TAPAS project (Feb 2020). Future exploitation after the project is being explored. At present this is likely to take the form of a public decision support platform with industry/producers, regulators and the public as its end-users. It will be web-based and freely available to all. A strategy for the different components of this framework and its future management is under development and will be presented.

Acknowledgements

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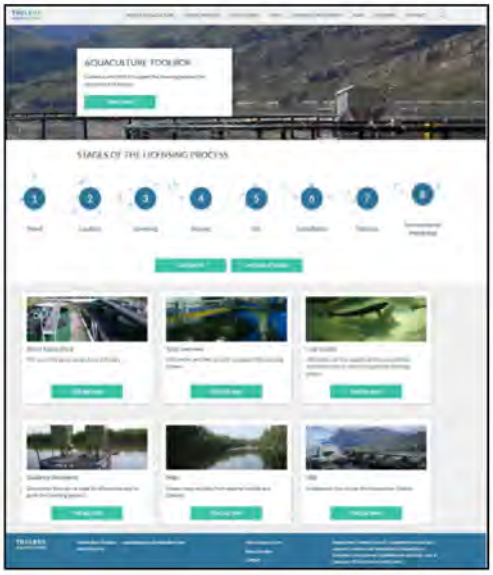

Fig 1. Prototype of Home page of the Aquaculture Sustainability Toolbox, showing the navigation path followed by users to use the tools at various points in the application and monitoring process.

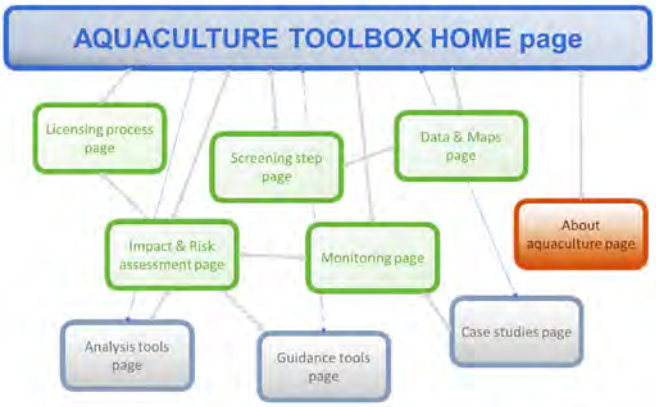

Fig 2. The Toolbox consist of a homepage and several subpages. The subpages belong to two types: subject pages (green) and tool pages (grey). In addition, the web portal comprises of an "About Aquaculture" page. The arrows illustrate the network making it possible to access all subpages from the homepage as well as other subpages.

GROWTH PERFORMANCE AND METABOLIC UTILIZATION OF METHIONINE IN TILAPIA FED DIETS SUPPLEMENTED WITH DIFFERENT METHIONINE SOURCES

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Introduction

Aquaculture is currently one of the most fast-growing animal production sectors. However, the expansion of the sector requires higher sustainable practices. Economic and environmental sustainable feeding practices in aquaculture may imply a higher inclusion of plant ingredients in fish diets. However, these sources are often limited in methionine (Met) and their use in fish diets requires its supplementation. This essential amino acid is involved in protein synthesis, transmethylation reactions and antioxidant defence. Therefore, DL-methionine (DL-Met), L-methionine (L-Met) and calcium bis-methionine hydroxyl analogue (MHA-Ca) are commonly methionine sources supplemented in animal diets. However, the biological efficiency of the different methionine sources may differ considerably. The biological efficiency of sources of methionine may be assessed by key performance indicators (like growth and diet utilization, among others) and methionine metabolic fate.

In this context, this work intended to 1) evaluate the effect of dietary supplementation with different methionine sources on growth and feed conversion and, 2) to understand how different dietary methionine sources are utilised by Nile tilapia juveniles.

Material and Methods

Nile tilapia (Silver Natural Male Tilapia™) juveniles were obtained from Til-Aqua International B.V. (The Netherlands) and transported to the Centre of Marine Sciences facilities (Faro, Portugal). Fish were maintained at 26ºC in a recirculating aquaculture system and fed a commercial diet during acclimation period.

After this period, fish were allocated into nine 100L cylindrical tanks (50 fish per tank, with an initial mean body weight of 2.27 ± 0.01g). Eight fish from the initial stock were sampled for analysis of proximate composition and amino acid content.

Triplicate tanks were randomly assigned to one of the three dietary treatments (REF, DL-Met and MHA). The experimental diets were formulated to be isonitrogenous (32% crude protein) and isoenergetic (19 MJ/kg gross energy), using only plant ingredients as protein sources. REF was a basal diet and no methionine was supplemented, DL-Met and MHA diets were supplemented with 0.11% of DL-methionine and 0.13% calcium bis-methionine hydroxyl analogue, respectively. Fish were fed by hand to visual satiety, three times a day and feed intake was recorded daily for 57 days.

At the end of the feeding trial, each tank was bulk weighed. In addition, 10 fish from each tank were individually weighed and measured, of which five fish were sampled for analysis of proximate composition and amino acid content, and the other five were individually weighed (total, liver and viscera weight) and measured to calculate the condition factor as well as the hepatosomatic and viscerosomatic indexes. Fish were fasted for 24h before initial and final samplings

At the end of the growth trial, to understand how different dietary methionine sources were absorbed and metabolised in tilapia juveniles, a time-course metabolic trial was performed. The DL-Met and MHA diets were labelled with ^{14}C-DL-methionine and ^{14}C-calcium bis-methionine hydroxyl analogue, respectively and tube-fed to fish from the correspondent dietary treatments. Dietary methionine source effect, based on the metabolic fate of the ^{14}C-DL-Met and ^{14}C-MHA was determined at 1, 2, 3, 4 and 6h after ingestion.

Results and Discussion

At the end of the experiment, fish fed the DL-Met diet presented a significant higher body weight and lower feed conversion ratio (FCR) than fish fed the REF diet. No significant differences were detected between fish fed the MHA and the REF diets regarding growth and FCR. There were no significant differences among treatments in feed intake, condition factor, viscerosomatic and hepatosomatic indexes. Dietary methionine source effect based on the metabolic fate of the ^{14}C-DL-

Met and ^{14}C-MHA in juvenile fish is currently under analysis. Our study demonstrates that DL–methionine in plant-based diets promotes growth and reduces FCR in Nile tilapia juveniles. A higher sustainability of aquaculture sector can be ensured through an optimise efficiency of diet utilisation by fis

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IMPACT OF INSECT PROTEIN IN THE DIET OF RAINBOW TROUT (*Oncorhynchus mykiss*) ON FISH GROWTH PERFORMANCE AND GUT MICROBIOME

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Introduction

Interest in using insects as animal feed is growing, mainly in the aquaculture sector. Compared to other animal protein sources, insects -- in particular flies -- have several advantages, which have attracted the attention of the aquafeed industry (Lock et al. 2018). Produced by dried insect larvae, insect meal is extremely rich in proteins (60-80%), essential amino acids (EAA), vitamins, and minerals (Henry et al. 2015). Insects are also a good source of lipids (31-43%); however, their total lipid content and fatty acid composition depend on rearing conditions and technological treatments (Meneguz et al. 2018) Accordingly, we investigated the effects of substitution of fishmeal (FM) with insect meal from *Hermetia illucens* larvae in the diet of rainbow trout (*Oncorhynchus mykiss*), on fish growth performance, and gut microbiota composition. High-throughput 16S rDNA sequencing (MiSeq platform, Illumina) was applied to characterize gut microbial community profile in trout. The use of Next Generation Sequencing (NGS) approaches permit to obtain a large number of sequence reads that are orders of magnitude larger than those produced by previous culture-independent techniques.

Materials and methods

H. illucens larvae were grown on a substrate of fruit and vegetables provided by the wholesale market. At the prepupal stage, larvae were harvested and then processed to be transformed into insect meal.

Four diets were formulated with increasing percentages of Hi larvae meal as substitutes for FM: one control diet with 0% (Hi 0) and 3 experimental diets with 10% (Hi 10), 20% (Hi 20), and 30% (Hi 30) of Hi meal as fed basis inclusion.

A 12-week feeding trial was performed on 348 rainbow trout of 66.5 ± 1.7 g initial mean body weight. Fish were randomly distributed in 12 indoor rectangular fibe -glass tanks of 1 m^3 connected to a flow-through open system supplied with artesian well water. The apparent digestibility coefficients (ADC) of DM, CP and EE of each diet were assessed through an in vivo digestibility trial.

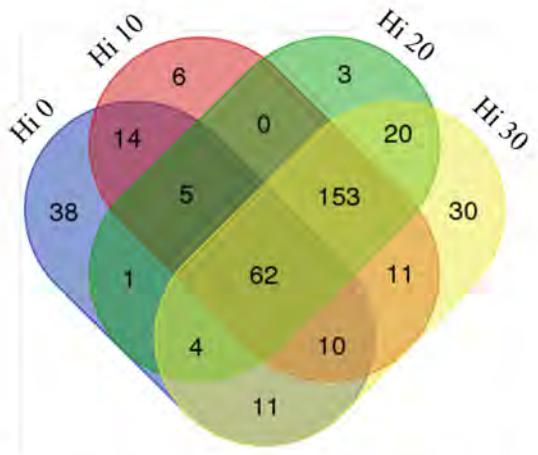

Figure 1. Venn diagram representing unique and shared OTUs among all dietary groups. For this study, the core microbiome was defined as the OTUs present in 80% of the samples regardless of diet.

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At the end of the trial, 6 fish/diet were sampled. The intestine was aseptically removed from each fish and squeezed to collect the fecal matter. For microbiome analysis, bacterial DNA was extracted from each faecal sample. The high-throughput sequencing of 16S rRNA gene was applied to analyse and characterize the gut microbial communities of rainbow trout. The obtained sequencing raw data were analyzed using the QIIME software. A Two-way ANOVA was applied to test for differences in mean values of bacterial taxa between the experimental groups using STATISTICA software (StatSoft, Inc.). A $p\text{-}value<0.05$ was considered significant.

Results and Discussion

During the 12 weeks of feeding fish promptly accepted the experimental diet and mortality was negligible, i.e., lower than 1%. At the end of the feeding trial, all fish had tripled their initial body weight, and growth performance parameters (WG and SGR) were not affected by diet composition. Similarly, FCR was comparable among the treatments and remained lower than one in all groups, meaning that all fish grew efficiently and including the H. illucens meal did not negatively affect diet palatability. No differences were reported among treatments for the feed digestibility. Crude protein digestibility was high and above 90% in all treatments.

The 24 fecal samples were sequenced on one paired-end MiSeq run (Illumina, Italy) and the sequencing raw generated were analysed using the QIIME pipeline. The number of reads taxonomically classified according to the Greengenes database was 1,140,534. We identified 450 OTUs at 97% identity in trout fecal samples; 62 OTUs constituted the core gut microbiota (Figure 1). *Actinobacteria*, *Firmicutes* and *Proteobacteria* represented the dominant phyla in both experimental groups. Among them, the abundance of *Actinobacteria* and *Proteobacteria* was significantly influenced by including insect meal in the diet.

In conclusion, our findings clearly indicated that insect meal positively modifies fish gut microbiota, increasing its richness and diversity and in particular, increasing the amount of beneficial lactic acid- and butyrate-producing bacteria, which contribute to the global health of the host. In addition, based on our present and previous studies, we believe that the prebiotic effect of insect meal is principally due to fermentable chitin.

Acknowledgements
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IMPACT HOTSPOT ANALYSIS OF THE NORWEGIAN FARMED SALMON VALUE CHAIN

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Introduction

The Norwegian farmed salmon chain is a global food value chain with farming based in Norway and production of feed ingredients, processing and consumption distributed around the world. A significant proportion of feed ingredients like soy protein concentrate, fish oil and fish meal for salmon are sourced from South America. The processing of whole fish into fillets and value-added products is carried out in hub-markets in Europe while end-consumers for Norwegian salmon are spread across the world with Europe being the biggest market (Norwegian Seafood Council, 2018). The production of Norwegian salmon is projected to increase to 5 million tonnes by 2050 which is a five-fold increase from today's level (Sintef, 2012) and therefore it is important to assess the impact of this projected increase. The climate change impact associated with salmon supply chain has previously been analysed (Zeigler et al., 2013) but there is a need to extend the assessments to include more environmental and social impacts. The goal of this study was to evaluate the overall impact and identify hotpots in the salmon value chain to provide a basis for strategic and operational advice for managers and policy makers.

Material and Methods

This study adopted a holistic approach from raw material extraction to waste disposal known as 'cradle-to-grave' based on ISO standard 14040:2006 on principles and framework for Life Cycle Assessment (ISO, 2006). The investigated environmental impact assessment focusses on key impacts governed by EU legislations: greenhouse gas emissions (European Climate Change Programme), freshwater eutrophication (Water and Nitrate Framework Directives), acidification (Air Quality Directive, Ammonia regulations). The social impacts are based on the UNEP-SETAC guidelines which include quantitative social indicators, such as working hours, health and safety, and public living conditions (Unep-setac, 2009). In order to identify the most relevant social indicators and rank them in order of importance, an opinion survey was conducted in Norway.

Results

The results identified feed production by terrestrial agriculture as an important environmental hotspot. Transporting fresh salmon by air freight was also an important factor that contributes to the total environmental footprint of Norwegian salmon. Domestic waste also plays an important role for all impact indicators.

About 50 respondents from the aquaculture industry in Norway including farming companies, researchers and consumers ranked the social indicators identifying the health and safety of workers as the most important. Based on the quantitative analysis the social impacts in the chain are mainly associated with the farming stage and were identified as an important hotspot.

Discussion and Conclusion

The environmental and social hotspots analysis provided a greater understanding of impacts from the salmon value chain and identified the supply chain stages and value chain actors that are affected by these. The results of the analysis provide insight for decision-makers such as regulatory bodies and businesses to identify the improvement opportunities for the salmon value chain.

This study has been conducted as part of the research activity in H2020 project VALUMICS where the hotspot analysis of the salmon chain will be provide input to the logistics optimisation models as well as agent based models focussing on fairness in food value chains and will be tested for various future scenarios forming a basis for policy recommendations for a more sustainable and resilient salmon value chain.

(Continued on next page)

Acknowledgements

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FUNCTIONALCHARACTERIZATION,ANTIVIRALCAPACITYANDTRANSCRIPTIONAL BEHAVIOUR OF IRF6 IN *Hippocamus Abdominalis*

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Introduction

Interferon regulatory factors (IRFs) are one of the central transcription factors which induce the interferon signaling (Lu, 2008)macrophages and dendritic cells. A series of recent studies have further demonstrated critical functions for IRF4 and 8 at several stages of B-cell development including pre-B-cell development, receptor editing, germinal center reaction and plasma cell generation. Collectively, these new studies provide molecular insights into the function of IRF4 and 8 and underscore a requirement for IRF4 and 8 throughout B-cell development. This review focuses on the recent advances on the roles of IRF4 and 8 in B-cell development.”,”author”:[{“dropping-particle”:””,”family”:”Lu”,”given”:”Runqing ”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””}],”containe -title”:”Trends in Immunology”,”id”:”ITEM-1”,”issue”:”10”,”issued”:{“date-parts”:[[“2008”,”10”,”1”]]},”page”:”487-492”,”publisher”:”Elsevier Current Trends”,”title”:”Interferon regulatory factor 4 and 8 in B-cell development”,”type”:”article-journal”,”volume”:”29”},”uris”:[“http://www.mendeley.com/documents/?uuid=515fb2d4-0a53-3238-bd42-3b589dcef25b”]}],”mendeley”:{“formattedCitation”:”(Lu, 2008. IRFs involve with main biological functions such as antimicrobial defense and antiviral defense, immune regulation and cell differentiation(Savitsky et al., 2010; Zhang et al., 2015)subsequent studies have revealed much broader functions performed by IRF members in host defense. In this review, we provide an update on the current knowledge of their roles in immune responses, immune cell development, and regulation of oncogenesis.”,”author”:[{“dropping-particle”:””,”family”:”Savitsky”,”given”:”David”,”non-dropping-particle”:””,”parse-names”:false,”suffix” ””},{-“dropping-particle”:””,”family”:”Tamura”,”given”:”Tomohiko”,”non-dropping-particle”:””,”parse-names”:false,”suffi ”:””},{“dropping-particle”:””,”family”:”Yanai”,”given”:”Hideyuki”,”non-dropping-particle”:””,”parse-names”:false,”su ffi ”:””},{“dropping-particle”:””,”family”:”Taniguchi”,”given”:”Tadatsugu”,”non-dropping-particle”:””,”parse-names”:false,”suffix”:””}],”containe -title”:”Cancer Immunology, Immunotherapy”,”id”:”ITEM-1”,”issue”:”4”,”issued”:{“date-parts”:[[“2010”]]},”page”:”489-510”,”title”:”Regulation of immunity and oncogenesis by the IRF transcription factor family”,”type”:”article-journal”,”volume”:”59”},”uris”:[“http://www.mendeley.com/documents/?uuid=060a70bd-85e2-482c-ad14-e1373e89a13e”]},{“id”:”ITEM-2”,”itemData”:{“DOI”:”10.1016/j.fsi.2015.02.033”,”ISSN”:”10959947”,”PMID”:”15712401”,”abstract”:”Interferon regulatory factors (IRFs. Mammalian IRF6 regulate the keratinocyte differentiation and maturation (Leslie et al., 2012).

Methodology

IRF6 of big belly seahorse (*Hippocampus abdominalis*) (HaIRF6) was molecularly and functionally analyzed for their sequence, transcription and antiviral capacities. Transcriptional levels with time defendant manner were detected upon Poly (I:C) LPS, and S*treptococcus iniae* and un challenged conditions. Subcellular localization were detected.

Results

Gene expression of HaIRF6 was detected in vivo against immune stimulants. At poly I:C challenge, expression was significantly induced at early time points with compared to the other two challenges in blood and gill. VHSV Viral gene transcription was significantly reduced at 24h and 48 h. cellular localization of HaIRF6 was observed in cytoplasm

Discussion and Conclusion

Fish IRF6 function is considerably deviated comparative to the function of mammalian IRF6. These findings revealed the importance of seahorse IRF6 in the immune defense system against pathogen and their antiviral tolerance against VHSV infection.

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BEHAVIORAL DIFFERENCES IN COPING STYLES BETWEEN WILD-TYPE AND GR-KNOCKOUT ZEBRAFISH GENERATED WITH THE CRISPR/CAS9 TECHNOLOGY

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Introduction

Stress response in teleosts is mediated by the Hypothalamus-Pituitary-Interrenal (HPI) axis whose final hormonal product is cortisol (corticosterone in rodents) (Mommsen *et al*., 1999). The effects of cortisol on the organism's stress response are regulated by two receptors, the mineralocorticoid (MR) and glucocorticoid (GR) receptors. These effects spread on a molecular, cellular, physiological and behavioral level (Prunet *et al*., 2006)a mineralocorticoid receptor (MR. Blocking the GR signaling has been known to lead to compromised cognitive abilities. In several teleosts there are two Gr isoforms, Gr1 and Gr2, while on the contrary zebrafish contain only one Gr isoform (Alsop & Vijayan, 2009)we examine whether molecular components involved in the functioning of the hypothalamus-pituitary-interrenal (HPI. Zebrafish Gr-KO lines have been generated with various genetic manipulation technologies; gr^{s357} zebrafish have shown chronically elevated cortisol levels and anxious behavioral phenotype (Ziv *et al*., 2012), CRISPR/Cas9 Gr-KO lines have displayed physiological responses linked to GC-resistance, such as overstimulation of basal HPI axis associated with unresponsiveness to a prolonged mechanical stressor (Facchinello *et al*., 2017). In our experiments we exposed heterozygous as well as homozygous adults of a Gr-KO zebrafish line to two behavioral tests, and assessed their response to novelty and their shoal cohesion

Materials & Methods

Experiments were performed at the installations of the Fish Physiology Laboratory of the University of Crete. Fish were provided by the laboratory of Luisa Dalla Valle (Facchinello *et al*., 2017) and housed in a stand-alone system (Tecniplast), water temperature set at 28 °C and photoperiod at 12D:12D. The novel tank test involves the introduction of each fish into a glass rectangular 2L tank. The tank consists a novel environment, given that the individuals had never been exposed to this apparatus. Fish were left to move freely and explore the tank for 6 minutes, while their behavior was recorded. At the end of each trial, fish were netted back to their mother tank. After 7 days shoal cohesion was tested and fish were separated in groups/shoals of 4, 6, 8 and 12 and left overnight to interact. The next day, their behavior was recorded for 10 minutes. The trials of both tests were performed between 10:00 and 14:00. Videos obtained from the ethological experiments were analyzed using the specialized behavioral analysis software Ethovision XT (Noldus). The analysis focused on the quantification of various behavioral parameters of exploratory and anxious behavio .

Results

The parameters analyzed for both tests (novel tank and social preference) were (i) time spent in the three zones of the tank (lower, medium, upper for the novel tank test and closer, medium and far from conspecifics for the second test), (ii) frequency of visits in the three zones, (iii) velocity, (iv) distance travelled and (v) freezing behavior, and representative traces of each trial were produced by the software as depicted in Figures 1 and 2. Differences in the above parameters were observed among the different genetic lines (WT, $gr^{+/-}$, $gr^{-/-}$) (data not shown).

Discussion

Novel tank test has been widely used as an ethological protocol to assess an animal's response to novelty, often considered a stressful stimulus. It has been previously shown that this response is hugely affected by numerous factors such as the genetic line, sex and specific pharmacological manipulations. A zebrafish s natural response to a novel tank includes an initial diving in the bottom of the tank and a gradual exploration of the rest of the tank. An increased latency to the upper part of the tank and reduced exploration of the upper part, along with increased duration of erratic swimming and freezing behavior draw a picture of significant levels of stress. These behaviors have been successfully replicated by exposing fish to anxiogenic stimuli, as alarm pheromone and exposure to a predator. Our results are in accordance with the stressful phenotype observed in gr^{s357} mutants (Ziv *et al*., 2012), where homozygous mutants exhibited significantly higher levels of freezing behavior and lower exploratory activity, when compared to the control group. Generally, this preliminary study shows the effects of gene editing on the behavior and social perception of adult zebrafish. Further analysis will hopefully shed more light on the implication of the HPI axis on the exploratory behavior and social abilities and tendencies of zebrafish.

(Continued on next page)

Fig 1. Representative traces of the WT (a), *gr*$^{-/-}$ (b) and *gr*$^{+/-}$ (c) adult zebrafish tested in the novel tank protocol and analyzed using video-tracking software Ethovision (Noldus Inc.).

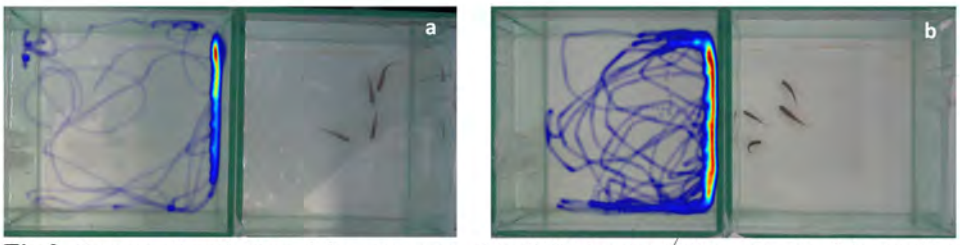

Fig 2. Heatmap visualization of the movement of the WT (a) and *gr*$^{-/-}$ (b) adult zebrafish tested in the social preference test and analyzed using video-tracking software Ethovision (Noldus Inc.)

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CHARACTERISATION OF INTERLEUKIN-11 OF REDLIP MULLET (*Liza haematocheila*) AND ITS REGULATORY FUNCTIONS ON STAT3 TRANSCRIPTION FACTOR

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Introduction

IL-11 is first identified as a pleiotropic cytokine with lympho-hematopoietic effect. Further studies have revealed the anti-inflammatory function of IL-11 by reducing inflammatory mediators such as TNFα, IL-1β, IL-12, IFN↑ , and nitric oxide (Trepicchio et al., 1996). IL-11 has been identifie as an active player in the host immune response in pathogenic infection of teleost fish. The IL-11 is an IL-6 family or gp130 family member cytokine which transduce the signaling through the JAK-STAT pathway. It activates the nuclear translocation of STAT3 and inhibits the NF-κB nuclear translocation (Trepicchio et al., 1997). The current study was aimed at cloning and functional characterization of IL-11 ortholog from redlip mullet (*L. haematocheila*) revealing its transcriptional expression during the pathogenic invasion and functional capacity to regulate the inflammation

Materials and methods

The coding sequence of IL-11 was identified from a previously prepared cDNA library of redlip mullet. Various software and online bioinformatics tools were utilized to characterize the coding nucleotide sequence and the predicted amino acid sequence of redlip mullet IL-11. Healthy redlip mullets were purchased and acclimatized to laboratory conditions. Eleven different tissues (peripheral blood cells, kidney, head kidney, spleen, liver, gill, intestine, brain, skin, heart, and stomach) were sampled from five fish and pooled for investigating the tissue-specific mRNA expression of IL-11. Groups of fish were challenged with LPS, Poly I:C and *Lactococcus garvieae.* Peripheral blood cells sampled from challenged fish were sampled in 0, 6, 24, 48, 72 hours after the challenge. Sampled tissues from both experiments were subjected to mRNA extraction followed by cDNA synthesis. The cDNA was used to evaluate the tissue-specific mRNA expression of IL-11 and investigate the transcriptional variation of IL-11during pathogenic invasion in fish. The open reading frame of redlip mullet IL-11sequance was amplified using sequence-specific primers and cloned to pMAL-c5X expression vector. After the confirmatio of sequence, successful clones were transformed into E*scherichia coli* BL21 strain. The recombinant IL-11 was overexpressed with Maltose Binding Protein (MBP) tag and purified using pMAL™ protein fusion and purification system. Purified *r*IL-11 was treated to redlip mullet kidney cells with 100.00 ngμL^{-1}concentration using MBP as the control. LPS (2.00 μgmL^{-1}) was used to induce inflamma ion in cells. Cells were lysed and subjected to mRNA extraction and cDNA synthesis in 0, 1, 3, 6 and 12 hours intervals. qPCR was performed to investigate the transcriptional variations of STAT3, TNFα, IL-1β in IL-11 treated redlip mullet kidney cells.

Results

The complete ORF of redlip mullet IL-11 was 603bp in length and the deduced amino acid sequence has 200 residues. The predicted molecular weight and isoelectric point (pI) of redlip mullet IL-11 were 23.16 kDa and 9.16 respectively. The three-dimensional modeling of redlip mullet IL-11 shows that it has four alpha-helix bundle structure. The polypeptide chain contains a 26bp long signal peptide. The multiple sequence alignment demonstrated that the sequence of the signal peptide, alpha helix A, alpha helix B, and D are barely conserved among fish but less conserved with other phyla. The phylogenetic analysis demonstrated that mullet IL-11 is cladding with other fish IL-11 sequences. The identity similarity analysis showed that redlip mullet IL-11 has 92.5% identity with *Tetraodon nigroviridis*. The tissue specific mRNA expression analysis showed that stomach and intestine have the highest IL-11 expression during ordinary conditions. IL-11 expression in PBC was upregulated by LPS and poly I:C in the acute phase of the challenge. Upregulation of IL-11 in *L. garvieae* challenge was observed in later phase of the challenge experiment. The transcription of STAT3 and NF-κB p65 was downregulated by IL-11 in mullet kidney cells. Similarly, the transcription of LPS induced TNFα and IL-1β were downregulated in IL-11 treated cells.

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Discussion and conclusion

Redlip mullet IL-11 sequence has four alpha-helix bundle structure as other members of IL-6 family cytokines. The IL-11 sequences of fish are barely conserved. The gastrointestinal tract is one of the primary sites that can make the entrance of pathogenic microbes. Thus, the cells may undergo continuous pro-inflammatory state, the IL-11 may use to control the inflammatory conditions in the gastrointestinal tract. IL- can inhibit the nuclear translocation of NF-κB, hence interrupt to the transcription of downstream pro-inflammatory cytokines such as TNFα and IL-1β (Trepicchio et al., 1997). LPS and poly I:C is pathogen-associated molecules and *L. garvieae* is live pathogen to redlip mullet. The challenge may induce an inflammator condition in the blood cells of mullet. It may upregulate the IL-11 transcription to prevent continuous pro-inflammatory state, due to the pathogenic stimulants. STAT 3 is the transcription factor which involves in signal transduction of IL-11 through JAK-STAT pathway. In the STAT3 transcription factors can bind into the promoter regions of pro-inflammatory cytokines such as TNFα, IL-6, and iNOS (Carpenter and Lo, 2014). In order to prevent continuous transcription of such pro-inflam atory cytokines by STAT 3, the transcription of STAT3 may be downregulated as a feedback controlling mechanism. The downregulation of TNFα and IL-1β may due to the blockade of NF-κB (Trepicchio et al., 1997) or reduced transcription by IL-11treatmant. Overall, the IL-11 can control the proinflammatory cytokine production in LPS induced redlip mullet kidney cells. This may use as a therapeutic measure in pathogen-induced inflammations in cultured fish.

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GONAD DEVELOPMENT AND PLASMA LEVELS OF SEX STEROIDS IN FARMED LUMPFISH (*Cyclopterus lumpus*) UNDER DIFFERENT PHOTOPERIOD AND TEMPERATURE REGIMES

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Introduction

To ensure year-round supply of right-sized juveniles for sea-lice control, off-season production of fertilized lumpfish eggs is important (Imsland et al., 2018)control of the sexual maturation cycle is critical for a sustainable production of the species. For year-round reliable production of juvenile lumpfish of the appropriate size for stocking salmon cages, there is a need for basic and applied knowledge on the control of sexual maturation in cultured lumpfish broodstock. Lumpfish (initial size 219g and 16.9cm. This will allow development of methods for management of broodstock. However, little is known about the reproductive biology and the subsequent control of sexual maturation in farmed lumpfish. To produce fertilized eggs year-round to the aquaculture industry, studies in teleost reproduction are important (Lubzens et al., 2010). Photoperiod and temperature are primary regulators of temperate finfis reproduction, and studies demonstrate that, manipulations of photoperiod and temperature affect sexual maturation and spawning in farmed species (Taranger et al., 2010). Subjecting lumpfish to continuous, and then compressed photoperiods resulted in higher growth and advanced spawning (Imsland et al., 2018)control of the sexual maturation cycle is critical for a sustainable production of the species. For year-round reliable production of juvenile lumpfish of the appropriate size for stocking salmon cages, there is a need for basic and applied knowledge on the control of sexual maturation in cultured lumpfish broodstock. Lumpfish (initial size 219g and 16.9cm. Imsland et al. (2019) found that, compressed and phase advanced natural photoperiods cause advanced spawning in lumpfish. Gametogenesis and its response to photoperiod and temperature manipulations in lumpfish is not yet known. This study was conducted to describe ovary and testis development stages, and temporal changes of plasma sex steroid levels in lumpfish under di ferent photoperiod and temperature regimes.

Materials and methods

Lumpfish acquired for this experiment were reared under continuous light from hatching. On 21st September 2017, the fish were 18 months old, weighing on average 697.9g (± 364.1g), and with average length of 25 cm (±3.8cm). Four tanks (1500 l each) were stocked with the fish at 75 fish tank^{-1}, and a M:F sex ratio of 1.08. The tanks were grouped into two (2) photoperiod groups, one continuous light (CDL0T) and one short day length (SDL0T, 8 hours light, 16 h darkness), both kept at ambient temperature (AT, 4–9 °C). Four months later, the temperature in one tank for each photoperiod was increased by 3°C (CDL3T and SDL3T), and photoperiod in both SDL tanks changed back to CDL. All tanks were maintained for further 1.5 months, before termination in March 2018. There were four sampling points, in which, body and gonad weights were measured. Blood plasma for changes in sex steroid levels and gonad sections for histological gonad development were also acquired. Based on oocyte types, ovaries were categorized into: pre-vitellogenesis, early vitellogenesis, late vitellogenesis, final maturation and ovulation. Testes were categorized based on the cell types: spermatogonia, spermatocytes, spermatids and spermatozoa.

Results

No significant difference in growth was observed between any of the groups. Females in the SDL groups generally had higher GSI than those in the CDL groups, and higher temperature resulted in higher GSI. Only pre-vitellogenic and early vitellogenic ovaries were observed at the start in September 2017. In January 2018, the most developed ovaries were at late vitellogenesis and final maturation in the CDLAT group. In February 2018, the SDLAT group had most ovaries in final maturation. In March 2018, the largest proportion of ovaries in ovulation was observed in the SDLAT group. Estradiol 17- β (E2) and Testosterone (T) levels in females increased with time and were higher in the SDLAT groups than CDLAT groups, even higher at high temperature. Testes showed a high variation in development, and mature males were observed from the start throughout the experiment. T and 11-Ketotestosterone (11-KT) levels in males were highest in February 2018 and decreased towards March 2018. The SDL groups had a shorter spawning period (February) than the CDL groups, which were spawning earlier and had a more prolonged spawning period (December – February). The ambient temperature groups spawned earlier than the elevated temperature groups during the last 1.5 months.

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Discussion and conclusion

The absence of difference in growth between the groups was different from the observations by Imsland et al. (2018)control of the sexual maturation cycle is critical for a sustainable production of the species. For year-round reliable production of juvenile lumpfish of the appropriate size for stocking salmon cages, there is a need for basic and applied knowledge on the control of sexual maturation in cultured lumpfish broodstock. Lumpfish (initial size 219g and 16.9cm, in which, continuous photoperiod accelerated somatic growth in lumpfish. Ovary development was higher in the short photoperiod groups, similar to previous observations that continuous photoperiods may delay or inhibit gonadal development (Davie et al., 2007). The low levels female sex steroids in the continuous photoperiods agree with low testosterone and estradiol 17-β levels related with exposure of haddock to continuous photoperiod (Davie et al., 2007). The role of temperature in timing of maturation and spawning observed in wolffish (Tveiten and Johnsen, 1999) tempts to suggest that, lumpfis ovary maturation is affected by temperature, due to the observed effects of increasing temperature. Spawning differences among the photoperiod groups agree with Imsland et al. (2019), who found that, lumpfish spawn sparsely under continuous photoperiod, but distinctly under compressed photoperiods. This is the first study to describe gonad development in farmed lumpfish histologically. It is further demonstrated that, photoperiod and temperature manipulations can be applied to control sexual maturation and spawning of broodstock, for better broodstock management and ensured year-round supply of right-sized lumpfish juveniles. This is a pilot study for a more comprehensive experiment which is currently running.

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THE POTENTIAL OF ARTIFICIAL INTELLIGENCE TO SUPPORT AQUACULTURE FARM MANAGERS: PRELIMINARY RESULTS OF THE APPLICATION OF MACHINE LEARNING ALGORITHMS FOR THE EARLY PREDICTION OF BATCH PERFORMANCE

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Introduction

Shrimp and fish farming are complex operations due in part to the large number of factors that affect performance of stocks in production. From biological and genetic to nutritional, water quality and environmental parameters to management, human performance and engineering factors, all affect the way a batch of fish or shrimp performs and therefore all affect the performance of the farm as a business.

Good managers have the capacity to, often subconsciously, process many of these factors and make good day-to-day decisions that aim at improving performance of their stock. However, it is not possible for humans to take into account, rationally, large sets of data for all factors mentioned and therefore there are limits to even the best managers in the industry.

Machine Learning (ML) algorithms allow Artificial Intelligence (AI) to be trained (learn) and then AI can be used to aid managers in their daily decisions. ML can process very large data sets and take into account hundreds or thousands of factors when interpreting results making them ideal tools for fish and shrimp farming data

The aquaculture production management software Aquanetix was designed as a Cloud service and is today one of the largest databases of day-to-day data for dozens of farmed species from many production systems in various countries.

Training of ML algorithms requires complete batch (from stocking to harvest) data sets where final performance is known and in Aquanetix we have thousands of such data sets for more than 20 different species of fish and shrimp. This is an ideal system therefore to test the application of ML to aquaculture data.

Methods

ML algorithms increase in their capacity to model complex data as we move from linear regression, logistic regression and to neural networks of varying complexity. In this paper we have focused on developing algorithms that predict performance of tilapia batches from data collected in the first 6 weeks of grow-out. We will present results from the three main ML methodologies and show how using Neural Networks we can develop predictive models that have accuracy greater than 90%.

Data used are stocking details (number of fish stocked, mean weight of fish), weight data and mortality data in the first 6 weeks as well as site, harvest year and harvest week and we predict final production cost (cost per kg) as well as final survival. These results can be used to recommend actions early in the lifecycle of production that will improve performance of batches by harvest time.

Discussion

There are many potential applications of AI to aquaculture operations, from risk avoidance (pathologies) to size dispersal control, performance forecast and adjustment and eventually to everyday advice for managers on what to feed, when to split/grade or even harvest fish or shrimp batches. In order to get there, we need to collect large real-life datasets using systems such as Aquanetix and to start developing learning models that will take into account the many factors involved. The use of IoT (Internet of Things) technologies such as environmental monitoring probes, video footage from cages during feeding and feeding system delivery data will enrich the dataset and add more information for ML algorithms to result in better, more sophisticated and precise models.

THE EFFECT OF DIFFERENT WATER SOURCES ON THE POTENTIAL H_2S-FORMATION WITHIN RAS

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Introduction

In the last years, several of the serious incidents involving acute fish mortalities in recirculating aquaculture systems (RAS) for Atlantic salmon (*Salmo salar*), have been caused by hydrogen sulphide (H_2S). These incidents have mainly occurred in seawater systems, e.g. post-smolt production. H_2S is formed by sulphate-reducing bacteria which uses sulphate (SO_4^{2-}) and organic material under anaerobic conditions (Muyzer and Stam, 2008). Seawater contains 1000 times more SO_4^{2-} than freshwater (Gerardi, 2006), increasing the potential risk for H_2S production. However, using seawater is pivotal to avoid desmoltification and preparing salmon for seawater transfer (Mortensen and Damsgård, 1998). The project where this preliminary study is from, propose the idea of removing sulphate from seawater through membrane filtration as a measure for reducing fish mortalities caused by $_2S$.

The aim of this preliminary study was to understand what microbial environments in RAS have the highest potential risk for H_2S-formation and to gain a better understanding of the dynamic between organic material and sulphate concentration for H_2S formation in RAS-water.

Materials and Methods

Three main environmental sources where H_2S could potentially form in a commercial RAS were selected: sludge, biofilter elements and RAS-water. A small-scale batch experiment was conducted where each of these three potential sources were exposed to seawater, freshwater, brackish water and treated water (freshwater + filtered seawater). The filtered seawater was provided by a membrane filtration technology developed during the same project that this study is a part of. The H_2S kinetics and production rate was measured for each test. The organic material was also measured in form of COD (chemical oxygen demand) and organic carbons as fatty acid. Anions such as NO_3^- and SO_4^{2-} and other water quality parameters were assessed.

Results

The results of this experiment are still under development and analysis and will therefore be presented at the European Aquaculture Society conference.

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PREVENTING MYCOTOXIN EFFECTS ON HEALTH STATUS OF NILE TILAPIA (*Oreochromis niloticus*) WITH MODIFIED ZEOLITE (CLINOPTILOLITE) FEED ADDITIVE

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Introduction

Feed additives to prevent mycotoxin effects on aquatic animals have been increasingly used since mycotoxin contamination of aquaculture feed and feed ingredients is an ongoing global problem. Recent chemical modification of zeolite (clinoptilolite E567/568; Minazel-Plus®) surface with addition of organic cations has been shown to increase selective adsorption of both polar and non-polar mycotoxins in contaminated feed. However, the effects of this new additive on Nile tilapia (*Oreochromis niloticus*) health and production parameters during mycotoxin exposure have not yet been studied. We report the preventive effects of Minazel Plus used as feed additive in diets naturally contaminated with aflatoxin and fed to freshwater young of the year tilapia.

Materials and Methods

To investigate effects of mycotoxin dietary exposure on fish health parameters, Nile tilapia were fed basal diet (control), and diet naturally contaminated with a mix of aflatoxins (AFs; AFB1and 2, AFG1 and 2) at level of 16 µg/kg (AFs group). To investigate safety and efficac of surface-modified clinoptilolite adsorber as part of both polar and non-polar mycotoxin control and prevention strategies, we supplemented control and aflatoxin contaminated diets with Minazel-Plus® at level of 2 g/kg (MZ, AFsMZ, respectively) for 8 weeks. Effects of AFs and the protective role of Minazel-Plus® on fish health were evaluated using growth performance and hepatosomatic index (HSI), hematological parameters, innate immune and antioxidant responses, bioaccumulation of mycotoxins in liver and musculature, and histopathological assessment of liver and kidney tissues.

Results and Conclusion

Significant differences in production and health parameters were observed between AF vs Control and MZ supplemented groups, including decrease of red blood cells/RBCs, hemoglobin/Hb, and blood packed cell volume (PCV), changes in dynamics of leukocyte counts (specifically neutrophils), and decrease in serum lysozyme and bactericidal activity. Further, increase in Malondialdehyde (MDA) and decrease in catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD) activity were observed in AFs group compared to other groups. Aflatoxin residues were detected in both liver and musculature at 1.292 and 0.263 µg/kg respectively. Supplementation of Minazel-Plus® decreased AFs residues to 0.09 (liver) and 0.022 (muscle) µg/kg. Histopathology showed marked changes in liver and kidney of fish from AFs group.

Reported results strongly suggest that supplementation of low-level aflatoxin contaminated feed with 2 g/kg of Minazel-Plus® prevents negative health effects of aflatoxicosis in Nile tilapia. The authors propose that feed-mill monitoring and prevention strategies for aflatoxin levels in fish feed ingredients need to be routinely implemented and strictly enforced due to possible illegal use of highly contaminated feed, and inadequate or missing regulations of safety level of aflatoxins in fish feed

SAPRISTI, AN INNOVATIVE MODULAR TOOL IN THE IMPLEMENTATION OF A METHODOLOGY FOR THE RECOVERY OF FISH FARMING EFFLUENT BY AQUAPONICS

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Introduction

The conversion of a traditional flow through system to a recirculated aquaculture system (RAS) allows a fish farm to drastically reduce water requirements and reach optimal growth conditions by controlling the physicochemical parameters of the water. To further improve these intensive fish farming systems, we have to address other challenges such as the recovery of RAS effluents (sludge and renewal water) which is a way to improve energy efficiency (water and sludge treatment) and decrease the environmental impact (discharges of effluents). Aquaponics is a technique which can meet these challenges as the discharged water and the sludge containing nutrients can be used for the growth of plants of commercial interest. From a fish farmer point of view, the interest of a coupled aquaponics is to improve biofiltration by removing nitrates transformed by bacterial communities. On the other hand, a decoupled aquaponics can support or replace effluent treatment. Within the Laboratory of Integrated and Urban Plant Pathology of the University of Liège, the study of these scenarios is now possible by the implementation of SAPRISTI (System Aquaponics and Pilot for Research and Innovation in Science and for Transfer to Industry). It is a modular tool that allows simultaneous comparison of decoupled aquaponics, coupled aquaponics, fish farming alone and hydroponics alone by simulating the physico-chemical parameters of the water encountered in a fish farm. This tool has been tested with the commercial company Belgian Quality Fish (BQF) producing sturgeons.

Materials and methods

SAPRISTI consists of three identical RAS-type units designed for the rearing of different species of temperate or tropical freshwater fish. A sludge collection system was installed in each RAS at the outlet of the drum filter to study its mineralization. Each element is by-passable (UV, biofilte , etc.) to study the impacts on the system, mainly on the bacterial communities that make it up. Each RAS forms a decoupled aquaponics system by the possible connection to a horticultural greenhouse where different hydroponic systems are available (DWC, NFT, Drop, Ebb & Flow). The water sent to this greenhouse can be sent back to fish production through a network of pipes and pumps, forming a decoupled aquaponics system. For the case study with BQF, the RAS were loaded with sturgeons (*Acipenser gueldenstaedtii*) with a density of 25kg/m³. Each hydroponic unit consisted of an NFT subunit, a DWC subunit and an Ebb & Flow subunit. One hydroponic unit had no connection with the fish, the other two were each connected to a RAS, either partially (decoupled aquaponics) or completely (coupled aquaponics). Finally, a RAS ran as a single fish farm unit

Results and conclusions

Two successive trials are underway. During these tests, the fresh and dry weight yield of lettuce (*Lactuca sativa*), their micro- and macronutrient content, as well as the monitoring of water quality and nutrients will be carried out, with a greater focus on nitrogen cycle. Water and energy flows related to the operation of aquaponic systems will also be monitored and compared to those same flows applied to fish farming and hydroponics alone These results will be presented.

SAPRISTI can be also used to develop a methodology for other species and for different farming practices, allowing advice on the most appropriate hydroponic technique to choose, and estimation of the expected yields and substantial savings in water and energy from fish farming

FLAT OYSTER (*Ostera edulis*) RESTORATION IN OFFSHORE WIND PARKS: AN OPPERTUNITY FOR INNOVATION OF SCOUR PROTECTION

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Introduction

The potential for offshore wind parks in the North Sea to contribute to nature-inclusive development and achieve biodiversity goals, restore ecosystem functions and enhance ecosystem services is gaining commitment through governmental policy aims. This includes restoration of flat oyster (*Ostrea edulis*) beds in the Dutch North Sea that once were an important species and habitat providing natural hard substrate (Kamermans *et al.*, 2018). The disappearance of flat oyster reefs is predominantly caused by overexploitation and subsequent habitat destruction by intensive bottom trawling (Gercken & Schmidt, 2014).

Several opportunities for the development of flat oyster populations (*Ostrea edulis*) in existing and planned wind farms in the Dutch section of the North Sea exist. However, knowledge that enables selecting the optimal moment and procedure for oyster spat treatments, prior to outplacement to offshore field locations, including optimizing survival of oysters and reducing the risk of outplacing non-native species is needed. The designation of Borssele V as an innovation site situated in the Borssele Wind Park, 20 km located off the coast of the Netherlands provides an opportunity for oyster reef development and to gain knowledge on best restoration practices.

Within the project plan Borssele V- EcoScour different outplacement methods for long-term establishment of live European flat oysters on the two scour protections of Borssele V are tested. For the success of a restoration project, selecting the right substrate is important. As part of the objective to test outplacements methods different substrates for oyster spat settlement will be tested to provide insight in which substrate is most likely to be successful in collecting spat.

The knowledge gained with these experiments contributes to the return of oyster beds. Restoring self-sustaining populations of wild oysters to a significan level benefits the supply of wild oyster larvae. In addition, insight in the factors that are important for reproduction, growth and survival of flat oysters in restoration projects can provide useful information for oyster aquaculture. Besides relieving the pressure from oyster fisheries, flat oyster aquaculture can assist oyster reef restoration by supplying in the high demand for live oysters necessary for reef restoration.

Methodology

Ten different substrates are tested with 5 replicates per type. The tests are carried out at 3 locations during the summer period when larvae are present and include an area with disease-free oysters.

To determine the right deployment moment for the substrates larval samples are collected and analyzed. Spat settlement is manually counted per substrate type.

Results and discussion

The results of oyster spat settlement on the experimental substrates in the three test locations will be discussed as well as the application for oyster reef restoration projects. These results will contribute to guidelines for innovative eco-designs of scour protection that may have positive effects on the maritime environment and practical guidelines for best restoration practices using oyster outplacement.

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VACCINES INDUCE A SIGNIFICANT IMMUNE RESPONSE BUT NOT A ROBUST NEUROENDOCRINE REACTION IN BRAIN AND PITUITARY TISSUES OF SEABREAM (*Sparus aurata*)

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Introduction

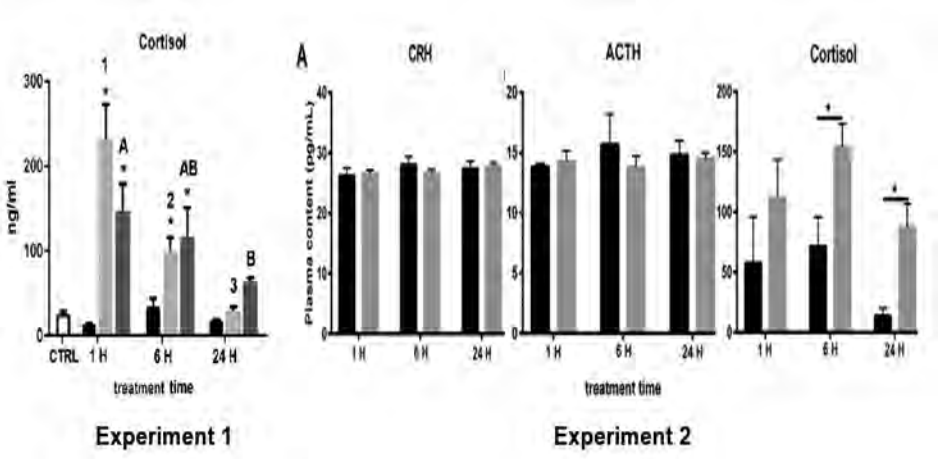
Vaccination is a widely used therapeutical strategy in aquaculture (Plant and LaPatra, 2011), but whether vaccination elicits stress responses in the central neuroendocrine system by itself and enhances the crosstalk between immune and endocrine systems in the brain or pituitary after vaccination remains unclear (Khansari et al., 2017, 2018). To answer this question, two experiments using two different vaccine exposure routes, i.e., waterborne vaccine bath or intraperitoneal injection, were carried out on gilthead seabream (Sparus aurata L.).

Materials and methods

Two types of vaccines were used in this study. In the first experiment, the stress responses of fish subjected to waterborne *Vibrio anguillarum* bacterin were analyzed and compared with responses obtained after air exposure or the combination of both procedures. In the second experiment, fish were subjected to an intraperitoneal injection of *Lactococcus garvieae* bacterin, the central stress response was assessed and we investigated whether or not a significant immune response was induced in brain and pituitary. Blood, brain and pituitary tissues were collected at 1, 6, and 24h post stress for hormone determination and gene expression analysis.

Results

Results indicated that bath vaccination induced a moderate central stress response compared to air exposure which stimulated both brain and pituitary stress genes. In the second experiment, vaccine injection showed unchanged plasma stress hormones except cortisol that raised at 6 and 24h time points. Similarly, non-significant or slight changes on the transcription of stress-related genes were recorded, including the hormone genes of the hypothalamic-pituitary-interrenal (HPI) axis and other stress gene indicators such as *hsp70*, *hsp90* and *mt* in either brain or pituitary. However, significant changes were observed in *crhbp* and *gr*. In this second experiment the immune genes *il1β*, *cox2* and *lys*, showed a robust expression in both pituitary and brain after vaccine administration, notably *il1β* which showed more than 10 fold increase.



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Discussion and conclusions

It is not precisely known what are the particular mechanisms of interaction between hormone elements and immune agents. Due to the protection of the blood brain barrier, pathogens can hardly access to the brain or pituitary. However, mediators such as cytokines could play a role of connecting antigens and response pathways (Banks et al., 1995). In previous works of our group we observed that both the medium from *the in vitro* cultured spleen and recombinant IL1β induced a significant effect on the immune response of trout pituitary tissue (Liu et al., 2019). Thus, the immune response in the central neuroendocrine system might be regulated by some mediators produced and released into the bloodstream by lymphoid organs as a response to the bacterin delivered by intraperitoneal injection.

Overall, vaccination procedures, although showing a clear cortisol response, did not induce other major stress responses in brain or pituitary, regardless the administration route and the type of vaccine at the doses tested. Other than these main results, the alteration of specific genes such as *crhbp* and *gr* suggests that these particular genes could play a relevant role in the feedback regulation of HPI axis after vaccination.

Moreover, from the results obtained in this work, it is also clearly demonstrated that the immune system maintains a high activity in both brain and pituitary tissues after vaccine injection, as shown by the enhanced activity of the relevant immune associated cytokine genes as well as the innate immune genes assessed in these tissues.

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CORTISOL IN PLASMA, SKIN MUCUS AND SCALES. A COMPARATIVE VIEW OF THE HYPOTHALAMIC -PITUITARY-INTERRENAL AXIS ACTIVITY IN DIFFERENT FISH MATRICES

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Introduction

The end hormone of the hypothalamus-pituitary-interrenal (HPI) axis, cortisol, is probably the most often measured indicator of stress in fish (Schreck and Tort, 2016). Nevertheless, obtaining blood involves certain degree of invasiveness which is not always possible or suitable and raises the consequent point of invasiveness and welfare issues. Over the last years, skin mucus has been developed, which is a far less invasive method, but as blood, this method potentially provides a brief window of information of the HPI axis activity. (Bertotto et al., 2010; De Mercado et al., 2018).The recently described cortisol measurement in fish scales provides another integrated measure of the HPI axis that may be useful for measuring long-term activity in this axis in fish (Aerts et al., 2015; Hanke et al., 2019).). Although cortisol in skin mucus or in the scales cortisol present practical advantages, there are still several issues that remain unclear related to their biological relevance, particularly under both acute and chronic stress situations. Therefore, we evaluated whether cortisol levels in skin mucus and scales can be a reliable measurement for stress response when subjecting fish to prolonged, continuous stressful conditions.

Materials and methods

A total of 64 rainbow trout were randomly divided into 8 groups. Five stress groups were confined at a high-density (30 kg/m3), each group in a different keepnet (45 cm×25 cm×25 cm) thus maintaining the confinement but also not modifying the total water volume. Three control non-stressed groups were kept undisturbed in different holding tanks at a low-density (2.6 kg/m3). One control group was sampled for blood, skin mucus and scales at the start of the experiment in order to obtain pre-stress levels (0 h). Three stress groups were sampled for blood and skin mucus at 1, 6 and 24 h after starting the confinemen stress (early phase of the stress response). The remaining four groups were kept under control or stressed conditions for 14 and 30 days (late phase of the stress response), and at each period, blood, skin mucus and scales were sampled from a control and a stress group. One individual from the 14th day control group died during the study.

Results

A significant increase in plasma and mucus was detected one hour after confinement stress. In plasma, cortisol levels remained significantly higher than pre-stress levels up to 24 h whereas in mucus, cortisol levels decreased significantly at 24 h compared to the initial peak, although remaining above pre-stress levels. At day 14 and 30 plasma and mucus decreased significantly compared to the cortisol at 1, 6 and 24 h with no differences at days 14 and 30. In mucus, no differences were detected between controls and stressed fish at day 14, while at day 30 stressed fish presented higher mucus cortisol than controls. Significant differences in scales were detected between stressed and control fish at day 14 and 30, although showing a decrease from day 14 to day 30 of the study. Finally, the control group displayed a significant increase in scale cortisol at day 14, but returned to pre-stress levels at day 30.

Discussion and conclusions

The present study shows that the measurement of cortisol in skin mucus reflects plasma cortisol concentrations when fish are subjected to a strong activation of the HPI axis. Our results also demonstrate that the cortisol content in scales strongly correlates with circulating plasma cortisol levels in fish subjected to chronic stressed. Our results also indicate that scales cortisol assessment may provide a retrospective measure of the past stress experience in fish. Overall, while this study offers a good basis for future research on the measurement of stress responses by applying the mucus and scales cortisol assessment, the present results open the question of whether these new matrices receive additional sources of cortisol other than plasma, and which should be the route of cortisol incorporation or diffusion in such a case. Further work should deep into the general robustness and stability of the cortisol content in scales cortisol when fish is subjected to prolonged stress. This will help to understand the dynamics of hormone fluctuations in this matrix

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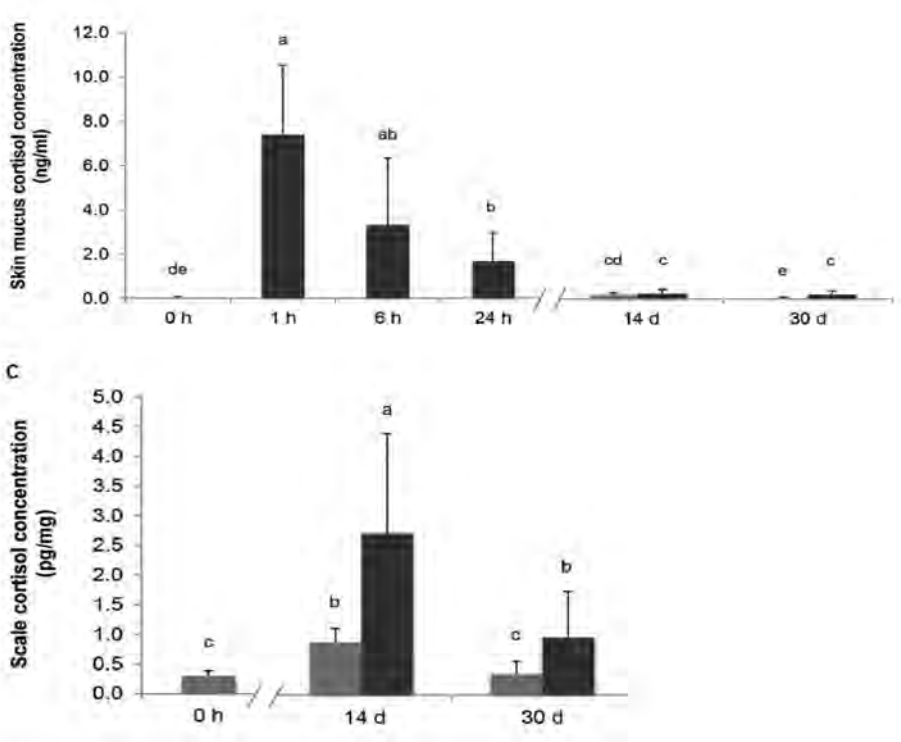

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OPTIMIZATION OF ANTIOXIDANT ACTIVITY IN SEA VEGETABLES GROWN IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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Introduction

The value of sea vegetables in a healthy human diet is becoming increasingly recognized and supported throughout Europe. Sea vegetables provide a rich source of vitamins, minerals, proteins, fatty acids, and antioxidants. Sustainable aquaculture of sea vegetables will be necessary to supply the increasing demand for these functional foods, as natural harvests are limited and cannot keep up with demand. Different methods can be used to cultivate sea vegetables, including flow through systems with natural seawater, or recirculating aquaculture systems (RAS) with artificial seawater. The latter provides strict control over the growth conditions and water quality in order to provide a high quality and traceable final product. Environmental conditions such as salinity, temperature, and light can be modified to optimize the concentration of functional ingredients in sea vegetables. To date, most research efforts have been focused on quantifying the concentrations of functional ingredients in different species from different geographic regions, as well as identifying seasonal trends in wild sea vegetables. There is less information available on how to optimize these functional ingredients in aquaculture. Therefore, we performed controlled experiments to optimize the activity of antioxidants in sea vegetables grown in RAS.

Materials and Methods

We investigated the effects of daylength and light quality on the growth, oxygen production and free radical scavenging activity of *Ulva* sp. using the ABTS radical cation decolorization assay (Re et al. 1999). Additionally, we investigated the effects of salinity, desiccation, daily photon dose, and UV light on the growth and free radical scavenging activity of *Gracilaria vermiculophylla*. Both species were grown under climate-controlled conditions (15°C) with artificial irradiance provided by LEDs.

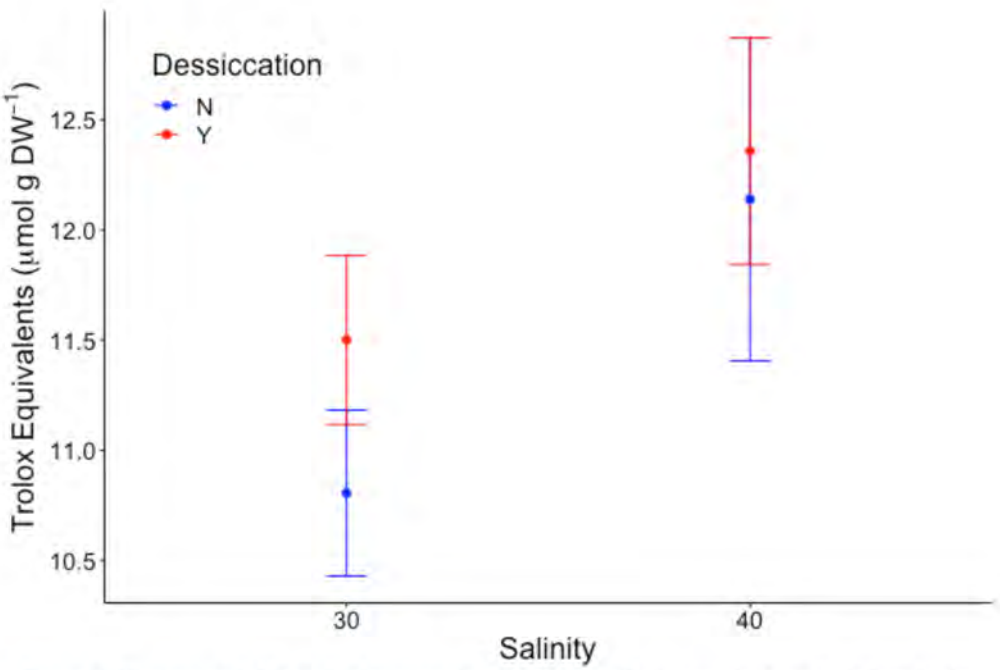

Figure 1. The mean (±SE) radical scavenging activity (as Trolox equivalents) of *Gracilaria vermiculophylla* under different salinity conditions with (Y) and without (N) exposure to desiccation.

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Results

We show that the conditions for optimal antioxidant activity are not necessarily optimal for growth or photosynthesis. For example, preliminary results show that high salinity combined with desiccation in *G. vermiculophylla* resulted in poor growth rates and quantum efficiency of Photosystem II ($_{v}/F_{m}$), but the highest antioxidant activity (Fig. 1).

Discussion

We show that antioxidant activity in two sea vegetables can be optimized in RAS culture. The environmental conditions should be optimized for growth to produce biomass, followed by a short acclimation phase to increase antioxidant activity. We discuss the implications of our experiments for future sea vegetable aquaculture activities, and provide recommendations for optimization of antioxidant activity in the two species investigated.

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EFFECTS OF FISH & PLANT OILS REPLACEMENT WITH MICRO-ALGAE OIL ON GUT MICROBIOME, GENE EXPRESSION AND GROWTH PERFORMANCES OF NILE TILAPIA FRY(*Oreochromis niloticus*)

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Aim:
This experiment will assess the effects of microalgae lipids on gut microbiota, gene expression and growth performances of Nile tilapia fry. While tilapia is the second most farmed fish on the planet information about its gut microbiota, especially on how it develops from early life stage, is still missing. In this experiment we will characterize the gut microbiome of Nile tilapia and we will observe how it is influenced by the change of lipid's origin in the diet. In particularly we will observe how the replacement of conventional fish and plant oil with micro-algae oil affects the microbiome and how it is associated with the health and fitness of the fish (growth performances, immune gene expression

Materials and methods:
At the start of the experiment 1620 tilapia fry (mixed sex) were purchased from Stirling University and housed in 18 tanks of 25 litres. Each tank will contain initially 90 fish. Three tanks were randomly assigned to each of the 6 experimental diets. The diets for the tilapias were made in CSAR following the same methodology illustrated in the study of Royes and Chapman (2003). The diet composition of the control diet (CON) is the same of the Nile tilapia "reference diet" used in the experiments of Teuling, Wierenga et al. (2019) and Teuling, Schrama et al. (2017). The positive control diet (+CON) is the same of CON but with 100% fish oil (no soya oil). The negative control diet (-CON) is the same of CON but with 100% soya oil (no fish oil). The microalgae diets are three, each one with a raising percentage of microalgae oil in it up to 100%. The microalgae used as oil source is Schizochytrium. This microalgae-oil not only doesn't affect negatively the welfare of tilapia, it also improves its growth performances as demonstrated in the studies of Watters, Rosner et al. (2013); Sarker, Gamble et al. (2016) and Sarker, Kapuscinski et al. (2016). The diet Algae33 is the same of CON but with 33% of fish&soya oil replaced by microalgae oil. The diet Algae66 is the same of CON but with 66% of fish&soya oil replaced by microalgae oil. Finally, the diet Algae100 will is the same of CON but with 100% of fish&soya oil replaced by microalgae oil. At the start of the experiment 3 fish per tank were sacrificed (using a Schedule1 method, overdose of anaesthetic) for initial microbiome-sampling and length &weight measuring. The fish are fed to satiation three times per day. The experiment will last 8 weeks, but it will be most likely extended to observe the long term development of the gut micro-biome. At day 21 of the experiment, 4 fish per tank were sacrificed for microbiome- sampling. After day 21, sampling of 4 fish per tank will occur every 2 weeks for the remaining duration of the experiment. At the end of the experiment, a final microbiome-sampling and length & weight measuring will be undertaken. The samples taken are gut and liver stored in RNA-later solution for both microbiome and gene expression analysis. Weight and length of the sampled fish is also recorded for assess the growth performances. The gut microbiome will be characterized with the 16S ribosomal RNA sequencing, meanwhile gene expression in both gut and liver will be evaluate via microarray analysis and reverse transcription polymerase chain reaction (RT-PCR). Because this trial is still underway at the moment, results and discussions are not yet available.

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OPTIMISING GENOMIC SELECTION WITH LOW DENSITY MARKERS IN FARMED ATLANTIC SALMON

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Introduction

Genomic selection is increasingly applied in aquaculture breeding to expedite genetic gain for key production traits such as disease resistance. Its effective application in breeding programmes depends on large training datasets of phenotypes and genotypes. In livestock breeding, genotype information on large populations can be achieved in a cost-effective manner through genotype imputation, which allows to infer high density genetic information from low density genotype data. In typical aquaculture breeding programmes where large full-sibling families sharing large genomic segments are available, the value of imputation is yet to be fully assessed.

The aim of this study was to evaluate strategies for the use of low density SNP panels and genotype imputation in Atlantic salmon breeding programmes. Our study focuses on two economically important traits, namely resistance to sea lice (sea lice count) and body weight. Sea lice is the most costly disease-related problem in all major salmon-producing countries (Gjerde et al., 2011). Previous studies (Tsai et al., 2015; Tsai et al., 2016) have shown the existence of genetic variation in resistance to sea lice (heritability of 0.22-0.33) and body weight (heritability of 0.5-0.6), and both traits were found to have a polygenic genetic architecture, hence lend themselves to genomic selection.

Materials and Methods

This study focused on a Scottish Atlantic salmon breeding programme population challenged with *L. salmonis* (Landcatch, UK) (Tsai et al., 2015). All samples were genotyped with the Affymetrix Axion 132K Atlantic salmon SNP chip (78362 SNP markers) (Houston et al., 2014). The analysis comprised three main parts: (a) identifying high density (HD) SNP panels with optimal density and composition; (b) testing the imputation accuracy when offspring were genotyped at a range of low densities (LD) consisting the imputation panels, and parents were genotyped at HD; and, (c) comparing the genomic prediction accuracies of each of the datasets, with and without imputation. All analyses were performed via an in-house built software pipeline, using R and Shell for selection of HD and LD SNP panels, calculation of genomic relationship matrices, and cross-validation; FImpute for genotype imputation; and, ASReml for estimating breeding values.

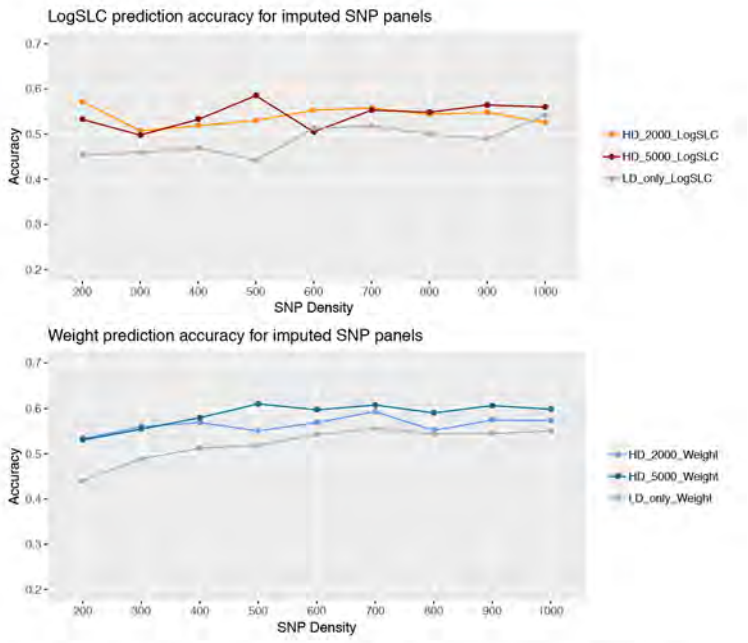

Figure 1. Mean cross-validated prediction accuracy for sea lice count and weight.

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Ten replicates of SNP panels of varying densities were constructed by random sampling of SNPs (i) across the genome, or, (ii) within each chromosome proportionally to its length. Mean genomic prediction accuracies were calculated over 50 replicates of 5-fold cross-validation as:, where was the correlation between phenotypes and predicted breeding values, and h the square root of the genomic heritability.

Results and Discussion

The heritability estimated using the genomic relationship matrix was 0.19 (0.07) for sea lice resistance, and 0.57 (0.07) for weight. Reducing the SNP panel density from the full HD SNP panel (76,488 SNPs) to 200 SNPs resulted in a 14.5% decrease in genomic prediction accuracy for sea lice resistance, and a 27.9% decrease for body weight. This loss of accuracy started between 2,000 and 5,000 SNPs for both traits, therefore these densities were tested as the optimal target HD SNP panels for imputation.

Imputation accuracy increased with increasing imputation SNP panels density. Genomic prediction accuracy using imputed genotypes was only marginally higher for body weight when increasing the density of the imputation SNP panel, and for sea lice resistance there was very little difference between lower and high density imputation panels (Figure 1). The benefits of imputation were highest at the lowest imputation panel densities. For example, genomic prediction accuracy using 200 SNPs without imputation for sea lice resistance was 0.45, whereas with imputation to 5000 SNPs it was 0.53, which corresponds to a 17.47% increase due to imputation (Figure 1).

In conclusion, genomic prediction using LD genotypes imputed to medium densities (e.g. 5000 SNPs) can provide better accuracies than using LD true genotype data alone. There is large variability in the genomic prediction accuracy from LD SNP panels, depending on the SNPs randomly selected on the panels, thus using LD SNP panels with imputation is likely to be cost-effective.

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DELTAMETHRIN RESISTANCE IN SALMON LICE: MITOCHONDRIAL AND NUCLEAR SINGLE NUCLEOTIDE MARKERS

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Introduction

The salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837) is a copepod fish parasite causing huge economic damage in the farming of Atlantic salmon. Salmon lice infections can be controlled by bath treatments with the pyrethroid deltamethrin (DTM, AMX®, PHARMAQ), which is believed to act by blocking voltage gated sodium channels (Na_v1). However, the use of DTM for salmon delousing is threatened by resistance development (Aaen *et al*., 2015). In insects, a common mechanism of DTM resistance involves target-site mutations rendering Na_v1 insensitive (knock down resistance, *kdr*). Alternatively, resistance can be based on increased metabolic detoxification due to up-regulation of cytochrome P450s or esterases (Carmona-Antoñanzas *et al*., 2017). In *L. salmonis,* DTM resistance has been shown to be mainly inherited maternally, and to be associated with mitochondrial (mt) mutations (Carmona-Antoñanzas *et al*., 2017; Bakke *et al*., 2018). This suggests a novel, yet uncharacterised, resistance mechanism and implies the presence of mt targets for toxicity in susceptible lice. In addition, a putative *kdr*-type mutation has been identified in one of three *L. salmonis* Na_v1 homologues (Carmona-Antoñanzas *et al*., 2018). In order to obtain insights into the mechanisms of DTM resistance in *L. salmonis,* pharmacological and genotyping approaches were combined in this study, investigating DTM susceptible and DTM resistant salmon lice.

Materials and methods

L. salmonis used in this study originated from laboratory strains (IoA-00: EC_{50} 0.28 µg l^{-1}; IoA-02: EC_{50} 40.1 µg l^{-1}), farm bioassays (Farm 1: EC_{50} 1.7 µg l^{-1}; Farm x: EC_{50} >>2 µg l^{-1}; Farm y: EC_{50} 12.63 µg l^{-1}), and a cross between a DTM resistant male and a DTM susceptible female. DTM susceptible and resistant *L. salmonis* were genotyped for selected single nucleotide polymorphisms (SNPs), which were associated with DTM resistance in previous studies (Carmona-Antoñanzas *et al*., 2018; Carmona-Antoñanzas *et al*., 2017). Genomic DNA was extracted from individual lice. Allele specific PCR assays were carried out using the KASP system (LGC Genomics) and designed to detect the mt SNPs t3338c (COX3), t8600c (COX1), a8134g (ND1), and a5889g (ND5) and the Na_v1 SNP a3041g. In addition, *L. salmonis* bioassays were performed with the pseudo-pyrethroid etofenprox, which cannot be metabolised by esterases. Bioassays were performed using adult male and preadult II female lice. Lice were exposed to toxicants (DTM: 30 min; etofenprox: 24 h) and allowed to recover in seawater (DTM: 24 h; etofenprox: no recovery) before being rated as affected or unaffected (Carmona-Antoñanzas *et al*., 2017). A seawater control and at least seven toxicant concentrations were tested (duplicates, five males and females each). Lice were classified DTM resistant when remaining unaffected after exposure to ≥3 µg l^{-1} DTM and classified susceptible when a fected after exposure to ≤1 µg l^{-1} DTM.

Results

The allele frequencies of the putative *kdr* mutation a3041g in Na_v1 did not differ ($p>0.05$) between DTM resistant and susceptible lice from farm sites and did not correlate with DTM resistance of F2 progenies from a cross between a DTM resistant male and a DTM susceptible female (Table I). Compared to the susceptible strain IoA-00, strain IoA-02 was resistant to DTM but susceptible to etofenprox (Table II).

The allele frequencies of the mt SNP alleles g3338a, t5889c, g8134a, and t8600c were higher in DTM resistant lice than in susceptible lice ($p<0.001$). Only SNP t8600c in COX1 was present in all DTM resistant lice but also in a few susceptible lice (Table III).

Discussion and conclusion

In insects, *kdr* mutations cause cross-resistance to etofenprox (Carnevale *et al*., 1999). In contrast, DTM resistant *L. salmonis* included in this study were not cross resistant to etofenprox. The allele frequency of the putative *kdr* mutation a3041g did not correlate with DTM resistance. Thus, DTM resistance in *L. salmonis* seems to be unrelated to target site mutations in Na_v1. Significantly higher allele frequencies of all tested mt SNPs in DTM resistant lice than in susceptible lice indicate an association with DTM resistance. However, while SNP t8600c, leading to the missense mutation Leu107Ser in COX1, was present in all DTM resistant lice, it was also found in some susceptible lice. A previous study showed that Leu107 is located on the surface of the COX1 protein structure and thus, might represent a potential DTM binding site (Bakke *et al*., 2018).

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Table I Genotyping of *L. salmonis* at the single nucleotide polymorphism locus a3041g

Origin *L. salmonis*	Allele frequency SNP a3041g		P-value (exact G-test) Pairwise comparison
	Susceptible	Resistant	
Farm site 1	0.81	0.75	0.71
Farm site y	0.50	0.75	0.10
F2 progenies [IoA-02 male x IoA-00 female]	0.30	0.42	0.26

Table II Half-maximal effective concentration EC_{50} of etofenprox and deltamethrin in *L. salmonis*

Strain	EC_{50} deltamethrin (95% CI) [µg L^{-1}] (no sex difference)	EC_{50} etofenprox (95% CI) [µg l^{-1}]	
		Female	Male
IoA-00	0.28 (0.23-0.36)	0.57 (0.40-0.74)	0.27 (0.20-0.36)
IoA-02	40.1 (22.1-158.9)	0.43 (0.30-0.56)	0.32 (0.23-0.40)
Resistance ratio	*143.21*	*0.75*	*1.20*

Table III Genotyping of *L. salmonis* at four mitochondrial single nucleotide polymorphisms (SNP)

Origin *L. salmonis*	Deltamethrin resistance	N	Allele frequency of SNP alleles			
			g3338a	t5889c	g8134a	t8600c
IoA-00	Susceptible	22	0	0	0	0
Farm site 1	Susceptible	16	0.25	0.25	0.25	0.25
Farm site y	Susceptible	6	0.33	0.5	0.5	0.5
IoA-02	Resistant	24	1	1	1	1
Farm site 1	Resistant	8	0.88	0.88	0.88	1
Farm site x	Resistant	13	0.70	0.92	0.92	1
Farm site y	Resistant	12	0.83	0.83	0.83	1

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THE CARBOXYLESTERASE FAMILY IN SALMON LICE AND THEIR ROLE IN DELTAMETHRIN RESISTANCE

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Introduction

The salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837) is a copepod fish parasite causing huge economic damage in the farming of Atlantic salmon. The pyrethroid deltamethrin (DTM, AMX®, PHARMAQ) is a licensed bath treatment for the control of salmon lice infections. However, the use of DTM for salmon delousing is threatened by resistance development (Aaen *et al*., 2015). In insects, pyrethroid resistance typically involves mutations of the compound's molecular target site or increased detoxification by enhanced expression of biotransformation enzymes such as carboxylesterases (CaEs), cytochrome P450s, or glutathione-S-transferases (Ranson *et al*., 2011).

The CaE gene family is functionally very diverse. CaEs possessing a catalytic triad, which can hydrolyse the central ester bond of pyrethroids, and thus promote its degradation (Montella *et al*., 2012). In *L. salmonis*, DTM metabolism involving cleavage of the central ester bond has been recently reported, with higher rates of metabolism in DTM resistant than susceptible lice (Bakke *et al*., 2016).

The aim of this study is to obtain insights into *L. salmonis* genes potentially involved in DTM hydrolysis. CaEs are identified in sequence databases and phylogenetic relationships are established for *L. salmonis* CaEs. Their transcript expression will be assessed in laboratory *L. salmonis* strains differing in susceptibility to DTM.

Materials and methods

L. salmonis CaEs homologues were identified by homology searches in an *L. salmonis* transcriptome assembly (EBI ENA reference ERS237607) and genome assembly LSalAtl2s (metazoan.ensembl.org), using the entire complement of *Drosophila melanogaster* CaEs as queries (E-value cutoff = 10^{-10}).

Phylogenetic analyses of *L. salmonis* CaEs sequences retrieved by this strategy took into account CaEs of *D. melanogaster* and *Apis mellifera*. Full and partial protein sequences were aligned using the programme Clustal Omega and then subjected to phylogenetic analysis using the RAxML package.

CaEs transcript expression is currently being studied in relation to DTM exposure in female and male *L. salmonis* from DTM resistant and susceptible laboratory-maintained strains.

Preliminary results and discussion

The. *D. melanogaster* and *A. mellifera* genomes contain 35 and 24 CaEs, organised in 11 and 9 clades, respectively, which assign to three major classes (Claudianos *et al*., 2006). The first class contains catalytically active intracellular enzymes covering dietary and detoxification functions (clades A-C). The second class contains enzymes that are catalytically active, secreted, and not membrane-bound (clades D-H), and the third, most ancient class, contains catalytically inactive membrane-associated proteins with neuro/developmental functions (clades L, M), uncharacterised proteins (clades I, K), and catalytic active acetolycholinesterases (clade K). CaEs conferring pesticide resistance occur in all three classes but most CaEs associated with metabolic resistance to pesticide belong to the first class (Montella *et al*., 2012).

In *L. salmonis*, 27 CaEs were identified, of which 17 CaEs occur in five clades in two classe

L. salmonis has 16 members of the third neuro/developmental class, including two putative achetylcholinesterases, 12 putative neuroligins, and one putative gliotactin and neurotactin. Thus, *L. salmonis* has twice as many neuroligins as *D. melanogaster* and *A. mellifera*. Neuroligins are postsynaptic cell adhesion proteins involved in formation and specificatio of synapses (Dean and Dresbach, 2005). *L. salmonis* has only one member of the second secreted catalytic class in the small clade D, containing integument esterases (Montella *et al*., 2012).

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None of the *L. salmonis* CaEs could be assigned to the first class, providing detoxificati n functions. Similarly, *L. salmonis* has been shown to possess a markedly reduced number of detoxifying ABC transporters (Carmona-Antoñanzas *et al*., 2015). The low number of detoxifying genes in *L. salmonis* may have arisen because salmon lice only ingest host product when feeding and are partially protected from environmental toxicants during host-attachment. Similarly, the human body louse *Pediculus humanus,* which is an obligate blood feeder, and *A. mellifera*, which maintains a mutualistic symbiotic relationship with flowering plants, possess only 3 and 9 CaEs in the detoxifying class, respectively (Lee *et al*., 2010; Claudianos *et al*., 2006). In contrast, the expansion of detoxifying class CaEs in *D. melanogaster* (13 CaEs), *Anopheles gambiae* (16 CaEs), *Bombyx mori* (55 CaEs), or *Tribolium castaneum* (26 CaEs) is likely due to their polyphagy throughout their life cycles (Oakeshott *et al*., 2010; Carmona-Antoñanzas *et al*., 2015).

CaE genes evolve rapidly, which complicates phylogenetic analyses (Montella *et al*. 2012). Ten out of 27 identified *L. salmonis* CaEs could not be assigned to clades, which are present in insects. Their function has yet to be clarified

Ongoing analyses will show whether certain identified CaEs are differentially expressed between DTM resistant and susceptible, and female and male *L. salmonis*, in the absence or presence of DTM exposure.

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GENE EXPRESSION LEVELS IN DISTINCT BRAIN AREAS OF CARPS AFTER POSITIVE AND NEGATIVE ACUTE STRESSORS

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Introduction

While the effects of stress on the mammalian brain are under thorough investigation, only few explored how distress and eustress affect the brain of teleosts (Schreck et al. 2016). Being the center of an elaborate nervous system, the fish brain is likely to be influenced by current and past stress and may also alter the perception of future stressors

Many stimuli which fish in aquaculture encounter are processed by the brain, making the latter the place where most stressors first affect the fish irrespective of their quality and quantity. A better understanding of this primary stress response may lead to deeper insights in how stress influences fish and how negative effects might be lessened while positive ones may be utilized.

For this purpose, the expression levels of several genes in carps were compared between different brain areas at different time points after the fish experienced either a positive, a negative or no stresso .

Methods

70 juvenile carps (*Cyprinus carpio*) from a local supplier were held in a recirculating aquaculture system till a mean weight of 58g at standard conditions. In addition to the commercial pellet feed, the fish were trained to receive mosquito larvae in the morning by hand feeding. 30 pairs of fish were transferred to a 40 l glass tank with a flow-through system for an acclimatization phase of 40h where water parameters were held stable and equal to before and fish were hand fed twice a day. Thereafter both carps in the tank were subject to the same of 10 treatment groups. Eustress groups were hand fed with mosquito larvae and sampled after 10min, 30min and 60min after the feeding. Distress group were exposed to air for 1min and sampled after 10min, 30min and 60min after the exposure. The control groups were only visually checked and sampled after 0min, 10min, 30min and 60min after the check. Sample size was 6 individual fish i.e. three pairs for each group

Fish were caught with hand nets and placed in a MS222 solution (150mg/L), after the loss of the swim reflex blood was sampled from the caudal vein within three minutes from capturing. Fish were measured and brains were dissected and stored in RNAlater while blood samples were centrifuged, and plasma kept at -80°C.

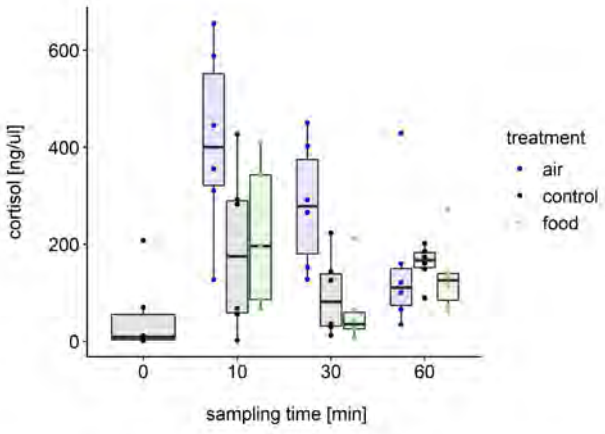

Fig. 1: Plasma cortisol levels for each treatment group. Air exposure: blue, food reward: green and control check: black. Sample size was 6 per treatment group, except for food10 (n=5).

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Cortisol values of the plasma were analyzed using HPLC methods. The brains were split in 4 parts, the telencephalon, the optical tectum, the hypothalamus and the cerebellum and gene expression levels were analyzed using quantitative real-time PCR. Relative gene expression levels were normalized based on three house-keeping genes and expressed as ddCt values. In the focus of the analyses were immediate early genes indicating activity of the corresponding brain area as well as genes known to be part of the hypothalamus-pituitary-interrenal (HPI) axis.

Results

Cortisol analyzes of the blood plasma show the effect of the distress treatment resulting in an increase of the stress hormone after 10min with a following decline over the later sampling points (fig. 1). Similar to the air exposure the feeding treatment and the control led to higher cortisol levels after 10min with a decline afterwards. However, the peak levels are higher in the distress group.

Statistical analysis and preliminary results of the qPCR measurements will be available for the second submission date in July and all data will be ready for presentation at the conference.

Discussion

Despite considerable individual differences the cortisol levels in the plasma show a stress response to the treatments which was stronger in the distress groups. The differential influence of the identity, the severity and the frequency of a stressor on the primary and secondary stress response in fish is known (Pavlidis et al. 2015). And now the gene level analyses will show the patterns in different brain areas after different stressors. The immediate early genes will reveal which of the four brain parts are activated by the different treatments and the expression of the stress related genes will shed light on the relevant brain parts.

The first preliminary results show how important the structuring of the teleost brain is when investigating the effects of stress on fish. Taking into account that different brain areas also have various functions and therefore may also be affected differently by stress is crucial to any future investigation as whole brain analyses are likely to cover relevant signal being restricted to specific areas

In a next step the relevant brain parts and genes will be analyzed in a chronic setting allowing a better understanding the effects of prolonged stress on fish. The data will pave the way to a potential bio indicator of stress in fish enabling a better assessment of handling practices and husbandry conditions and their effect on fish welfare in aquaculture

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EFFECT OF THE DIETARY SUPPLEMENTATION OF ZINC ON GROWTH, SURVIVAL AND BONE DEVELOPMENT IN GILTHEAD SEA BREAM LARVAE

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Introduction

\Gilthead sea bream (*Sparus aurata*) is an important species in the Mediterranean market among other cultured marine fish. Still, the occurrence of skeletal anomalies and reduced growth are problems to be solved in the production of juveniles of this species. The stages of larval development are crucial for the recognition and identification of abnormalities in skeletal structures. Also, during this developmental phase, major changes occur in the skeletal system. Zinc (Zn) is an essential trace element that intervenes in a wide series of metabolic processes, including ossification, bone development and growth (Yamaguchi, 1998). Dietary deficiency of Zn in fish causes reduced growth, high mortality, low tissue Zn and dwarfism (Ogino and Yang, 1978; Watanabe et al., 1997). In larvae of another sparid, the red sea bream (*Pagrus major*), Zn deficient larval diets increased the incidence of bone anomalies (Nguyen et al., 2008). However, the information about mineral nutrition in marine fish larvae, and particularly gilthead seabream is still scarce. This study aims to investigate the effects of the dietary supplementation of Zn on skeleton health and growth performance in gilthead sea bream larvae.

Materials and Methods

Diets. Four isoenergetic and isonitrogenous diets were supplemented with different levels of Zn at concentrations of 0, 20, 40 and 200 mg/kg.

Fish and experimental conditions. Gilthead sea bream larvae were randomly distributed into twelve light grey color fibreglass tanks (2200 individuals/ 200-L FRP tank) filled until 170 L. Initial mean body length and weight were 8.96 ± 0.86 mm and 0.78 ± 0.11 mg. Fish larvae were fed with one of the 4 experimental microdiets every 45 min from 8:00-20:00. The total amount of diet fed daily was 4 g and increased 0.5 g each week. A siphon was used to clean the bottom of tank every day at 14:00 and uneaten feed was weighted and recorded during the feeding period. Photoperiod was kept at 12h light: 12h dark at light intensity of 1700 lux. Flow of filtered seawater was maintained at an increasing rate of 0.3 – 1 L/min along the feeding trial. Larval tanks were continuously aerated at 125mL/min to attain 5-8 g/L dissolved O_2 and saturation ranged between 60% – 80%.

Growth was monitored by measuring the weight and length of larvae after 8, 15 and 21 days (Final sampling) of feeding. At day 8,15 and 21 of samples were also collected for gene expression relative to bone and mineral metabolism (30 larvae/ tank) and cartilage stain (30 larvae/tank). At the end of the study live larvae were sampled for weight and length (30 larvae/ tank), histology (15 larvae/tank), whole mount stain with alizarin red for calcified skeletal structures (100 larvae/tank), gene expression (30 larvae/tank), TBARS (300 larvae/tank) and remaining larvae for mineral analyse. Daily mortality was calculated for survival rate. Experimental diets and whole larvae of biochemical composition were analyzed following standard procedures. Mineral analysis of diets and whole larvae were conducted using an inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Scientific, Massachusetts, USA)

Statistics. All data were tested for normality and homogeneity of variances and means compared by Ducan test and one-way ANOVA ($P < 0.05$).

Results

After the 21 days of feeding survival rate was no significantly different ($P > 0.05$) among larvae fed the different Zn dietary levels (Figure 1). Fish fed a diet without supplemented Zn showed the lowest body length and weight, whereas increase in dietary supplementation up to 20 and 40 mg/kg significantly increased growth (Figure 2, 3). No significant differences were observed in growth of larvae fed diets supplemented with 20, 40 and 200 mg/kg of Zn.

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Figure 1. Daily mortality of gilthead sea bream larvae fed diets with different supplementation level of Zinc

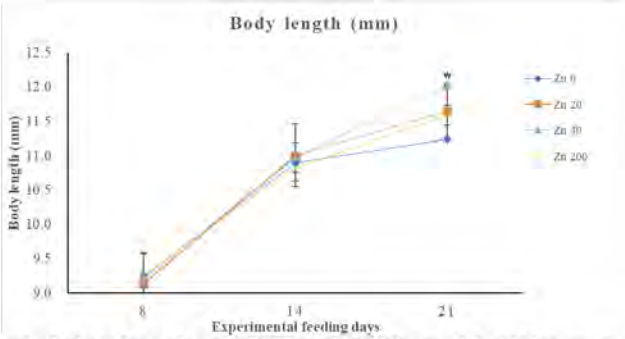

Figure 2. Body length of gilthead sea bream larvae fed diets with different supplementation level of Zinc

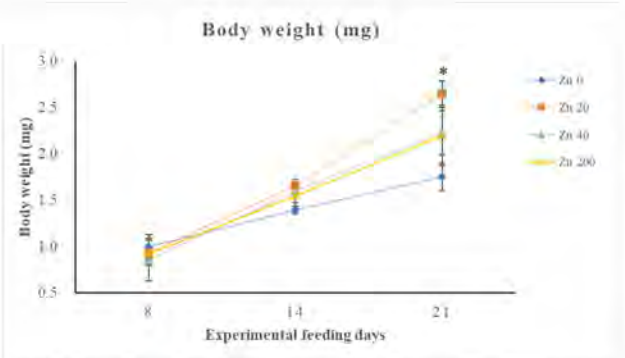

Figure 3. Body weight of gilthead sea bream larvae fed diets with different supplementation level of Zinc

Discussion and conclusion

The present study showed the positive effect of Zn supplementation on microdiets for gilthead sea bream. These results are in agreement with the positive effect of increased dietary Zn levels found in other sparids (Nguyen et al., 2008), and the increased body weight and length found in first feeding rainbow trout (*Oncorhynchus mykiss*) (Shahpar and Johari, 2018). In juveniles of malabar grouper (*Epinephelus malabaricus*) increased dietary Zn levels up to 28.9-33.7 mg/kg also increased weight gain (Chen et al., 2014). Our previous study in gilthead sea bream has shown that supplementation of microdiets with a combination of Zn, selenium (Se) and Manganese (Mn) also led to an increase in total length compared to larvae fed the unsupplemented diet (Izquierdo et al., 2016). Besides, dietary minerals supplemented group showed a better mineralization pattern than non-supplemented group and reduced malformations in bones that form around a cartilaginous anlage. However, in that study it was not possible to identify which of these minerals were responsible for seabream performance improvement. The present study confirms the importance of Zn supplementation in microdiets to improve sea bream growth, complementing the previous study. Studies on skeletal anomalies, bone mineralization and larval morphology will provide more information about the effects of Zn on larval gilthead sea bream.

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STUDYING GENETIC DIVERSITY IN PARASITES WITH HIGH IMPACT ON THE MEDITERRANEAN AQUACULTURE INDUSTRY USING MODERN GENOMIC APPROACHES: THE CASE OF *Ceratothoa oestroides* and *Sparicotyle chrysophrii*

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Introduction

The most frequent and detrimental ectoparasites of Mediterranean cage-reared gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) are the monogenean *Sparicotyle chrysophrii* and the cymothoid isopod *Ceratothoa oestroides*. In order to better understand their transmission and epidemiology, knowledge pertaining to the genetic structure of parasite assemblages infecting wild and farmed hosts is critical. In the current study, Mediterranean-wide genetic structure of *S. chrysophrii* and *C. oestroides* collected from the wild and farmed hosts was evaluated using genome-wide SNP markers generated by ddRADseq (Peterson et al., 2012), trying also to identify and validate distinctive markers to differentiate the two ectoparasites across wild and farmed fish populations. This is the first time in parasite epidemiology, and across an extensive geographic area.

Materials and Methods

Sampling of parasites sampling was carried out across four large Mediterranean farming areas in Spain, Italy, Croatia and Greece. DNAs were extracted using QIAamp DNA Mini kits (Qiagen), and checked for quality and quantity by agarose gel electrophoresis and fluorimetr , respectively. The ddRAD library preparation protocol used has been that detailed elsewhere (Manousaki et al., 2016). Five libraries were constructed in total following exactly the same procedure. Particularly, 310 *C. oestroides* (16x parental generation, 138 Croatian/Farmed and 156 Croatian/Wild) and 330 *S. chrysophrii* (54 Croatian/Farmed, 39 Croatian/Wild, 69 Greek/Farmed, 10 Greek/Wild, 58 Italian/Farmed, 22 Italian/Wild and 78 Spanish/Farmed). For each sample c. 20 ng DNA were co-digested by two high fidelity restriction enzymes: *SbfI & SphI,* barcoded adapters were ligated and size-selected fragments (400 bp to 700 bp) were PCR amplified (15 cycles), column purified and finally quanified by fluorimetr . Each ddRAD library was sequenced on a separate Illumina MiSeq run (300 cycle kit, 162 bp paired end reads). The raw sequence data produced were analysed with STACKS (v2.3) (Catchen et al., 2013). SNPs surviving the filtering criteria were imported to STRUCTURE (Falush et al., 2003) to infer the most likely population structure. PCA analysis was conducted to assess the relatedness of individuals.

Results

SNPs that were detected in more than 30% of the parasites were used for population analysis , i.e. 246 SNPs for *S. chrysophrii* and 313 SNPs in *C. oestroides*.

Discussion and conclusions

In *S. chrysophrii*, taking relatedness indices into account, each sampled farmed population from Greece, Italy, Croatia and Spain was genetically differentiated, whereas all wild samples closely clustered, showing the probability that they might be at the source of any sample encountered at the ends of the distribution. In *C. oestroides*, it seems that there is no clear genetic differentiation between isopods found in aquaculture and wild fish either using population structure or relatedness analyses. Taken together, the generated knowledge on the population structure of the two parasites suggests that water marine currents and distance among fish cages holding fish with different age/size classes in the aquaculture site have to be carefully considered to mitigate propagation of two parasite species. In addition, placement of cages at higher depth and management of wild fish stock could help to decrease parasitic incidence

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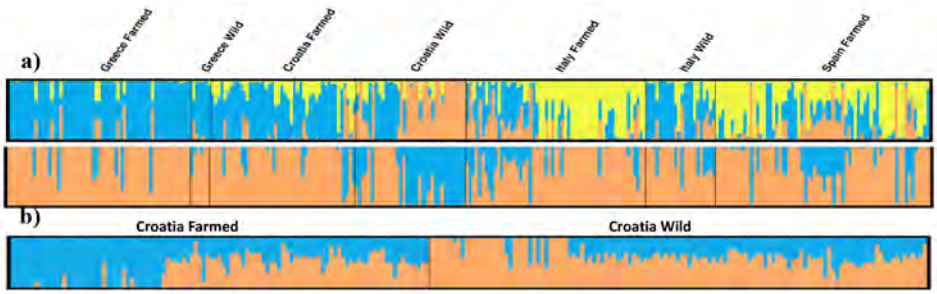

Figure 1: a) Structure analysis for *S. chrysophrii* assuming three (top) or two (bottom) genetic clusters. Individuals are categorized according to their origin to farmed from Greek, Croatian, Italian and Spanish farms and wild from Greece, Croatia and Italy. **b)** Structure analysis for *C. oestroides* assuming two genetic clusters. Individuals are categorized according to their origin to farmed and wild Croatian.

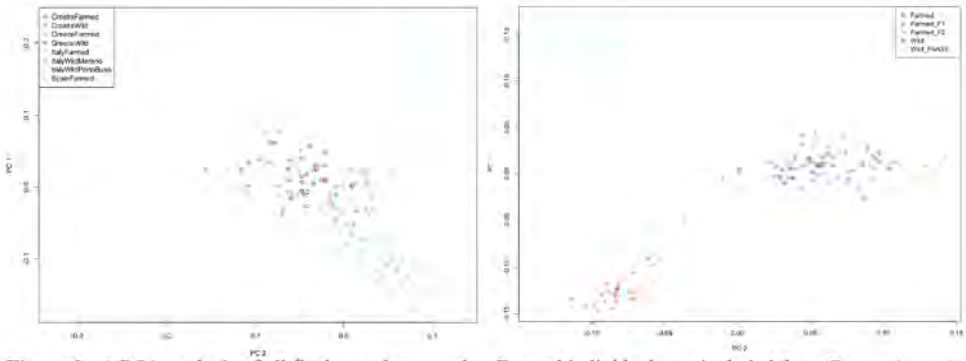

Figure 2: a) PCA analysis of all *S. chrysophrii* samples. Farmed individuals are included from Greece (green), Croatia (black), Italy (light blue) and Spain (grey). Wild individuals are included from Greece (dark blue) and two Italian populations (purple and yellow). **b)** PCA analysis of all *C. oestroides* samples. Different individual origins are shown in different colours.

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SCANNING OF GENETIC VARIANTS AND GENETIC MAPPING OF PHENOTYPIC TRAITS IN GILTHEAD SEABREAM THROUGH DDRAD SEQUENCING

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Introduction
Gilthead seabream (*Sparusaurata*) is a teleost of considerable economic importance in Southern European aquaculture. The aquaculture industry shows a growing interest in the application of genetic methods that can locate phenotype-genotype associations with high economic impact. Through selective breeding, the aquaculture industry can exploit this information to maximize the financial yield. One of the main avenues to genetically improve the cultured stock is to identify associations between genetic variants and traits of interest, such as growth, disease resistance, fat content, etc. Here, we conducted a Genome Wide Association Study (GWAS) Through double digest Random Amplified DNA (ddRAD) Sequencing, we generated a per-sample genetic profile and tested for association with phenotypes related to growth

Materials and Methods
The experiment was conducted on 112 samples belonging to seven different seabream families collected from a Greek commercial aquaculture company. Through a ddRAD protocol (Manousaki et al. 2016) we sequenced the samples on an Illumina Hiseq4000 instrument. Then, ddRAD data were analysed using the software STACKS (Catchen et al. 2013) to obtainedhigh quality Single Nucleotide Polymorphisms (SNPs). These profiles were tested for association with four phenotypes of major financial importance: Fat, Weight, Tag Weight and the Length to Width ratio. Prior to the association analysis, we conducted a clustering of the individuals based on their genetic profile to ensure that genotyping results agree with the family relationships of the studied individuals. Then, we applied two methods of association analysis. The first is the typical single-SNP to phenotype test, and the second is a feature selection (FS) method through two novel algorithms that are employed for the first time in aquaculture genomics and produce groups with multiple-SNPs associated to a phenotype (Tsagris et al. 2018).

Results
Data analysis led to the discovery of 2,258 high quality SNPs. Based on those we clustered the individuals revealing the expected structure based on the seven families (Figure1). Then, our association analysis revealed multiple significant SNPs. In total, we identified nine single-SNPs and six groups of SNPs associated with weight related phenotypes (Weight and Tag Weight), two groups associated with Fat, and 16 groups associated with the Length to Width ratio (Figure 2). Six identified loci were present in genes associated with growth in other teleosts or even mammals, such as semaphorin-3A, and neurotrophin-3.

Discussion and conclusions
Our study presents, in a small scale example, the availability of the tools necessary for the application of Next Generation Sequencing results (i.e. GWAS using SNP markers) in an important Mediterranean aquaculture species. Moreover, the combination of the novel loci, presented in the results, may lead to the selection of brooders based on specific genetic signatures and demonstrate the feasibility of Genomic Selection (GS), a selection method that can have a great effect on the efficiency of the aquaculture.

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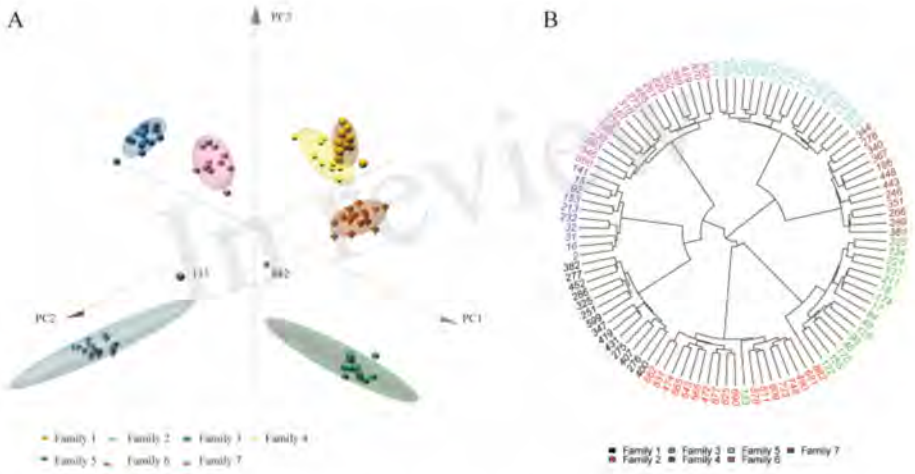

Figure 1:(A) Principal component analysis of the genetic profiles of the 105 individual progeny for all families. The total explained variation of the three components is 30% (11% Principal Component (PC) 1, 9% PC2, 8.9% PC3) (B) Cluster dendrogram of the 105 individuals based on the Euclidean Distance of the genotypes. The Sample-ids are colored based on the tagging family id.

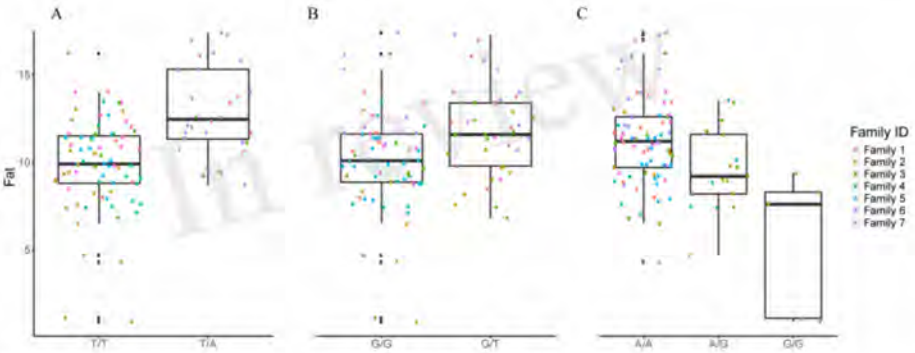

Figure 2: The effect of each of the selected SES SNPs associated with fat content. (A-C) Boxplots of selected SNPs. A) chr8:1385781 , B) chr13:1098152 , C) chr21:19924408.

Acknowledgements

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IMPROVING THE GILTHEAD SEABREAM AND EUROPEAN SEABASS GENOMES USING OXFORD NANOPORE TECHNOLOGY

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Introduction

In the era of next generation sequencing, new genomes are being sequenced and published in an unprecedented pace. However, sequencing a genome using solely short reads in high depth has limited the contiguity of newly built genomes leading mostly to draft assemblies comprised of thousands short contigs. This drawback is being tackled by employing long read technologies that can be used to fill the gaps and lead to high quality chromosome-level assemblies. Here, we have focused on the two most significant fish members of Mediterranean aquaculture, the Gilthead seabream (*Sparus aurata*) and the European seabass (*Dicentrarchus labrax*). Both species have been sequenced at a draft assembly level. The first genome draft for sea bass has been released in 2014 (Tine et al.), while a seabream draft genome was produced in 2018 (Pauletto et al.). In either case, the assembly quality is not sufficient to have large contiguous chromosome-level scaffolds, which is essential for spatial organization of genes, SNPs identification, and mapping of SNPs obtained through genotyping-by-sequencing.

Materials & Methods

DNA was extracted with a Qiagen QIAamp mini kit from fresh blood from one seabream and one seabass individual. Size was estimated by gel electrophoresis in a low-percentage megabase agarose at a low voltage. Standard ONT library preparation protocol was followed. The DNA was sheared only in a few runs to increase the quantity of reads obtained, while when not sheared the reads obtained had greater length. Then, six flow cells were run on a MinION machine for seabream and four for seabass. All runs have been base-called with Albacore v233 (https://github.com/dvera/albacore) on IMBBC, HCMR high performance computing platform. Nanoplot (https://github.com/wdecoster/NanoPlot) was used to assess the basic statistics for each run.

Table 1. Sequencing statistics for gilthead seabream and European seabass. Filtered reads have a quality threshold of 7.

Seabream	Sequencing kit	shearing rpm	raw Gbases	>Q7 Gbases
Run1	108	6500	13.81	12.14
Run2	108	6000	9.87	8.94
Run3	108	6500	13.38	11.45
Run4	109	no	15.47	13.78
Run5	109	no	10.21	8.77
Run6	109	no	6.55	5.87
Sum			**69.28**	**60.95**
Seabass				
Run1	109	no	6.69	6.26
Run2	108	5200	19.299	17.10
Run3	109	no	11.596	10.81
Run4	109	no	8.340	7.86
Sum			**45.93**	**42.03**

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Results

We built a dataset of Oxford Nanopore sequencing for both focal species yielding a high coverage genomic resource. Especially for gilthead seabream, we obtained ~60Gb sequencing throughput leading to >60x coverage of the genome (Table 1). For European seabass we obtained 42Gb leading to >40x coverage of the genome respectively (Table 1).

Conclusions

The long reads produced will be used to produce an improved genome assembly for both species leading to an improved gene annotation, SNP discovery, QTL mapping. This development will allow a better genomic selection and selective breeding programmes for the two species, ultimately developing the Mediterranean aquaculture sector.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 652831 (AQUAEXCEL2020). This publication reflects the views of the authors only, and the European Commission cannot be held responsible for any use which may be made of the information contained therein.

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MODELLING THE PRESERVATIVE EFFECT OF MODIFIED ATMOSPHERE PACKAGING ON FRESH FISH QUALITY AND SHELF LIFE

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Introduction

The limited and variable shelf life of chilled fish, mainly due to bacterial activity, is a major problem for their quality assurance and commercial viability. Modified atmosphere packaging (MAP) can effectively alter and delay the spoilage process and extend the shelf life of fresh fish (Tsironi and Taoukis, 2018). CO_2 inhibits the development of the respiratory organisms like *Pseudomonas* spp. and *Shewanella putrefaciens*. Despite the increasing importance of MAP technology in fish industry and the several studies evaluating the effect of MAP on fish products, a limited number of predictive models for quality deterioration and shelf life have been proposed, including the combined effect of temperature and gas concentration in the packaging environment (Koutsoumanis et al., 2000). An Arrhenius-type model has been developed by Tsironi et al. (2011) as an effective tool for predicting gilthead seabream (*Sparus aurata*) fillet quality and shelf life under different chilled storage temperatures (0-15°C) and modified atmospheres (20-80% CO_2) and its applicability has been validated in the real cold chain. On the other hand, a disadvantage of ordinary MAP is its demand for high gas to product volume ratio. Increased concentration of CO_2 in the headspace and high gas to product volume ratio may result in increased dissolution of CO_2 in the fish flesh, due to a higher partial pressure of CO_2 (Devlieghere et al., 1998). Optimal gas mixture depends on the type of the packed food.

The objective of the study was to select appropriate MAP parameters for farmed gilthead seabream and European sea bass and to develop predictive models for quality deterioration and shelf life of chilled MAP fish

Materials and methods

Whole, gutted, gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax)* packed under modified atmospheres ($40\%CO_2/40\%N_2/20\%O_2$) were provided by Selonda S.A. Two alternative packaging types were tested, i.e. 1-3 specimens/package, corresponding to different levels of gas to product volume ratio in the package headspace. Samples were stored at controlled isothermal conditions in the refrigerated range (0-10°C) in high-precision (±0.2°C) low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan) for shelf life evaluation. Temperature in the incubators was constantly monitored with electronic, programmable miniature data-loggers (COX TRACER®, Belmont, NC). The composition of the headspace of package was measured using CheckMate 9900 O_2/CO_2 (PBI Dansensor, Ringsted, Denmark) gas analyzer. Quality assessment of fish was based on microbiological analysis (total viable count, *Pseudomonas* spp., lactobacilli, *Enterobacteriaceae* spp. and H_2S-producing bacteria), pH and sensory scoring. Samples were taken at appropriate time intervals to allow for efficient kinetic analysis of quality deterioration. Values of the different measured indices were plotted versus time for all temperatures studied and the apparent order of quality loss was determined based on the least square statistical fit. The experimental data for microbial growth were fitted to the Baranyi model (Baranyi and Roberts, 1995), using DMfit software of IFR (Institute of Food Research, Reading, UK), and the kinetic parameters of microbial growth were estimated (maximal growth rate, k and lag phase duration, lag). Temperature dependence of the deterioration rate constants, k, was modelled by the Arrhenius equation (Taoukis et al., 1997).

Results

CO_2 concentration in the package headspace decreased during the initial hours of storage (up to final CO_2 level of 20%), which was related to CO_2 dissolution in the fish flesh, especially at the lower gas to product volume ratios. Afterwards, $\%CO_2$ level increased, due to metabolic activity of spoilage bacteria, reaching highest levels at the end of storage period. With regards to O_2 concentration, it showed a descending trend, while at the end of shelf life O_2 concentration exhibited zero levels, which was related to increased microbial population of total viable counts. Shelf life estimation of MAP fish was performed based on the correlation of microbial load of total viable counts and the sensory evaluation of the samples. At all studied conditions, the time of sensory rejection coincided with an average total viable count of 10^7 cfu/g. The developed mathematical models were adapted into user friendly worksheets that allow the prediction of microbial

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growth and shelf life of gutted gilthead sea bream and European sea bass packed in MA and stored under different time-temperature conditions. It is apparent that the selection of optimal MAP parameters (i.e. initial headspace gas composition and gas to product volume ratio) is a complex issue and is a very important step in the design of MAP systems for fish products. Selection depends on the effect on microbial growth, desired food quality and shelf life, as well as the appearance of a package. CO_2 emitters, which produce CO_2 in contact with water that is obtained from liquid leaking from the fish flesh may enable reduced gas to product volume ratios in MAP fish and further increase shelf life.

Discussion and conclusion

Since shelf life extension of MAP fish requires in pack CO_2 concentration maintenance, a smart label monitoring CO_2 levels in package headspace would ensure this. The combined use of the developed shelf life models with an indicator with function of CO_2 detection and a Time Temperature Indicator (TTI) would provide useful information on the probability of the quality deterioration of packed fish, allowing better management and optimization of the cold chain from manufacture to consumption.

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QUALITY ENHANCEMENT AND SHELF LIFE EXTENSION OF FRESH MEDITERANNEAN FISH BY NONTHERMAL AND MINIMAL PROCESSING

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Introduction

Fish begins to spoil immediately after it has been harvested, caused by rapid rigor mortis, enzyme activity and bacteria. Fish preservation methods are mostly based on storage temperature decrease, i.e. refrigerated ice storage in the range 0 to 4°C, super-chilled storage in the range -4 to 0°C and frozen storage at -18°C. The development of new fish processing and packaging methods or novel combinations of existing technologies is sought by the industry in the pursuit of producing alternative products, achieving shelf life extension, and management and reducing food waste (Taoukis and Tsironi, 2013). The advantages of nonthermal processing over classical thermal methods include the better retention of nutritional and sensory properties. Several studies have been conducted recently on the efficacy of washing and sanitizing treatments in reducing microbial populations on food products (Gil et al., 2009; Thi et al., 2015). Osmotic dehydration (OD) is a technique used to reduce water activity (a_w) in foods in order to improve nutritional, sensorial and functional properties of food. It consists of an immersion of the product into a concentrated solution (i.e. sugar, salt, sucralose etc.). By reducing the water activity of the food matrix, microbial growth is reduced or inhibited. Additionally, processing in concentrated aqueous solutions can decrease the microbial load due to decontamination induced by high solute concentrations at the product/solution interface. Pulsed electric fields (PEF) treatment is a novel nonthermal processing technique which has the potential to alleviate the need for thermal processing of foods. PEF involves the application of high-voltage pulses to electroconductive foods which are placed between the electrodes. The resultant electric field induces movements of ions and permeabilization of cell membranes called electroporation (Tsironi and Taoukis, 2019).

The objective of the study was to investigate the effect of nonthermal i.e. osmotic dehydration (OD), pulsed electric fields (PEF), and minimal processing methods (i.e. surface decontamination of fish) on the quality and shelf life of farmed gilthead seabream and European sea bass during refrigerated storage.

Materials and methods

Gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) were treated using OD or PEF (Elcrack-5kW, DIL, Germany) as alternative approaches to the conventional post-harvest fish processing methods The incorporation of natural organic acids (i.e. lactic acid, citric acid) at different concentrations in the washing water was also tested for its efficacy to reduce initial microbial load and prolong shelf life. Untreated (Control) and samples treated with the alternative methods were stored under controlled isothermal and variable conditions (0-15°C). Samples were taken in appropriate time intervals to allow for efficient kinetic analysis of quality deterioration. Quality assessment was based on microbiological analysis (total viable count, *Pseudomonas* spp., *Enterobacteriaceae* spp., lactic acid bacteria, etc.), chemical quality indices (pH, lipid oxidation, total volatile basic nitrogen), colour and texture measurement and sensory scoring.

The microbial growth was modeled using the Baranyi Growth Model (Baranyi and Roberts, 1995). For curve fitting the program DMFit was used (available at http://www.combase.cc/index.php/en/). Kinetic parameters such as the rate (k) of the microbial growth were estimated. Values of the different measured indices were plotted vs time for all temperatures studied and the apparent order of quality loss was determined based on the least square statistical fit. Temperature-dependence of the deterioration rate, k, was modeled by the Arrhenius equation (Taoukis et al., 1997).

Results

The results of the study indicated that the application of OD led to improved quality stability during subsequent refrigerated storage in terms of microbial growth, physicochemical and organoleptic degradation of the fillets and significant shelf life extension of fish (7 days and up to 18 days for untreated and osmo-treated samples at 5°C, respectively). PEF did not affect significantly the initial microbial load, flesh quality and shelf life of fish fillets. Initial surface decontamination up to 1.5 logcfu/g by the addition of organic acids in the washing water resulted in 2-3 days shelf life extension of fish fillets at 0°C. At all temperatures studied, the time of sensory rejection coincided with a *Pseudomonas* spp. level of 10^7 cfu/g for whole fish and 1 ^{6} cfu/g for fillets

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Discussion and conclusion

The results of the study indicated that the application of nonthermal and minimal processing led to improved quality stability during subsequent refrigerated storage and significant shelf life extension, in terms of microbial growth, physicochemical and organoleptic degradation of the fillets

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NUTRIENT INPUT IN A SMALL SCALE AQUAPONIC SYSTEM: EFFECT ON LETTUCE FUNCTIONAL RESPONSES AND TILAPIA GROWTH

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Introduction

Aquaponics is a closed-loop system which combines fish and plants production through recirculating aquaculture system (RAS) and hydroponics. In aquaponics, nutrients which are important for plant growth are often lower than hydroponics and several studies have shown that the addition of fertilizers in aquaponics, such as potassium (K) and iron (Fe) favors plant growth and yield (Delaide et al., 2016; Rafiee et al. 2019). Nevertheless, one of the main advantages of the aquaponics is nutrient recovery and re-use between the three components (i.e. fish, bacteria, plant), thus minimum external inputs would benefit the equilibrium as well as the environmental footprint of the system. This is a multifaceted issue since it depends on fish and plant species involved, stock density and system physicochemical parameters yet requires an extensive study of fish and plant physiology under the prevailing conditions. In regard to plants, the relevant literature is restricted to growth and yield evaluation. Although effective measures of the productivity potential of an aquaponic system, these are inadequate to describe alone the performance of the system when several variables change. Alternatively, the study of the functional responses of plants could address the above-described issues by identifying system constraints and indicating possible management practices. In this frame we investigated the functional responses of lettuce (*Lactuca sativa* L. var. Romana) and the growth of red tilapia (*Oreochromis* sp) in an aquaponic system when no fertilizers were applied in comparison with Fe and Fe+K addition.

Materials and Methods

The experiment took place at the University of Thessaly from March 1st to April 15th of 2019. It was conducted in three indoor aquaponic systems. Each system had the following characteristics: total water volume of 630l comprising of a fish tank (400l), mechanical and biomechanical filte , water pump, media bed (clay) hydroponic unit of 1m^2 (50l), air pump to oxygenate the water and one HPS light bulb 600W over each grow-bed (PAR 450-500μmol m^{-2} sec^{-1}; photoperiod 10L:14D). Thirty red tilapias were placed in each fish tank and after 10-day acclimatization, they were weighed and that was considered the initial weight of the experiment. The same day eight 15-day-old plants were placed in each grow-bed. Fishes were fed twice a day (10:00 and 16:00) *ad libitum* for thirty minutes with commercial fish food of 47% protein content. The food was weighed before and after each meal and the daily amount administered was calculated. Each system comprised a treatment, thus 3 treatments were realized: a) control (C), where no fertilizer was added, b) Fe, where only Fe was added in Fe-DTPA form in order to reach the target water concentration set by hydroponics practice for lettuce (i.e. 40umol L^{-1}, Sonneveld and Straver (1994)) and c) Fe+K, where Fe was added as in the previous treatment along with K in K_2SO_4 form (10mmol l^{-1}). During the experimental period pH was measured on a daily basis, photosynthesis (Li-Cor 6400XT, Li-Cor Inc.) and chlorophyll a *in vivo* fluorescence (as an early indicator of stress, Handy-PEA, Hansatech) on a weekly basis and fish body weight on biweekly basis. The experiment lasted for 45 days and at the final harvest, various plant growth parameters were measured (leaves and roots fresh weight, leaf area, biomass) and the final weight of the fishes was recorded.

Results

pH showed a downward trend in all three systems. In order to keep pH constant and with-in tolerance limits of fish, water changes were performed and almost half of the water volume (300l) was changed in each system. The mean value of pH of the whole experimental period was 7.22, 7.10 and 7.12 for C, Fe+K and Fe treatment respectively. Initial and final weights of fishes did not show statistically significant differences between treatments, while no mortality was evident. Tilapias had an initial average weight of about 16 grams and reached about 60 grams, exhibiting a specific growth rate (SGR%/day) of 2.68, 2.98 and 2.81%/day for C, Fe+K and Fe groups respectively. The daily amount of fish food supplied was almost equal between groups and ranged from 19 to 22g/day. At the last days of the experiment, the fishes in the control tank were reproduced, while eggs were observed in female's mouth in the Fe+K treatment.

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Statistically significant differences in plant growth were observed between treatments. Plants treated with Fe+K had an average fresh weight of 493g while 250g and 220g were measured in the Fe group and control respectively. In total, 3.9kg of lettuce was produced in the Fe+K treatment, while 2kg in the Fe and 1.7 kg in the control group. The same pattern appeared in the roots, leaf area and biomass.

Discussion and conclusion

The downward trend of pH reflects a robust nitrification process which produces acid according to Rakocy et al. (2004) who reported a daily pH decrease in their aquaponic system. Consistent with ours are the results of Rafiee et al. (2018) as they observed a decrease in pH (below 6) mainly in the first 4 weeks. Delaide et al. (2016) compared the effects of aquaponic solution (AP) and complemented aquaponic (CAP) in which several nutrients were added. Their principal finding was that CAP solution promoted shoots and roots growth outperforming AP, which is in agreement with the results of the present experiment.

The results of the present study highlight the beneficial effect of the addition of Fe+K on lettuce growth in a small-scale aquaponic system, in comparison with no external inputs. Ongoing analysis of photosynthesis and fluorescence parameters will shed light into the functional responses of the photosynthetic apparatus to the various treatments and contribute to the identification of the constraints imposed by the system as they were reflected in the control treatmen

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DOES AQUAPONICS WORK IN PRACTICE OR IS IT JUST A VIRTUAL INDUSTRY? STATUS OF COMMERCIAL AQUAPONICS IN THE CZECH REPUBLIC

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Introduction

Yearly fish production in Czechia fluctuates between 20,4 – 21,0 thousand tons per year (Ženíšková et al., 2017) with majority of the production originating from ponds covering an area of 52 000 ha (Adámek et al., 2012). Since aquaponics technologically consists of a recirculating aquaculture system (RAS) and hydroponic system, succesful introduction of aquaponics on the market may be dependent on functionality and establishment of RAS and hydroponics. Estimated annual production from RAS in Czechia is around 500 t (Kouřil et al., 2015). There are only five large hydroponic farms specializing in tomato production (ČT, 2018). Our research question was if there are functioning, profitable aquaponic businesses in Czechia. The minor part of our research was related to the status of organic aquaponics in Europe.

Materials and methods

For carrying out the national survey, we have used a questionnaire for aquaponics entrepreneurs according to Love et al. (2014). To find relevant respondents, online desktop research was carried out using the Google Search engine set up for Czech interface. We entered the following keywords in Czech language: "akvaponie" [aquaponics]; "akvaponické systémy"[aquaponics system]; "akvaponická farma"[aquaponics farm]. The search was done on 25. 6. 2019. Obtained results from Google search were sorted into four categories: Producers, Services, Media and Blogs, Education. Respondents in category Producers were chosen for the questionnaire survey. Producers were further size-classified according to Palm et al. (2018). Questionnaires were filled in person. Organic certification possibilities were reviewed using EU Commission legislation for the organic sector. Additionally, results were consulted with relevant stakeholders at the Ministry of Agriculture of the Czech Republic.

Results

We found 117 results, from which only four were producers (3 %). The major category was media and blogs (69 %), followed by service providers (24 %) and education (4 %).

Two large-scale commercial farms, one intermediate system and one domestic, however commercial system were found. The only intermediate system was profitable in the last 12 months, as the only respondent incorporating also non-production related services. This respondent is also the only services provider who has built commercial-scale aquaponics in Czechia. The largest system in Czechia has a 215 m^3 volume in the aquaculture part of the system and 1 000 m^2 of plant area. The farm has reached the production of 3 t of fish and 226 kg of plants. The profitability in this enterprise is expected in the next 12 months.

Graph 1 Google Search Results sorted out in following categories: Producers, Services, Media and Blogs, Education

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According to EU Commission legislation Section 3, Article 25g of EU regulation 710/2009 recirculating aquaculture cannot be certified as organic food production, citing "**Closed recirculation aquaculture animal production facilities are prohibited**". To certify plant production as organic, plants must be grown in soil. Therefore **hydroponic production is prohibited** (EU regulation 2018/848 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007, Annex II, Part I, paragraph1.2).

Discussion and conclusion

From 117 results only one was a commercial aquaponics producer (and yet not-profitable), suggesting "media bubble" around aquaponics and it´s attractiveness. Other farms had negligible production. Villaroel et al. (2016) only one respondent had production higher than 10 000 kg of fish per annum. Love et al. (2015) stated 9 respondents having higher production than 4 537 kg. **The system recorded in the presented study, now has in stock 17 000 t of fish, therefore could be considered as one of the biggest aquaponic systems in Europe.**

Following EU regulation 710/2009 and 2018/848 **aquaponics in Europe cannot be recognized as organic.** Without soil nutrition, we can talk of "plants on drips". However, in U.S. the USDA National Organic Standards Board has labelled aquaponics production in 2008 as organic (Miličić et al., 2017). Nowadays The Coalition for Sustainable Organics Center for Food Safety delivered a petition (<http://www.coalitionforsustainableorganics.org/>) to the U.S. Department of Agriculture to end organic certification of hydroponic and aquaponic production.

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DEVELOPMENT OF BSC AND MSC FISHERIES AND AQUACULTURE DEGREE PROGRAMS IN MYANMAR

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Myanmar is one of the top fifteen countries on fisheries and aquaculture production and it has considerable future aquaculture potential due to its geography and abundance of natural resources. However, traditional extensive farming methods are practiced and the production is generally low when it is compared to neighboring countries such as Thailand and Bangladesh. To improve the aquaculture sector in a sustainable way, one important obligation is to develop the capacity of human resources who have strong knowledge in aquaculture and fisheries. Therefore, universities and vocational training centres play an important role in support of developing a sustainable aquaculture and fisheries sector. Until 2013, specialization for fisheries and aquaculture had not been established in Universities in Myanmar. In most universities, syllabus regarding ichthyology is extremely limited and embedded in the Departments of Zoology.

In 2014, USAID's project 'Developing a Sustainable Seafood Industry Infrastructure in Myanmar' had been launched at University of Yangon (UY) to expand the capacity of higher education institutions in Myanmar to support the development of an intensive marine and inland fisheries sector. During the three years of the project, we developed a Seafood Safety Laboratory in UY. That laboratory has been established not only as a teaching and research laboratory, but also for training and laboratory service for faculty, students and farmers. More than 450 faculty members and students from 44 universities have been trained. A total of 12 faculty members and students have been supported to attend short-term training in Bogor Agricultural University (Indonesia), Cantho University (Vietnam), Nong Lam University (Vietnam), Universiti Tunku Abdul Rahman (Malaysia), and the Tamil Nadu Fisheries University (India). Through the USAID project, we were able to select the human resources who can conduct the fisheries and aquaculture education in universities across Myanma .

In 2015, the Dutch public-private capacity building (Knowledge to Knowledge or K2K) program provided short-term trainings to selected faculty members of Department of Zoology on how to develope competency-based curriculum for MSc Aquaculture based on the stakeholder needs. The faculty members were taught how to collect the needs of stakeholders, how to develop the job profiles based on the needs and how to develop the courses

In 2017, according to the country needs, Ministry of Education and Ministry of Agriculture, Livestock and Irrigation selected UY to establish a new undergraduate study program for Bachelor of Science (BSc) in Fisheries and Aquaculture within the Academic Year 2018. This is the first Fisheries and Aquaculture program in tertiary education in the country.

Based on the needs of Government and private sector, Deutsche Gesellschaft für international Zusammenarbeit (GIZ) launched the 'Myanmar Sustainable Aquaculture Programme (MYSAP)'. Main targets of MYSAP in education sectors are the promotion of vocational and university education and strengthening universities' research capacities. Thus, it supports the development and establishment of a Bachelor of Science (BSc) and a Master of Science (MSc) degree curriculum in Fisheries and Aquaculture. Within the 4 years implementation phase (2018-2021), the programme has assisted a series of workshops for selected faculty members from seven universities to facilitate fisheries and aquaculture bachelor and master degree classes. MYSAP has invited international experts to develop the competency based curriculum together with faculty members from UY, staff from Department of Fisheries (Ministry of Agriculture, Livestock and Irrigation) as well as the private sector, on both, local and international levels. Furthermore, the programme assists to establish national research networks and to connect to international aquaculture as well as education networks such as ASEAN Fisheries Education Network.

MYSAP equipped the teaching facilities such as two classrooms, three laboratories and the wastewater treatment system for the laboratories at the Yangon University Rearch Center. The private sector, Myanmar Fisheries Federation, has donated a fully-equipped computer laboratory.

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In December 2018, the first bachelor degree program (BSc Fisheries and Aquaculture) was launched in University of Yangon with 40 students. The curriculum in the new courses have been based on competency which is divergent from our traditional teaching method and guarantees to improve the communication skills, lifelong learning activities, and presentation skills of the students. In addition to the laboratory trainings and field trips, various practical trainings opportunities and internships in cooperation with private sector like in hatcheries, on farms, and processing industry are included. The program has been selected as a model program in University of Yangon for the other departments to enhance the modification of traditional curriculum to competency-based curriculum.

Recently, application for the second intake of the bachelor degree program has been opened. The MSc Fisheries and Aquaculture program will be launched in 2020 academic year for the students who have graduated with in Zoology or Marine Science. In the near future, globally competent faculty and students from UY will coordinate research, teaching and service activity to develop the sustainable fisheries and aquaculture sectors in Myanma .

NUTRITIONAL PROGRAMMING OF LIPID-METABOLISM IN GILTHEAD SEA BREAM: TRANSGENERATIONAL EFFECTS OF BROODSTOCK SELECTION AND FEEDING

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Introduction

Nutritional programming is described as the "events during critical or sensitive periods of development that may "program" long-term or life-time structure or function of the organism" (Lucas, 1998). The "nutritional programming" concept presumes that alterations of the environment during the early life stages of an organism affect its phenotype at later-life stages. Environmental changes during critical early periods, such as embryogenesis or early development (referred as developmental plasticity periods), provide a tool to the organism to forecast potential environmental challenges in later life and give the opportunity to adjust its metabolism for a better adaptation increasing its chances to survive and reproduce.

These adaptational changes can be used in favour to obtain individuals better adapted to the feeds used in animal production systems. In production systems, diets are formulated to supply the necessary nutritional components, selecting appropriate ingredients based on their nutritional characteristics, cost and availability. Thus, restrictions in the availability of a high-quality feedstuff lead to the search for alternative ingredients. Consequently, there has been an increasing interest in aquaculture to find out different ways to maximize the capacity of fish to use alternative sources in a species dependent manner. Recent studies in commercially important aquaculture species have been showed, nutritional programming may be a new novel strategy to create individuals have better capacity of using the alternative ingredients in feeds. In some marine fish species, complete replacement of fish oil (FO) is still a main challenge in aquaculture diets, due to their low ability to synthesize n-3 LC-PUFA and the lack of these fatty acids in vegetable oils (VO). For this reason, studies on nutritional programming have also aimed to increase marine fish capacity to utilize low n-3 LC-PUFA diets. This review summarizes the data obtained from six years and five different broodstock groups. The results include offspring growth gene expression on stress-related genes (Turkmen et al, 2019), lipid metabolism related genes (Izquierdo et al., 2015; Turkmen et al., 2017) and potential epigenetic mechanisms involved (Turkmen et al, submitted).

Materials and methods

Briefl , in each trial a nutritional stimulus was applied to the broodstock fish during the spawning period, at least for 1 month before seeding the eggs for rearing the progeny, in order to modify the egg fatty acid profiles. Those eggs obtained from differently fed broodstock groups were produced under the same conditions and fed commercial feeds and feeding protocols until the desired juvenile stage. Afterwards, juveniles were nutritionally challenged with low FM FO diets in order to test the performance of the offspring during the juvenile stage. In all studies, the gilthead sea bream used were produced and reared in different facilities of the EcoAqua Institute (Telde, Canary Islands, Spain). A general schematic presentation of the experimental design showed in Figure 1.

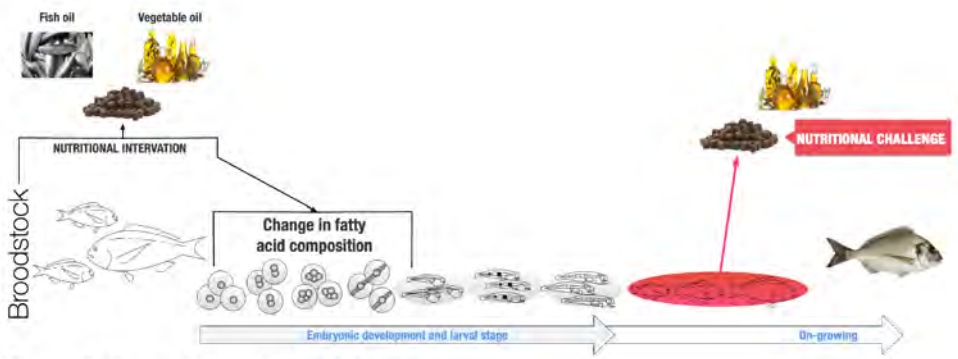

Figure 1. General experimental design

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Results and discussion

FO replacement by VO equal or higher than 80% in gilthead seabream broodstock diets may have negative consequences for spawn quality, larval growth and even juveniles. Thus, FO replacement by LO up to 80–100% in broodstock diets for gilthead sea bream not only markedly reduce fecundity and spawn quality, but also the size of 45 days after hatch (dah) and 4-month-old juveniles, as well as the *fads2* expression in larvae. On the contrary, 60% replacement of FO by VO, leading to a reduction in LC-PUFA and an increase in the LA and ALA precursors, did not negatively affected offspring production, demonstrating the interesting potential of early nutritional programming of marine fish by broodstock feeding to improve long-term performance of the progeny. Parental feeding with FO replacement by VO induced long-term changes in PUFA metabolism, leading to an increased production of PUFA and the up-regulation of *fads2* expression, affecting also the expression of other lipid metabolism related genes. For instance, replacement of FO with LO in parental diets affected offspring transcription of *lpl*, *cpt1* and *elov6*, genes related to regulation of energy metabolism in liver, allowing a better utilization of diets high in VO and VM. The persistent long-term effect of nutritional programming of gilthead sea bream through broodstock feeding and the efficiency of feeding a 'reminder' diet during juvenile stages to improve utilization of low-FM/FO diets.

Selection of gilthead sea bream broodstock with high *fads2* expression when fed a low FM and FO diet produces offspring that performs better, even when they are challenged with a low FM and FO diet. Besides, selection of high *fads2* broodstock induced regulation of genes such as *elovl6* and *cpt1* in juvenile stage if challenged with a very low FM FO diet. The results showed that methylation of CpG islands in the *fads2* promotor, particularly in positions CpG2 and CpG3, are among the potential epigenetic mechanisms for regulation of gene expression in nutritionally programmed fish

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EFFECT OF DIETARY VITAMIN D_3 IN GROWTH, SURVIVAL AND SKELETAL DEVELOPMENT OF GILTHEAD SEA BREAM LARVAE (*Sparus aurata*)

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Introduction

Mortality and skeletal anomalies in cultured marine fish larvae are among the major drawbacks in hatcheries to produce high quality juveniles at more effective production costs. Nutritional unbalances regarding micronutrients may be partly responsible of these losses due to the scarce knowledge available regarding the optimum levels of these nutrients on larval diets. Vitamin D is a fat-soluble micronutrient that has various functions in bone development, calcium absorption, mineralization and cellular proliferation. Vitamin D_3 (cholecalciferol) is the most active form, which gets hydroxylased in liver of fish to form 1,25(OH)D3 (calcitriol) to bind Vitamin D receptors. Unfortunately, information regarding optimum dietary Vitamin D levels in diets for gilthead sea bream larvae is very scarce. Hence, considering this research gap, the main aim of the present study was to evaluate the effect of several levels of Vitamin D_3 in practical microdiets on survival and skeletal development of gilthead sea bream larvae.

Materials and Methods

Diets: Four different isoenergetic and isonitrogenous supplemental diets were formulated with a varying level of Vitamin D_3 such as 0IU (D1), 1500IU (D2), 2000IU (D3) and 20000IU/Kg (D4) of diet. *Fish and experimental conditions:* 2100 gilthead sea bream larvae obtained from one single spawn from the GIA-Ecoaqua Progensa fast growing selected broodstock were randomly distributed into twelve 170L circular tanks. Initial (26 day post hatching (dph)) mean total length 8.84 ± 0.86 mm; body weight 0.86 ± 0.83 mg (mean ± s.d). *Sampling:* Growth was monitored by measuring the total length (TL) and weight of larvae for every 8 days interval. Daily mortality was monitored and at the end of the study (47 dph) all the live larvae were counted and sampled for TL, weight, histology, whole mount stain, gene expression, Thiobarbituric Acid Reactive Substances (TBARS) and High Performance Liquid Chromatography (HPLC). *Statistics:* All data were tested for normality and homogeneity of variances and means compared by Tukey test ($P < 0.05$).

Figure 1: Final biomass of gilthead sea bream larvae fed diets with different level of vitamin D_3

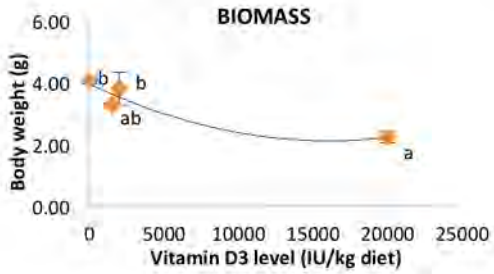
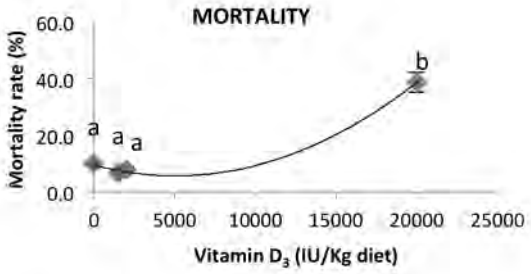


Figure 2: Mortality rate of gilthead sea bream larvae fed diets with different level of vitamin D_3 for 21 days



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Results

After 20 days of feeding the experimental diet there was a dropping mortality found in larvae fed the highest level of Vitamin D_3 (D4) that lasted until the end of the trial and reduced survival rate. However, larvae fed with other levels of Vitamin D_3 showed no significant differences in length, weight and survival. The final biomass (Fig. 1) and daily mortality rate (Fig. 2) of each treatment were calculated and it shows significant difference in D4 with other treatments. A high incidence of skeletal anomalies was observed in fish fed diet D4. Biochemical and molecular studies are being conducted to further understand the physiological mechanisms involved.

Discussion and conclusion

Despite the importance of feeding marine fish larvae with formulated inert microdiets to substitute rotifers and artemia (Kolkovski *et al*., 2009), the optimum nutrient contents of these diets have been only determined for few nutrients (Hamre *et al*., 2013). Paticularly, there is an almost complete lack of studies aiming to determine the optimum dietary levels of vitamin D in microdiets for marine fish larvae. Besides, there is a large variation in the nutrient composition of commercial rotifer diets and a resulting large variation in the nutrient composition of rotifers fed to marine fish larvae at an industrial scale (Hamre, 2016). Our study has shown that the highest larval biomass is obtained with a diet not supplemented with vit D_3 suggesting that the basal levels were sufficient to fulfil the vit D requirements of gilthead sea bream larvae. However, supplementation with vit D_3 up to 20,000 IU/Kg diet lead to high mortalities and skeletal anomalies. These results are in agreement with the higher incidence of skeletal anomalies found in Japanese flounder larvae fed over 20,000 IU supplemented as vitamin D_3 (Haga et al., 2004). The results also agree with the higher incidence of skeletal anomalies found in european seabass fed over 40,000 IU (Darias et al., 2010), although the levels were double than those supplemented in the present study. Vitamin D_3 level in rotifers enrichment diets markedly differ for different marine fish hatcheries and ranges from 2.8 IU to 15200 IU/kg (Hamre, 2016), which is extremely higher than the requirements stablished for juvenile fish (NRC, 2011). Hence, the present study has shown that increase in Vitamin D_3 supplementation up to 2000 IU/kg of diet has no significant effects on growth and survival. But the highest level of supplemented Vitamin D_3 (20000 IU/kg of diet) reduced final survival and total biomass, indicating a potential toxicity in gilthead sea bream larvae. Further analyses are being undergone to understand the effects of vitamin D_3 supplementation.

Acknowledgment

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CLEANER FISH BEHAVIOUR IN FULL SCALE SALMON PRODUCTON UNITS – AN ACOUSTIC TELEMETRY APPROACH

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Introduction

In order to control the infection level of the parasitic sea louse, *Lepeophteirus salmonis* (Krøyer, 1873) in aquaculture production of Atlantic salmon (*Salmo salar*), cleaner fish, and especially lumpfish (*Cyclopterus lumpus*), have seen an exponentially growth in productions numbers over the last few years (McVicar, 2004; Krkosek et al 2005; 2006; Imsland, at al 2014). In Norway a total of 27 038 000 lumpfish were used for combatting sea-lice in 2017, amounting to a first hand investment cost of approx. €50 mill. (www.fiskeridi .no). Lumpfish are a relatively new species in aquaculture. Nevertheless, as for all species in aquaculture, preservation of good animal welfare through fulfillment of the requirements of the animal welfare act must be ensured. New knowledge on all aspects of the fish biology and requirements must be established when introducing a new species in commercial-scale production. The objectives of this study were to conduct behavioral studies of lumpfish to assess their performance and welfare in fully stocked industrial-scale salmon cages. The study also involved development and adaptation of an acoustic fish telemetry system to obtain observations of individual fish behavior in a challenging environment

Material and methods

The study was performed in a commercial salmon farming site in Norway (64° 36.165' N: 10° 51.220' E, Kråkholmen, Bjørøya AS) using standard circular production cages (circumference: 160m; depth: 15 – 20m). Implantation of acoustic tags in six lumpfish followed a well-documented surgical protocol described e.g. in Mulcahy (2003) and Urke et al. (2011).

The fish horizontal position was obtained by using GNSS time-synchronized acoustic receivers and employing time difference of arrival (TDoA) calculations to signals received in parallel on three receivers placed in known locations along the fish cage perimeter (Fang, 1990). Fish swimming depth was determined by a pressure sensor embedded in the tag with its measurements conveyed through digital modulation of the acoustic signals. Benchmarking using test tags at known locations demonstrated a typical positioning accuracy of 1.1m. Fig 1. shows an example of the data output by the fish positioning system.

Results

Data on lumpfish spatial behaviour were collected from a commercially stocked salmon production cage from early November until late December 2016. No fish died or showed signs of unnatural behaviour caused by the surgical implantation and handling. Cleaner fish hides were deployed over the north, west and south sides of the sea cage during the period, and there was a strong correlation between the individual fish preferred location and the locations of the hides (Fig 2.). Moreover, of a total of 16 357 positions acquired during the study, 95% remained closer than seven meter from the net wall, indicating a tendency of the lumpfish to reside close to the sea cage perimeter and almost never dwell in the central parts of the cage. The observations also showed that lumpfish were inclined to occupy the north-western areas of the cage. This specific aquaculture site has a dominating northerly current direction, which may contribute to the observed distribution of the fish

Discussion

Results confirm the lumpfish preference of hides, staying almost 50% of the time at rest at the same depth within the hide locations. The clear tendency towards fish detections in the northern and western areas suggests that tide and currents may have a strong impact on the overall distribution of lumpfish in sea cages. The results indicate the importance of cleaner fish hides for lumpfish and emphasise the need for further investigations on lumpfish behaviour to assess its efficiency as a sea lice relief strategy and to secure animal welfare in aquaculture.

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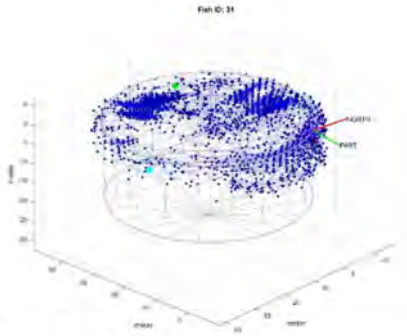

Fig. 1. Typical output of the acoustic fish positioning system with each blue dot representing a single lumpfish position in the cage at a specific time.

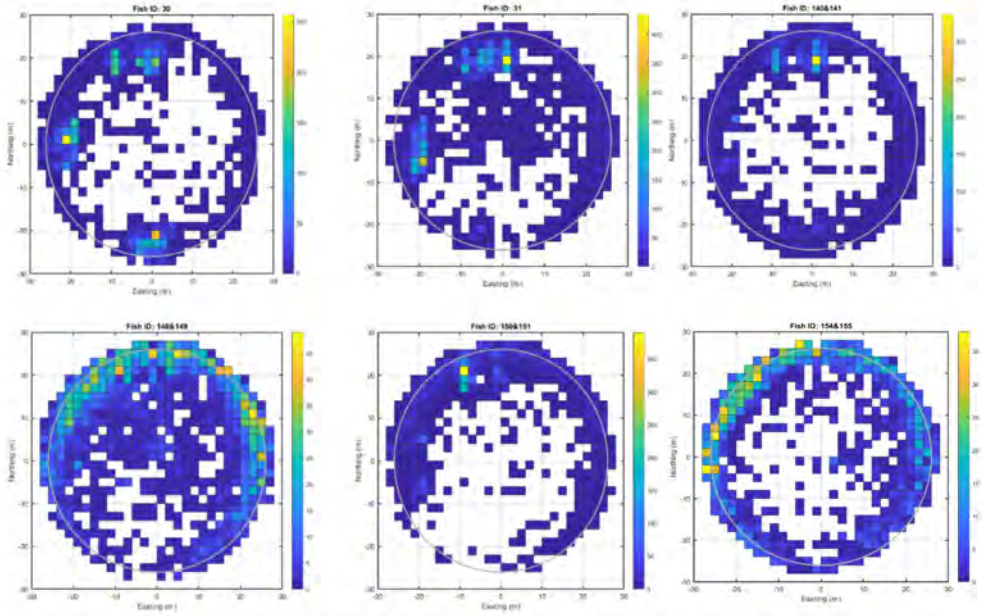

Fig 2. Heatmap showing horizontal distribution of six lumpfish during the study period. The circle represents the sea cage perimeter (north facing up) and each cell is 2m × 2m. Cells with lighter colours are more used.

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IMPROVING PROTEIN NUTRITION STATUS THROUGH FISH CONSUMPTION: AN ASSESSMENT OF KNOWLEDGE, ATTITUDE AND PRACTICES OF LOW INCOME EARNERS IN NIGERIA

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Introduction

Fish is among the healthiest foods on the planet which is packed with important nutrients, such as protein, vitamin D and omega-3 fatty acids, which are highly important for human body and brain (Ruxton *et al*., 2004). Fish is generally considered to be among the best foods to eat for a healthy heart. Studies have shown that people who eat fish regularly seem to have a lower risk of heart attacks, strokes and death from heart disease (Buscemi *et al*., 2014). Therefore this research seeks to generate scientific information that will inform policy for improvement of nutrition among low income earners by determining of the knowledge of the target population concerning fish based nutrition, evaluating the attitudes and assessing the practices of target population regarding fish consumption

Materials and methods

Seventy pieces of questionnaires were created based on the scientific information that will determine of the knowledge, evaluate the attitudes and assess the practices of target population regarding fish consumption. These questionnaires were distributed among the low income earners in the Abakaliki metropolis of Ebonyi state. The data collected via the questionnaires was analyzed using the methods developed at McMaster University Canada (Johnson and Lavis 2009). The main parameter measured was participants' perceptions of their own knowledge/understanding. The analysis is based on mean rating (MNR), median rating (MDR) and range. For instance the figures represent Likert rating scale of 1–5 points, where 1 point = grossly inadequate; 2 points = inadequate; 3 points = fairly adequate; and 4 points = adequate; and 5 points=very adequate.

Results

The outcome of the respondents' knowledge of fish nutritional value, individual attitude towards fish consumption and practices regarding fish consumption is presented in Table 1 below.

Discussion and Conclusion

This study indicated sufficient knowledge about the nutritional value of fish which is in agreement with an analysis of 20 studies involving hundreds of thousands of participants who indicated that eating approximately one to two 3-ounce servings of fatty fish (salmon, herring, mackerel, anchovies, or sardines) a week reduces the risk of dying from heart disease by 36 percent (Mozaffarian and Rimm (2006). However, there was insufficient knowledge on effort/policy of the government to improve fish availability for consumption. A positive attitude towards fish consumption was indicated and in line with the findings of FAO (2009) which stated that fish is still nutritionally important in many African countries as well as in Asia and Oceania and in other words, a large majority (73%) of the countries where fish is an important source of animal protein are poor and food deficient countries. Low frequency in consumption was in agreement with this statement by UNSCN (2009), that poor households have generally limited income for food consumption therefore they tend to prioritize certain food items to purchase. Also inadequate effort of the government to encourage small scale production to enhance local availability was indicated.

The most important factors responsible for low frequency in consumption and advocacy are availability, accessibility and affordability of fish for consumption. The effort and policy of the government will improve fish availability for consumption and the establishment of more government owned fish ponds /farms for fish production (aquaculture) will boost access to fish products for consumption. Policy makers in all related fisheries organisations should make policies that will make fish products more abundant and reachable for consumption by the poor masses.

Table 1: Scientific information that will inform policy for improvement of nutrition among low income earners.

Knowledge of fish nutritional value Question	Range	Median	Mean
1. To what extent can you rate your knowledge about nutritional value of fish?	1-5	4	3.81
2. How would describe your understanding about the cost of fish-based meal?	1-5	3	3.11
3. How would you describe your knowledge of preparation of fish-based meal?	1-5	4	3.50
4. How would describe your knowledge of the source of procurement of fish for consumption?	1-5	3	3.28
5. How would you describe your knowledge of the effort/policy the government to improve fish availability for consumption?	1-5	2	2.66
Attitude towards fish consumption Question			
1. How would you describe your willingness to eat fish-based meal as main source of protein?	1-5	4	3.98
2. How would you describe your willingness to buy fish at present cost for consumption instead of other animal sources of protein?	1-5	4	3.58
3. How would you describe your willingness to support the effort/policy of the government to make fish available for the population?	1-5	4	3.50
Practices regarding fish consumption Question			
1. How would you describe the frequency of your fish consumption?	1-5	3	3.01
2. How would you describe the frequency of use of fish for your meal preparation?	2-5	3	3.39
3. How would you describe the effort of the government to encourage small scale production to enhance local availability?	1-5	2	2.80
4. How would you describe the frequency of your advocacy for fish consumption?	1-5	3	3.16

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INTRODUCTION OF THE ENVIRONMENT AND FOOD SAFETY RISKS OF POND FISH PRODUCTION – CAN CARP CONSUMERS BE SATISFIED?

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Introduction

The main aim of „Development of a new risk management model system for increasing water and food safety in fish production line", or so called „HappyFish" project, supported by the National Research, Development and Innovation office is to understand and define the criteria of good quality fishmeat and to recommend limits for further legislative regulations. These unfortunately are missing in the case of fish products.

Traditionally, pond aquaculture is done in earthen ponds, and producers have very limited information about the quality and complexity of the soil of the ponds (mud, sediment, soil) or the quality parameters of the producing medium, the water. The majority of the produced fish is carp (close to 85%), and its meat quality (texture and taste) depends greatly on the production technology, and the water and soil parameters.

Materials and methods

The project is halfway through, and here are the main statements:
More than 400 pesticides and 100 medicine residues have been tested in the waters of fish ponds, in the water, in the sediments and in the fishmeat
The most frequent and highest concentration of pesticide in fish pond water and sediment is glyphosate, but its concentration – where there is existing regulation – is always below the limit in the sediment;
Almost every fish sample and still the water samples contained traces of DDT: the metabolites (DDD, DDE) of this insecticide that has been banned for half a century are still present in the ecosystem;
There are no determined limits for the majority of pesticides and medicine residues found in concentration near detection limits in pond water and fish meat
The controlled environment and technologies applied in fish ponds – the removal of mud, among others – may contribute to maintaining the self-cleaning abilities of the fish ponds and to avoid pesticides and medicine residues posing a threat to the ecosystem and to human health.

Results

The results of the project support the expectations of producers and consumers that fish is a healthy and saf product. The further tests will examine this area in an even wider range, with deeper analyses and with more high-tech technology.

Acknowledgements

Our work is supported by NVKP_16-1-2016-0023, HappyFish project. The presentation is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project and TUDFO/51757/2019-ITM. The project is co-financed by the European Union and the European Social Fund.

INTRODUCTION OF THE ENVIRONMENTAL AND FOOD SAFETY RISKS OF POND FISH PRODUCTION – CAN CARP CONSUMERS BE SATISFIED?

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The main aim of „Development of a new risk management model system for increasing water and food safety in fish production line", or so called „HappyFish" project, supported by the National Research, Development and Innovation office is to understand and define the criteria of good quality fishmeat and to recommend limits for further legislative regulations. These unfortunately are missing in the case of fish products.

Traditionally, pond aquaculture is done in earthen ponds, and producers have very limited information about the quality and complexity of the soil of the ponds (mud, sediment, soil) or the quality parameters of the producing medium, the water. The majority of the produced fish is carp (close to 85%), and its meat quality (texture and taste) depends greatly on the production technology, and the water and soil parameters.

The project is halfway through, and here are the main statements:

- More than 400 pesticides and 100 medicine residues have been tested in the waters of fish ponds, in the water, in the sediments and in the fishmeat
- The most frequent and highest concentration of pesticide in fish pond water and sediment is glyphosate, but its concentration – where there is existing regulation – is always below the limit in the sediment;
- Almost every fish sample and still the water samples contained traces of DDT: the metabolites (DDD, DDE) of this insecticide that has been banned for half a century are still present in the ecosystem;
- There are no determined limits for the majority of pesticides and medicine residues found in concentration near detection limits in pond water and fish meat
- The controlled environment and technologies applied in fish ponds – the removal of mud, among others – may contribute to maintaining the self-cleaning abilities of the fish ponds and to avoid pesticides and medicine residues posing a threat to the ecosystem and to human health.

The results of the project support the expectations of producers and consumers that fish is a healthy and saf product. The further tests will examine this area in an even wider range, with deeper analyses and with more high-tech technology.

Our work is supported by NVKP_16-1-2016-0023, HappyFish project. The presentation is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

OPTIMAL EXTRACTION METHODS FOR BEST ANTIOXIDANT YIELD IN MICROALGAE FROM DIFFERENT ORIGIN

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Introduction

Research for natural alternatives of currently used synthetic antioxidants have been and are a major topic in the food industry. As a rich source of carotenoids, vitamins, and phenols, microalgae could provide novel and food-grade approved antioxidant molecules with the potential in the prevention of lipid peroxidation and extending shelf-life of food products (Goiris et al., 2012). The value of target compounds obtained from microalgae could easily be enhanced by their relatively fast and easy growth, high yields, use of low quality (waste)water, CO_2 consumption and possibilities of future genetic improvements. However, insufficient knowledge and limited reports concerning the relationship between microalgae phenolic content and antioxidant activity is still a barrier in understanding their metabolism and implementation, using them as an alternative for current large-scale industries and making them economically viable. Further research in the future must be made to provide solutions for sustainable microalgae industry. The main objective of this study was to determine the antioxidant activity of marine and freshwater microalgae extracts, depending on their water content and used extraction solvents.

Materials and methods

Microalgae *Dunaliella tertiolecta* and *Chlorella kessleri* were cultured in f/2 enriched seawater medium (Guillard and Ryther, 1962) and Bold's Basal Medium (Bischoft and Bold, 1963), respectively, in batch bioprocess, under constant illumination of $6.5x10^3$lux, linear shaking 100rpm and 25°C. After 20 days, cultures were harvested and extraction was performed using two solvents, distilled water, and methanol, in a bead beading homogenizer for 3min, $1.5x10^4$rpm for both wet and dry biomass samples (dried at 70°C until constant mass). Obtained extracts were centrifuged and supernatants were used for further analysis. Total phenolic content in microalgae extracts was determined using the Folin-Ciocalteu (FC) method according to Singleton and Rossi (1965). Absorbance was measured at 750nm and results were expressed in gallic acid equivalents (GAE) per 1g of biomass. The ability of the extract to prevent the bleaching of β-carotene was determined according to the β-carotene linoleic acid assay (BCLAA) (Koleva et al., 2002).

Results

All of the *D. tertiolecta* extracts showed similar total phenolic content in mg of gallic acid equivalents (GAE) per 1g of biomass as can be seen in Figure 1. *C. kessleri* dry biomass extracts showed higher total phenolic content in comparison to their wet biomass extracts. Higher β-carotene preservation rate was obtained for wet *C. kessleri* biomass water extracts (76.35%) in comparison to their dry biomass extracts (71.38%). Conversely, for methanol extracts a higher percentage of β-carotene preservation rate was observed in dry *C. kessleri* biomass in comparison to dry biomass extracts (92.01% vs. 81.30%). A similar pattern was obtained for *D. tertiolecta* water extracts (88.53% β-carotene preservation rate for wet extracts in comparison to 63.04% for dry extracts), and methanol extracts (72.08% β-carotene preservation rate for dry extracts in comparison to 63.04% for wet extracts).

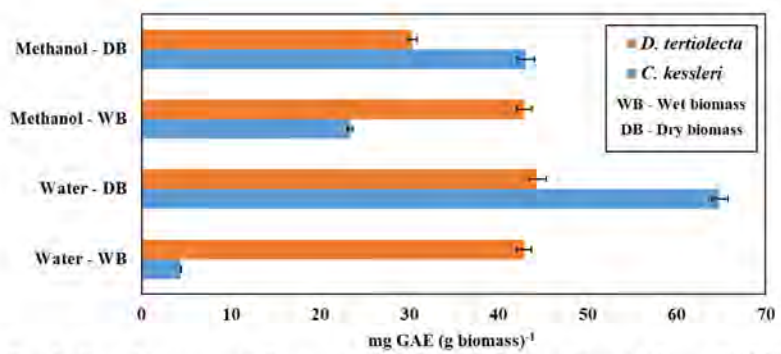

Fig.1. Total phenolic content of extracts in mg of gallic acid equivalents (GAE) per 1g of biomass depending on the microalgae biomass and used extraction solvent.

(Continued on next page)

Discussion and conclusion

Results obtained using FC method provided information of total phenolic content in different extracts. The results differ depending on the extraction conditions and microalgae morphology. *D. tertiolecta* is a cell wall lacking microalga in comparison to *C. kessleri* (Oukarroum et al., 2012). Since the cell wall is a strong mechanical structure it firstly has to be destroyed in order to provide a good extraction of microalga content. Obtained results for wet *C. kessleri* biomass had shown that extraction was time dependent, i.e. extraction was not long enough to break the cell wall and provide good extraction results. The drying process has induced the disruption of the cell wall of *C. kessleri* and enabled better extraction of total phenols. Using the BCLAA method, the oxidation of linoleic acid generates hydroperoxide free radicals which oxidize the highly unsaturated β-carotene and causes loss of colour. Oxidation of β-carotene by hydroperoxides can be neutralized by the presence of antioxidants in extracts (Kumaran and Karunakaran, 2006). *C. kessleri* extracts in all cases had a higher β-carotene preservation rate then extracts obtained from *D. tertiolecta* and therefore a higher antioxidant capacity. Results obtained using BCLAA are in accordance with results of FC method for all tested extracts. Phenols contribute to the total antioxidant capacity of extracts and result in a higher β-carotene preservation rate.

Further studies should involve different extraction methods for the analysis of total phenols and their antioxidant activity in different microalgae. The acquired data could provide a better understanding of metabolic processes and their secondary metabolites which could improve current industrial production and increase the yield of high-value products.

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LOCALLY PROCESSED LAND ANIMAL BY-PRODUCTS AS POTENTIAL CANDIDATES TO REPLACE FISHMEAL AND FISH OIL IN PRACTICAL DIETS FOR EUROPEAN SEABASS

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Introduction

The sustainability of European aquaculture, both economic and environmental, relies on the feed industry's ability to produce high quality diets able to respond to consumer's demand, at the most competitive prices, while respecting the environment. Moreover, the agro-food industry generates large amounts of by-products that after proper processing can be valid fishmeal and fish oil substitutes in aquafeeds. Using these locally generated by-products as feedstuffs would not only increase aquaculture sustainability, but also contribute to a functional circular economy by reintroducing such by-products into valued production chains. The ANIMAL4Aqua project aimed at the identification and valorization of processed land animal by-products to replace the traditional, unsustainable and expensive fishmeal and fish oil in diets for European seabass (*Dicentrarchus labrax*).

Materials and methods

Locally processed animal by-products were selected as candidates to replace fishmeal (FM) and fish oil (FO) in practical diets for seabass and characterized in terms of nutrient composition and availability: poultry by-product meal, steam and enzymatically hydrolyzed feather meal, FO from rendering fish by-products, poultry fat (PF) and mammal fat (MF, about 70% lard and 30% beef tallow), also from rendering by-products. The most promising by-products as protein or lipid sources were then incorporated in practical diets as FM or FO replacement, respectively, and evaluated in growth trials with seabass juveniles to establish the maximal level of replacement while assuring good growth performance, nutrient utilization and tissue fatty acid (FA) profile

Results

The protein and the essential amino acids' apparent digestibility coefficients (ADCs) were high in all tested alternative protein sources (> 84%). Considering these results, market availability and price, steam hydrolyzed feather meal (HF) was selected as the best candidate to replace FM in seabass diets. The replacement of 28, 55 and 76% FM by HF in an 18 weeks' growth trial did not affect growth performance, nutrient gain, muscle fatty acid composition and humoral non-specific immune parameters of seabass juveniles. Also, the dietary inclusion of HF improved phosphorus ADC, significantly decreasing P emissions into the environment (Campos et al., 2017).

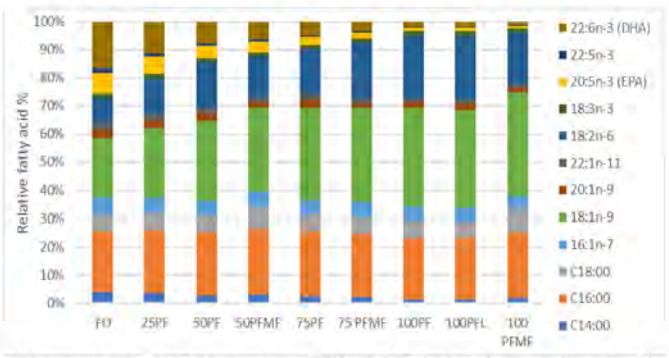

Figure 1 Flesh fatty acid profile of fish fed increasing levels (25-50-75-100%) of PF and/or MF.

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Both PF and MF had good ADC results (>86, 87 and 92% for energy, lipids and protein, respectively), and an overall high ADC for individual FA was reported in PF (90%). Both fats were further tested as FO replacement in two growth trials: one using PF to replace 25, 50, 75 and 100% FO, and the other one using a PF and MF mixture (50:50, PFMF) as 50, 75 and 100% FO replacement (Monteiro et al, 2018; Campos et al, 2019). An emulsifier (soy lecithin) was also tested in the 100PF diet (100PFL).

FO was completely replaced by PF without impairing feed intake, growth performance and nutrient utilization, but this was only possible up to 75% with PFMF (37.5% PF + 37.5% MF). The inclusion of soy lecithin in 100PFL attenuated liver lipid deposition and improved triglycerides digestibility. The FA profiles of the analyzed tissues generally reflected those of the diets, increasing MUFA and decreasing PUFA content. However, despite the changes in muscle FA profiles (Fig 1), seabass fed diets with up to 75% FO replacement (by either PF or PFMF) still provided the EPA and DHA levels recommended for human consumption (0.25-0.5 g per 100 g portion of fish)

Discussion and conclusion

In conclusion, HF is well digested by European seabass and can replace 76% of the dietary FM without impairing growth, nutrient gain, immune status or EPA and DHA levels in the muscle, suggesting that this can be a very good protein source for this species.

PF seems a better alternative lipid source than MF and can totally replace FO without impairing feed intake, growth performance and nutrient utilization. Yet, the FO replacement should not overpass 75% in order to assure the recommended levels of EPA and DHA for human consumption. Using rendered by-products as fishmeal and fish oil substitutes will reintroduce them into the local economy, contributing to a circular economy and reducing the environmental impacts of the aquaculture industry.

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EFFECT OF DIFFERENT CONCENTRATIONS OF NITRATE IN THE PRODUCTION OF TOMATO (*Solanum lycopersicum* cv Grape) AND SHRIMP (*Penaeus vannamei*) IN LOW SALINITY WATER

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The objective of the present work was to evaluate the technical feasibility of using shrimp effluents (*Penaeus vannamei*) with low salinity to irrigate tomato (*Solanum Lycopersicum* L cv Grape) under an aquaponics system supplying different concentrations of nitrates. An alternative to food production is the use of aquaculture recirculation systems, which are intensive systems whose main characteristic is that the rate of water exchange is less than 10% of the total volume until it even operates in zero replacement. These operating conditions are carried out promoting the recycling of nutrients, which allows the constant reconditioning of water. Four treatments were evaluated, three with shrimp effluents compensating the deficiencies with synthetic fertilization in three of them, to which they were given 0, 100 and 200 mg l^{-1} of KNO_3^{-}, while the remaining treatment was administered Steiner solution. The flow of water in the system was continuous at a rate of 1 l/m. For the experimental design, a completely randomized treatment was used in the aquaculture component while in the hydroponic component a randomized complete block treatment was applied; both treatments were carried out with three repetitions. Tomato seedlings were transplanted in a zigzag arrangement at a density of 3.5 plants per linear meter. The densities in the system were 100 shrimp m^{-3}. In the aquaculture system, physicochemical variables, weight, length, survival and mortality were evaluated, and in vegetative variable plants, macronutrients present in each stage of the crop and fruit production. A record of the physicochemical parameters was taken out of normal and a final weight of 18 g was obtained for shrimp. Shrimp survival was 40% and the highest yield was at the level of 744 NO_3^- (1 kg m^{-2}) on average and the lowest (0.6 kg m^{-2}) at the level of 0 mg l^{-1} of NO_3^-, even if the grape tomato is small fruits of lower demand for nitrates. Further work to obtain information on the density of organisms required per plant, since the integration of fruit horticultural crops with nutritious shrimp solutions by adding synthetic fertilizers optimizes the use of water resources, food sources are produced in two different integrated systems, synthetic fertilizers are saved and the impact on the environment is minimized by aquaculture discharges.

THE EFFECT OF ENCAPSULATED *Lactobacillus plantarum* KC426951 AS A PROBIOTIC IN DIET ON GROWTH AND BLOOD FACTORS OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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In this research, the probiotic effect of *Lactobacillus plantarum* KC426951 on growth and blood factors of rainbow trout (*Oncorhynchus mykiss*) were investigated. The bacteria were combined into diets of 3 different treatments including two treatments with 10^7 and 10^8 cfu/g *Lactobacillus plantarum* (T_1 and T_2 respectively) coated with calcium alginate and a control diet without *Lactobacillus plantarum* bacteria (T_0). 90 Fish with an average weight of 12.94±0.35 were randomly assigned to 9 tanks of 500 liters (10 fish per tank) and fed with different diets for eight weeks. For each treatment, three replicates were considered. The fish were fed twice a day at 5% body weight in the first month and 3% in the second month. According to the results of comparison of T_1 and T_2 treatments with control treatment, T_1 treatment significantly had the highest weight gain (WG), weight gain percentage (PWG), lowest food conversion ratio (FCR) and the highest Specific Growth rate (SGR) ($P < 0.05$). However, there was no significant difference in survival percentage among treatments ($P > 0.05$). The results of this study showed that the number of white blood cells (WBC) in T_1 and T_2 treatments was significantly higher than control ($p < 0.05$). However, there was no significant difference in the number of red blood cells (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean hemoglobin concentration in red blood cells (MCHC) ($P > 0.05$). According to the results obtained in this study, it can be suggested using of encapsulated *L. plantarum* with 10^7 cfu/g in rainbow trout diet for improving the growth indices and some blood factors.

SUITABILITY OF MICROALGAE CULTURED IN NITROGEN-LIMITED MEDIUM FOR ROTIFER PRODUCTION

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Introduction

Marine finfis hatchery production requires the production of other organisms to be delivered as live prey during the early larval stages. Rotifers are the first live prey for the main finfis species. Its availability during the first critical period of finfis development, the onset of the exogenous feeding, depends on a suitable management of its mass scale production. Rotifer production is based on the use of microalgae and baker's yeast as food, and currently several microalgae-based commercial products are available. Microalgae species commonly used are *Tetraselmis* sp., *Isochrysis* sp. and *Nannochloropsis* sp., due also to their contribution to rotifer's lipid and fatty acid composition. Microalgae total lipid and fatty acid profile can be modified by changing culturing conditions such as light, temperature, salinity or culture media (Converti et al. 2009); but these modifications can also change other chemical profiles (Ribalet et al. 2007), with potential consequences on rotifer culture growth. In the present study, rotifer culture growth based on microalgae cultured in standard conditions or in nitrogen-limited conditions has been compared.

Materials and methods

Three experimental cycles were performed, one per microalgae species: *Nannochloropsis oculata*, *Tetraselmis suecica* and *Isochrysis galbana*. Microalgae were cultured with Guillard's culture medium in two 120L annular photobioreactors. Additional nutrients were added one week after the onset of microalgae culture in photobioreactors: the standard composition in one photobioreactor, the standard composition without sodium nitrate ($NaNO_3$) in the other. Four days later microalgae were used for feeding rotifer cultures in 6L containers, 5 containers per experimental group: control (fed with S.Parkle®, INVE Technologies, Dendermonde, Belgium; 0.5g per million of rotifers), standard (fed with microalgae cultured in standard medium; 7 x 10^6 cells mL^{-1}) and modified (fed with microalgae cultured in medium without $NaNO_3$; 7 x 10^6 cells mL^{-1}). Two days before the onset of rotifer growth experiments, rotifers (*Brachionus plicatilis*) were stocked in clean seawater and fed with 0.75g yeast per million of rotifers. One day before the onset of rotifer growth experiments, rotifers were stocked in clean seawater and kept starved. Rotifer growth experiments started in 2L of seawater containing 200 rotifers mL^{-1} plus 0.2g S.Parkle® or 14 x 10^9 microalgae cells. A total volume of 0.5L of seawater containing the amount of S.Parkle® or microalgae cells, based on microalgae and rotifer densities, was added daily to each container. Microalgae density, rotifer growth, temperature, salinity, oxygen and pH were monitored daily for 7 days. Microalgae density was counted with a Neubauer chamber at the inverted microscope (Leica DM IRB, Leica, Weitzlar, Germany). Total rotifers, total females with eggs and total females with 1, 2, 3, 4 or 5 eggs were counted in subsamples of known volume at the stereomicroscope (Leica MZ8, Leica, Weitzlar, Germany). Temperature, salinity and oxygen were measured with a multiparametric probe (Hach HQ40d multi, Hach, Loveland, CO), pH was measured with a pHmeter (Crison pHmeter 507, Hach, Loveland, CO).

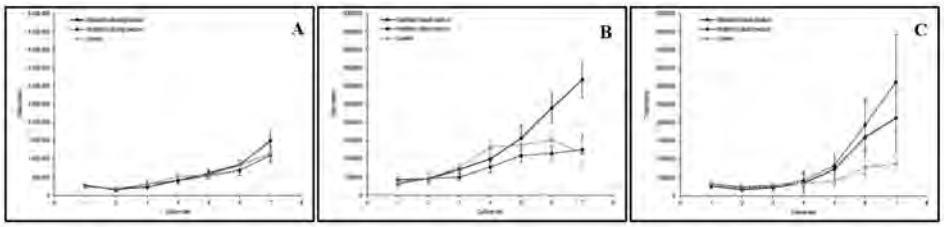

Figure 1. Total rotifers (mean ± SD) based on *N. oculata* (A), on *I. galbana* (B) or on *T. suecica* (C) cultured in standard or modified Guillard's medium. Control groups were fed with S.Parkle®.

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Results

Rotifer growth based on *N. oculata* did not differ between microalgae culturing conditions neither to control growth, and was lower than the obtained with the other two microalgae species (Figure 1A). *Isochrysis galbana* cultured in standard medium brought a higher growth than the same species in modified culture medium or the control groups (Figure 1B). Rotifer growth based on *T. suecica* was more variable between containers, higher than in control groups, and better in those fed with microalgae grown in modified culture medium (Figure 1C).

The maximum percentage of females with eggs was reached on day 1 when fed with *I. galbana* (41% average in standard groups and 37% average in modified groups), on day 2 when fed with *N. oculata* (36% in standard groups and 44% in modified groups) or *T. suecica* (43% in standard groups and 42% in modified groups) and on day 2 or 3 when fed with S.Parkle® (44% average). Females with 3 and 4 eggs were present in rotifer cultures fed with *T. suecica* from day 3 onwards; females in cultures fed with *N. oculata* and *I. galbana* presented a maximum of 2 eggs.

Discussion and conclusion

Nitrogen limitation in microalgal culture medium had a different impact on rotifer growth depending on the microalgae species. *Nannochloropsis oculata* cultured in nitrogen limitation conditions doubles its lipid content (Converti et al. 2009) but this change does not improve neither reduce rotifer growth. The composition of *I. galbana* and *T. suecica* cultured in standard medium is more suitable for rotifer growth, although the performance obtained with *T. suecica* is variable among experimental replicates and is in agreement with the unexpected crashes in rotifer cultures observed during large-scale production (pers. obs.). The presence of toxic compounds (Ribalet et al 2007) in *I. galbana* cultured in nitrogen limited conditions should be explored.

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HATCHERY PRODUCTION OF FLATHEAD GREY MULLET, *Mugil cephaluS,* JUVENILES

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Introduction

Mediterranean aquaculture diversification has targeted flathead grey mullet *Mugil cephalus*, an euryhaline omnivorous fish with a world-wide distribution, as a species of interest. A Sardinian regional project contributed to develop aquaculture techniques for this species by designing protocols and a production pipeline suitable for Sardinia specificities. Fully mature wild females (oocyte diameter 550 – 600 mm) were induced to spawn (63.6% success), and survival to 30 mm total length (TL) juveniles ranged from 1 to 6% in indoor conditions (Vallainc 2017).

The availability of wild females in tertiary yolk stage advanced vitellogenic oocytes (550 – 600 mm) is unpredictable, while secondary yolk stage vitellogenic oocytes (<550mm) are more abundant in coastal lagoons during the spawning season. Juvenile growth up to 60 mm TL was obtained in outdoor conditions, and little is known about growth and survival of hatchery produced individuals in indoor conditions. The present work focuses on investigating the spawning induction of females in secondary yolk stage vitellogenesis, and provides additional data on egg quality, growth and survival of juveniles up to 60 mm TL, underlining the frailties encountered in rearing the flathead grey mullet indoo .

Materials and methods

All procedures regarding broodstock capture, transport, spawning induction, egg incubation, larval and juveniles rearing followed the protocols reported in Vallainc (2017). Twelve females were captured during the spawning season and induced to spawn in an indoor recirculating aquaculture system (RAS). The 50% of the females were induced to spawn with a single hormone injection, the remaining were treated twice. Oocyte maturation was monitored by ovarian biopsies up to a maximum of 122 h after treatment.

Spawned eggs were incubated in a RAS and a sample of fertilized eggs was used to determine egg quality by culture plate (EIA plate). Larvae were reared in two indoor RAS, each one consisting of three circular fibe glass tanks of 2,000 L volume. Individuals were considered to shift from larval to juvenile stage at 34 days post hatching (DPH).

Fish were periodically sampled for growth measurement. Survival was determined by image analysis at 170 DPH. Dead juveniles older than 90 DPH were examined under a light stereoscope.

Results

Two treated females spawned but it was not possible to practice the biopsy before the treatment. In both cases the spawning occurred spontaneously 22-24 h after a single dose treatment. The female with bulging abdomen and red papilla (Female 1) spawned a good quality batch: 100% hatching rate, ~55% mortality at 3 DPH (Figure 1), 5% deformities. The other female (Female 2) did not show the external features and spawned a batch of lower quality: 45% hatching rate, more than 90% mortality at 3 DPH (Figure 1), 11% deformities. All the other females failed in spawning and showed bad quality pre-ovulation oocytes.

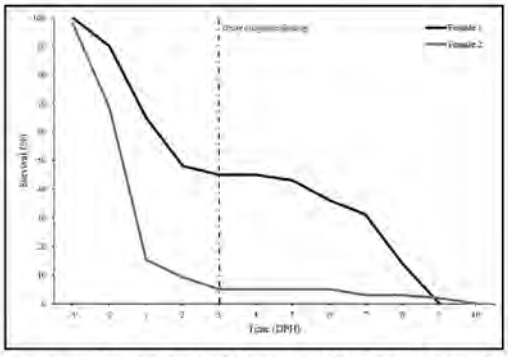

Figure 1. Hatching rate and survival of larvae obtained from two spawning females. Dotted line highlights 3 DPH, the onset of exogenous feeding.

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A total of 1.5 ± 0.6 (mean ± SD) million eggs were incubated and showed growth and survival similar to previous batches. Juvenile daily growth rate (GR) was 0.14 ± 0.04 mm day^{-1} TL and 0.003 ± 0.002 g day^{-1} WW. Survival at 170 DPH (~ 34 mm TL) was ~2%.

Dead juveniles presented different characteristics: 1) small and mainly deformed individuals with evident signs of starvation, 2) bulging abdomen and mainly protruded intestine and 3) big and seemingly healthy individuals with pale gills and steatosis -like liver. No pathogen could be linked to the mortality recorded during the juvenile stage.

Discussion

Maturation stage influenced the quality of the eggs. The female not showing the external advanced maturation features spawned lower quality eggs in contrast to the one featuring a bulging abdomen and red papilla. No spawning neither good quality pre-ovulation oocytes were obtained by double treating the females, and the only batch of eggs obtained from a not fully mature female was by a single injection treatment.

Female maturation stage and stress play a crucial role in spawning success (Crosetti and Blaber 2015). The hormone treatment was successful in inducing the maturation toward the tertiary yolk stage but the quality obtained was too low. Stress due to capturing and handling should be reduced, as confirmed by the poorer results obtained by double treatments.

Growth and survival of juveniles up to 30 mm was similar to previous batches managed with the same protocol (Vallainc 2007). Previous growth rates were 0.18 ± 0.04 and 0.2 ± 0.03 mm day^{-1}. The features of dead juveniles and our personal observations point to feed availability and characteristics, feed formulation and rearing parameters as the most likely points to improve during this stage.

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EUROPEAN SEAWEED VALUE CHAINS IN AN INTERNATIONAL PERSPECTIVE

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Introduction

The global seaweed industry is currently dominated by Asian countries, with China and Indonesia being the main producers of raw material. Processing facilities are increasingly concentrated in China and a large part of the seaweed biomass produced in Indonesia and Chile is now exported as raw material to China for further processing (Bindu and Levine 2011). The European seaweed sector historically relied on the harvesting of wild seaweeds, particularly in France, Ireland and Norway. Harvested seaweed biomass is used by companies such as Dupont, Algaia and FMC for the production of alginates.

Recently, interest in the cultivation of seaweeds in European seas has grown considerable. Now that there is evidence that seaweed aquaculture in various European seas is feasible, seaweed cultivation is recognized as an important future source of agricultural production. Start-ups and established companies have stepped into this sector, developing new production systems and machinery required for efficient large-scale production and harvesting, and exploring new consumer products, feed and non-food applications. The increasing interest in seaweed is also reflected in (policy) documents, such as the draft "Klimaatakkoord" and Noordzee 2030, and in the financial support for research and upscaling of production provided by European governments through research projects such as the GENIALG (www.genialgproject.eu) and ProSeaweed (www.proseaweed.eu).

From an economic and value-chain perspective, the inevitable question is how the nascent European cultivated seaweed sector relates to the dominant global producers, concentrated in Asia. Earlier studies have pointed to the significant differences in cost of production, warranted by selling the products in high value niche markets (van den Burg, 2019). Large-scale seaweed production in Europe means accessing the global seaweed value chain.

Objective

This paper report on recent investigations into the joint development of sustainable and circular seaweed production systems for the Chinese and Dutch seaweed sectors. It addressed the question if there is an opportunity to strengthen ties with the Chinese seaweed sector, identify and discuss shared solutions that will ensure a sustainable development of the seaweed sector in both the Europe, with a focus on the Netherlands, and China.

Methodology

The global value chains framework is used to analyse how local actors (firms, communities, workers) are linked to and affected by major transformations in the global economy, using the core concepts of governance and upgrading – activities aimed at generating higher value for a product (Gereffi and Fernandez-Stark 2016). Upgrading strategies followed in Europe and in China are identified. These are assessed by looking closer at the value chain through the features of the market, governance and context.

Various phases of data collection are identified. In the first phase, a review of literature and policy documents was be conducted to gain insight into the key issues at hand for both the European and Chinese seaweed sector. A social network analysis on the Chinese seaweed sector was conducted, seeking out the different stakeholders involved in seaweed research, production and processing in China, and the actors who are key nodes in the network (Borgatti et al. 2009).

The second phase identified priority areas for further investigations. The network of companies partnering within the Stichting Noordzeeboerderij (+/- 90 organizations) were be consulted through interviews to identify key opportunities for collaborations from a Dutch perspective. Confronting the priorities of the Dutch seaweed sector with the social network analysis on China, most relevant stakeholders were identified and approached

In the third phase, a field trip to China was organised to visit key Chinese stakeholders. Through interviews with these stakeholders, key priorities of the Chinese seaweed sector were identified and conditions for cooperation with the Dutch sector were discussed. In the fourth phase, the data collected in document analysis and interviews in Netherlands and China was used to conduct a SWOT to identify the overlapping areas of interest on which consortium building must focus.

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Results

The European sector currently focusses on into niche markets and development of new high-value products and new markets. Developing new standards for seaweed, e.g. for sustainability and product quality , is another attempt differentiate from ‘global’ seaweeds.

A first analysis of strengths and challenges in respectively Netherlands and China highlights the long-standing experience of China in seaweed breeding and cultivation, and availability of an infrastructure for production and processing. The European and Chinese seaweed sectors appear to be worlds apart, with their own challenges for the future. However, on closer examination the solutions to overcome these challenges may not be so dissimilar. The European seaweed sector is young and in its early stages of development. Upscaling will depend on the availability of high quality seeding material, mechanisations to reduce costs and positive perception by the public. To achieve the latter, and to satisfy consumers and retailers alike, the sector will need to demonstrate low or positive environmental impacts of its production processes with a high degree of transparency. The Chinese seaweed sector, on the other hand, is well established but it has shown little innovation in recent years. It still relies on heavy manual labour and has a troubled track-record in terms of environmental impact and sustainability.

Based on the preliminary analysis, there is ample room for cooperation between China and the European seaweed sector, particularly in developing sustainable practices of breeding, production and harvesting. Shared concerns are the improvement of production processes through technological advancements, higher quality seedlings and transparency in the value chain are needed.

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POTENTIAL FOR USING MUSSEL FARMING (*Mytilus edulis)* IN NUTRIENT REMEDIATION IN ESTUARIES OF THE UNITED KINGDOM

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Introduction

The Nitrates Directive (Council Directive 91/676/EEC) aims to reduce water pollution from agricultural sources and to prevent further such pollution, together with the Water Framework Directive (WFD) is one of the key instruments in the protection of waters against agricultural pressures. While ground and surface water quality in the EU have improved over the last decade, there is an ongoing need for improvements and assessment of mitigation measures (European Commission, 2018).

Bivalve molluscs can benefit coastal ecosystems by providing ecologically and economically important ecosystem services (Dalrymple and Carmichael, 2015; van der Schatte Olivier et al., 2018)we compared N in tissue, shell, and biodeposits between juvenile and adult oysters. Juvenile oysters assimilated 165 ↑ 8 mg (↑ SE. These include nutrient uptake, mediated by filter feeding on particulate organic matter including phytoplankton (Newell et al., 2005; Saurel et al., 2014) and their biodeposits (feces and pseudofeces. The use of bivalves as a mechanism of reducing the impact of catchment-derived nutrients in estuaries and coastal waters has been proposed (Rose et al. 2014) and demonstrated as cost-effective in sheltered fjord environments (Petersen et al., 2014)Denmark where biological and economic parameters related to nutrient removal was monitored throughout a full production cycle (1yr. The UK represents an interesting case for investigating the potential for shellfish farming in coastal nutrient remediation, due to the wide ranges of conditions including nutrient loading in catchments, hydrodynamics and coastal morphology.

To quantify the nutrient removal by shellfish, one approach is to estimate the total nutrient content of harvested stock and to consider that as removed from the marine system. This has been applied to global estimation of ecosystem services provided by bivalve shellfish farming (van de Schatte Olivier et al 2018). To achieve this, total biomass yields can be estimated from national or farm-level harvest aquaculture statistics but there are significant gaps in information on the nutrient content of shellfish and how these vary seasonally as well as across sites with different nutrient loading and environmental conditions. Hence, the effects of environmental nutrient loading on uptake rate in mussels and seasonal variation in nutrient composition of mussels were investigated, prior to a nationwide survey of potential nutrient removal by mussels across a range of sites.

To investigate the effects of environmental nutrient loading and seasonality, two sites were selected in the same geographic region in North Wales; one low nutrient (Menai Strait) and one high nutrient (Afon Briant), see Figure 1. At each site, samples of mussels were collected monthly from January 2018 to January 2019. Mussels were also sampled from a further 12 sites with varying nutrient loads and conditions around the UK, within a single seasonal period between September and October 2018.

Figure 1 Location of mussel and nutrient sampling sites around the UK. The temporal sites are marked with a star and the other sites are marked with circles.

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Discussion and conclusion

Initial results show significant seasonal trends in nitrogen and phosphate content of mussels, that can inform timing of harvest to optimise nutrient removal from the environment. There are also significant differences in nitrogen and phosphate content of mussels between the high and low nutrient loading sites. These latter results will be integrated with the UK-wide survey of nitrogen and phosphate content in mussels. This can then be used as a basis for estimation of current nutrient removal at all sites based on aquaculture production and fishery landings, with cost-benefit projections for potential future strategic increases in nutrient mitigation using shellfish farms under a range of environmental settings.

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HOW TO GENETICALLY INCREASE FILLET YIELD IN FISH: RELEVANT GENETIC PARAMETERS AND METHODS FOR THE ESTIMATION OF GENETIC GAIN

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Introduction

Fillet yield (i.e. the proportion of edible muscle in a fish) is a key economic trait for species sold as fillets. Its genetic improvement is complicated by several of its characteristics 1) it is a ratio trait, 2) its numerator (fillet weight) and denominator (body weight) are strongly correlated (correlations in the range 0.89-0.99) 3) it offers little phenotypic variation and 4) it cannot be measured on a live breeding candidates.We formerly showed that it could still be improved by selection, using fillet yield, residual fillet weight or a ratio-specific linear index as selection indices. However, previous research showed that the genetic parameters of ratio traits do not permit a reliable prediction of genetic gains. As predictability of genetic gains is a key requirement to define breeding programs, we investigated how genetic gains in fillet yield could be predicted by the genetic parameters of fillet yield, residual fillet weight and of the component traits of the linear index. To this end, we compared simulated genetic gains with those estimated by classical prediction methods.

Material and methods

In order to perform simulations of fillet weight, body weight and waste weight with realistic values, we first estimated their genetic and phenotypic parameters, using 9 datasets of carcass traits recorded during 7 experiments on 4 species: Europeans sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*). All fish used to estimate parameters were from factorial or partly factorial designs with mixed families, with pedigree recovered by microsatellites genotyping. The estimations of genetic parameters were performed using an animal model with VCE version 6.0.2, with specific fixed fects for each population.

We simulated, using the genetic parameters specific to each dataset
- non-trimmed fillet weight (FW) and waste weight (WW), which were correlated with additive genetic correlation $r_A$fw_ww and phenotypic correlation rPfw_ww, waste being composed of viscera, head and bones of the fish
- non-trimmed fillet weight (FW) and body weight (BW), which were correlated with additive genetic correlation $r_A$fw_bw and phenotypic correlation rPfw_bw.

Phenotypes were simulated for the initial (G_0) generation, phenotypic selection applied and a next generation of G_1 offspring produced, with 5 selection indices:
- Fillet yield FY=FW/BW
- Fillet ratio FR=FW/WW
- An linear index LIN_FY=αFW+βBW optimising response in FY, following linear index theory (Lin, 1980)
- An linear index LIN_FY=γFW+δWW optimising response in FR (Lin, 1980)
- resFW, the residual of the regression of FW on BW.

Then, we compared simulated gains (average FY in G_1-average FY in G_0) with expected genetic gains obtained from:
- selection index theory on ratios, as proposed by Lin and Aggrey(2013)
- phenotypic variance and heritability of FY in the base population, using the breeder's equation
- phenotypic variance and heritability of resFW in the base population

As Gunsett (1987) showed that the reliability of ratio traits heritability was dependent on selection intensity, all this was performed with 3 selection intensities for each set of genetic parameters (p=50%, p=20%, p=5%), thus 27 data points were available for each index tested (3 intensities * 9 fish populations)

Results

The simulated genetic gain was best with the linear indices, and hardly lower (95-96% of linear index value) with FY and resFW.

Linear index theory was extremely efficient in predicting genetic gains, as the correlation between predicted and simulated gains was always higher than 0.995, with a regression coefficient between 0.99 and 0.999

Prediction from the genetic parameters of FY was much less efficient, the simulated gain was on average only 82% of predicted gain. In addition, this was quite population specific, as the simulated gain could range from 65% to 135% of the predicted gain

Prediction from the genetic parameters of resFW was more efficient albeit imperfect, as the simulated gain was on average 93% of predicted gain. This was much less population specific, as the simulated gain ranged from 80% to 102% of the predicted gain.

Discussion and conclusions

The present study, using real genetic parameters from 9 fish populations in simulations, showed that as expected, the phenotypic and genetic parameters of fillet yield are not reliable to predict genetic gains for this trait. On the contrary, linear index theory predicted gains with a high efficienc , irrespective of the fact that the genetic and phenotypic parameters used were related to FW and WW or to FW and BW. One point of attention is that, especially for FW and BW, the phenotypic and genetic correlations are extremely high (0.972-0.994 and 0.982-0.997, respectively) and must thus be estimated with high precision for the estimated gains to be meaningful.

An alternative phenotype, residual fillet weight, provided genetic and phenotypic parameters that led to gave reasonable estimates of predicted gains in fillet yield. As it is simpler to use than composite indices, and easier to correlate to potential predictors, residual fillet yield may be an appropriate surrogate for fillet yield in the context of fish selective breedi

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THE INVESTIGATION OF THE REPRODUCTIVE BIOLOGY OF HÉVÍZ DWARF CARP (*Cyprinus carpio carpio morpha hungaricus*)

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Introduction

Common carp is in the largest quantity produced freshwater fish species in Hungary. Wild populations of *Cyprinus carpio* (the European subspecies) are continuously decreasing due to hybridization with domesticated stocks. The wild common carp is listed on the IUCN Red List as '*vulnerable*' since 2008 but the River Danube subpopulation (*Cyprinus carpio carpio morpha hungaricus* Linnaeus, 1758) belongs to the '*critically endangered*' status (Freyhof & Kottelat 2008). Lake Hévíz is the biggest, biologically active natural thermal lake (38 degree) in Hungary. The high temperature and the individual chemical composition of Hévíz Lake resulted of a unique biological community (Ponyi, 2002). According to our knowledge, one of the last genetically pure *Cyprinus carpio carpio morpha hungaricus* population can be found isolated in the lake. The improvement of the artifici l propagation can support the conservation of this important wild carp subpopulation. The subspecies conformed to the special environment with a small adult size (Hévíz "dwarf" carp). The aim of the study was to improve sperm cryopreservation methods and to establish spermbank in the mentioned carp subspecies. The optimized methods can support the reintroduction and protection of the native population at Lake Hévíz (Cabrita et al. 2010).

Materials and methods

In our work, 2 different experiments were carried out to establish a spermbank, optimized the cryopreservation method and to avoid the sperm agglutination following thawing in stress sensitive *Cyprinus carpio carpio morpha hungaricus (N*=23*)*. The spermiation was hormonally induced using carp pituitary. Sperm was collected with syringes. The progressive motility (pMOT) in both fresh and cryopreserved samples was recorded with a CASA system (Computer-assisted Sperm Analysis, Sperm VisionTM v. 3.7.4., Minitube of America, Venture Court Verona, USA) using 50mM NaCl solution with 1% BSA. In our study 2 different extenders were compared Grayling extender (200 mM glucose, 40 mM KCl, 30 mM Tris, pH: 8.0 ± 0.2, Horváth et al., 2012, Bernáth et al., 2016) and the Pike extender (150 mM glucose, 75 mM NaCl, 30 mM KCl, 1 mM Na2HPO4×12H2O, 1 mM MgCl2×6H2O, 1 mM CaCl2×2H2O, 20 mM Tris, and 0.5% BSA, pH: 8.0 ± 0.2, Várkonyi et al. 2018). Diluted sperm was loaded in 1:9 dilution ratio with 10% methanol as an intracellular cryoprotectant into 0.5 straws (Minitube GmbH, Tiefenbach, Germany). In our study 2 different cryopreservation method was used in Polystyrene box (P.box) at 3 cm above the surface of liquid nitrogen for 3 minutes (Horváth et al. 2003) and in a controlled-rate freezer (CRF (cooling program (7,5ºC to -160ºC, cooling rate: 56ºC/min)), (IceCube 14 s, IceCube Series v. 2.24; Sy-Lab, Neupurkersdorf, Austria). The straws were thawed at 40 °C using a waterbath for 13 seconds (Horváth et al. 2012).

Experiment 1. Sperm cryopreservation in RAS kept broodstock

Samples (*N*=9) were diluted with Grayling extender and loaded into straws. Freezing was carried out in a Polystyrene box. Motility parameters was compared in fresh and cryopreserved groups as well groups.

Experiment 2. The optimization of the cryopreservation method

Freshly stripped samples were diluted with Grayling and Pike extender. Diluted sperm was cryopreserved in Polystyrene box and in controlled-rate freezer. Motility was compared in all groups (CRF-Grayling (CG), CRF-Pike (CP) extender; P. box-Grayling extender(BG) P. box-Pike extender (BP).

Results

In *Experiment 1.* significantly higher pMOT (94±3) was recorded in the fresh control group compare to the cryopreserved (65±9%). In *Experiment 2.* Moderate pMOT wasrecorded in all groups CP (14±3%), CG (33±12%), BP (24±8%) and BG (38±14%). No significant difference was recorded between the two cryopreservation methods iusing the two extenders. A significantly higher pMOT was observed using the Grayling extender with the CRF in comparison with the Pike extender. Sperm agglutination phenomenon was observed in our experiment using Grayling extender compare to the Pike extender which showed a regular pure homogenous sperm suspension after thawing.

(Continued on next page)

Discussion and conclusion

In conclusion, sperm cryopreservation was successfully carried out in the stress sensitive and genetically pure population of *Cyprinus carpio carpio morpha hungaricus*. According to our results, both the Polystyrene box and the controlled-rate freezer are suitable for the Hévíz dwarf carp sperm cryopreservation. In our experiments, both Grayling and Pike extenders showed a similar efficienc . However, Pike extender successfully eliminated the sperm agglutination following thawing.

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EFFECTS OF NOVEL INGREDIENTS ON GROWTH PERFORMANCE IN EUROPEAN SEA BASS, *Dicentrarchus labrax*

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Introduction

Limited availability of ingredients in aquaculture feeds is crucial in order to maintain the increasing demands of aquaculture industry Gamboa-Delgado & Márquez-Reyes 2018). However, to safeguard sustainable exploitation of natural resources, the use of capture fisheries-based fishmeal and fish oil needs to be reduced in conventional fish feeds (Tacon & Metian, 2015). Accordingly sources with high quality protein and essential nutrients are imperative need otherwise fish performance (Kousoulaki et al., 2012), health status and final product quality (Kousoulaki et al., 2016) may be jeopardized when substituting dietary fish meal by alternative ingredients of lower nutritional value. The main objective of this study is to test ingredients and design formulations for commercially relevant tailored-made aqua feeds, ensuring high performance, maintaining or enhancing nutritional value and environmental friendliness.

Materials and methods

Ten experimental feed mixes were prepared (Table I). The levels of conventional and trimmings fish meal and oil and that of the novel feed ingredients, balanced to same proximate composition, EPA+DHA and total phospholipid content with rapeseed or fish oil, soy lecithin, wheat and wheat gluten, were predetermined by a chosen range in the applied three component mixture design. Juvenile European sea bass of an initial average body weight of 5.72±0.72g were fed the experimental diets. Fish were weighted individually at the beginning and end of the experimental trial under mid anesthesia with clove oil. Fish were fed *at libitum* twice a day. Mortalities and feed consumption were recorded daily in order to be able to evaluate accurately values for feed utilization (FCR, SGR, % daily feed consumption).

Results and Discussion

The results so far show that there is a significantly positive effect in sea bass performance of moderate dietary inclusion of innovative ingredients in the presence of minimum 10% conventional fish meal and 2.7% conventional fish oil. The inclusion of fish trimmings at any level had negative effect on fish performance. In the absence of any kind of fish meal, and the highest inclusion level of bacterial, yeast, microalgae and sunflower meal, feed intake was lowest (Figure 1- able II).

Acknowledgement

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Figure 1. Preliminary mixture models (R^2>0.87, p<0.01) for SGR (a), feed intake (b) and FCR (c).

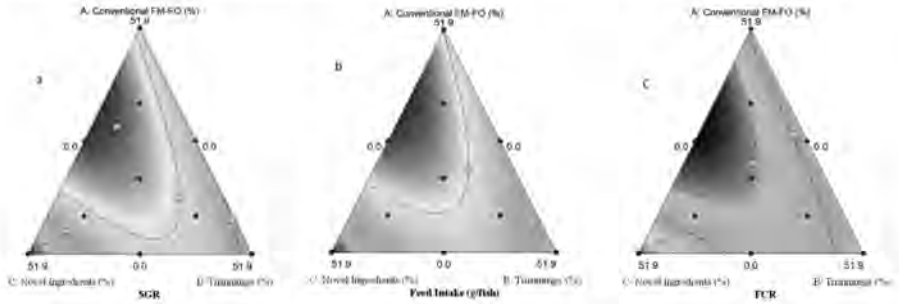

Table I. Diet formulation and nutrient composition of the experimental diets %.

Diets	1	2	3	4	5	6	7	8	9	10
Fish Meal	-	-	-	10.0	20.0	10.0	6.7	3.3	13.3	3.33
Fish Oil	-	-	-	2.7	5.5	2.7	1.8	0.9	3.6	0.9
Trimmings FM	20.0	10.0	-	-	-	10.0	6.7	3.3	3.3	13.3
Trimmings FO	5.50	7.90	10.3	5.15	-	2.75	5.27	7.78	2.63	5.38
Bacterial protein	-	7.00	14.0	7.0	-	-	4.7	9.3	2.3	2.3
Yeast meal	-	3.00	6.00	3.00	-	-	2.0	4.0	1.0	1.0
Microalgae	-	3.7	7.5	3.7	-	-	2.5	5.0	1.2	1.2
Sunflower meal	-	6.4	11.6	6.40	-	-	4.2	8.5	2.1	2.1
Wheat	19.3	9.6	-	9.64	19.3	19.3	13.2	7.1	15.5	15.7
Soya lecithin	1.3	1.9	2.6	1.9	1.3	1.3	1.7	2.1	1.5	1.5
Rapeseed oil	9.5	5.5	1.6	5.5	9.5	9.5	6.8	4.2	8.1	8.1
Wheat gluten	13.5	11.7	10.0	11.7	13.5	13.5	12.3	11.1	12.9	12.9
SPC	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0
Maize gluten	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5

Table II. Growth performance parameters and feed utilization.

Diets	1	2	3	4	5	6	7	8	9	10
Initial Weight	5.53	5.49	5.94	5.78	5.75	5.69	5.83	5.73	5.45	5.73
Final Weight	14.9	16.2	13.8	19.0	16.7	16.2	17.7	17.2	16.8	16.7
Weight increase	9.4	10.7	7.87	13.3	11.0	10.5	11.9	11.5	11.4	10.9
FCR	0.94	0.89	1.00	0.81	0.93	0.88	0.85	0.86	0.86	0.8
SGR	2.55	2.79	2.16	3.06	2.74	2.69	2.86	2.82	2.90	2.7
% Consumption	2.3	2.3	2.1	2.3	2.4	2.2	2.3	2.3	2.3	2.3

EFFECTS OF DIFFERENT DIETS AND INCORPORATION OF PROBIOTICS ON THE SKIN MORPHOLOGY OF ATLANTIC SALMON (*Salmo salar*)

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Introduction

Commercial salmon feeds used in Norwegian aquaculture industry are based on a mixture of plant and marine ingredients. Over the last decades the proportion of the plant ingredients in the feeds have increased while marine ingredients decreased. The current ratio of plant : marine ingredient composition is 70:30 (Ytrestøyl et al., 2015). Plant proteins used in the feeds are derived from protein concentrates, usually with reduced content of antinutritional factors (ANFs). Nevertheless, amino acid imbalance and presence of ANFs are still a concern of the aquafeed industry. Furthermore, plant lipids have an unfavorable ratio of n-3:n-6 and does not provide the recommended EPA and DHA to the fish. Hence, ingredient composition of feed may have an effect on the health of the fish (Torrecillas et al., 2015). In addition, dietary administration of probiotics can influence the mucosal immune system of the fish (Hoseinifar et al., 2015). Skin, which is the largest first line defense organ of the fish, plays a key role in protecting the fish from the surrounding environment which is rife with opportunistic pathogens. Therefore, skin mucous morphology (Vatsos et al., 2010) which is considered as an indicator of fish s skin health, may be affected by several environmental factors including those linked to feeds. This study investigated the histo-morphological alterations of the skin of Atlantic salmon (*Salmo salar*) fed different diets with or without probiotics.

Material and methods

A feeding experiment was conducted with Atlantic salmon (mean weight of 146.97 ± 4.9g SD). The fish were fed three types of feeds with different basal diets with or without probiotics. The ingredient composition of the diets were, Diet 1: fish meal/ fish oil based, Diet 2: a commercial like diet dominated by plant ingredients (plant : marine is 70:30), and Diet 3: a fish meal/ fish oil based diet in which soybean meal replaced 20% of the fish meal. Dietary probiotic was cultured in the laboratory and vacuum coated on the diets. Dorsal skin samples were collected from 12 fish (mean weight 201.4 ± 37.8g SD) per treatment and fixed in 4% formalin. Samples were decalcified with 10% formic acid for 5 hours, prior to processing. After paraffin embedding, tissue sections of 4µm were prepared and stained with Hematoxylin and Eosin (H&E) and Alcian Blue – Periodic Acid Schiff (AB-PAS). Images (n= 9/ fish, N=108/ diet) from different locations of skin were acquired using light microscope. Quantitative analysis of skin morphology was performed; average area of mucous cells (AAM), ratio between total area of mucous cells and total area of epithelium (TAM/ TAE), and number of mucous cells per epithelium (M/E) were determined. The analysis was done using Image J (1.52a).

Results and discussion

The results did not reveal any significant differences in the average area of the mucous cells (AAM), and ratio between total area of mucous cells and total area of epithelium (TAM/TAE). However, the number of mucous cells per epithelium (M/E) was significantly influenced by diet and probiotics. Fish fed Diet 2 and 3 had significantly more M/E compared to those fed Diet 1. Addition of probiotics to Diet 1 increased the M/E, an indication of a strengthened skin barrier.

Conclusion

The results of this study revealed that the ingredient composition of diets directly influence the number of mucous cells per epithelium. Dietary administration of probiotics increased the number of mucous producing cells per epithelium, and this increase may have improved the barrier function of the skin of Atlantic salmon.

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NON-IONIZED AMMONIA ASSESSMENT MODEL IN *Chirostoma estor estor* INTENSIVE CULTURE USING ARTIFICIAL NEURAL NETWORKS

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Introduction

The *Chirostoma estor estor*, commonly known as white fish, is an endemic species of great importance in the area of Lake Patzcuaro, located in the state of Michoacan, Mexico (Llorente & Ocegueda, 2008). This represents a large income in the local economy, unfortunately it is currently in danger of extinction due to the pollution of the lake and its excessive fishing (Guevara et al., 2015). National institutions, through aquaculture farming, have made efforts in the conservation of this type of fish, however there is still a lack of penetration of the scientific sector in this problem (INAPESCA, 2016). This paper presents a computational model for the evaluation of non-ionized ammonium which is highly toxic and of vital importance in aquaculture farming systems. Using an Artificial Neural Network (ANN), a relationship is established between non-ionized ammonium (NIA) and parameters such as pH, temperature and total ammonia (TAN). Data bases obtained from measurements in culture ponds and generated by similar models have been proposed to generate efficient RN training.

Materials and methods

The computational model is divided into four stages (figure 1), in the first a database was simulated using the model proposed by Kennet et al., (1975). Which made a table at different temperatures at different pH concentrations, calculating the percentage of NIA for each case, this database, together with the measurements obtained in culture ponds, were used in the training of the ANN. In the second stage it has a pre-processing so that the efficiency of the ANN is not diminished by a poor selection of patterns (Principe et al., 2000, Carbajal et al., 2017). The third stage consists of an ANN whose topology is given by the expression , which indicates that it consists of two inputs, a number of hidden layers with neurons in each hidden layer and one output. Finally, in the last stage the value of NIA is obtained.

Results

The lowest MSE was the criterion for the choice of ANN configuration, both in the training set and the test set, and this was obtained with the topology , which indicates that it consists of only two hidden layers and twenty neurons in each of them. The results obtained by the training of the RNA show a learning rate of 99.91% of success in the approach. In the visualization of these results it is important to underline that each parameter (temperature and pH) individually affects differently the behavior of the NIA, for which the evaluation of the RNA for each of these was carried out individually (figure 2)

Discussion and conclusion

In this work, a new computational model has been designed to evaluate the non-ionized ammonium in white fish culture systems based on artificial neural networks. Given that there are currently no systems, sensors or methods to calculate this important and toxic parameter, this work represents a great innovation in the aquaculture area. Taking into account the factor of 99.91% accuracy, it points to this system as a viable, accurate and economical option in obtaining the NIA, calculating it from two parameters on which it depends enormously and which are easy to obtain: temperature, and the pH. It is of great importance to point out that the system can be considered as a full-fledged virtual sensor, since it is enough to obtain values from the aforementioned two parameters, introduce them to the artificial neural network already trained and this will deliver an accurate value of NIA. Finally, it is proposed for future work the implementation of this computational model, in field measurements to obtain more results of this toxic paramete .

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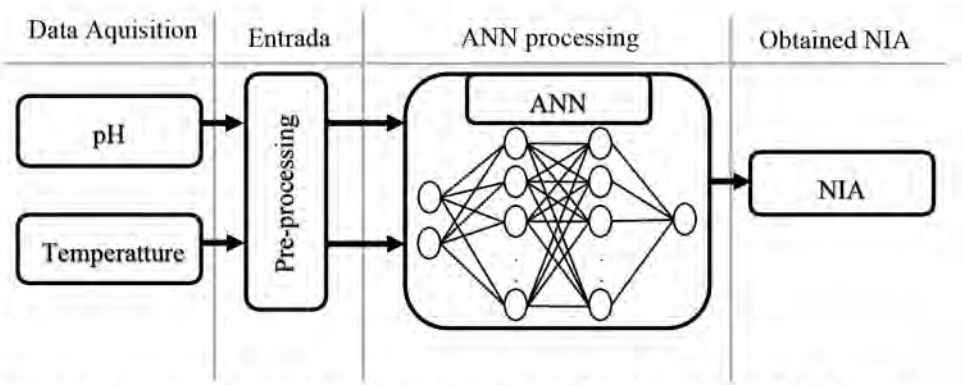

Fig. 1. Computational model for the evaluation of the NIA based on artificial neural networks.

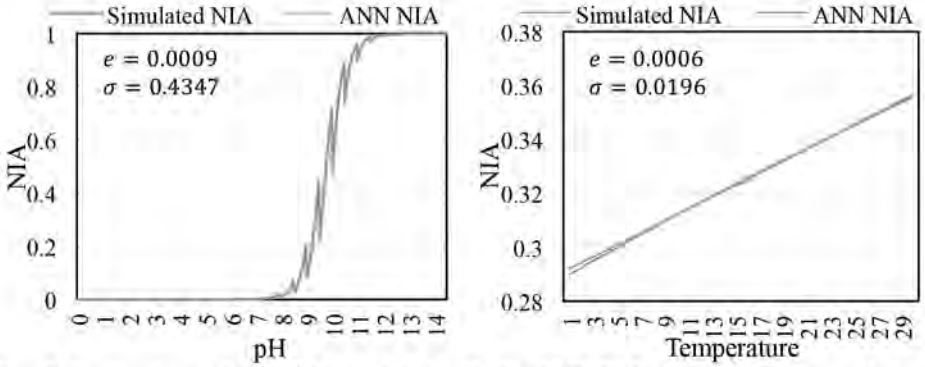

Fig. 2. Comparison of the NIA simulated with the ANN NIA, for pH and temperature.

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LEVELS OF BIOACTIVE COMPOUNDS IN *Ulva* spp. GROWN IN DIFFERENT INTEGRATED SYSTEMS

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Introduction

Ulva spp. grow in sheltered, eutrophic coastal waters, covering large areas and being sometimes considered a nuisance. However, they have valuable bioremediation capabilities that, associated with the fast growth rate, provide important ecosystem services mainly in respect to waste nutrient impact mitigation (Buschmann et al., 2017). Other very important *Ulva* properties are associated to its biochemical composition characterized by several compounds that can be used in fields like medicine (antioxidant, anti-inflammator , anti-fungal, and anti-cancer), nutraceuticals, animal feed, and bio-material construction (e.g. bioplastic) (Holdt and Kraan et al., 2011; Helmes et al., 2018). Their cultivation efficiency in closed systems (Lamprianidou et al. 2015, Neveux et al. 2017) and efficient nutrient uptake (particularly ammonium) makes them valuable macroalgae for integrated multitrophic aquaculture (IMTA). The present work used two different water sources with different nutrient concentration and two different IMTA production systems (semi-intensive and extensive) to study the temporal and spatial effect of nutrient concentration and production system on the productivity, nutrient uptake, and bioactive properties of *Ulva* spp. biomass.

Materials and Methods

Ulva spp. biomass was cultured in 0.7 m^3 floating cages (extensive production) placed at a reservoir (R) and settling pond (SP) of an aquaculture research station. In order to compare the efficiency of these floating cages against raceway tanks for *Ulva* semi-intensive production, water from the SP was also pumped into 6.3 m^3 fibre glass raceway tanks (K) coupled with bottom centred aeration. Growth, nutrient uptake, proximate composition, polyphenol content, antioxidant and anti-inflam atory activity were estimated in *Ulva* from the floating cages and raceways. Water environmental parameters (temperature, dissolved oxygen, turbidity, salinity and irradiation) were daily recorded. *Ulva* samples grown from 30/01/2019 to 06/02/2019 (T1), 06/02 to 13/02/2019 (T2), 27/03 to 03/04//2019 (T3) and 03/04 to 11/04 /2019 (T4), were analysed as representative of Winter and early Spring growth conditions. T1 and T2 culture were kept initially at 1.0 kg/m^3 with 50% day^{-1} intermittent water renewal in the raceways, whereas in T3 and T4, were at 0.5 kg/m^3 with 100% day^{-1} continuous water renewal. Ferric Ion Reducing Antioxidant Power (FRAP) technique was applied to *Ulva* spp. biomass in accordance to Benzie and Strain (1996).

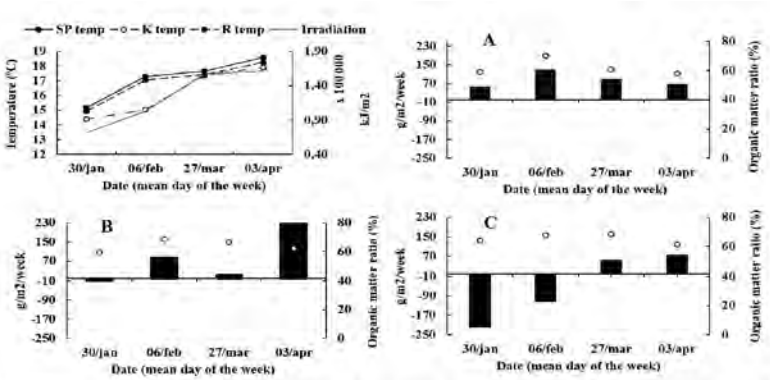

Fig. 1. Mean *Ulva* productivity during the sampling period: A – settling pond (SP); B – raceways (K); C – reservoir (R). Circles correspond to organic matter (%) and columns to growth (g/m^2/week). Top left: Left axis: Temperature; Right axis: irradiation during the sampling period.

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Results and Discussion

Results show higher growth rate in early Spring than in Winter, which is probably related to seasonal temperature and irradiation increase (Fig. 1, top left). Differences in growth were observed between settling pond and reservoir at T1 and T2 ($p < 0.05$) and within T1, T2 and T4 in the raceways ($p < 0.05$). A correlation was observed between growth and organic matter percentage on SP samples (R^2: 0.98). The FRAP antioxidant power of the aqueous *Ulva* extracts was correlated with temperature, radiation and growth (R^2: 0.92; R^2: 0.94; R^2: 0.95, respectively) in the reservoir samples.

The analysis of bioactive compounds in the samples from SP and K is still under way and the results will be presented in the poster. Though there is still scarce information on the spatial and temporal influence on bioactivities and composition of *Ulva* spp., it is expected to observe differences in some aspects of the chemical composition like the polyphenol content and trace metals between the systems and time as indicated in the literature (Helmes et al., 2018; Roleda et al., 2018).

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ADVANCED SELECTIVE BREEDING FOR DISEASE RESISTANCE IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Introduction

Aquaculture industry is expected to fulfill an increased demand of fish for human consumption, and challenges in long term sustainability for this industry, such as diseases outbreaks, water pollution and global warming, need to be met. Viral nervous necrosis (VNN) also known as viral encephalopathy and retinopathy (VER) is one of the main infectious diseases affecting marine aquaculture. Caused by a *Betanodavirus* of the family *Nodaviridae* (OIE, 2016), VNN is endemic in the Mediterranean and affects wild and farmed populations of European sea bass (*Dicentrarchus labrax*), which is one of the most valuable farmed Mediterranean fish with aquaculture production valued at approx. 1.1 billion USD during 2017 (FAO, 2019). Despite the approval of a commercial vaccine to protect sea bass against the most common genotype of VNN in the Mediterranean, its use on mass scale is still limited due to its cost as single vaccine, technical and logistic problems presented when combining with other vaccinations against Vibriosis and pasteurellosis. In addition, global warming trend and temperature dependent nature of VNN remains a treat for the sustainable production of this species (Costa and Thompson, 2016)the causative agent of viral encephalopathy and retinopathy (VER. Selective breeding has been proved to be an effective tool to accumulate genetic gain on a desired trait such as disease resistance (Gjedrem and Rye, 2018) , and the use of new tools for marker assisted selection (MAS) have provided ways to implement individual selection in disease resistance traits leading to higher genetic gains compared to more traditional methods (Dekkers, 2007).

In our work, we present results of a challenge test against RGNNV in a commercial European sea bass breeding program performed during two consecutive years. In addition, tissue samples of challenged fish were sent to genotype on high-density SNP array specific to the species. Results of accuracy using traditional and MAS methods will be presented including expected genetic gains.

Material and Methods

A challenge test was performed during two consecutive years on a captive population of Sea bass under selection. During the year 2017, approx. 30 individuals from each of 89 families were grown separately till individual tagging at body weight 11g, and sent to be challenged, using a sea bass RGNNV isolate, virus was isolated from a commercial fish farm outbreak in 2013, via intramuscular injection. Average weight of the fish challenged was of 15g. Mortalities were recorded every 4 hours during 28 days after infection. Same procedure was followed during 2018 where ~30 individuals from 92 families were sent to be challenged against VNN with an average weight at challenge of 25.4g. In addition, prior to challenge, tissue samples from each individual fish was taken and preserve in 95% ethanol and stored at -4°C

After finalizing the second challenge test, a subsample of tissue from dead and survival fish of 30 and 32 families from challenge test 2017 and 2018 respectively was sent to genotype using a 57K SNP array specific for this species.

Genetic parameters were estimated for survival at end of test using BLUP methodology. In addition, a GWAS analysis was performed for each marker including the marker as random effect and the polygenic effect using a relationship matrix constructed with all markers except for the marker under test.

Finally, accuracy of EBVs and GEBVs were tested using fivefold cross validation. Expected genetic gains using each of the methods were estimated.

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Table I: Genetic parameters for survival at end of VNN challenge test

Dataset	Animal linear model		Threshold sire-dam model	
	h^2	c^2	h^2	c^2
Year 2017	0.15±0.04	0.00±0.00	0.21±0.04	0.00±0.00
Year 2018	0.15±0.06	0.02±0.02	0.20±0.08	0.03±0.03
Year 2017+2018	0.18±0.03	0.00±0.00	0.24±0.03	0.00±0.00

Table II: Genetic parameters and predictive ability of end of VNN challenge test using GBLUP

	N	Heritability	Predictive Ability
GBLUP (Gmat)	1795	0.17±0.03	0.26
GBLUP (Hmat)	4851	0.18±0.02	0.27

Results

Mortalities started to appear during the first and second day after infection in 2017 and 2018, respectively, reaching a peak during day 5 for both challenge tests. Mortalities ceased before termination of trial approx. on day 20 after challenge, when accumulated mortality had reached respective 58.2 and 58.8% in 2017 and 2018.

Genetic parameters estimated based on traditional pedigree evaluations are presented in Table I including the effect of common rearing of full-sibs prior tagging (c^2). Results for genomic BLUP (GBLUP) and predictive ability from five-fold cross validation are presented in Table II.

Additional, expected genetic gains of selection candidates using either traditional or MAS methods will be presented.

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MICROALGAE *(Isochrysis galbana)* IN DIETS FOR JUVENILE AMBERJACK (*Seriola dumerili,* Risso 1810); EFFECTS OF ON GROWTH, COLOR PERFORMANCE, BODY COMPOSITION AND LIVER MORPHOLOPHY

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Introduction

Microalgae are increasingly being used in aquaculture feed for marine organisms, since they are a natural source of protein, essential polyunsaturated fatty acids, pigments, antioxidants and other bioactive compounds, which give them apart from nutritional also functional properties (Spolaore *et al*., 2006). In this contest, the *Isochrysis* sp. combines medium-high level and quality of protein with high lipid and DHA contents (Sánchez *et al.,* 2000). *Seriola dumerili* (Risso 1810), has a great potential for the global industry and in order to the diversification of aquaculture owing to its rapid-growing and adaptive characteristics (Mazzola *et al*., 2000). Although some research belongs to nutritional aspects are reported in the last years, there is a lack of functional ingredient knowledge for this species. Therefore, in this study the growth performance, biometry, and colour parameters, proximal composition and liver morphology of amberjack fed increased levels of a freeze-dried biomass of *Isochrysis* sp. were determined.

Materials and methods

-*Feeding conditions:* Four diets with 4 levels of *Isochrysis galbana* were formulated (CONTROL 0%, ISO 3%, ISO 5% and ISO 10%). Thirty amberjack juveniles, having a mean initial body weight of 5.53±1.08 g, were randomly allocated in triplicate groups in 150L fibreglass tanks. Fish were fed the experimental diets to apparent satiation (five times a day) for 30 days. Water temperature and dissolved oxygen were monitored.

-*Analysed parameters:* Growth and biometric was recorded in all fish along the trial. At initial and final samplings, 12 fish were sacrificed and immediately subjected to individual skin colour measurements. All specimens were then subjected to biometry measurements in order to evaluate yield individual. Finally, the objective tissues were used for biochemical and histological analysis.

Results and discussion

Water temperature and dissolved oxygen measured along the trial were 23.3±0.67 °C and 5.55 ± 0.34 mg l^{-1}, respectively. Lowest growth values were found for those fish fed 10% ISO diet, with 5% ISO responses close to control diet ($P<0.05$, table 1). In the case of the specific growth rate (SGR) feeding up to 5% ISO give equal responses respect to the control diet being the smaller only for 10% ISO SGR ($P <0.05$, table 1). Regarding feed ingestion ratio (FIR), a trend for lowering values was observed as ISO increase in the diets with significan ly lower intake for those fish fed 10%ISO respect to 3ISO and 5%ISO. Accordingly, feed conversion responses (FCR) were in parallel to intake in growth, except for the 5%ISO which shows similar growth and FCR responses respect to the control diets, although with significantly lower feed intake ($P>0.05$, table 1).

Regarding skin colour, all parameters were significantly affected by feeding ISO respect to CONTROL (table 2). Higher lightness (L*) was observed in fish fed the control diet, following the 5%ISO treatment ($P < 0.05$). Relative to controls, there was a marked increase in the a* (redness), b* (yellowness) and colour saturation (Chroma) in fish fed the ISO diets ($P < 0.05$), with the highest values in 10% ISO fish $P < 0.05$).

(Continued on next page)

Table 1: Growth performance and feed utilization of juvenile amberjack fed test diets for 30 days

	Diets				
	Control	**3% ISO**	**5% ISO**	**10% ISO**	**Sign**
Initial weight (g)	5.42±0.24	5.60±0.14	5.55±0.26	5.53±0.10	NS
Final weight (g)	12.95^{a} ± 0.62	11.07^{b} ± 0.77	11.74^{ab} ± 0.86	8.99^{c} ± 0.39	**ANOVA
Final survival (%)	87.78 ± 5.29	80±3.79	86.67±3.51	74.44 ± 3.00	NS
SGR	2.90^{a} ±0.07	2.26^{a} ±0.28	2.49^{a} ±0.21	1.62^{b} ±0.20	**ANOVA
FCR	1.53^{b} ±0.15	1.94^{a} ±0.23	1.60^{ab} ±0.24	2.03^{a} ±0.36	**ANOVA
FIR	3.54^{a} ±0.28	3.21^{ab} ±0.14	2.99^{b} ±0.07	2.12^{c} ±0.1	**ANOVA

Values represent mean ± SE. Means followed by the same superscript do not differ at $P < 0.05$
The data with **ANOVA are significantly different (P<0.05)
Specific growth rate (% day^{-1}), SGR 1/4 ln (final weight/initial weight)/days
Feed conversion ratio, FCR 1/4 feed consumption (g)/biomass gain (g).
Feed intake ratio (% bw), FIR 1/4 100 · feed consumption (g)/average biomass (g) · days.

Table 2: Colour parameters (L*, a*, b*, C*, H*) in the skin dorsal regions of yellowtail fed the test diets over 30 days

	Diets			
	Control	**3% ISO**	**5% ISO**	**10% ISO**
L*	75.60^{a}	62.87^{b}	69.75^{a}	61.74^{b}
a*	-3.43^{c}	0.15^{b}	0.18^{b}	1.73^{a}
b*	10.46^{c}	29.31^{b}	31.68^{a}	32.2^{a}
Chroma	65.92^{c}	446.46^{b}	517.79^{a}	529.47^{a}
Hue	-1.21^{c}	0.31^{b}	-0.10^{d}	1.31^{a}

All results obtained will be presented and discussed.

Acknowledgments

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DEVELOPMENT OF A PRACTICAL REPETITIVE STRESS CHALLENGE MODEL FOR SEAWATER ATLANTIC SALMON (*Salmo salar*)

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Increased reliance on non-medicinal health interventions has unequivocal benefits but may impose frequent exposure to severe stressors susceptible to chronically compromise health and performance. Developing strategies that mitigate physiological stress and bolster mucosal robustness is therefore critical to support non-medicinal treatments and practices. The study benchmarked the impact of a repetitive exposure to severe stressors on the oxidative, immune and mucosal status of the stock under controlled conditions. The aim was to obtain a commercially relevant repetitive-stress challenge model that goes beyond the primary response to a single stressor and allows exploring mitigation measures.

A 10-week, triplicated tank-based trial was performed using 500 g Atlantic salmon (37 fish / tank). Following a 5-week resting period, the "repeated-stress" (RS) group was crowded and netted-out at weekly interval for immersion into 2 freshwater bath (1h) prior a hydrogen peroxide bath (H_2O_2; 30min; 1,500 mg/L) then given a 2-week recovery period. The "single-stress" (SS) group was exposed to the H_2O_2 bath only. Sampling was performed at the end of the resting-period (T_1); prior to H_2O_2 exposure (T_2), 1 h after (T_3), 48 h after (T_4) and 2-week after (T_5, trial end) H_2O_2 exposure. A range of biological parameters were investigated targeting growth, feed performance, stress response, anti-oxidant status, mucosal integrity, innate immune parameters and plasma biochemistry.

Prior exposure to repetitive stressors significantly elevated chronic plasma cortisol level (T_2, Fig 1) but lessened the acute primary response to an immediate stressor (T_3, Fig 1). The antioxidant status appeared significantly altered with, e.g., a depletion of super-oxide dismutase (SOD) at circulating (Fig 2) and skin level (Fig 3). Some innate immune defenses appeared damped down by RS based on blood and skin mucus biochemistry. Growth was not statistically affected but feed intake tended to be lower in the RS group. The other parameters assayed will be discussed including mucosal integrity and molecular work.

The study describes the effect of repetitive stressors on salmon physiology and suggests that prior RS exposure reduce the capacity of the stock to cope with a subsequent intervention or health challenge. A "repeated-stress" challenge model is proposed along with selected biomarkers towards the testing of improved practices and mitigation solutions.

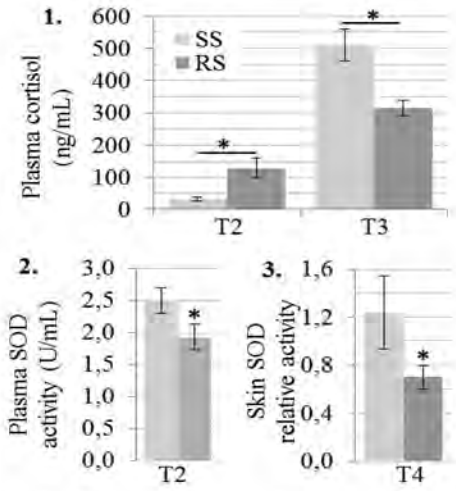

Fig 1. Plasma cortisol; **2.** plasma SOD activity at T_5 and **3.** skin SOD activity at T_4

QUANTITATIVE CHARACTERIZATION OF THE RAINBOW TROUT *(Oncorhynchus mykiss)* INTESTINAL EPITHELIUM IN RESPONSE TO DIET CHANGES DURING THE FIRST YEAR OF DEVELOPMENT

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Introduction

Intestinal epithelium is characterized by a rapid cell turnover to ensure an effective barrier function in the harsh intestinal environment allowing, at the same time, the absorption of all the necessary nutritional principles (Barker et al., 2012). This implies a constant adaptation not only in response to pathological situations but also to less stressful stimuli like those induced by diet changes. Whereas qualitative changes have been well characterized in the past, quantitative measurements are not so widely available in aquaculture species. Furthermore, limited information is available on the mechanisms at play. In particular intestinal stem cells (ISC) have been characterised only in Medaka (Oryzias latipes, Aghaallaei et al., 2016). Aim of this work was to quantify the changes taking place in the rainbow trout intestinal epithelium in response to growth and diet changes and to broaden the array of molecular tools required for the characterization of ISC and their cell niche. The data will help to define more accurately even subtle changes in response to different diets and, when specific tools will be fully developed, to widen our understanding of the cellular and molecular mechanisms.

Materials and methods

Segments of anterior intestine were collected from 5 rainbow trout of 7, 10 and 12 months weighing approximatively 50, 150 and 500 g, respectively. These stages correspond to stepwise increase of digestible energy: 18 MJ/kg up to 50 g, 18.5 MJ/kg from 50 to 150 g and 19 MJ/kg up to 500 gr (Optiline, Skretting). After fixation, dehydration and paraffin processing, sections were stained with hematoxylin and eosin (HE) for morphological investigations and stereological quantifications; periodic acid-Schiff (PAS)-Alcian Blue (AB) for the characterization of goblet cell mucus content; phloxine tartrazine stain for the identification of Paneth cells; immunohistochemical staining of Proliferative Cell Nuclear Antigen (PCNA) for the localization of proliferating cells; endogenous Alkaline Phosphatase (AP) activity for the determination of fully differentiated enterocytes in the epithelium. Villi height and villi width were measured with NanoZoomer Digital Pathology 2.7.2 software. Transverse sections stained with HE were used to estimate the volume density of each intestinal layer using a point-count stereological grid following the Delesse principle. Expression of LGR5 and SOX9, well-known intestinal stem cell markers, was investigated by qualitative PCR using sequences annotated in ncbi.nlm.nih.gov/gene/.

Results

Given the wide variation among their length, villi were arbitrarily divided in two categories: below or above 400 µm. Short villi were longest at 150 g while long villi were significantly longer and more branched at 500 g (Table I).

Table I: Proximal intestine villi morphometry

	Proximal intestine			
Trout weight	Short villi length (µm)	Long villi length (µm)	Villi width (µm)	Villi branching
50g	$251.0^{a} \pm 6.0$	$469.2^{a} \pm 30.5$	$94.3^{a} \pm 5.5$	-
150g	$310.3^{b} \pm 19.9$	$555.8^{a} \pm 28.7$	$122.2^{b} \pm 3.9$	+
500g	$245.6^{a} \pm 16.9$	$660.3^{b} \pm 21.4$	$114.7^{b} \pm 6.2$	++

$^{a-b}$ Different superscripts in the same column indicate significant differences ($P<0.05$). Values are expressed as means ± SEM.

Table II: Histochemical characterization of Goblet cells mucus composition.

	Goblet cells mucus composition		
Trout weight	Neutral	Acid	Combination of neutral and acid
50g	$20.8^{a} \pm 3.4$	$79.2^{a} \pm 3.4$	-
150g	$6.0^{b} \pm 1.3$	$94.0^{b} \pm 1.3$	-
500g	$7.2^{b} \pm 0.9$	$92.7^{b} \pm 0.9$	-

$^{a-b}$ Different superscripts in the same column indicate significant differences ($P<0.05$). Values are expressed as means ± SEM

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Stereological evaluation showed that mucosa is the predominant layer of the proximal intestine and the epithelium:lamina propria ratio remained unchanged along development. PAS/AB stain indicated that goblet cells contain either neutral (bright magenta) or acid (blue) mucus, whereas no cells containing a mixture of the two were observed. At 150 g we observed a sharp decrease of neutral mucus containing cells (Table II). Phloxine tartrazine positive cells were detected within the upper portion of villi epithelium, in the lamina propria and in the submucosa, irrespectively of the fish age. Fully differentiated enterocytes were localized in the upper part of the villi and did not superimpose with the proliferative compartment localised in the bottom part and $PCNA^{+}$. Both LGR5 and SOX9 were expressed in the intestine at all stages of development.

Discussion and conclusion

In this study, we provide a detailed quantitative analysis of rainbow trout intestinal mucosa morphology during the first year of development. Dividing villi in two populations, based on their length, enabled the identification of changes along development that otherwise would have been unnoticed. Since the two types of villi change in an independent way during growth and in response to the changes of diet, they may play a different function. Furthermore, long villi became progressively more branched from 150 g and, at the same time, we observed a sharp increase of goblet cells containing acid mucus. Whereas in mammals these changes have been associated with alterations in the intestinal homeostasis, we observed a constant epithelium:lamina propria ratio that indicates a healthy intestinal tract. This suggests that diet changes taking place in this experiment, induced some stress, possibly due to the increased lipid content, but did not lead to a pathological state. Phloxine tartrazine positive cells in the submucosa were previously described also in the Atlantic salmon (Sveinbjornsson et al., 1996), but their localization within the upper intestinal epithelium and in the lamina propria was not described before. However, none of these locations is expected for *bona fidae* Paneth cells. Finally, the expression of LGR5 and SOX9 indicate that these ISC markers can be used also in rainbow trout. However, due to the presence of many paralogs further work is necessary to define which ones are more relevant in the intestine.

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QUANTIFICATION OF STRESS AND DEVELOPMENTAL KEY GENES DURING EARLY ONTOGENESIS OF PIKEPERCH (*Sander lucioperca*)

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Introduction

In recent years, efforts have been made to establish pikeperch (*Sander lucioperca* L. 1758) as a farmed fish in recirculation aquaculture systems (RAS) in European aquaculture. However, the development is still in its initial stages and is accompanied by high losses in the early ontogenesis, especially through the conversion to exogenous feeding and intensified cannibalism. Furthermore, pikeperch is considered a highly stress-susceptible species (Baekelandt et al., 2018). The choice of optimal husbandry conditions, including central factors such as water temperature, at least for juvenile fish, is more focused on economic considerations than on welfare concerns. For instance, optimal growth rates in juveniles have been reported at temperatures up to 28°C (Frisk et al., 2012; Rónyai and Csengeri, 2008). Here, energy allocation to maintain homeostasis is optimal, allowing maximal energy to be allocated to growth. But, in contrast to the established salmonid aquaculture with a selection of trait-based breeding lines, so far, there are no accompanying genetic studies on development and stress physiology in pikeperch that could enable and support marker-based breeding and the instant querying of their welfare status.

Using temperature changes from 15°C to 25°C, we have investigated a set of genes for the purpose of selecting an informative panel of inducible genes that will contribute to the establishment of a multiplex assay for stress detection in finfis aquaculture. Moreover, we aimed to examine the general expression patterns of key genes during the early ontogenesis of pikeperch.

Material & Methods

All fish used in this study were reared at the pikeperch facility of the Mecklenburg-Vorpommern Research Centre for Agriculture and Fisheries (Hohen Wangelin, Germany). For analyses of gene expression pattern during pikeperch development, we sampled fertilized eggs, yolk sack larvae (4dph), larvae fed with artemia (7dph), larvae weaned on dry food (18dph), as well as liver tissue from 3- and 5-month-old fingerlings. For the temperature experiment, 8-week-old fish were transferred to the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (Berlin, Germany) and distributed randomly into 50-L freshwater tanks. After acclimation the water temperature was gradually elevated from 15°C to 25°C by 1°C on a daily basis. Every day, the gills and liver of 3 fish each were sampled.

Real-time qPCR gene expression analysis of a gene set (development: 23, temperature challenge; 38) was performed by using the Roche LightCycler96 or Biomark HD systems. In addition, for the temperature experiment liquid chromatography/ mass spectrometry was used to assess the individual levels of glucocorticoids.

Results

We created a gene-profiling panel for different developmental stages and the detection of stress in pikeperch. It included genes involved in development, metabolism, stress and immune response (heat-shock and acute-phase proteins, hypoxia-induced genes, stress hormones, adaptive immune system), as well as transcription factors. In addition, we evaluated suitable reference genes for transcriptional analysis in juvenile pikeperch illustrated in Fig. 1.

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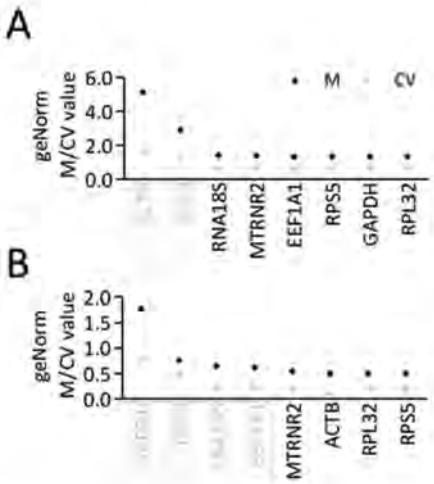

Fig. 1. **Evaluation of suitable reference genes for transcriptomic analyses in pikeperch.** Reference genes were evaluated for each particular set of samples from (**A**) liver and (**B**) gills based on the geNorm parameters M (black dots) and CV (grey dots). Reference genes that passed quality check are indicated by black gene symbols; others are indicated by grey gene symbols.

Discussion & Conclusions

In order to reduce the high losses in the early ontogenesis of pikeperch and to optimise their rearing conditions, it is necessary to gain basic knowledge of the physiology and underlying genetics of this species. On the one hand, our study provides basic expression patterns of development-specific marker genes in pikeperch. It also provides an extensive set of stress biomarkers for future assessment studies on the imbalanced homeostasis of the fish by means of molecular tools

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METABOLIC UTILIZATION OF DIETARY GLYCEROL BY RAINBOW TROUT (*Oncorhynchus mykiss*): A MULTI-TRIAL EVALUATION

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Background:
Glycerol is a simple compound, water-soluble, almost colourless, odourless, viscous, hygroscopic liquid that can be found in triglycerides, acting as a structural backbone. It is virtually nontoxic to human health and environment, when administered at physiological levels, and it is a by-product of biodiesel production. Once absorbed, glycerol can be metabolized as a glycolytic, gluconeogenic or lipogenic substrate for subsequent metabolism. This potential of glycerol to be used as a dietary alternative source of energy was already tested in several farmed terrestrial animals (pigs, poultry and bulls), but also fish species such as Nile tilapia, catfish and gilthead seabream. Rainbow trout (*Oncorhynchus mykiss*) is an important carnivorous fish species in aquaculture which is adapted to high levels of dietary protein, but with recent studies unravelling its capacity to utilizing dietary carbohydrates. We hypothesize that glycerol could effectively compete with dietary amino acids for gluconeogenic carbons thereby sparing their conversion to glucose/glycogen. This was evaluated by a series of sequential trials involving the evaluation of zootechnical parameters, growth performance and feed conversion efficienc , but also metabolic, metabolomic and enzymatic analysis.

Methods:
Three isoproteic, isolipid and isoenergetic diets were formulated while fulfilling the known nutritional requirements of the species (Sparos Lda., Loulé, Portugal). Briefl , a control diet with no glycerol (D0); and two experimental diets, supplemented with 2.5% (D2.5) or 5.0% (D5.0) glycerol (at the expense of cellulose) were tested.

Nine groups of 25 fish (average body weight 20.2 ± 0.1 g) were randomly distributed between 9 fibe glass tanks (volume 300 L, water flow rate: 120 L h^{-1}), in a flow-through freshwater system (15±1°C temperature; 6.7±0.1 pH; exposed to natural photoperiod). The diets were randomly assigned to 3 tanks each and fish were hand-fed twice daily until apparent satiety. At the end of 8 weeks, classical zootechnical parameters were evaluated. Fish were also sampled 6 and 24 h after last meal and sampled for plasma and liver tissue to evaluate the enzymatic regulation of the main metabolic pathways. The remaining 72 fish (24 per diet; 8 from each replicate tank) were kept in tanks with fresh water enriched with ~4% ^2H_2O, in a well-aerated recirculation system equipped with an external filtering unit and UV unit for another 6 days of feeding. Twenty-four hours after the last meal, animals were anesthetized, measured, weighed and sampled for several tissues. These were freeze-clamped in liquid nitrogen and kept at -80°C until further processing.

Results and Discussion:
Growth performance was overall unaffected by the dietary inclusion of glycerol after the 8-week feeding trial, although voluntary fed intake was significantly higher in trout fed a 5% glycerol diet. Liver glycogen and hepatosomatic index were affected by diet while plasma glucose, triglyceride and glycerol levels remained unchanged. Most genes coding for enzymes evaluated from the main metabolic pathways (glycolysis, gluconeogenesis and lipogenesis) responded significantly for post-prandial timing (6 vs. 24 h) but not for dietary glycerol. Incorporation of ^2H-labelling into several metabolites was evaluated by NMR. While sources of blood glucose remained unaltered, sources of hepatic glycogen significantly increased at the triose-phosphate level, indicating the utilization of dietary glycerol. Labelling of triacylglycerol in the liver, indicative of *de novo* lipogenesis was also unaltered by increased dietary glycerol. The same was observed in the muscle, where newly synthesised triacylglycerol was not significantly di ferent with glycerol supplementation.

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Conclusion:
Rainbow trout are able to efficiently utilize dietary glycerol without compromising growth and metabolic performance. Glycerol, which is considered a lipogenic substrate, did not enhance lipid accumulation, both in liver and muscle. This may represent a novel and economically viable strategy for minimizing the catabolism of dietary protein in farmed carnivorous fish

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PHOSPHORUS REMOVAL FROM RECIRCULATING AQUACULTURE SYSTEMS (RAS) EFFLUENT BY REACTIVE COLUMN FILTERS

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Introduction

RAS farms typically discharge wastewater streams containing increased concentrations of phosphorus (P)[1]. Drum filter and biofilter backwash water, tank sludge cones and various types of settling columns, swirl separators or radial settlers collecting sludge, are units emitting P. These wastewater streams have high solids concentration and thus contains most of the P not retained in fish growth. Another wastewater stream has low solids levels but contains most of the nitrogen not retained in fish growth. Albeit being the major nitrogen source, this stream is still high in P, and due to the stringent effluent regulation, RAS farms would benefit from further P removal. However, P is present mainly in soluble form, and cannot be efficiently removed by conventional coagulants and flocculants We report phosphorus removal of an up-flow packed bed reactor (PBR) filled with three reactive filter media; the calcium-silicate-hydrate Sorbulite, the calcium-silicate Polonite and, Polonite in combination with Vermiculite.

Material and methods

The column experiment was performed at RAS research systems of Luke's Laukaa fish farm, Finland, during 6 weeks from 24 April to 5 June. The pilot scale RAS run at 500 liters of replacement water per kg feed delivered the wastewater to the duplicate PBR's, of four treatments. Treatment 1 consisted of Sorbulite, treatment 2 of Polonite, treatment 3 of Sorbulite and Vermiculite (1:1 by volume) and, treatment 4 consisted of Polonite and Vermiculite (1:1 by volume). Each column was filled with approximately 6 of material. The Vermiculite was placed over the Polonite and Sorbulite materials.

Peristaltic pumps delivered wastewater to the columns with a continuous average flow of 9.1 mL/minute. Column effluent grab samples were taken one to three times per week and at the same time samples on the influent wastewater. Total-P and dissolved phosphate phosphorus (PO_4-P) concentration were analyzed using the SEAL Auto Analyzer (AA3) instrument.

Results and discussion

The results are shown in figure 1a and 1b. Total-P concentrations of the influent wastewater varied between 1.43 to 2.27 mg/L while PO_4-P concentrations ranged from 1.22 to 2.02 mg/L. The filter media Polonite and Polonite+Vermiculite efficiently removed Total-P and PO_4-P to concentrations of 50.8 µg/L and 9.4 µg/L respectively at the end of the experiment. The Sorbulite medium alone and with Vermiculite did not remove P very well. Column effluents had concentrations of Total-P as high as 1.93 mg/L.

The filter media Polonite has a well-documented efficiency to remove P from wastewater [e.g. 2] and in the case of fish farm wastewater it does not seem to be an exception. It should rather be an advantage since the used wastewater has a very low concentration of organic matter which can disturb the P sorption. Phosphorus breakthrough was not expected because of the short trial period. Complete breakthrough was not either shown for the Sorbulite medium, however the influent and effluent concentrations were close to each othe .

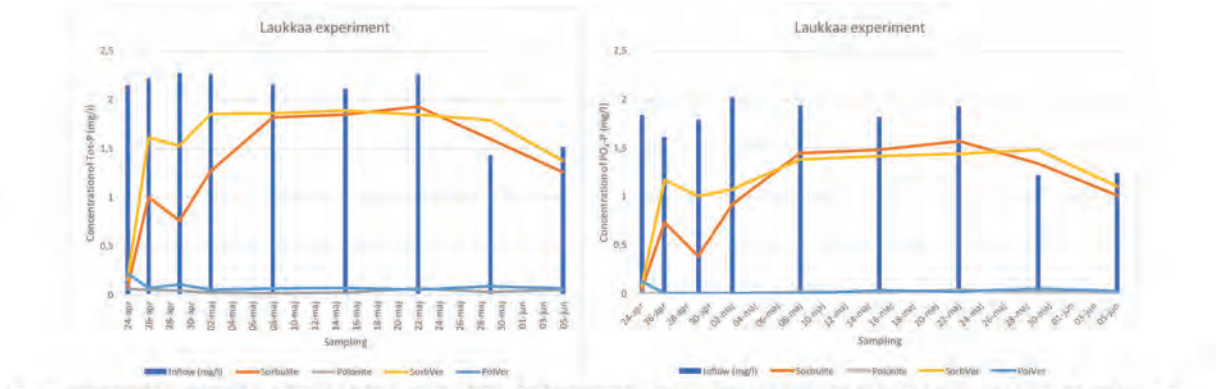

Figure 1: a) Removal of Total P and b) PO_4-P from fish-farm wastewater during a period of six weeks.

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Vermiculite was introduced in the filter system because of its known capacity to remove nitrogen (N). The removal data on N will be published elsewhere. The P removal efficiency of Vermiculite was probably very limited which is found from the results in figure 1a and 1b. Higher efficiency was expected by Sorbulite but this material has a long reaction time for P sorption and the retention time for wastewater in the columns was probably too short.

Conclusion

It is concluded that the reactive filter media Polonite is able to remove P from RAS effluent, where the P concentration is generally low, to reactive phosphate concentrations which can meet very stringent effluent criteria for discharge to sensitive water bodies. Sand beds after Polonite filters can be applied to reduce effluent Total-P concentration as small calcium particles with bound P leave the filters. A study with pilot-scale filter beds should be done to investigate the long-term performance, creating basis for cost estimations of a full-scale system.

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WATER QUALITY INDICATOR FOR *AQUACULTURE* IN INTENSIVE CULTURE USING A FUZZY ANALYTICAL HIERARCHY PROCESS

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Aquaculture is the activity focused on the culture of aquatic organisms for its consumption or for the preservation of a species that are endangered due to the high levels of pollution seen in its ecosystem and severe overfishing. A fuzzy analytical hierarchy model for assessing water condition (subsequently FAHP-WQI) from whitefish in intensive culture ponds was developed. FAHP-WQI combines analytic hierarchy process and fuzzy logic.

Temperature, pH, dissolved oxygen and ammonia were considered. Based on the measurements of them, FAHP-WQI obtained an indicator of regarding the water quality condition as a good or bad. It was tested and evaluated in intensive ponds. Additionally, individual weights are computed and allocated according to the parameter importance on the water evaluation.

Water Quality Modelling

n aquaculture, environmental parameters have some concentration limits, where low or high concentrations (depending of the parameter) that can be harmful for the organism. Following these behaviors, it is possible to implement a fuzzy hierarchical model considering that those limits and changes in the ecosystem can be used for determining when a concentration is good or bad for fish, and how the combination between them, affects the water quality stability in the habitat. This strategy will be helpful for detecting potential crisis of cultured ponds in order to prevent stress in the organism and reduce mortality rates. The computational model can be consulted in Fig. 1.

This was compared with similar models and the proposed index shows a good performance in real environments. Due to index proposed by National Sanitation Foundation (NSF) provides a good basis for the freshwater quality assessment because a weight is assigned to each parameter according to their importance. In Fig.2 is possible to appreciate that NSF shows greater quality, the origin of this difference is in the importance assigned to dissolved oxygen, where practically the result depends primarily on this parameter.

Then, FAHP-WQI was compared with Canadian Council of Ministers of Environment Water-Quality Index (CCME), which is not composed of a weight system. CCME is calculated using different equations, which they take into account the scope, frequency and breadth. As reflected in Fig.3, CCME shows quality in maximums values, this is because it is an average of all the parameters involved is done, which is why can compensate bad conditions of one parameter with the good ones of another.

This model emerges as an alternative, suitable and reliable tool for the aquaculture of any aquatic species, only ranges must be set up for each species.

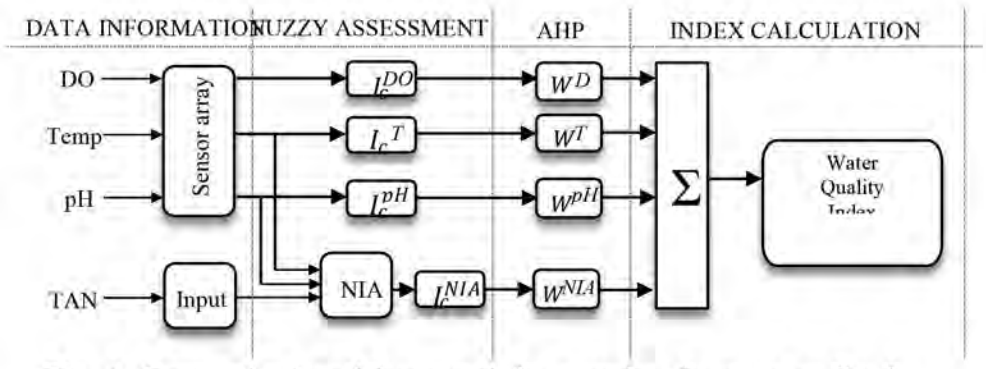

Fig. 1. Computational model proposed for assessing the water quality in intensive cultured systems

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Fig.2. FAHP-WQI vs NSF-WQI

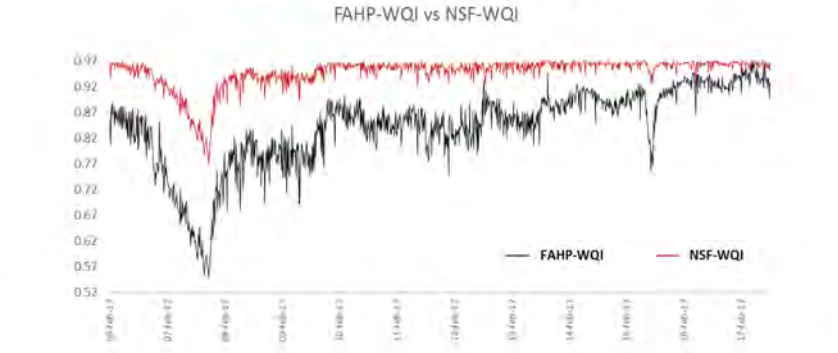
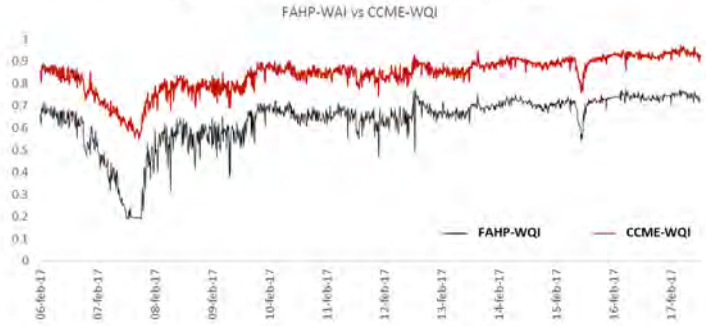


Fig.3. FAHP-WQI vs CCME-WQI.



SELECTION FOR STRESS RESISTANCE AT EARLY STAGES OF DEVELOPMENT IN COMMON CARP:AQUACULTURAL AND BIOLOGICAL CHARACTERISTICS OF OFFSPRING

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Introduction

The success of fish farming depends on the ability of the young to withstand the effects of biotic, abiotic and man-made factors. To reduce the effects of stress, new methods of broodstock formation with increased stress resistance are being developed. (Lomakina, Chernorotov, 1998). New breeding methods based on the selection of resistant groups in the early stages of development have been created. (Simonov, Vinogradov, 2012).

The aim of this work is to determine how the artificial selection for resistance to dehydration at the larval stage of development affects the aquacultural and biological characteristics of the offspring.

Material and methods

A material of this study was mature fish, eggs, larvae, fry and two years old carps. Three females and three males of each breed were crossed in a complete diallel pattern. Nine families were obtained. Part of each family in triple repetitions (1000 larvae) was subjected to dehydration. Three progeny with high survival after exposure were designated as experimental groups, and families with low survival were mixed and designated as controls. Rearing was carried out in separate ponds with the same conditions. After the catch, the fish characteristics of the each offspring were taken into account. Their resistance to acute hypoxia (Klyashtorin, 1982; Urbakh, 1964), as well as fluctuation of glucose level during and after handling (Romanova, 2004), were evaluated. Morphological differences were determined (Pravdin, 1966; Tyurin, 2010). Statistical processing was performed using Statistica 8.0 (ANOVA, Discriminant analysis).

Results

The average weight of the fingerlings did not have serious differences, for experimental groups it was 34.6 ± 0.74 g., and for control - 31.8 ± 1.18 g. The survival rate of the control group was 26.79 ± 2.56%, (4823 pcs.), which is almost two times lower than the experimental groups, 50.05 ± 3.69 (9090 pcs.). Hence, the low fish productivity of the control was 297.6 ± 17.8 kg / ha, while the experimental groups had 618.93 ± 50.39 kg / ha. On average, two-year-olds of all groups had no significant differences in either average weight or survival. This was due to the low growth rate and the survival rate of one of the experimental offspring, whose performance was lower than that of the control. However, the other two groups had an average weight advantage of 12%.Thus, despite the lag of one experimental group, the growth rate of control was lower than that of experience.

The resistance of the offspring to acute hypoxia was expressed after the time of death of 50% of the individuals. LT-50 for experimental groups was 191.2 ± 14.32 minutes, and for control -261.25 ± 18.26 minutes. Survival rate of the control under conditions of autogenous acute hypoxia was significantly higher than that of the experimental groups. The reaction to handling was determined by the fluctuation of blood glucose in the intact and stressed condition. In the intact state, the glucose level in the blood of all groups was the same and amounted to 26.2 ± 2.39 mg / dL and 27.5 ± 4.92 mg / dL for the experiment and control, respectively. After exposure, the level of stress in control was significantly higher, the glucose level was 45.6 ± 6.64 mg / dL and in the experimental group -32.65 ± 4.57 mg / dL. According these data, stress in control group at the equal impact was higher than in experimental group. Differences between groups were evaluated by morphotype based on measurements and calculations of body indices by Mahalanobis distance (Table1).

Discussion and conclusion

Based on the results of the work, it should be noted that the selection for resistance to the effects of stress at the larval stage of development leads to an increase in aquacultural properties after rearing in ponds. Also, the selection affects the physiological resistance of the organism to the adverse factors of natural and anthropogenic nature, as well as the morphometric parameters of farmed fish

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Table 1 – Mahalanobis distances between groups

	Exp 1	Exp 2	Exp 3	Control
Exp I	0,0	3,46	8,68	1,27
Exp 2	3,46	0,0	11,35	3,52
Exp 3	8,68	11,35	0,0	7,98
Control	1,27	3,52	7,98	0,0

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EVALUATION OF HEALTH STATUS IN EUROPEAN SEA BASS (*Dicentrarchus labrax* L.) JUVENILES FED DIETS WITH PARTIAL REPLACEMENT OF FISH MEAL BY MICROALGAE MEAL

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Introduction

The aquaculture sector has rapidly expanded worldwide to meet the increasing demand for seafood; consequently, increasing amounts of raw feed materials are needed by this industry to sustain this rapid growth (Ganga et al. 2015). To date, fish meal (FM) and fish oils (FO) has been the most important protein source in aquafeeds, especially for carnivorous fish species, because it is an excellent source of high quality protein, essential nutrients, attractants, and potentially unidentified growth factors (Hardy, 1996). Consequently, the aquaculture feed industry requires suitable and sustainable ingredients alternative to FM and FO. Unfortunately, few studies have reported the effect of FM replacement by microalgae in fish innate immune parameters after bacterial challenge. Therefore, the aim of this study attempts to evaluate the immunostimulatory impact on several immune-related enzymes and bactericidal activity in plasma and skin mucus in European sea bass (*Dicentrarchus labrax* L.) fed diets that replaced fishmeal with *Chlorella* sp. and *Nannochloropsis* sp. during 12 weeks.

Material and methods

Healthy European sea bass (*Dicentrarchus labrax*) (24 ± 1,2 g mean body weight) were obtained from a commercial hatchery (Acuinuga, Spain). Fish were randomly distributed into 24 identical tanks (25 fish per tank) where the following groups were established in quadruplicate. Six diets were formulated, positive diet (positive control, PC) formulated with 30% FM and fish oil as the only lipid source; negative diet (negative control, NC) based on plant ingredients (15%) and low FM (15%), including a mixture of fish and vegetable oil (40/60) as the lipid source. Four test diets were formulated similarly to the negative control, replacing 7.5% and 15% FM by freeze-dried biomass of microalgae *Chlorella* sp. (Chrl7.5 and Chrl15) and *Nannochloropsis* sp (Nanno7.5 and Nanno15). The fish were fed until saturation, during 12 weeks. Fish were sampled after 1 and 12 weeks of feeding. At the end of trial, the fish were anaesthetized and inoculated by intraperitoneal injection (i.p.) with killed *Photobacterium damselae* subsp. *piscicida* (Phdp, strain PP3), while the same groups were i.p. injected with sterile PBS (control groups). The bacteria concentration was 10^5 cfu/per fish. Challenged and unchallenged fish were sampled for plasma and skin mucus at 4, 24 and 48 h post injection. Fish were maintained in the same system and same conditions as during the growth trial but in separate tanks. The methodology of all immune-related activities is described in detail elsewhere (Guardiola et al. 2016).

Results

Our results revealed that the lysozyme, protease and antiprotease activities were unaffected in plasma of fish fed all experimental diets after 1 and 12 weeks. After 1 week of feeding, a significant increase in peroxidase activity plasma was observed in fish fed Chlrl15 diet respect to the values found in plasma of fish fed Nanno15 diet. Interestingly, the bactericidal activity against *P. damselae* was higher in plasma of fish fed Nanno7.5 compared to the other experimental groups. At the end of the trial (12 weeks), an increase in the bactericidal activity against *Vibrio anguillarum* and *P. damselae* was observed in the plasma of fish fed Chlr7.5 and Chlrl15 diets respect to NC and PC diets, respectively. The complement activity was higher in fish fed Nanno 15 compared to the results observed in the fish fed PC diet and both Chlr diets (7.5 and 15)

After bacterial challenge, any variations were observed in the activities measured at 4 hours post-challenge. However, the bactericidal activity against *P. damselae* was higher in the plasma of challenged fish fed Nanno15 respect to unchallenged ones, after 24h, and in challenged fish fed Chlrl 7.5 decreased respect to the fish fed Nanno 15, after 48h. In the challenged fish a decrease in the lysozyme plasma in fish fed Chlr7.5, after 24 hours was observed. The antiprotease activity was higher

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in challenged fish fed Chlr7.5 diet respect to PC diet after 24h. Interestingly, decrease in the bactericidal activity against *V. anguillarum* after 4 h were observed in the unchallenged fish fed Chlrl 7.5 diet respect to the fish fed NC diet, and the challenged fish fed Nanno 7.5 diet respect to the fish fed PC diet. Interestingly, the increase in peroxidase activity were observed in challenged and unchallenged fish fed Chlrl 7.5 respect to the fish fed PC diet, after 24 and 48h. Haemolytic complement activity increased in the challenged fish fed Nanno 15 diet, compare to the fish fed PC diet. Interestingly, nitric oxide activity increased in the challenged and unchallenged fish fed Chlrl 7.5 diet respect to the fish fed PC diet, after 24h. The values of protease activity increased in the unchallenged fish, fed Chlrl 15 diet respect to the challenged one. In the case of skin mucus, the values of lysozyme activity decreased in the fish fed Chlrl diets respect to other experimental groups at 1 week, whilst the peroxidase activity showed an increase in the fish fed Chlrl 15 diet respect to other experimental groups after 12 weeks of feeding. However, the antiprotease activity increased in fish fed Chlrl 7.5 compared to the other experimental groups at the end of the trial (12 weeks). Interestingly, the bactericidal activity against *Vibrio anguillarum* exhibited a decrease in skin mucus of fish fed Chlrl 15 diet respect to control diet at both sampling times.

Discussion and conclusions

Nannochloropis sp. with 7.5 and 15 % replacement of fishmeal, did not have any negative effects in the systemic and local immune response. In fact, in the present work has even been observed that some immune parameters were improved in fish fed Nanno diets. Therefore, Nanno diets could be considered a good fishmeal partial replacement whilst Chlr diets with 7.5 % and 15% replacements of fish meal seem to be inadequate for aquafeeds

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DESIGN, AQUASCAPE AND CONDITIONING OF A BRACKISH AQUAPONICS SYSTEM

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Introduction

An aquaponic system combines a closed aquaculture system with hydroponics in a symbiotic environment with invisible beneficial bacteria. Thus, an aquaponics system has the fish, the plants and the crucial bacteria. Water containing fish waste provides to the plant's valuable nutrients and in exchange "excess nitrogen-free" water returns to the fish tanks in a repetitive circle (Somerville et al. 2014). The nitrification process which takes place in the bio filter first converts the toxic ammonia from fish waste to nitrite and then the nitrite is converted to nitrate with the help of the «*Nitrosomonas sp.*» and the «*Nitrobacter sp.*» bacteria. The biofilter provides a large surface area, proper temperature, pH and dissolved oxygen for the bacteria to accommodate them and keep the system healthy. The aim of this study was the set up and the conditioning of a small-scale brackish water aquaponic system under two different salinities (8 ppt and 20 ppt).

Material and Methods

The study was carried out at the aquaponics lab of the University of Thessaly where six autonomous aquaponics systems were designed and constructed. Two metal benches were built into which the aquariums of the fish and the grow beds of the plants were placed in a parallel arrangement, thereby allowing the water to flow due to gravity from the grow bed to the fish tank and from there in turn to the sump filte . For each aquaponic system a 55 L fish tank, a 55 L raft type grow bed and a 30 L biological sump filter was constructed as described by Vlahos et al. (2004).The sump filter was divided into three different parts with surface areas (455 cm², 214.5 cm² and 214.5 cm²)for the mechanical filte , biological filter and reservoir tank respectively. All of the piping circuits used in each aquaponic system for the water flow were PVC pipes (32mm). The water flow of all the systems was continuously flowing thereby creating a constant flow rate of 1496 cm^3 / min and a filtration rate of 2.24 cm / min. For each aquaponic system air pumps were used and oxygen was supplied by aeration through an air stone to ensure better diffusion of air into the water for both fish tank and plant grow beds. The lighting was provided using sodium vapor lamps which were placed in reflectors. The photoperiod was set at 12 hours dark 12 hours light. The filter conditioning of each aquaponic system was achieved through the methodology described by Vlahos et al. (2013) and Mente et al. (2016). Initially, in order to achieve complete evaporation of chlorine from the water, the aquaponic systems were operated for 24 hours and then 2-3 grains of a filter media (e.g lava grain) from an aquarium of satisfactory functioning were added into the filters for the growth of the bacteria of the biological filte . To initiate the filter procedure, 0.2 g of NH_4Cl was added as a source of ammonia to the filter (Vlahos et al. 2004). Total ammonium nitrogen (T.A.N), nitrite (NO_2^-) and nitrate ions (NO_3^-), pH and O_2 were measured twice a week. The oxidizing capacity, the active depth of the filter and the waste load of the fish were calculated as described by Hirayama (1966) and by Spotte (1992). Active depth of the filter is the depth in which half of the ammonia produced is oxidized to non-toxic nitrate ions per water recirculation. Data were analyzed using the independent t-test to determine the significant differences between the means of all groups at $p<0.05$. Data were checked for normality and homogeneity test through Levene' s test (Zar 1999).

Table 1: Functional characteristics of a brackish aquaponic system under two different salinities. Data were expressed as means ± S.E.M (n=6).

	8 ppt	20 ppt
Hydraulic Loading Rate (HLR) (m/day)	1.85±0.005 [a]	1.85±0.005[a]
Rerciculation rate (r) (min)	0.014±0.003[a]	0.014±0.003[a]
Water Retention time (min)	9.7±0.01[a]	9.7±0.02[a]
Specific Surface area (SSA) (m^2/m^3)	162±0.30[a]	224±0.31[b]
Volume media (V_{media}), (m^3)	6.73±[a]	6.94±0.08[b]
Filter's Volume (V) (L)	32.13±0.001[a]	32.13±0.001[a]

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The results showed that the conditioning of the aquaponic systems was observed when TAN eliminated to 0.05 mg/Land nitrate ion was continuously increased until reach their maximum value which was 160 mg/L. The oxidizing capacity of the filter was 0.17 mg O_2 and the active depth of filter bed was 4.5±0.05cm for all aquaponic systems. Spotte (1992) reported that the half depth of the filter bed when TAN was reduced by 50% was lower than 10 cm depth.

The active depth was characterized as the ability of the filter to remove the maximum produced waste pollutants of the fish due to their metabolism (Table 1). In addition, the hydraulic loading rate (HLR), the recirculation rate, the retention time of the water into the filter (t), the specific surface area of the filter (SSA), the volume of filter media (V_{media}) and the filter volume (V)were summarized on Table 1.Endut et al. (2009) suggest that the best HLR for a fresh water aquaponic system was 1.28 m/day because it provides the best production performance for fish (SGR: 1.80%/day)and plant growth (1.75 cm/day). In the present study the HLR (1.85 m/day) was higher. However, it provides also higher growth performance for fish (SGR: 3.17%/day) and plants (PGR:1.87 cm/day for 20 ppt and 0.99 cm/day for 8 ppt, respectively) in a brackish aquaponic system.

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N-methyl-D-aspartate RECEPTOR AND NITRIC OXIDE PATHWAYS AS POTENTIAL REGULATORS OF LARVAL METAMORPHOSIS IN BIVALVE SPECIES: INVESTIGATION OF NEW PATHWAYS FOR COMMERCIAL HATCHERY APPLICATIONS

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Introduction

In bivalve species, metamorphosis, the transition from a free-living larva to a juvenile (spat), is regulated by a complex interaction of neurotransmitters and neurohormones. The actual regulatory pathways, however, are still poorly understood. Catecholamines such as epinephrine are neurotransmitters routinely used in oyster hatcheries to induce metamorphosis and settlement when single-seed production (spat not attached to a substrate) is desirable. However, epinephrine only induces metamorphosis in certain species of bivalve molluscs, and there is limited understanding of the pathways involved (Joyce and Vogeler, 2018), thus making it difficult to apply existing knowledge when developing hatchery protocols for new species. Therefore, our current work aims to better understand the signalling pathways involved in regulating bivalve metamorphosis by investigating the N-methyl-D-aspartate (NMDA) receptor pathway and the nitric oxide (NO) pathway as two potential regulatory drivers of metamorphosis; two concepts that have not been considered previously for bivalve development.

Methods

Competent larvae (approx. 100 pediveliger larvae ready for metamorphosis) of the Pacific oyster (*Crassostrea gigas*), Eastern oyster (*Crassostrea virginica*), hard-clam (*Mercenaria mercenaria*) and soft-shell clam (*Mya arenaria*) were exposed to known vertebrate NMDA receptor antagonists, MK-801 and ifenprodil, and to epinephrine. Metamorphosis success was assessed after 24h. Pacific oyster larvae were also exposed to known nitric oxide synthase inhibitors (NOS, key enzyme involved in NO production) such as s-methylisothiourea sulfate (SMIS), aminoguanidine hemisulfate salt (AGH) and 7-nitroindazole (7-NI) as well as to ODQ, an inhibitor to the guanylyl cyclase, which is an enzyme for the production of cGMP and activated by nitric oxide. NMDA receptor subunits were cloned and their gene expressions were quantified in competent Pacific oyster larvae and larvae treated with NMD receptor antagonists by quantitative real-time PCR. The NMDA receptor subunit CgNR1 was localised in competent Pacific oyster larvae by *in-situ* hybridisation.

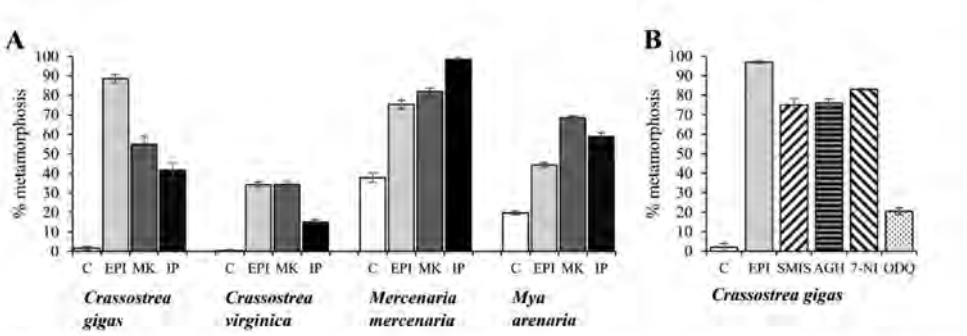

Fig. 1: Percentage of metamorphosis after 24h in (**A**) oyster or clam larvae after exposure to epinephrine at 10^{-4}M (EPI), MK-801 at 10^{-4}M (MK) and ifenprodil at 10^{-7}M (*Crassostrea. virginica*) or 10^{-6}M (remaining species), and in (**B**) *Crassostrea gigas* larvae after exposure to SMIS, AGH, 7-NI and ODQ. All treatments were significantly different to each species' respective no-treatment control (C).

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Results

Exposure of competent larvae to the NMDA receptor antagonists significantly induced metamorphosis in all four bivalve species with optimal concentrations ranging from 10^{-4}M to 10^{-7}M (Figure 1A). In particular, both clam species were highly responsive to the NMDA receptor antagonists with e.g. *M. mercenaria* displaying almost 100% induction to ifenprodil at a final concentration of 10^{-5}M and 10^{-6}M. In *C. gigas*, the three NOS inhibitors as well as the guanylyl cyclase inhibitor ODQ induced metamorphosis with the NOS inhibitors resulting in the strongest effects with metamorphosis rates ranging from 75% to 83% (Figure 1B). The NMDA receptor subunits, CgNR1 and two CgNR2 (CgNR2A and CgNR2B) were successfully cloned and expression studies showed all subunits are expressed in competent oyster larvae, although their expression was not affected by the different treatments. *In situ* hybridisation has shown that the NMDA receptor subunit CgNR1 is mainly expressed in areas where the pedal nerve fibres and apical ganglion are located in competent larvae

Discussion

NMDA receptors are ligand gated ion channel receptors located in signalling cell membranes in the nervous system. Based on the exposure experiments it seems that metamorphosis is negatively regulated by an NMDA receptor pathway given that metamorphosis can be induced after exposure to NMDA receptor antagonists. Furthermore, NMDA receptor subunits in competent Pacific oyster larvae are located in nerve structures, which are connected to the larval foot and apical organ (at the base of the velum), two organs lost during metamorphosis and suspected to partially function as sensing organs for external settlement cues. NMDA receptors are known to regulate specific signalling pathways by increasing the intracellular calcium concentration. We hypothesise that this increase in intracellular calcium concentration is activating the NOS, the key enzyme for the production of NO. Nitric oxide, a gaseous signalling molecule and neurotransmitter, potentially inhibits bivalve's metamorphosis through a series of negative cell responses (i.e. inhibition of apoptosis) as it has been previously proposed for other invertebrate species (Leise et al, 2004; Comes et al, 2007). However, to our knowledge the effect of NO on bivalve larvae has not been investigated and our theory is supported by the increasing metamorphosis rate in Pacific oyster larvae after exposure to different NO pathway inhibitors. This new concept of the NMDA receptor and NO pathway regulating bivalve metamorphosis could potentially provide the missing information to identify new external chemical inducers for metamorphosis in different bivalves to optimize hatchery productivity.

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A GIS SUITABILITY ANALYSIS FOR SELECTING MUSSEL FARM SITES IN THE SOUTH-WESTERN BALTIC SEA

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Introduction

Mussel farming is one of the most sustainable forms of aquaculture due to the mussels' filtering capacity and may be used as a mitigation measure for eutrophication or to mitigate some of the negative impacts of fish farms. In Denmark there is a halt for new fish farm permits in marine areas due to environmental concerns regarding eutrophication, which is, in particular, a problem in the Baltic Sea. Therefore, mussel farming has increasingly come into focus. This study presents a GIS based suitability analysis that is combined with an ecological model. It shows where mussel farming is possible in terms of ecological suitability and spatial availability and investigates the identified sites in more detail to determine which site has the highest potential in terms of mussel harvest and effects on water clarity. The latter is an important criterion for site selection in eutrophied seas, such as the Baltic Sea.

Methods

The suitability analysis was applied to a case study in the south-western Baltic Sea. It consists of a Multi-Criteria analysis that is implemented in GIS using raster data. Criteria include existing maritime activities, as they put constraints on the available area, and environmental conditions that determine mussel growth. For each criterion a parameter-specific suitability function (PSSF) (Longdill et al. 2008) was identifie on a scale between 0 and 1 (unsuitable-suitable) or on a binary scale (unsuitable or suitable). The suitability maps created for the area were used to identify potential suitable locations for mussel farms. These locations were further analysed with a dynamic energy budget model (DEB) that is implemented into a 3D farm scale model. The model simulates the dry weight and shell length of blue mussels. These parameters were used to estimate the amount of nutrients removed by harvest and impacts on water clarity.

Results

The GIS analysis shows that large parts of the case study area are unsuitable for mussel farming due to spatial restrictions and insufficient environmental conditions. The suitability maps pinpoint to the area of Hjelm Bay in Denmark as a potential location for mussel farms. The three sites selected for investigation in the ecological model show a potential harvest of up to 4t ha^{-1} after 2 years and an increase in water transparency of up to 50% in the summer time.

Discussion and conclusion

The GIS analysis serves well for screening marine areas with regard to suitable locations for mussel farming. It requires data on maritime activities and environmental conditions in the areas of interest, which can be an obstacle for the application in marine areas with low data availability. This was not the case in the study area, however. The screening was followed by a more thorough analysis of the identified potential suitable sites to get an idea of the potential mussel harvest from these locations and to estimate the effect on water clarity. This two-step approach – screening for potential locations of mussel farms and more detailed investigation of identified sites – is a suitable method to support mussel farm site selection. The GIS analysis will be converted to a user-friendly tool as part of the Decision-Support System developed by the BONUS BASMATI project. Via this platform, end-users will be able to access it and apply it to different areas.

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THE DIETARY INCLUSION OF GRAPE MARC EXTRACT MODULATES THE HEPATIC EXPRESION OF GENES ASSOCIATED WITH ANTIOXIDANT DEFENSES AND OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC) IN RAINBOW TROUT PLASMA

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Introduction

The capacity of a fish to deal with oxidative stress depends among other factors on the contribution of nutrients or dietary compounds that via different mechanisms define anti-oxidative defenses (Birnie-Gauvin et al., 2017)in favour of the former. In recent years, the associationbetweenoxidativeprocesses,environmentalchangeandlifehistorieshas receivedmuchattention.However,moststudieshavefocusedonavianandmammalian taxonomicgroups,withlessattentiongiv entofish,despitetheirecologicalandsocio-economicrelevance.Herewepresentareviewoftheextrinsicandintrinsicfactors that influence oxidative processes in fish, using a comparative and evolutionary approach. We demonstrate that oxidative stress plays a key role in shaping fish s responses to environmental change as well as life history strategies. We focus on representative examples to compare and contrast how levels of oxidative stress respondtochangesintemperature,salinityand oxygenavailability.Furthermore,we describe how emerging threats (i.e. pollution. Feeding carnivorous fish, like salmonids, with diets rich in plant derived ingredients have shown to disrupt the oxidative balance in different tissues, poiting to an increase in the demand for dietary antioxidants (Wacyk et al., 2012)"ISSN":"00448486","abstract":"The objective of the study was to evaluate the effect of dietary protein source on fish growth, nutrient utilization, plasma variables and hepatic gene expression in juvenile rainbow trout (Oncorhynchus mykiss. The inclusion of polyphenol rich products has shown to improve antioxidant defenses in different tissues of mammals and chickens (Brenes et al., 2016), however information regarding similar benefits in carnivorous fish fed diets supplemented with this type of ingredients, is lacking. The objective of the present work was to evaluate changes in hepatic gene expression and antioxidant capacity (ORAC) in the plasma of rainbow trout (RBT) in response to the dietary inclusion of a grape marc extract in plant rich diets.

Material and Methods

Red wine grape marc microencapsulated using maltodextrin as wall material and used to formulate three experimental diets. Four hundred and fifty RBT juveniles (90 g) were randomly distributed to nine 380 L tanks and fed the three experimental diet. One plant-based control (0% grape marc), and two plant-based diets with 1 and 2% grape marc inclusion were extruded using industry settings. Diets were randomly distributed and delivered three times a day (2% BW) during the experimental period. Hepatic and blood samples were taken from 3 fish per tank at 0, 4, 8 and 12 weeks.

Results

Preliminary results, (first 24 hours) show that the inclusion of grape marc in rainbow trout diets is able to cause significant changes in plasma antioxidant capacity (Fig 1. a). The inclusion of 2% grape marc consistently elevated ORAC in the plasma of the sampled fish in this period when compared with the rest of the experimental treatments. Results also show, significant differences in terms of circulating iron (Fig 1. b), however, not a clear trend of the inclusion of grape marc over the plasma levels of this mineral. Similar response was observed for other plasma parameters evaluated in the present study.

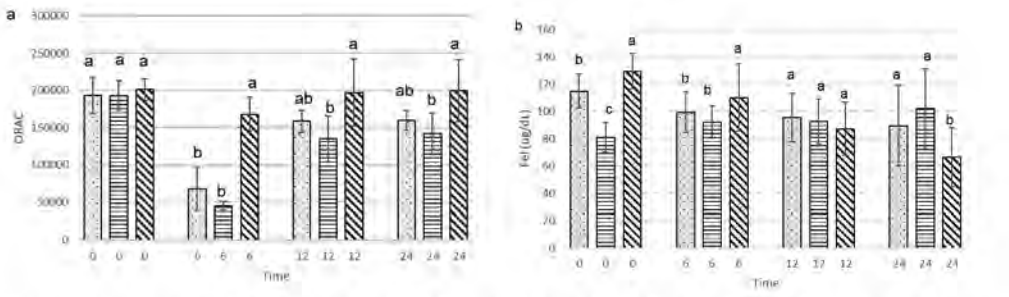

Fig. 1. Plasma antioxidant capacity (ORAC) and Iron (Fel) levels in Rainbow trout plasma fed diets with 0% (▒) 1% (▤) 2% (▨) grape marc inclusion. n=3, different letters indicate statistical difference (p<0,05). Time in hours post feeding. ORAC units umol Equiv. Trolox.

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Discussion and conclusion.

The present data indicate that the inclusion of grape marc microencapsulated has the potential to improve plasma antioxidant capacity without altering other variables of fish plasma. This seems to be in agreement with increases in antioxidative defenses previously reported in chicken for this type of product (Chamorro et al., 2015). There are just few reports in fish using polyphenol rich product from grape, with no direct evaluation over antioxidant defenses, nonetheless reporting, improvements in growth and immune response (Magrone et al., 2016; Zhai et al., 2014). Current data on the use of polyphenol rich products derived from grape, including grape marc indicate that seem to be suitable ingredients to be use in carnivorous fish diets. Further research is needed to better evaluate effect over the fish and optimize the product inclusion in the diets.

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APROPOSAL TO INCREASE CARRYING CAPACITY OF HIROSHIM BAY ECOSYSTEM TO INCREASE OYSTER CULTURE PRODUCTION WITH SIMULTANEOUS MAINTAINING THE SPECIES DIVERSITY

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Introduction

Hiroshima Bay (HB) has a long history of Pacific oyster or Japanese oyster (*Crassostrea gigas*) culture. The way of hanging oysters under a floating raft has been used for culture since 1958 (Kusuki,1981). The raft used for oyster culture is rectangular in shape with 20 m x 10 m. It is suggested that a large number of oyster rafts (ca. 12,000) placed in northern Hiroshima Bay (nHB) is supposed to be working as "artificial hanging reef" (Matsuda et al., 2000). Ecologically, oysters are providing a wide range of ecosystem services, by improving water quality through their filtering activity (Newell et al., 2004), and provisioning habitat for fishes (Peterson et al., 2003). The objective of the present study is to evaluate quantitatively the biomass of organisms attached and associating with oyster rafts. We also estimate how to function the oyster raft as an artificial reef in terms of the material cycle using the prey-predator numerical model

.

Materials and methods

Biomass of organisms was estimated by field observations for those associating with oyster rafts at three oyster rafts in addition to those at 7 stations at shoreline in June, July and August 2016. Animals attached on hanging oyster clusters and those attached on concrete walls and natural rocks in the shoreline were identified and counted. Fish gathering under the oyster rafts were also identified and counted by video recordings

To understand material cycles in nHB, we constructed a numerical model to express phosphorus flows in prey-predator interactions. The dominant organisms whose biomass was higher than 5% of the total weight were selected to be incorporated in the model. As a result, 22 compartments were placed in the model. The model was developed using a software Stella Architect (version 1.4.3), running with a time step of 0.02 days by the fourth-order Runge-Kutta method.

Results

The video camera records showed three commercial fish species (black seabream, pufferfish, and black rockfish) were dominant under the oyster rafts. Among the animals attached on the oyster clusters, oysters were largest in weight (67.2±2.2 g) compared to mussels (0.4±0.2 g) and barnacles (3.4±1.7 g) but were less abundant in numbers compared to the latter twos.

As shown in Table 1, the increasing of oyster (OYS) biomass decreased the biomass of all animals such as attached organisms and fish associating with the oyster rafts. Analyses of material flow revealed that larger amount of materials produced by phytoplankton go to OYS and the rest were shared by the other filter feeders

Table I. Summary of percentage responses of animals attached (OYS: oyster, MUSS: mussel, BARN: barnacle) and associating dominant fish (BSE: black seabream, PFI: puffer fish, BRO: black rock fish) to increasing/decreasing of OYS biomass.

Compartment	Present value (mg P m^{-3})	OYS biomass (0.141 Ind. m^{-3})					
		+30%	+50%	+100%	-30%	-50%	-100%
MUSS	0.118	-4%	-7%	-12%	+2%	+4%	+10%
BARN	0.002	-3%	-7%	-12%	+2%	+4%	+8%
BSE	0.019	-3%	-6%	-10%	+2%	+3%	+8%
TSF	0.053	-4%	-7%	-12%	+2%	+2%	+11%
PFI	0.100	-4%	-7%	-12%	+2%	+2%	+11%
JSB	2.819	-1%	-3%	-6%	+1%	+2%	+3%
BRO	1.22	-1%	-3%	-6%	+1%	+2%	+3%

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Discussion and conclusion

It was revealed that increasing of oyster biomass may decrease the biomass of all the other living things by monopolizing the products by phytoplankton. Since mussels and barnacles are food sources for some fish species (BSE, TSF, and PFI), the decrease of mussel and barnacle production also effect on fish production. This could lead to low species diversity even if the oyster production can be increased.

Cultured oyster production in HB has been decreasing to 2/3 since the 1980s in addition to the decrease of fish catch. It is pointed out that the government measure to reduce phosphorus and nitrogen loads since 1980s is the major cause of the decrease of fisheries production (Yamamoto, 2003). Since the phosphorus load is very small (880 kg P day^{-1}in 2016) compared to those in 1980s (~80 ton P day^{-1}), we cannot attain the level of large oyster production as we had in 1980s unless the input of phosphorus and nitrogen are increased, because of the shrinkage of carrying capacity of the bay ecosystem. In the other areas, relaxation of sewage effluent from treatment plants has started, but the effect is limited because of dilution with the large volume of seawater. Since the government is nervous on the risk of red tide generation by the operation, the relaxation is practically conducted to the level of the upper limit of allowance, whereas fishermen s request to remove the allowance limit.

An alternative is to apply fertilizer in a raft scale, which may be efficient as proposed by Yamamoto et al. (2017). They actually increased ca. 20% in the oyster individual weight in average by an application of fertilizer. We would like to propose a measure to increase the carrying capacity of the bay by application of fertilizer in addition to the relaxation of sewage effluent which can increase oyster production with simultaneously sustaining the species diversity in the ba .

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BIOINFORMATIC ANALYSIS AND IMMUNE-STIMULATED TRANSCRIPTIONAL ANALYSIS OF THE IFP35 COUNTERPART FROM ROCK BREAM (*Oplegnathus fasciatus*)

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Introduction

Interferon-induced protein-35 (IFP35) is a 35kDa protein that was first discovered in HeLa cells stimulated with IFN-γ(Bange et al., 1994)named IFP 35, whose expression is regulated by interferons (IFN. It is a leucine zipper protein, and it is induced in the regulation of gene expression(Bange et al., 1994)named IFP 35, whose expression is regulated by interferons (IFN. However, the physiological and biological roles of the IFP35 are not known. IFNs are major cytokines in the immune reaction regulations and regulating the immune and inflammatory reactions(Schneider et al., 2014). There are three types of IFNs have been identified according to their amino acid sequences(Randall and Goodbourn, 2008)if not all, virus infections in the absence of adaptive immunity. However, viruses can still replicate and cause disease in vivo, because they have some strategy for at least partially circumventing the IFN response. We reviewed this topic in 2000 [Goodbourn, S., Didcock, L. & Randall, R. E. (2000. IFP35 initially found in mitochondrial located in the cytoplasm. This protein can translocate to nucleus from the cytoplasm through stimulating the IFNs(Chen et al., 2000). When viruses are attacked to the cell, IFNs are secreted into the extra cellular matrix to bind their related receptor on neighboring cells(Altmann et al., 2003)IFN-beta, IFN-omega, IFN-delta, IFN-kappa, and IFN-tau is a large group of cytokines involved in the innate immune response against various microorganisms. Genes for IFN have been cloned from a variety of mammalian and avian species; however, IFN genes from lower-order vertebrates have not been forthcoming. Here, we report the cloning and characterization of the IFN gene from the zebrafish, Danio rerio. Zebrafish IFN (zfIFN. This activated signal stimulates a group of antiviral proteins such as antigen presentation proteins, apoptotic proteins, GTPases, chemokines and heat shock proteins(Sen, 2000).

Materials and methods

Rock bream (*Oplegnathus fasciatus*) Interferon-induced protein-35 (*RbIFP35*) homolog was identified, and its biological functions were characterized. Rock bream complete cDNA of IFP35 got from rockfish cDNA transcriptomic database. *RbIFP35* protein characteristic and structural features were analyzed by several bioinformatic tools, such as ExPASy PROSITE, NCBI BLAST programme, and SignalP online server. The expression level of mRNA in rock bream was analyzed by quantitative real-time PCR technique. Conserved domain parts of the *RbIFP35* were determined by using multiple sequence alignment tool, Clustal W. The phylogenetic tree analysis was constructed according to the Neighbor-joining (NJ) method by MEGA 5.0 software. The putative 3D structure of the *RbIFP35* was constructed by I-TASSER server. To measure the immune response of *RbIFP35* on stimulant or pathogens, healthy rock breams were used to experiment. Each group of rock bream was injected with rock bream iridovirus, *Edwardsiella tarda*, *Streptococcus iniae*, lipopolysaccharide and polyinosinic: polycytidylic acid. Phosphate buffered saline (PBS) was injected into the control group. For each treatment, various tissues were collected from the challenged rock bream from different time intervals. Then total RNA was extracted from each tissue and did the qPCR assay.

Results

The two NID domains in the *RbIFP35* open reading frame(ORF) were identified by using online software tools. The *RbIFP35* mRNA was highly expressed in rock bream gill tissue compared with others. *Lates calcarifer* IFP35 gene was aligned with *RbIFP35* with more than 70.6% identity in the pairwise homology analysis Phylogenetic tree analysis represented that the *Larimichthys crocea* IFP35 and *RbIFP35* have a closer evolutionary relationship with each other. The putative 3D structure of the *RbIFP35* was constructed by I-TASSER, and the two NID domain parts were identified. The significant increment of the mRNA expression level of *RbIFP35* in blood tissue after the post challenged. In response to the infection with Poly: IC, *RbIFP35* mRNA expression was significantly increased from 6 to 24h, peaking at 12h. Following the LPS administration, mRNA expression upregulated to at 24h. *E. tarda* infection, mRNA expression level upregulated at 12h. RBIV infection and *S. iniae* significantly increased *RbIFP35* expression, with the peak of 2.47-fold and 2.26-fold increases in expression levels peaking at 24h, respectively.

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Discussion and conclusion

We analyzed the functional aspects and molecular structure of recombinant *RbIFP35*. *RbIFP35* is a protein composed of 368 aa. The presence of two NID domains in *RbIFP35* suggested its Nmi-Nmi protein interaction ability(Chen et al., 2000). *RbIFP35* represent high aa sequence identity with fish homologs, but low identity with reptiles, mammals and bird's homolog. We find the evolutionary stage of that gene in vertebrate evolution. We identified the *RbIFP35* mRNA content in various tissues and assessed that was upregulated with the certain immune stimulants and pathogens. Findings of the current study represent the overall informative information related to the IFP35 gene of the rock bream.

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SUSTAINABLE AQUACULTURE OF ATLANTIC SALMON (*Salmo salar*) ON BOARD A LARGE SAIL SHIP

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Introduction

Atlantic salmon is a core product of the European seafood trade. The European aquaculture production of Atlantic salmon is exceeding landings from fisheries by 700 times, or 1000 times in view to the global scale. It is desirable to change and improve an aquaculture technology which is more and more faced with severe environmental issues.

Most of European salmon aquaculture is situated along the coast. The coast lines are affected by climate change and previously favorable hydrographic conditions change for the worse. The biological temperature preferences of aquaculture fish species such as Atlantic salmon (*Salmo salar*) may not be met anymore (Wade et al. 2019). Thermal stress in fish affect metabolic expenditure and eventually weakens the immune response of fish living in dense aquaculture populations.

The attempt to bring European salmon aquaculture on land into RAS is resource-intensive. A production area of several thousand hectares is required if a safe RAS technology (Orellana et al. 2014) is employed. The energy demand to maintain a cold-water environment for salmon is tremendous. Thus, the idea emerged to bring salmon aquaculture into the ocean in quantities that fit the market requirements. The attempt in the industry to produce bigger and bigger cohorts in giant oceanic installations is in conflict with the environment because they represent point sources of pollution which is a core problem of industrialization. In contrast, our key idea is to develop an environmentally sound and sustainable novel aquaculture technology. This includes the use of wind power to move the aquaculture ship and to run the process technology on board.

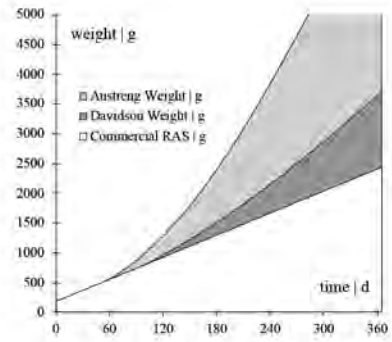

Fig. 1: Evaluation of Atlantic salmon growth redrawn with data from Austreng et al. (1987), marine net cage; Davidson et al. (2016), fresh-water RAS; commercial fresh water RAS.

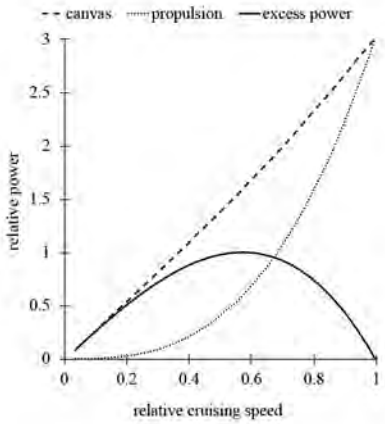

Fig. 2: Relative wind power allocation on a sail ship cruising half wind.

Method

The proposed sail ship is an aquaculture process ship tailored to the need of Atlantic salmon. A growth model based on literature data (Fig. 1) was developed, basic operations and an appropriate process chain were defined, and mass flows and energy consumption for both the fish and the technical processes were calculated. Following aspects were so far considered in the process:

- Smolt supply and transfer process, RAS for inspection of incoming fis
- Process tank, hydrodynamics, water exchange, gassing, degassing, lighting
- By-product discharge, particles, nitrogen, phosphorus, carbon dioxide
- Preliminary ship design. Ship stability and propulsion.
- Seawater intake, passive system through forward movement of ship vs. pumps
- Wind power and power allocation
- Internal ship logistics, storage and distribution of auxiliaries, e.g. feed, oxygen
- Supply logistics, bunkering, material plan, energy plan
- Economic and ecological operation, energy efficienc , emissions

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Results

The evaluation of Atlantic salmon growth in different types of aquaculture operations as shown in Fig. 1. Austreng et al. (1987), investigated the growth in a net cage operation having an optimal thermal environment of around 14°C. Davidson et al. (2016) were growing salmon at slightly higher temperature (15 - 17°C). The commercial RAS (pers. comm.) had severe temperature problems (> 18°C). The growth performance of Atlantic salmon negatively correlates with temperature so that the growth performance in an ocean-going aquaculture can be expected to match the growth observed by Austreng et al. (1987). The harvest weight is expected between 4 and 6 kilograms after one year starting with smolt of 0.1 - 0.2 kilograms stocking weight.

All further calculations were carried out based on Austreng's data because the sail ship will cruise in ocean water at optimal temperature. The cruising area will be in the North of the North Atlantic during summer and in the South during winter.

The interdisciplinary approach starting from the biology of the fish brought deep new insights into the operation. This made it possible to investigate the wind power allocation for a typical half wind course. In Fig. 2 the wind power (apparent wind) is shown in dependence from the cruising speed which is a result of vector addition. The power needed to propel the ship at a given speed was subtracted to estimate the excess power available to supply power to the process technology on board. This difference is shown by the dome shaped curve in Fig. 2 emphasizing that at intermediate speed a maximum share of wind power would be available for the aquaculture processes.

Discussion and Conclusions

Is salmon aquaculture justifiable in the ocean or is it again another kind of unsustainable industrial production process?

Open ocean aquaculture taking place in the North Atlantic is a minor discharge of organic carbon into the ocean compared to the organic carbon loss through the fisheries of seven to nine million tons every year. This applies also to losses of nitrogen, phosphorous, or other elements. Thus, a part of the European salmon aquaculture can be relocated in the open ocean using sail ships.

At the end, as in any aquaculture, severe effects, for instance genetic alterations and/or pathogen transfer, must be avoided. The escape of fish can be prevented through appropriate process technology that retain the fish safely inside the ship hull. Bio-secure breeding centres supplying pathogen free fish is by all means desirable in order to safeguard production and marine life. This, however, should be common to all aquaculture seeking for sustainable and responsible production.

Aquaculture on board sail ships can likely be powered by renewable energy and become independent from fossil energy to large extent. However, it is obvious to have additional engines on board, which can take over the energy supply in emergencies or at low wind. In the next stage of the project, photovoltaic coating on sails, wind and water powered generators, and surplus energy storage cater for an integrated and almost closed energy system.

The proposed sail ship aquaculture will be applicable to many other fish species heavily exploited by fisheries and produced in aquaculture today.

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Davidson et al. 2016: Aquacult. Eng. 74:1–16
Orellana et al. 2014: Aquacult. Eng. 58:20–28
Wade et al. 2019: Journal of Thermal Biology, 80: 64-74

BLACK SOLDIER FLY MEAL AS SUSTAINABLE FEED INGREDIENT FOR RAINBOW TROUT

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In pursuit of a viable alternative, an eight-week feeding trial was conducted to evaluate the feasibility of including reared black solider fly (BSF, *Hermetia illucens*) larvae into rainbow trout diet. A total of five diets were formulated with one being a fish meal-based basal diet (CTRL), two levels of BSF (15 %, BSF15; 30 % BSF30), and a further two diets with a supplementary protease enzyme were assigned to test potential dietary enhancement (15 %, BSF15P; 30 % BSF30P). At the conclusion of the trial there were no significant growth performance differences between the dietary treatments ($P>0.05$). However, condition factor in fish fed on 15 % BSF inclusion diet was significantly higher than 30 % +protease inclusion ($P=0.025$). Postharvest fillet quality was assessed and showed significant changes in fatty acid composition, colour and shelf-life stability ($P<0.05$). Trout fed BSF had a significantly lower ($P<0.001$) level of lipid peroxidation, with the level of malondialdehyde present decreasing with increased dietary BSF, regardless of protease. As the inclusion of black soldier fly meal (BSFM) increased the saturated fatty acid (SFA) content of the fillet significantly increased ($P<0.001$) and the monounsaturated fatty acid (MUFA) significantly decreased ($P<0.001$),

RAINBOW TROUT (*Oncorhynchus mykiss*) INTESTINAL EPITHELIAL CELLS AS A MODEL FOR STUDING GUT IMMUNE FUNCTION AND EFFECTS OF FEED ADDITIVES

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Introduction
Current knowledge regarding mechanisms underlying effects of feed additives on fish gut health and function is limited largely due to lack of targeted research tools. The use of *in vitro* approaches, such as appropriate cell lines, would facilitate investigation of basic functions of the digestive tract as well as effects of feed additives. Such *in vitro* tools would strengthen the understanding of intestinal immune responses, epithelial barrier and digestive function. The objective of this study was to evaluate the suitability of the rainbow trout intestinal epithelial cell line (RTgutGC) as an *in vitro* model for studies of immune function and effects of commercially relevant aquafeed additives.

Materials and methods
Effects of lipopolysaccharide (LPS), nucleotides, mannan oligosaccharides (MOS) and beta-glucans were evaluated in RTgutGC cells grown on conventional culture plates and transwell membranes. The analyses included indicators of cell viability and cell proliferation, brush border digestive enzyme activity, tight junctions, albumin transport across the epithelial layer, reactive oxygen species (ROS) production, morphology and relevant gene and protein expression.

Results
Permeation of fluorescent albumin (Figure 1), trans-epithelial electrical resistance (TEER) (Figure 2), and tight junction protein expression confirmed the barrier function of the cells.

Brush border membrane enzyme activities were detected in the RTgutGC cells and immune related gene responses were measured at levels comparable to those observed *in vivo* in rainbow trout distal intestine. LPS produced markedly elevated expression of the genes coding for the pro-inflammatory cytokines interleukin 1β, interleukin 6, interleukin 8, and tumor necrosis factor alpha but had no clear effect on ROS production. MOS seemed to be the most potent modulator of RTgutGC immune and barrier function among the feed additives (Figure 3 and 4).

Discussion and conclusion
Our TEER measurements and fluorescent albumin translocation confirmed earlier reports (Minghetti et al. 2017, Drieschner et al. 2017) showing that RTgutGC cells grown on transwell membranes strongly attenuate fluorescent model molecules' translocation from apical to basolateral chamber. Presence of the leucine amino peptidase (LAP) and maltase activity confirmed that the cells harbor, at least some, typical brush border functions. LPS, a common model pathogen-associated molecular pattern, markedly elevated cytokine gene expression, which shows that the RTgutGC cell line isolated from the distal intestine has capacity for a range of typical mucosal immune functions (Kawano et al. 2011). The increasing albumin translocation and lower *myd88* expression induced by MOS indicate how MOS may act as homeostatic balancer of barrier function also *in vitro* (Torrecillas et al. 2015).

Conclusion: Our findings suggest that RTgutGC cells possess features characteristic of functional intestinal epithelial cells indicating a potential for use as an efficient *in vitro* model to evaluate effects of bioactive feed additives on gut immune and barrier functions and their underlying cellular mechanisms.

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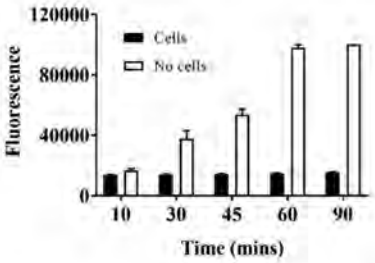

Fig 1. Fluorescent levels in basolateral media after fluorescent albumin exposure into the apical chamber of 24-well-transwell plates with or without RTgutGC

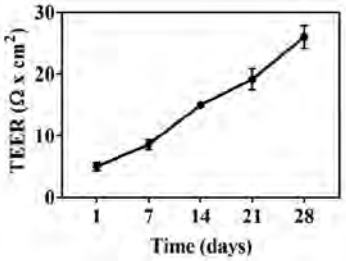

Fig 2. TEER of RTgutGC cells grown up to 4 weeks in 24-well-culture plates with inserts.

MOS seemed to be the most potent modulator of RTgutGC immune and barrier function among the feed additives (Figure 3 and 4).

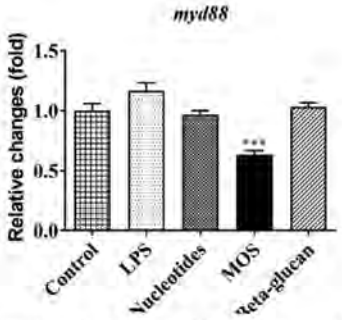

Fig 3. Myeloid differentiation factor 88 (*myd88*) expression in RTgutGC cells grown on 6-well transwell membranes and exposed to feed additives.

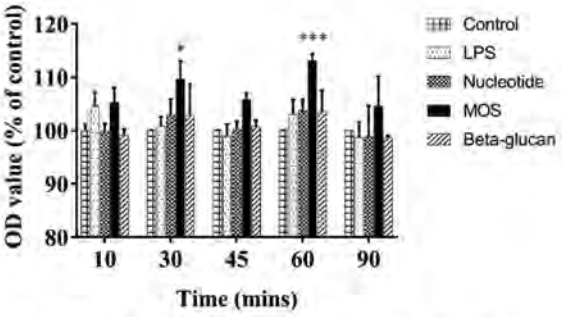

Fig 4. Basolateral fluorescence levels after fluorescent albumin exposure in apical chamber of 24-well transwell membranes with RTgutGC cells exposed to feed additives for 6 h.

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INFLUENCE OF PHOTOPERIOD AND TEMPERATURE ON REPRODUCTIVE DEVELOPMENT AND OOCYTE GROWTH IN THE POLYCHAETE *Hediste diversicolor*

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Introduction

Reproductive development, including gonad development and gametogenesis, is the initial stages of the reproductive process, whereas gamete maturation and spawning are the final stages. Both endogenous factors and environmental cues can influence different stages of the reproductive process (Bentley & Pacey, 1992). The aims of this study were to observe (1) the influence of an accelerated photoperiod transition (2.5 times fast-forward) on reproductive development, (2) effect of temperature on oocyte maturation and (3) combined effects of photoperiod and temperature on oocyte growth of the polychaete *H. diversicolor*.

Material & Methods

The study comprised three individual experiments. **Exp1:** Worms were cultivated at a constant temperature (8 °C) with mimicked (MP) or sped-up (SP) photoperiods lasting 118 and 46 days, respectively (46d). Histological analyses (n=8) and in-vitro oocyte size measurement (n=8) were performed eight times throughout the experiment. **Exp2:** To assess the effect of temperature on oocyte maturation, worms were collected in March and kept in the lab at low (2 °C, "LT") and high (8°C, "HT") temperatures. Sampling was performed 5 times throughout the experiment at lab. In Exp1 and Exp2, treatments were compared to field samplings from natural populations on coinciding day lengths (NP). **Exp3:** Worms were cultivated at long day length (16L:8D) at constant temperature (16°C, "ThPh"), or long day length at decreasing temperatures (13 to 7°C, "TlPh"), short daylength (8L:16D) at constant temperature (16°C, "ThPl"), and short daylength at decreasing temperatures ("TlPl"). Each treatment comprised 6 worms, and repeated oocyte measurements were conducted 8 times over the experimental period of 85 days.

Results

Exp1: The oocytes in the SP treatment matured significantly less than the oocytes in the MP and NP treatment (Fig. 1). **Exp2:** High temperature was found to have positive effects on oocyte maturation. The oocyte shape found for worms kept in the lab at constant temperature were circular in shape at the first sampling, whereas the natural worms displayed irregular oocyte shapes throughout. The size of maturated oocytes was Ø=196.34±3.75 µm. **Exp3:** Oocyte growth was fastest at short daylengths and decreasing temperatures (TlPl). Constant temperature and long daylength (ThPh) suppressed oocyte growth (Fig. 2). The variability in oocyte size decreased over time.

Discussion & Conclusion

The effect of sped-up photoperiods on gametogenesis were inconclusive and need further investigations. **Exp2:** Constant temperature stimulated oocyte maturation. During the final stage of oocyte maturation, increasing the temperature can boost the reproductive process. **Exp3:** The photoperiod transition from long day to short day in conjunction with reducing temperature favoured oocyte growth. This is consistent with the in-situ results of Olive et al. (1998) that photoperiod substantially controls the reproductive timing of *Nereis virens*, while temperature can also impose seasonality on the life cycle to control gametogenesis (Olive et al., 1997).

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SOME CONSIDERATIONS ON THE SUSTAINABLE DEVELOPMENT OF AQUACULTURE —CHALLENGES, CONSTRAINTS AND PROSPECTS

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The importance of aquaculture in food security, nutrition, poverty alleviation, income generation and so on are increasing, and its potential to meet the growing demand for aquatic products is well recognized worldwide. 《The 2030 Agenda》of United Nations presented the sustainable development goal, and aquaculture is facing more challenges to implement this goal.

Sustainable development is a broad topic, especially when aquaculture as an industry to be considered in the framework of social and economic development. In China, "Aquaculture first" policy was presented in 1980s', and tremendous achievements were made in the last 4 decades. In 2017, total aquaculture yield in China mounted to 49 million tons, accounted for 76.1% of total aquatic production in China and 60% of world aquaculture yield. The contribution of aquaculture for improving livelihood and economic development were well recognized, while its impact on local environment and ecosystem as well as its assorting with other economic sectors also raised concern widely.

In the past years, more attentions were paid on the yields and economic outputs in aquaculture. In some lakes and bays, overexploitation was observed, and natural landscape was changed. Overstocking occurred in pond farming, diseases and high mortality frequently happened. All of these evidences warned that production modes of aquaculture have to be transformed from yield and scale to quality and efficienc .

Aquaculture has to be planned and implemented as a component part of social and economic development, and sustainable development need to address properly the challenges and constraints facing the industry. This paper presents the status and developing trends of aquaculture in China based on data collected in last decades, analyzes challenges and constraints currently impacting aquaculture development, and prospects are discussed.

FOLLOWING THE INFECTION PROCESS OF VIBRIOSIS IN PACIFIC OYSTER (*Crassostrea gigas*) AND BLUE MUSSEL (*Mytilus edulis*) LARVAE USING FLUORESCENCE LABELING AND HISTOPATHOLOGY METHODS

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Pathogens, especially vibrios, are largely responsible for larval diseases in shellfish aquaculture. In order to understand how the infection process occurs in Pacific oyster (*Crassostrea gigas*) and blue mussel (*Mytilus edulis*) larvae, it is of great importance that the anatomy of the animals is well understood. In such a way, the sequence in which the internal structures are destroyed by the invading bacteria can be unraveled. In this study, three techniques were used in combination in order to have the full picture of what is happening during the infection process. After exposure of the larvae to different pathogens during challenge tests, clinical signs such as abnormal swimming behavior and destruction of the velum were observed under the inverted microscope. At the same time, by labeling *Vibrio hemicentroti* (ME09) and *V. anguillarum* (NB10) with the Green-Fluorescence Protein (GFP), we found a proliferation of the bacteria within the visceral cavity and subsequent necrosis of digestive organs, until a completed occupied body cavity. In addition, histological sectioning confirmed the damage on the level of individual organs and cells in function of exposure time. Interestingly, ME09 and NB10 had different lethality risk in Pacific oyster and blue mussel larvae but with the same progression in the infection process. This is the first time that the invasive pathways and infection dynamics of these two *Vibrio* pathogens have been investigated in Pacific oyster and blue mussel larvae. Understanding the infection process will help improve bio-control strategies and enhance the prospects of viable larviculture for oysters and mussels.

LOW CONCENTRATION PMS/UVA-LED COMBINATION AS A POTENTIAL ALTERNATIVE DISINFECTION METHOD FOR RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

Effective water disinfection played an important role in preventing the introduction and accumulation of fish pathogens in recirculating aquaculture systems (RAS)(Summerfelt et al. 2009). UV/peroxymonosulfate(PMS) based advanced oxidation was considered as a promising method in inactivating pathogenic microorganisms(Xiao et al. 2019). The present study was conducted to evaluate the feasibility of UVA-LED and 1mg·L^{-1} PMS in combination to disinfect water in RAS.

Materials and methods

Water samples (BF effluent) were collected from a RAS (*Orechromis mossambicus*) biofilter outlet. 20mL water sample containing 1mg·L^{-1} PMS(HSO_5^-) was added into a 6cm diameter petri dish and irradiated at 2cm from a UVA-LED(365nm) chip. A magnetitic stirrer was used to ensure a homogeneous solution during illumination (Fig. 1a). 0.5mL water sample was collected every 12min, quenched with 0.1M $Na_2S_2O_3$, followed by steps of 10-folds dilution with sterilized saline water, plated on a nutrient agar and incubated for 48h at 37°C to determine total bacteria.

Results

Preliminary results showed that the number of total bacteria decreased with increasing UVA-LED/PMS treatment time (Fig. 1b). Moreover, UVA-LED and 1mg·L^{-1} PMS in combination significantly reduced total bacteria number in RAS after 24↑ 60min treatment compared to original RAS biofilter effluent. Results also showed high concentration total bacteria still existed after 60min UVA-LED/PMS treatment.

Discussion and conclusion

The total bacteria inactivation performance might be due to sulfate radicals (SO_4^-) and hydroxyl radicals (·HO) generated in UVA-LED/PMS process and caused oxidative stress to bacteria (Rodríguez-Chueca et al. 2017). The high concentration total bacteria still existed after 60 min treatment may attribute to water organic compounds and alkalinity as radical scavengers. In conclusion, UVA-LED/PMS can produce free radicals, which might be used as a green, effective method to degrade pollutants and inactivate pathogens in RAS.

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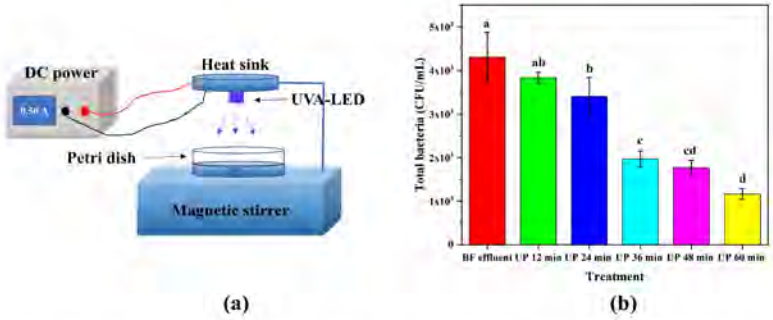

Fig. 1. (a) Schematic diagram of experimental setup and (b) Number of total bacteria after application of UV-LED/PMS (UP) at different time. Different subscripts represent the statistically differences using Duncan's post hoc comparisons ($p<0.05$).

REDUCTION OF STOCKING DENSITY THROUGH PARTIAL HARVEST: EFFECTS ON COMPENSATORY GROWTH AND PRODUCTION PARAMETERS OF *Litopenaeus vannamei* REARED IN BIOFLOC SYSTEM

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Introduction

Several researchers have reported that aquatic organisms may exhibit compensatory growth following increased growth if they are farmed under suitable conditions (Nicieza & Metcalfe 1997; Ali et al. 2003; Oh et al. 2007). This increase in growth rates is the result of strategies adopted by the organisms, including a higher food intake in the post-stress period (hyperphagia), increased feed efficienc , reduced metabolic wastes and reduced locomotion (Quinton & Blake 1990; Jobling et al. 1994; Ali et al. 2003). Wu et al. (2000) and Wu & Dong (2002) reported compensatory growth in *Fenneropenaeus chinensis* after periods of food restriction, a protein-deficient diet and low temperature. Wasielesky et al (2013) detected compensatory growth in nurseries after the decrease of stocking densities. The purpose of this study was to evaluate the effect of the effect of partial harvest and possible occurrence of compensatory growth in white shrimp *Litopenaeus vannamei* in the growout phase reared in a biofloc culture system.

Materials and Methods

The experiment was performed in a greenhouse with twelve experimental raceways, with 35 m^2 each. The tanks were supplied with saltwater from Cassino beach and densities of 600 shrimp/m^2 were stocked at an average weight of 0.22 g. After 98 days of culture the tanks were divided in four experimental treatments in which the treatment was named by the amount of partial harvests on it, then the first partial harvest for each treatment were made. The treatment T0 had no partial harvest and was used as control treatment. Treatment T1 had one partial harvest (50% of total biomass), treatment T2 had two partial harvest (33% of total biomass each harvest) after 28 days, treatment T4 had four partial harvest (20% of total biomass) every 14 days. Also, one day before the partial harvest and on the days 1, 4 and 8 biological samples of muscle, hepatopancreas and hemolymph of three shrimps per tank were collected, and then stored at -80ºC until further processing. The samples were analyzed to obtain the amount of glucose, glycogen, lactate and triglycerides in order to increase our knowledge on how energy is mobilized in order to compensatory growth can occur. The shrimps were kept at optimal water parameters conditions and were feed three times a day. The biofloc culture was maintained a at C:N ratio of 15:1 were feed represent 9:1 from total carbon proportion an measles. The suspended solids resultant from biofloc production was controlled trough clarification if it exceeds the amount of 500 mg $^{-1}$.

Results

Our results had significant differences in the production parameters as biomass production, survival and final growth between the control treatment and the treatments with partial harvests. A quadratic regression was made in order to maximize the biomass production ($R^2 = 0.99$) that could be obtained by obtaining the best percent of partial harvest. It was detected that the use of partial harvest strategy in a superintensive biofloc culture system can improve zootechnical parameters ($P<0.05$). Results confirm that biomass production and productivity were significantly higher in treatments T2, T3 and T4 (Table 1). Also, there is evidence of significant differences between treatments in the biological sample analyses, which could give us a better understanding of how compensatory growth occurs.

Table 1. Production parameters of *L. vannamei* after 156 experimental days with different percent of partial harvest.

Treatments	Partial Harvest (%)	Survival	Biomass production (Kg)	Productivity (Kg/m^2)
T0	0%	48.23%	135.02	3.85
T1	50%	90.95%	205.09	5.85
T2	33%	90.56%	217.90	6.22
T4	20%	85.07%	214.89	6.14

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Discussion and Conclusion

The results of this study indicate that the decrease in stocking density in the partial harvests increased the growth rates and survival of shrimp in the nursery phase. These results are in agreement with those presented by Wasielesky et al (2013) that obtained better results when shrimp were transferred to lower stocking densities in nursery phase of white shrimp production. In conclusion, our results indicate that shrimp farming can increase its production not by the increase of culture densities but through maximizing growth with compensatory growth by diminishing periodically the culture densities.

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NEWDEPOMOD – A MODELLING TOOL FOR BENTHIC IMPACT PREDICTION BENEATH MARINE CAGE FISH FARMS

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Introduction

NewDEPOMOD is a particle tracking modelling software, developed by the Scottish Association for Marine Science (SAMS), in conjunction with the aquaculture industry and the Scottish Environmental Protection Industry (SEPA). Its predecessor, DEPOMOD (developed between 1997 and 1999), was designed to predict impacts of marine cage fish farm discharges on the seabed and its benthic macrofauna, as a result of fish faeces and waste feed settling. Impacts depend upon the configuration of the cages and feeding rate, as well as environmental factors, such as bathymetry and water currents

AutoDEPOMOD (developed 2000-2002) iteratively runs DEPOMOD from a given starting farm biomass to obtain a compliant stocking solution, and was also adapted to incorporate residues of medicines such as Emamectin benzoate (EmBZ). AutoDEPOMOD's use has been prescribed by SEPA for fish farm development and expansion applications in Scotland for more than a decade.

The vision behind NewDEPOMOD was to remove third party software dependencies, and rewrite the AutoDEPOMOD software code in a modern programming language (Java) to allow easier maintenance and development thereafter. In addition, field and lab studies were used to improve the understanding of resuspension processes around marine cages, to enhance the predictive capabilities of the model. This was thought to be important for more dispersive sites, which are known to be of interest to the salmon farming industry as they look to expand their farmed biomass. Subsequently, the revised model was tested by SEPA to examine whether the resultant product allowed for increased accuracy in predictions, increased flexibility in its configuration, and would allow for expansion in the finfis aquaculture industry. A hindcasting approach was used to validate the model's performance against recorded seabed impacts.

Updated Resuspension Module: Fieldwork

In order to examine erosion and resuspension properties of the seabed around a variety of fish farm sites, state of the art benthic flumes were deployed by Partrac Ltd, in conjunction with the Scottish Association for Marine Science (SAMS). These 8 sites, all on the west coast of Scotland, were also previously mapped in detail using multi-beam bathymetry. A custom-built hydrodynamic lander, comprising of an acoustic Doppler current meter (ADCM), a downward looking ADC profiler (ADCP; 1m above the bed), and an upward looking ADCP (c. 2m above the bed), was also deployed above the seabed to measure its frictional effect on water movements. Deployment of this setup for a tidal cycle at each of the 8 sites allowed an estimate of bed roughness, which was discovered to be a key input parameter for enhanced accuracy of NewDEPOMOD model runs.

The results of this fieldwork allowed for a series of considerations and recommendations to be made, in order to advance the modelling of resuspension processes, known to play a significant role in more highly dispersive environments

Testing by SEPA of the revised model assessed its suitability for environmental risk assessment and determination of consent limits, to inform aquaculture-related discharge licences. The model aimed to be more accurate, more highly configurable and supportive of industry expansion. The resultant model allows for a much enhanced variety of scenarios that can be modelled, and therefore a much wider range of outcomes than AutoDEPOMOD.

Due to this high level of configurabilit , the model is capable of accurate deposition predictions, but requires an informed set up and configuration in order to achieve this. Validation work by SEPA was therefore carried out with 3 main aims: to appraise the original model (hindcast to compare AutoDEPOMOD output with site data), calibrate the new model (hindcast to compare NewDEPOMOD with site data by testing configurat on options), test consenting implications (use the outcome of aim 2 to test the implications of the new model for SEPA's consenting approach).

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The hindcasting approach provided a means to test the absolute accuracy of the model against empirical data, but had significant restrictions due to the number of well sampled sites. Testing of consenting applications therefore allowed practical questions regarding the future policy implications for SEPA to be answered, whilst adding >50 sites to the test suite. This allows for a wide range of tested scenarios with respect to environmental and operational conditions, e.g. varying flow regime and bathymetr , or cage layout and biomass etc. respectively.

Future Development

As with AutoDEPOMOD, NewDEPOMOD will be distributed by SAMS. Unlike AutoDEPOMOD, NewDEPOMOD software code can be acquired on an open source basis under a developer's licence, to encourage future development in new environments around the globe. This is with an aim to establishing a Global User and Researcher Platform, to ensure that the model will remain fit for purpose across a range of environmental and regulatory settings for the foreseeable future, as well as helping to ensure the sustainable growth of the Scottish finfish sect .

Recent and existing projects at SAMS are further developing NewDEPOMOD to improve usability and functionality, allowing closer integration with other modelling packages (such as coastal hydrodynamic models). It is expected that NewDEPOMOD will play a major role in the Scottish finfis sector in the upcoming months and years, with a view to informing sustainable regulation and expansion.

EFFECT OF A PHYTOGENIC FEED ADDITIVE ON THE GROWTH PERFORMANCE AND IMMUNITY OF PACIFIC WHITE LEG SHRIMP *Litopenaeus vannamei*, FED A LOW FISHMEAL DIET

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Introduction

Fishmeal has long been one of the most important ingredients in formulated aquafeeds, due to its high protein content, excellent composition of essential amino acids, and high digestibility. The aquaculture industry is currently facing sustainability and economic challenges driven by the reduced availability and concomitant increased price of fishmeal (FM) and fish oil. Consequently, FM is now used more selectively and at lower inclusion rates, while great efforts are being made to find alternative protein sources that promote more profitable and sustainable aquaculture endeavors (FAO 2018; Slater et al. 2018). Plant-derived products have been suggested to partially replace FM in aquafeeds (Olsen and Hasan 2012), however often at decreased growth performance, inflammator responses, and increased susceptibility to diseases (e.g. Sudaryono et al. 1999; Enterria et al. 2011; Zhu et al. 2013). This study assessed the effects of the commercial phytogenic feed additive Digestarom® PEP MGE on growth, nutritional performance, and immune response of *Litopenaeus vannamei*.

Materials and methods

After the acclimation period, 540 shrimp showing normal feeding behavior and no apparent sign of disease were selected, and randomly placed in 36 100-L tanks held in the same recirculating aquaculture system. Each tank was stocked with 15 mixed-sex individuals that weighed approximately 3 g. Four experimental groups were randomly assigned to the tanks (nine replicates per group), each receiving one of the following diets: i) standard formulation (control, 24 % FM), ii) low fishmeal diet (5 % FM), iii) low fishmeal diet plus 0.2 g/kg Digestarom® PEP MGE, and iv) low fishmeal diet plus 0.4 g/kg Digestarom® PEP MGE. The performance trial took 63 days. Shrimp were fed to apparent satiety six times per day. Feeding behavior, feed intake, and mortality were recorded for each tank during each meal during the day, and then used to calculate the amount of feed that should be provided in the subsequent meal. The following performance parameter were assessed: Fulton`s condition factor (FCF), Feed conversion ratio (FCR), Protein retention (PR), Protein efficiency ratio (PER), hepatosomatic index (HPI), body composition. Hemolymph was collected from 16 randomly selected shrimps in each group to determine immunity parameters: i) total hemocyte (THC), hyaline cells (HCs), semi-granular cells (SCs), and granular cells (GCs); ii) phenoloxidase (PO) activity; and iii) respiratory burst (RB).

Generalized Linear Model (GLM) (parametric) and Kruskal-Wallis tests (non-parametric) were used to analyze the significance of differences between treated groups. The relationship between the relative content of differentiated cells and other plasma parameters was obtained from linear regression analysis. All statistical analyses were performed in R-3.5.1. The significance level was defined as < 0.05

Results

Overall, shrimp fed the low FM diet showed a lower performance than shrimp fed the control diet. Particularly, survival and feed intake significantly dropped by 50 % and 28 %, respectively, with the reduction in FM level, whereas FCR, PER, and protein retention showed a significant increase of 65%, 46%, and 41%, respectively ($P < 0.05$). The PEP MGE supplementation of the low FM diet notably increased survival and feed intake, and decreased FCR, PER, and protein retention compared to the low FM treatment to levels similar to those observed in shrimp fed the control diet regardless of supplementation dosage. Similar shrimp growth performance and condition (FCF and HPI) were recorded among experimental groups ($P \geq 0.10$).

Shrimp whole-body composition was relatively similar among experimental treatments. Nevertheless, *L. vannamei* body composition changed throughout the trial duration; in particular, there was an increase of 13-17 % in protein content, a decrease of 30-34 % in ash content, and a notable decrease in carbohydrate content from 1.82 g/100 g to non-detectable levels.

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Total hemocyte count showed a 30 % decrease with the reduction in FM level ($P < 0.01$). However, PEP MGE supplementation counterbalanced such detrimental effects of low FM and higher plant-protein content up to ~$10x10^6$ cell/mL regardless of supplementation level. Hemocyte cell differentiation was similar among the four experimental treatments, as similar relative proportions of HCs, SCs, and GCs were observed regardless of FM level and PEP MGE in shrimp feed ($P \geq 0.11$). The activity of PO was also similar among groups, and ranged from 0.07 to 0.09 U/mg protein ($P = 0.12$). Similar RB results were also recorded in shrimp fed the control and LFM diets, but the inclusion of PEP MGE resulted in an increased RB response with increasing dosage of PEP MGE up to 43 % compared to the LFM treatment. In addition, PO activity was positively associated with the relative amount of GCs ($P < 0.05$), whereas RB was positively associated with the relative amount of SCs ($P < 0.05$).

Discussion and conclusion

In conclusion, the present study validates an alternative and nature-based solution for compensating the negative effects of partially replacing FM with plant-based protein in shrimp feeds. Although not directly tested, it is possible that this blend of essential oils improved gut health and performance. This would lead to the improved survival, feeding, and feed utilization observed here, despite the absence of direct effects on individual growth performance. Nevertheless, the immune stimulation of shrimp fed a low FM diet supplemented with Digestarom® PEP MGE suggests that this phytogenic can be successfully incorporated into FM diets containing as low as 5 % FM to improve *L. vannamei* health to the levels recorded in control shrimp, receiving diets containing 24 % FM.

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ASSESMENT OF CHRONIC STRESS AND WELFARE STATUS IN RAINBOW TROUT (*Oncorhynchus mykiss*) AT DIFFERENT STOCKING DENSITIES IN COMMERCIAL FLOW-THROUGH SYSTEMS IN GERMANY

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Introduction

Scientific evidence and awareness regarding the capabilities of fish experiencing pain and distress has raised the urgent need to quantify fish welfare more accurately by implementing standard procedures for welfare assessment in aquaculture. Next to commonly used welfare indicators such as fin damage, biomarkers on stress, in particular on chronic stress taking into account its deleterious effects on fish performance, reproduction, and susceptibility to disease, should be included to gain a more in-depth insight on the effect of a lifetime exposure of fish to the different aquaculture housing and management conditions.

To evaluate overall welfare of rainbow trout (*Oncorhynchus mykiss*) in commercial German aquaculture (ranging from extensive to intensive flow-through systems) fish will be examined from a behavioural, pathological-morphological, pathological-histological, microbiological, and stress physiological point of view. Hereby, chronic stress will be quantified using scale cortisol (Aerts et al., 2015; Zeytin et al., 2017; Hanke et al., 2019).

In this study, we will correlate for the first time quantitative data on chronic stress levels to a broad set of health and behavioural parameters in commercial aquaculture systems with different stocking densities to determine the overall fish welfare.

Materials and methods

On nine commercial trout farms, all equipped with flow-through systems but differing in stocking density, across Germany, 30 fish during grow-out (± 400g) per farm will be sampled for welfare evaluation in May 2019. Water quality parameters measured include O_2, pH, hardness, nitrogen compounds, iron and total bacterial load. Behaviour observations for social and health traits are conducted by three independent observers. Further, 30 fish per farm will be examined for outer morphological impairment and 10 fish will be sampled for plasma glucocorticoids, glucose, lactate, as well as for histological analysis of all organs. Ontogenetic scales will be sampled for scale cortisol.

Results and discussion

All different laboratory analyses will be performed in June 2019, after which the welfare in rainbow trout at nine commercial flow-through systems in Germany will be modelled. Correlations between and quality of particular welfare indicators as well as the impact of single indicators on overall welfare will be evaluated and presented at the conference.

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TOWARDS AN ECOSYSTEM APPROACH TO AQUACULTURE: DEVELOPING A HOLISTIC FRAMEWORK FOR CARRYING CAPACITY OF SALMON AQUACULTURE IN CANADA

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Introduction

The management and governance of aquaculture continues to face emerging challenges across diverse environmental and social aspects and the growing need to manage multiple objectives in increasingly crowded aquatic ecosystems. The need to take a more holistic approach to management of marine systems has prompted the development of the ecosystem approach to aquaculture (Soto et al. 2008). In recent years, carrying capacity has emerged as a potential interdisciplinary tool for the holistic analysis of pressures on the coastal zone and its ecosystem services. While carrying capacity traditionally encompasses physical, production, social, and ecological components (McKindsey et al. 2006), these components are most often studied in isolation. Despite some recent progress towards holistic assessments for shellfish (e.g. Byron et al. 2011), this approach has not been developed for finfis like Atlantic salmon (*Salmo salar*). The present research seeks to understand the requirements and capabilities of approaches for salmon aquaculture carrying capacity in Atlantic Canada to define a holistic framework based on the needs and priorities of an ecosystem approach to aquaculture.

Applying an interdisciplinary approach to build a holistic framework

This research applies interdisciplinary tools to understand the drivers of change within the social and ecological system of salmon aquaculture in Atlantic Canada (Fig. 1). A framework for holistic decision-making will be built by 1) formulating guidelines for carrying capacity based on stakeholder needs and considerations in the context of ecosystem-based management and marine spatial planning, 2) assembling information on carrying capacity indicators and thresholds, and their relationships with aquaculture, and 3) assessing the utility of new tools and approaches for integrated carrying capacity. Local case studies across different areas within Atlantic Canada will also variations in socio-economic contexts, cultures, and history of aquaculture interactions.

Preliminary results

Drawing from a review and gap analysis of carrying capacity within the literature, several principles and research needs were identified to support an agenda for better aligning carrying capacity with ecosystem-based management (able 1).

Preliminary results also emerge from discussions with key informants on the needs, values, and priorities for carrying capacity to identify relevant indicators, methods, and considerations to continue to build the framework. Further research will explore the utility of tools like expert judgement, ecosystem services, and spatial applications for holistic decision-making in Atlantic Canada. In addition, given knowledge gaps for social research in aquaculture, this work is exploring aspects of social carrying capacity, community-based participation and stakeholder engagement.

Implications for management and policy

Carrying capacity is a potentially powerful tool often considered critical to overall area management but is poorly implemented in many areas (Corner et al. 2018). Developing a framework for carrying capacity can provide managers and planners with a holistic tool to guide their decisions, such as determining suitable fish farming locations, setting appropriate monitoring thresholds, and making sustainable resource management policies (Ross et al. 2013). This research identifies relevant information about social, ecological, and economic indicators and thresholds, and tests new approaches and methods to build a holistic framework for carrying capacity. Where social aspects of aquaculture are a major challenge and still poorly studied, this research can foster a better understanding of the perceptions of local communities to aquaculture, identify conflicts, and help inform decision-makers about social acceptance issues. This work draws from experts both within and outside Atlantic Canada and can thus have applicability to management in other areas of the world, where aquaculture is also rapidly developing and where countries are increasingly recognizing the need for adopting an ecosystem approach to aquaculture.

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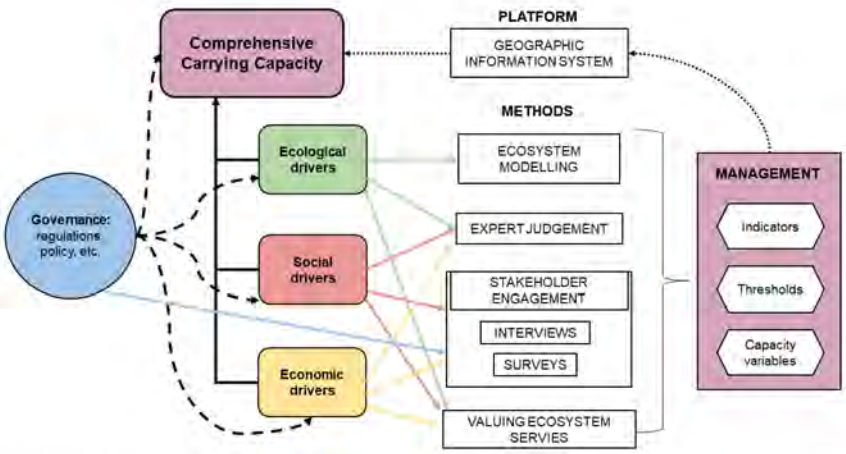

Fig. 1. Research framework to support the development of a comprehensive carrying capacity framework that integrates ecological, social, and economic drivers within the context of relevant governance factors in Atlantic Canada.

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ENVIRONMENTAL IMPACTS OF AQUACUTLURE: ASSESSING THE RISK OF FURTHER GENETIC INTROGERSSION OF DOMESTICATED SALMON IN WILD POPULATIONS IN NORWAY

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Farmed Atlantic salmon are domesticated, and where significant interbreeding with wild conspecifics occurs, this may lead to changes in fitness traits, and ultimatel , less productive wild populations (Reviewed by Glover *et al.* 2017).
Widespread and in some cases extensive introgression of domesticated Atlantic salmon is already documented in wild Norwegian populations. However, one of the central questions is whether further introgression is likely to occur in the future also.
Annual risk assessments of the environmental impacts of Norwegian aquaculture have been conducted by the Institute of Marine Research and collaborators since 2010. However, recently, the method for assessing risk was upgraded. In this talk we present the methods used, and results of the risk assessment in Norway in 2019.

In total, we categorized 7, 3 and 3 of the 13 aquaculture production zones, covering the entire Norwegian coastline, as having high, moderate and low risk of further genetic introgression in native populations respectively.

Review article "open access":

Glover K.A., Solberg M.F., McGinnity P., Hindar K., Verspoor E., Coulson M.W., Hansen M.M., Araki H., Skaala Ø. & Svåsand T. (2017) Half a century of genetic interaction between farmed and wild Atlan tic salmon: status of knowledge and unanswered questions. *Fish and Fisheries* **18**, 890-927.

NEW ANIMAL WELFARE STANDARDS IN SWITZERLAND: NOVEL APPROACHES FOR A FAIRER KILLING OF CRUSTACEANS

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Aquaculture production, import and diversification of aquatic species is continuously growing, also in Switzerland. Meanwhile, a rising concern on animal welfare in farmed animals has expanded through the last decades. Several studies on pain research have proved this concern, describing the potential suffering of many species, including the long forgotten crustaceans. On the 19th of September 2015 the topic reached the Swiss Federal Council through a Motion, aiming to ban the import of alive crustaceans for food purposes into Switzerland.

Due to the bilateral veterinary agreement with the EU (Annex 11 to the Agreement on trade in agricultural products; RS 0.916.026.81), it was not feasible to ban the import of alive crustaceans. Specificall , from the point of view of trade law (WTO and free trade agreements) high demands are placed on an import ban. Still, the Swiss Federal Council adapted the legislation in order to enhance welfare during the import and killing of crustaceans in the Swiss gastronomy. These modifications include the ban of transporting crayfish on ice, and when crayfish are hold for commercial purposes, it is required to pass specific training and hold a cantonal permit for the commercial husbandry of wildlife animals. Reaching a further level, crayfish can now only be killed when previously stunned. A new device to stun crustaceans was tested with different working settings and species of crayfish. After this, two different stunning devices are available on the Swiss market.

In this way, Switzerland has become the first country to ban the boiling of crayfish without previous stunning, being followed by Germany and parts of Italy. The changes allow a significant enhancement on animal welfare standards and promote fairer approaches to produce and consume aquatic invertebrates.

THE EFFECT OF DIETARY BLACK SOLDIER FLY LARVAE *Hermetia illucens* MEAL AND PASTE ON GROWTH PERFORMANCES AND IMMUNE PARAMETERS IN ATLANTIC SALMON *Salmo salar*

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Introduction

The development of novel feed ingredients with functional properties offers solutions to sustainable and health concerns in salmon industry. In the last decade, there has been a growing interest in using insects as a protein source for aqua feed production. One of the most promising insect species for feed purposes is black soldier fly (BSF) (*Hermetia illucens*). It has been reported that BSF larvae comprise chitin, medium chained fatty acids, antimicrobial peptides (Caligiani et al. (2018), Vogel et al. (2018)), all of which are destined to promote both growth and health of fish (Henry et al. (2018)). BSF larvae might, therefore, be considered a functional feed ingredient. In this sense, investigation of potential functional properties of BSF larvae that provide growth and health benefits beyond traditional feeds, represents a great opportunity to guarantee the sustainable future of salmon aquaculture. Therefore, in this experiment, we studied the effect of two dietary BSF larvae products produced by two different processing methods, i.e. insect meal and insect paste, on growth performances and immune parameters in Atlantic salmon.

Materials and methods

The insect meal contained 38.0% and 28.9% of crude protein and fat respectively, whereas insect paste contained 11.3% and 9.3% of crude protein and fat respectively with 28% of dry matter content. Further, the insect paste was preserved with 2.5% inclusion level of formic acid. A total of 1260 Atlantic salmon (*Salmo salar*) with 34 g of mean initial weight were distributed into 21 fibe glass tanks (60 fish per tank), and with 3 tanks per dietary treatment. The fish were fed *ad libitum* (i.e. 10% excess) for 7 weeks. The 7 dietary treatments include 3 diets where 6.25% (6.25_IM), 12.5% (12.5_IM) and 25% (25_IM) of dietary protein was replaced by insect meal; 2 diets, in which 6.25% (6.25_IP) and 12.5% (12.5_IP) of dietary protein was replaced by insect paste and 2 control diets named Control_1 (positive control based on fishmeal, soy protein concentrate, and fish oil) & Control_2 (negative control with 0.875% formic acid equal to the formic acid level in the 12.5_IP diet). At the end of the feeding period, feces and organs/tissue of fish were collected. Spleen leukocytes were isolated and stained with different antibodies targeting IgM, IgD and CD8. The number of B and T cells were measured using flow cytometry. Macrophages were isolated from head kidney, cultured and infected with CFSE labelled *Piscericketssia salmonis* for one hour. The capability of macrophages to incorporate the pathogenic bacteria were assessed by flow cytometry and confocal microscopy. Analysis of nutrient composition of feed and feces, plasma immune responses, proteomics of skin mucus, intestinal histology and 16S rRNA sequencing of gut microbiota are ongoing.

Results and discussion

Growth performances: The fish fed diets containing insect meal and insect paste showed similar growth performances as for the control diets. The feed conversion ratio and relative feeding rate of fish were ranged from 0.70-0.75 and 1.47-1.71, respectively, and were similar among the diets.

Immune parameters: Diet 12.5_IM present an increase in the number of IgD+ cells in the spleen. However, this increase was more significant in the fish fed diet Control_2, which contained formic acid. The same trend was observed on the number of T lymphocytes, where diets Control_2 presented the higher number of CD8+ cells in the spleen. On the other hand, macrophages isolated from fish fed 12.5_IM diet were more prone to incorporated labeled-bacteria, which suggest a higher phagocytic capacity compared to the other diets. The incorporation of *P. salmonis* was confirmed by confocal microscopy.

Conclusions

Based on growth performances, both insect meal and insect paste can potentially partly replace the current protein sources used in Atlantic salmon diets. Our ongoing analyses aim to confirm its functional e fect beyond the nutritional value.

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Table I: Performance indicators of fish fed experimental diets for 7 weeks

Performance indicator	Control_1	6.25_IM	12.5_IM	25_IM	Control_2	6.25_IP	12.5_IP	SEM	P value
Initial body weight (g)	34.4	34.3	34.3	34.3	34.3	34.4	34.3	0.03	0.934
Final body weight (g)	94.4	92.4	93.9	89.1	85.2	94.8	89.3	1.13	0.184
Specific growth rate (%body weight d-1)	2.2	2.2	2.2	2.1	2.0	2.3	2.1	0.03	0.252
Body weight gain (g)	60.0	58.1	59.6	54.8	50.9	60.5	55.0	1.13	0.199

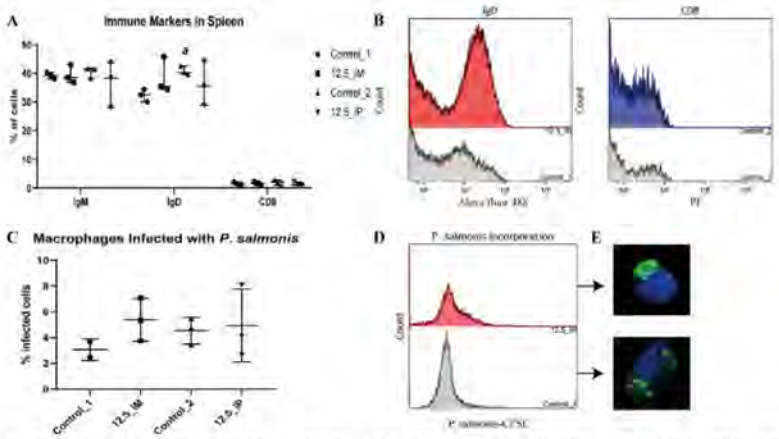

Figure 1: Immune parameters. **A)** Flow cytometry showing the number of spleen leukocytes expressing IgM, IgD or CD8. **B)** representative histogram showing the intensity on the fluorescence of IgD and CD8. **C)** number of macrophages able to incorporate labeled-bacteria (*P. salmonis*). **D)** representative histogram of intensity of fluorescence of CFSE. **E)** confocal microscopy confirming the incorporation of *P. salmonis*. Each measurement was performed in triplicate using 6 fish per tank.

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EFFICIENT GUIDE TO PRODUCERS FOR GOOD AQUACULTURE PRACTICES AT ALL STAGES OF PRODUCTION

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The GLOBALG.A.P. Aquaculture Standard brings to the market a complete solution for buyers and suppliers, based on current market demands, covering the key sustainability aspects that the animal production for human consumption is required to achieve. Operating since 2004, certified farms feedback reflects that this certification turns into a practical guide to their operations through detailed criteria written in a friendly and clear manner satisfying GLOBALG.A.P. purpose on working hand in hand with producers. Although the ultimate goal is to obtain certification, the standard is the best tool the aquaculture sector can adopt to properly manage their operations. Currently there are 38 species certified for the finfish crustaceans and molluscs in 32 countries worldwide.

The aspects covered are those stipulated by the FAO Technical Guidelines on Aquaculture certification. With strong governance reliability, including the robust Integrity Program. This pioneering program is the first of its kind in food certification, and is designed to ensure consistent delivery and implementation of the standard worldwide. It acts as a feedback mechanism that serves the ongoing improvement of the GLOBALG.A.P. System in all its aspects, promoting transparency and integrity.

The key elements of the GLOBALG.A.P. Aquaculture standard are currently recognized and evaluated.

- Food safety: is the ONLY certifi ation scheme recognized by the Global Food Safety Initiative – GFSI for farming of fish.
- Environment: recognized by the Global Seafood Sustainability Initiative – GSSI Apr 2018.
- Animal Health: the OIE Aquatic Animal Health Code criteria for farms is covered by the standard. The robust Veterinary Health Plan requirements is a reliable backing that farms give value.
- Animal Welfare: on top of animal health, GLOBALG.A.P. Aquaculture has been recognized as the only international private standard outside the UK that covers animal welfare practices at harvest and slaughter. Further animal welfare innovative criteria is spread for all production stages.
- Workers Occupational Health & Safety: workers are key to efficient operations, appropriate training is included in the requirements.
- Workers Welfare: GLOBALG.A.P. Risk Assessment on Social Practices is compulsory to assess.

The scope covers full production chain verification of: Broodstock, Seedlings, Feed, Farming Post harvest activities up to the point of sale for final consumers.

INFLUENCE OF PHYTOGENIC FLAVONOIDS ON GROWTH (LENGTH AND BODY WEIGHT) OF YOUNG RED STRAIN ALL-MALE TILAPIA IN INDOOR RAS SYSTEM

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A feeding trial with 468 all-male tilapia fingerlings was conducted in an in-house aquaculture facility. Aim of the trial was to assess the effect of a phytogenic feed additive (AO) rich in polyphenols on growth performance compared to a negative control (N). The feed additive (Anta®Ox Aqua, Dr. Eckel Animal Nutrition, Germany) is a natural phytogenic feed additive made from grapes, hops and green tea delivering a specific amount of flavonoids (>10%). The feed additive was added at a dose of 500 g/t of feed. Fish were kept in fresh water at 28°C in two separate indoor RAS (recirculating aquaculture system) with big (125 L) and small (40 L) glass tanks. Feed was provided manually three times per day on workdays and two times per day on weekends. Fish growth was measured at three time points (day 0, 22 and 68) by sampling 10 fish/tank and measuring individually standard-length and body weight.

Overall growth was within expected values. During the 68 days trial period fish grew on average from 0.41 g to 6.25 g live body weight and from 22.8 mm to 56.7 mm mean body length (see Fig. 1). Specific growth rate (SGR) was 3.92 % per day. Dispersion of values was within expected ranges: Coefficient of variation was in the range of 25 to 30 % for body weight and 8 to 10 % for body length.

A difference in growth performance was observed between fish kept in big and small tanks. Furthermore, growth performance (weight and length) was better in fish of the AO treatment group than in the N control group (see Fig. 2 A and B). Compared their mean bodyweight of day 0 fish of the AO group achieved a higher mean body weight than the negative control group (+16% in the small tanks and +14% in the big tanks). Analysis of variance of a linear model explaining the weight with 'system' and 'feed' as explanatory factors revealed that the 'system' effect was highly significant ($p<0.001$) and the influence of the 'feed treatment is also significant at $p<0.02$.

From the results of the present study, we conclude that addition of plant-derived flavonoids from grapes, hops and green tea can improve growth of Tilapia fingerings. The results are in line with former studies with polyphenol-rich feed additives in shrimp (Niyamosatha et al. 2015, Chuchird et al. 2017). The study results are of interest for tilapia hatcheries and hatchery feed producers worldwide. Further research could clarify which flavonoid sub-group has the biggest influence on growt

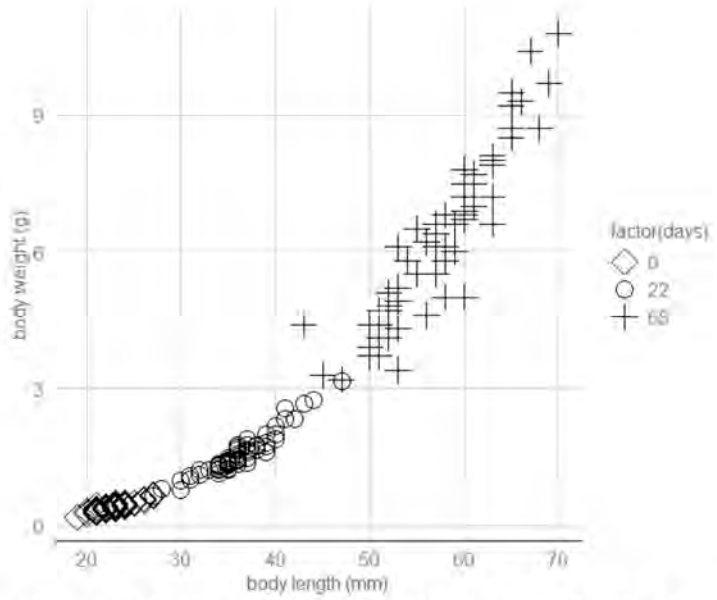

Fig. 1. Relationship between body length and body weight of individual Tilapia fingerlings during 68 days trial period. Colors representing sampling time points.

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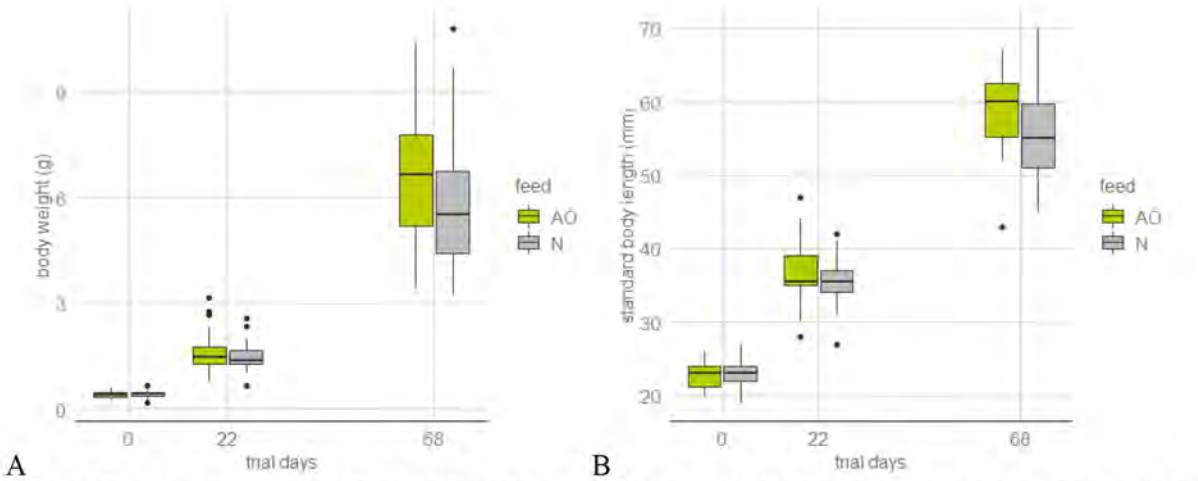

A B

Fig. 2. Development of body weight (A) and body length during 68 days trial period, split by feed treatment. Colors representing feed treatments.

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PARENTAL AND DIRECT FEEDING EFFECTS OF DIETARY SELENIUM IN RAINBOW TROUT (*Oncorhynchus mykiss*) FRY

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Introduction

Selenium (Se) is an important micronutrient needed for the production of selenoproteins involved in antioxidant metabolism. Total Se levels in animal feeds are regulated within the EU to a maximum of 0.5ppm. Due to the low Se content in plant protein sources, the replacement of fish meal in aquafeeds warrants an increasing attention on dietary Se supplementation over the full life cycle. Studies in poultry have shown a positive influence of parental Se transfer by supplementation of low basal Se diets on the oxidative status in progeny, especially when highly available organic Se sources were used (Zhang et al., 2014). In fish little is known about transgenerational e fects of Se (Wischhusen et al., 2019).

Material and Methods

The progeny of three groups of rainbow trout broodstock, fed either a control diet (Bctr: 0.3ppm Se) or diets containing a minimum of 0.6ppm Se by supplementation of either 0.3ppm sodium selenite (Bss) or 0.3ppm hydroxy-selenomethionine (Bso), over a 6 month period prior to spawning, were used in this feeding trial (Wischhusen et al., 2019). At the beginning of exogenous feeding, swim-up fry from each of the three parental groups were sub-divided into three groups (3 parental groups x 3 fry dietary groups, resulting in 9 treatments) and fed fry diets containing similar Se levels and sources compared to the parental diets (Fctr: 0.3ppm, Fss: 0.6ppm, Fso: 0.6ppm). Fish were sampled as whole fry at the end of an eleven-week feeding period for Se and antioxidant measures, followed by two-way ANOVA to study parental and direct feeding main effects.

Results

Neither nutritional history, nor direct Se feeding showed any significant effect on survival (97.6±0.34%) and final body weight (4.64±0.04g). However, the weight gain was higher in offspring coming from broodstock fed Bss and Bso compared to offspring of Bctr group (66±0.8 and 66±1 vs. 60±1%, respectively), without any significant effect of direct Se feeding (Fctr: 66±1; Fss: 63±1; Fso: 63±2%). Total Se content in whole-body fry was not significantly different according to the nutritional history, but was significantly increased by feeding Se supplemented diets to the fry ($P<0.01$; Table I); compared to fry fed Fctr, fry fed Fss showed +24% of whole-body Se while fry fed Fso showed +76% of whole-body Se. Preliminary results on markers of antioxidant metabolism show significant effect of both parental nutritional history and direct Se feeding. The GPX activity was increased by direct feeding of sodium selenite, but reduced in fry originating from the parental group fed sodium selenite. A similar reduction by parental Se-supplementation was noticed for GST (especially when fed directly sodium selenite) and CAT, while GST activity was also reduced by dietary organic Se supplementation of fry (especially in offspring of the Bctr group).

Discussion and conclusion

The maternal transfer of Se has been confirmed in rainbow trout recently (Wischhusen et al., 2019). In that study, Se concentration in the swim-up fry before first-feeding was the highest when the parental groups were fed an organic Se source compared to an inorganic Se source with the lowest concentrations in the negative control group. In the present study, after 11 weeks of exogenous feeding, the effect of parental Se nutrition on Se status of offspring was not detectable anymore. Nevertheless, similar to earlier studies (Fontagné-Dicharry et al., 2015), the Se status in the fry was elevated by direct feeding of Se supplemented diets with the highest level in hydroxy-selenomethionine-fed-group. Low Se levels in rainbow trout at early life stages have been associated with an increase of oxidative stress parameters (Fontagné-Dicharry et al., 2015). The present study shows a long-term parental effect in fry on the antioxidant metabolism, independent of the Se status, with a reduced activity of antioxidant enzymes, including the selenoprotein GPX, when originating from parental groups fed Se supplemented diets, in contrast to direct Se feeding and short-term parental effects described by Wischhusen et al. (2019). Pending results on oxidative stress parameters will help to ordinate the obtained results. The mechanisms behind the observed changes of this nutritional programming, possibly through epigenetic marking alteration, await further investigation.

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Table I. Parental and direct feeding effect on the body composition (µg kg fw^{-1}) and antioxidant enzyme activity (mU mg prot^{-1} except for CAT and SOD, U mg prot^{-1}) of whole-body rainbow trout fry sampled after 11-weeks of exogenous feeding.

	Parental effect			Direct feeding effect			p-value		
	Betr	Bss	Bso	Fetr	Fss	Fso	PE	DF	int
Body composition									
Se	774 ± 69	732 ± 64	717 ± 64	556 ± 18^{c}	691 ± 29^{b}	977 ± 17^{a}	0.20	**0.00**	0.87
Enzyme activity									
TotGPX	28 ± 2^{a}	23 ± 1^{b}	24 ± 1^{ab}	23 ± 1^{b}	27 ± 2^{a}	25 ± 1^{ab}	**0.03**	**0.03**	0.89
SeGPX	19 ± 2	17 ± 1	16 ± 1	16 ± 2	19 ± 1	17 ± 1	0.13	0.30	0.12
CAT	61 ± 4^{a}	51 ± 6^{ab}	44 ± 2^{b}	54 ± 5	51 ± 4	52 ± 5	**0.02**	0.82	0.51
SOD	8 ± 0	7 ± 1	7 ± 0	8 ± 1	7 ± 1	8 ± 1	0.07	0.55	0.74
GST	125 ± 7^{a}	111 ± 7^{ab}	97 ± 7^{b}	121 ± 5^{a}	109 ± 9^{ab}	102 ± 7^{b}	**0.01**	0.05	**0.03**
GR	14 ± 1	11 ± 1	12 ± 1	13 ± 1	13 ± 1	11 ± 1	0.13	0.43	0.43

Values are means ± SEM of nine rearing tanks (three broodstock or fry diets). Within rows and for each diet-related effect: parental (PE) or direct feeding (DF), means not sharing a common superscript letter are significantly different according to two-way ANOVA followed by TukeyHSD test. Significant parental effect × direct feeding interactions (int) are highlighted in bold type. totGPX, total GPX; SeGPX, seleno-dependent GPX; CAT, catalase; SOD, superoxide dismutase; GST, glutathione-S-transferase; GR, glutathione reductase.

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FINANCIAL RISK EVALUATION OF COMMERCIAL PRODUCTION OF NILE TILAPIA (*Oreochromis niloticus*) IN NET-CAGES, SÃO PAULO, BRAZIL

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Aquaculture is a sound economic alternative in the consumption of high quality protein, especially in developing countries, where food demand have presented an exceptional growth in the last years. Since it deals with the production of living organisms, the activity faces innumerable production risks, from environmental conditions to fluctuations in input prices, which may cause financial losses for the rural producer. This study aimed to evaluate the financial risk of commercial production of Nile tilapia (*Oreochromis niloticus*) in three production scenarios.

The farm comprised of 18 net-cages (43 m^3 each) located in Chavantes reservoir, São Paulo, Brazil (644.481 E, 7.413.975 N) which was used as a model during six production cycles (180 days). Productive and economic data were collected and three elaborated production scenarios were developed: scenario I (C1): production volume of 1,000m^3, scenario II (C2): production volume of 5,000m^3 and scenario III (C3): production volume of 10,000m^3. We evaluated the probability of occurrence of financial risks (N = 10,000 random interactions) in relation to the economic indicators, such as internal rate of return (IRR), net present value (NPV) and payback of capital. By means of applying the Monte Carlo Simulation method (MCS) using the @risk Software, from interactions between the productive and economic variables. The input variables productive were: production cycle, water temperature, mortality, feed conversion; and economics were: fish selling price, fry price, ration price and attractive minimum rate.

For all scenarios, the fluctuations of the variables such as fish selling price, fry price, ration price and attractive minimum rate were 1.14 to 1.52 US$.kg-¹, 0.03 to 0.07 US$.kg-¹, 0.48 to 0.61 US$.kg-¹ and 4.4 to 15.0% respectively. The initial investment value for C1 was US$ 124,522.03, for C2 of US$ 270.301,40 and C3 of US$ 444,138.00. Regarding the output variables, the probability of occurrence of values below 8% for IRR was 86.7% for C1, 47.3% C2 and 43.2% C3. For the variable NPV was 91.8% for C1, 60.2% for C2 and 55.7% for C3 in relation to values below zero. On the other hand, return of capital over 10 years, was 86.3% for C1, 44.8% C2 and 38.5% for C3 (Figure 1).

Within these results, we can conclude that aquaculture properties with a production volume of less than 5,000 m^3 presented a high financial risk (p 88.3%), while properties above 5,000 m^3 of production volume medium to low financial risk (p 48.3%), thus making it more financially sustainable and profitable

(FAPESP, CNPq, Capes)

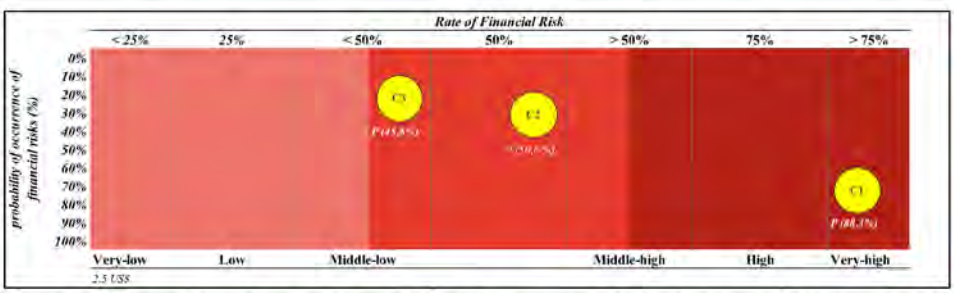

Figure 1. Financial risk evaluation of tilapia commercial production with Monte Carlo Simulation method.

IMPROVED USE OF LOW FM AND LOW FO DIETS IN GILTHEAD SEABREAM (*Sparus Aurata*) JUVENILES OBTAINED BY COMBINED BROODSTOCK SELECTION AND NUTRITIONAL PROGRAMING

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Introduction
Gilthead sea bream, as other marine fish species, has a restricted ability to synthesize 20:5n-3 and 22:6n-3 from their 18C fatty acid precursor, 18:3n-3, that is high in plant oils. Since this process is greatly limited by the activity of the fatty acyl desaturase 2 (fads2) enzyme, producing fish with a higher fads2 activity would improve their ability to utilize diets low in fishmeal (FM) and fish oil (FO) and containing plant oils. This could be achieved by nutritionally programing fish through broodstock nutrition as it has been previously demonstrated in sea bream (Izquierdo et al., 2015; Turkmen et al., 2017) or selecting broodstock that have a higher *fads2* activity. The later has not received sufficient attention and, therefore, the aim of this study was to determine the combined effect of selection of broodstock for their high or low fads2 gene (*fads2*) expression and feeding broodstock with diets differing in their fatty acid profiles.

Materials and methods
Sea bream juveniles were obtained from broodstock with either high (H groups) or low (L groups) *fads2* expression in blood, after being fed one month before the spawning season with low FM and low FO diets, and fed during the spawning season a diet with 100% FO (FO groups) or 20%FO/80%RO (RO groups). These four groups of juveniles (HFO, HRO, LFO and LRO) (2.3±0.01 g initial body weight, mean ± SD) were randomly distributed into 12 x 250 L tanks in triplicate and challenged with a diet containing only 7.5% FM and no FO for 45 days. At the end of the trial, liver samples were collected for biochemical and molecular analysis.

Results
The highest growth (final body weight and SGR) was found in juveniles from broodstock with a high *fads2* expression and fed RO diet (HRO) and the lowest in those from broodstock with a low *fads2* expression and fed RO diet (LRO) (Table 1). Those fish (HRO) had also the best FCR, being significant y improved in comparison to HFO juveniles. Besides, in juveniles from low *fads2* broodstock, growth was higher when broodstock had been fed FO (LFO). Therefore, there was a combined significant effect of selection of broodstock for their high or low *fads2* expression and the fatty acid profile of broodstock diets. Besides, in offspring from broodstock fed 100% FO diet, there was a higher level of 18:4n-3, 20:4n-6 and 20:5n-3 when broodstock was selected for its high *fads2* expression (Table 2).

Conclusion
In conclusion, selection based on high blood *fads2* expression and nutritional intervention of broodstock can be an effective way to increase the low FM/FO diet utilization of the progenies.

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Table 1. Growth of offspring juveniles from broodstock with high or low *fads2* expression and fed either FO or RO diets, after challenged with a low FM and FO diet for 45 days

		HFO		HRO		LFO		LRO		*Two-way ANOVA*		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	*Selection*	*Broodstock diet*	*Selection* diet*
Initial weight(g)	Mean	2.3	0.01	2.3	0.00	2.3	0.01	2.3	0.01			
Final Weight(g)	Mean	5.5	0.35	6.3^{A}	0.03	$^{1}6.1$	0.23	$^{2}5.3^{B}$	0.33	*n.d.*	*n.d.*	*0.001*
SGR (%)	Mean	2.0	0.15	2.3^{a}	0.02	$^{1}2.2$	0.09	$^{2}1.9^{a}$	0.14	*n.d.*	*n.d.*	*0.001*
FCR (%)	Mean	$^{a}1.6$	0.11	$^{b}1.4$	0.06	1.5	0.15	1.6	0.20	*n.d.*	*n.d.*	*0.023*

Two-way ANOVA was used to analyze the effect of selection and broodstock diet (n=3). T-test was used to analyses the data between different groups. $^{a, b}$ in front of the value mean there is significant difference between the offspring come from high *fads2* broodstock ^{1,2} in front of the value mean there is significant difference between the offspring come from low selection broodstock group. $^{A, B}$ in the back of the value mean there is significant difference between the offspring come from broodstock fed with diet FO but with different *fads2* expression. $^{a, a}$ in the back of the value mean there is significant difference between the offspring come broodstock fed with diet VO diet but with different *fads2* expression.

Table 2. Hepatic contents in selected fatty acids in juvenile seabream from broodstock with high or low *fads2* expression and fed either FO or RO diets, after challenged with a low FM and low FO diet for 45 days.

	HFO		HRO		LFO		LRO		*Two-way ANOVA*		
Name	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	*Selection*	*Diet*	*Selection* Diet*
18:2n-6	14.07	1.66	15.44	0.97	14.33	0.79	14.73	1.69	*N.D.*	*N.D.*	*N.D.*
18:3n-3	2.37	0.27	2.33	0.24	2.13	0.16	2.24	0.33	N.D.	N.D.	N.D.
18:4n-3	0.99^{A}	0.13	0.95	0.04	20.68^{B}	0.07	10.86	0.30	0.047	N.D.	0.013
20:2n-6	0.47	0.09	0.28	0.13	0.27	0.03	0.33	0.19	N.D.	N.D.	N.D.
20:4n-6	0.65^{A}	0.24	0.26	0.10	0.22^{B}	0.01	0.34	0.08	N.D.	N.D.	0.012
20:5n-3	0.86^{A}	0.22	0.45	0.22	0.36^{B}	0.05	0.67	0.38	N.D.	N.D.	0.037
22:6n-3	3.23	1.72	1.88	1.16	$^{1}1.43$	0.03	$^{1}2.30$	0.23	N.D.	N.D.	N.D.

Two-way ANOVA was used to analyze the effect of selection and broodstock diet (n=3). T-test was used to analyses the data between different groups. $^{1, 2}$ in front of the value mean there is significant difference (p<0.05) between the offspring come from same selection broodstock group (Low) but the broodstocks were fed with different diet. $^{A, B}$ in the back of the value mean there is significant difference (p<0.05) between the offspring come from broodstocks fed with diet FO but with different *fads2* expression.

Acknowledgements

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IDENTIFICATION OF *Chlorella* sp. USING THE INTERNAL TRANSCRIBED SPACER SEQUENCE AND ITS ROLE AS INHIBITOR STRESS CAUSED BY RNA *Viral nervous necrosis* INFECTION ON THE HUMPBACK GROUPER

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Introduction

Indonesia has a great diversity of microalgae, but has not yet received much study nor has this diversity been used for applied technology. However, relatively little information is available about microalgal resources despite their potential to be used as antiviral in the fish. *Chlorella sp.* is known as one of the fastest growing microalgae because it able to grow doubling its biomass weight in a few hours of sunlight and contains a triacylglycerol as much as 14-30%. This microalgae can yield the oil till 75% of dry weight under different condition. Algal organisms contain a rich of a novel source of biologically secondary metabolites and active primary. This metabolite may be an attractive bioactive compound for the pharmaceutical industry. Chlorella sp. contains lipid, protein, carbohydrates, minerals, and vitamins. Many functions also use *chlorella* sp. for medicine such as such as antifungal, antibacterial, and antiviral activity. The study aims to identify the Chlorella sp. from local isolate Using the Internal Transcribed Spacer (ITS) and to know its role as inhibitor stress caused by RNA *Viral Nervous Necrosis* (VNN) infection on the Humpback grouper

Methods

One way to determine *Chlorella* sp. local isolate was identified it using a gene analysis with the sequences of ITS. This study aims to determine the *Chlorella* sp. isolate and its potential as an anti-VNN virus in the Humpback grouper (*Cromileptes altivelis*). The methods were in vivo test through an administration Chlorella sp orally to the fish with treatment (A): control = normal fish, (B): fish + *Chlorella sp.*, (C): fish + VNN, and (D): fish + VNN + *Chlorella* sp.*);* analysis of *Chlorella* sp. was conducted by PCR using sequence ITS with IST-1 primer (Forward: TCCGTAGGTGAACCTGCGG) and ITS-4 (Reverse: TCCTCCGCTTATTGATGC). The stages of PCR amplification ITS included initial denaturation at 95ºC for 3 min., denaturation at 98ºC for 10 s., annealing at 53ºC for 30 s., and extension at 68ºC for 45 s. The PCR stage was carried out for 35 cycles. The sequencing results are compared and adjusted according to the order stored in the NCBI GenBank database using BLAST. The computed counts are then manually checked and corrected. The pair evaluation distance is calculated using the equations of Jukes and Cantor implemented in the MEGA6 program. Analysis of VNN Infection using a target of 294 bp with primer (Forward: CGTGTCAGTCATGTGTCGCT; Reverse: CGAGTCAACACGGGTGAAGA). The stages of PCR amplificatio of VNN included denaturation at 94ºC for 30 s., annealing at 60ºC for 30 s., and extension at 72ºC for 45 s. The PCR stage was carried out for 40 cycles. Tissue inflammatory response was conducted by the immunohistochemistry (IHC) on the kidneys tissue using IgG anti-HSP primary antibody cross-reaction with fish. The stress inhibitor response includes detecting inflammation in the tissue by IHC methods that quantified using the immunoratio technique.

Results and Discussion

Based on the results of the sequencing analysis, it is known that Chlorella sp. with ITS markers have a similarity level of 92-93% but the total score is still low, namely 738 compared to Chlorella sp. at the GenBank. Long sequence of base pairs Chlorella sp. with ITS marker which is 556 bp. PCR analyses showed VNN infection at 294bp in kidney organs. In vivo test results *Chlorella* sp., for anti-VNN, showed an inflammatory response to VNN infection through HSP-70 expression in the kidneys. The qualitative result using immunohistochemistry shows a positive anti-inflammatory reaction on the kidney tissues. Administration of Chlorella sp. to the fish increase HSP-70 expression in treatment A, B, C, and D were 19.7%, 50.0%, 63.9%, and 60.3%, respectively. Some studies mention, synthesis of HSP70 and protein coil as sitoprotektif cells that result in increased by osmotic stress stimulus (Deane dan Woo, 2004). Inflammatory reactions can also be characterized by the expression of Hsp (Yanuhar et al., 2015). The results of histopathological tests on the kidneys showed that in treatment D there was less tissue damage compared to treatments D. Damage that occurs includes necrosis, vacuolation, hyperplasia, and edema.

Conclusion

The conclusion is the identifica ion of Chlorella sp. molecularly can use ITS markers with a low score. Chlorella sp. has the potential as an antiviral VNN indicated by an increase in HSP-70 expression in Humpback Grouper kidney organs.

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THE ASEAN FISHERIES EDUCATION NETWORK; ESTABLISHMENT, EXPANSION AND EXTENSION

Yeong Yik Sung, Happy Nursyam, Truong Quoc Phu, Tan Min Pau, Najiah Musa, Siti Azizah Mohd. Nor, Mieke Eggermont, David Bassett, Marieke Reuver & Patrick Sorgeloos

The ASEAN Fisheries Education Network, referred to as ASEAN-FEN, represents a consortium of university based academics, drawn from those institutions with an emphasis on fisheries and aquaculture across south east Asia. ASEAN-FEN was established in 2011 by agreement of 9 founding members for the purpose of supporting and enhancing the fisheries and aquaculture sector through education, research, and public outreach across the ASEAN region. ASEAN-FEN supports and facilitates activities of educators, scientists, and agencies responding to local, regional, national, and international issues on fisheries and aquaculture. ASEAN-FEN has developed quickly, expanding from 9 members in 2011 to representing 32 institutions in 2019. Current membership comprises academic institutions from; Malaysia, Vietnam, Thailand, Indonesia, The Philippines, Myanmar, Cambodia and Laos. As the secretariat of ASEAN-FEN, Universiti Malaysia Terengganu (UMT) has lead and coordinated academic collaboration projects from both the European Union and China, aimed at promoting capacity building, academic exchange and research collaboration. This session will consider the establishment, expansion and outreach work of ASEAN-FEN, particularly with reference to the EC Horizon 2020 funded EURASTiP project, addressing capacity building between Europe and se-Asia in aquaculture education.

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AN INVESTIGATION OF HOW THE CLASSIFICATION STATUS OF SHELLFISH PRODUCTION AREAS MAY BE AFFECTED BY THE NUMBER OF *Escherichia coli* RESULTS ASSESSED

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The classificati n of shellfish production areas (SPAs) in many shellfish producing countries is based on monitoring for faecal indicator organisms. This monitoring assesses the risk of contamination with pathogens and determines the level of post-harvest treatment necessary for the shellfish prior to sale for human consumption

This study investigated the effect of the number of *E. coli* monitoring results on the classification status of SPAs using the A, B and C classification criteria prescribed in the European Food Hygiene Regulations. The assessment was based on a database of *E. coli* results obtained from monitoring in shellfish from seven production areas (>255 sample results/SPA) on the coast of Santa Catarina (Brazil). It was found that six SPAs would be classified as B and one as C if all the available results were considered. Ten series of 50 data samples were randomly extracted from each production area dataset (12–120 results/sample, in multiples of 12). Classifications given to each data sample resulted in two production areas that had been given B status based on the full database being classified more times as A than as B when data samples with 12 results were considered. In general, the number of data samples compliant with class A decreased with the higher the number of *E. coli* results that were assessed per data sample.

The results indicate that areas with class B status can be misclassified as A during the initial classification when fewer results are available. Furthermore, areas with 'prohibited' status can be misclassified as C during the initial classification, when 12 results are considered in compliance assessments.

This study did not identify the same potential misclassification issue in relation to SPAs with classifications ranging between B and C. These classes share the same maximum limit (46,000 MPN/100g), therefore, compliance with 4,600 MPN/100g is the legal standard that differentiates the two.

This study identifies two factors that may lead to misclassific tion of a production area: the varying number of results considered in the compliance assessment; and the consideration of maximum *E. coli* result as a legislative standard. Therefore, possible ways of minimising the risk of misclassificati n include the adoption of other statistics as microbiological legislative standards rather than maximum *E. coli* result or the consistent use of a fixed amount of results to classifying SPAs during initial and ongoing monitoring.

These results emphasise the need to consider long-term monitoring datasets in compliance assessments to ensure that the classification status of S As truly reflects environmental contamination levels

HOW DIET COMPOSITION AND ENVIRONMENTAL FACTORS AFFECT THE UPTAKE AND METABOLISM OF ASTAXANTHIN IN ATLANTIC SALMON (*Salmo salar*)

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Introduction:
The red flesh colour is a hallmark of salmon products, and essential for consumers' perception. Pigmentation of skeletal muscle and eggs is determined by the content of xanthophylls – oxygen containing carotenoids such as astaxanthin. Wild salmon accumulate carotenoids produced by algae and transferred via the food chain. Farmed salmon receive feeds with added astaxanthin. However, only about 1/10 of the astaxanthin added in feeds is retained in the salmon fillet, and lately there have been increasing problems with poor colour on salmon fillets in Norwegian salmon farming. The combination of low utilisation efficiency and crucial importance for product quality stimulates research on astaxanthin metabolism and factors that determine its retention and functional role in salmon. An important goal of the project was to enhance the understanding of dietary factors that affect astaxanthin uptake, metabolism and retention, since little is known about the mechanisms that regulate the deposition of astaxanthin in the salmon muscle. Astaxanthin is a precursor of retinol in salmon, but salmonids also have a reductive metabolism of astaxanthin. Astaxanthin is also a powerful antioxidant, and stress may have a negative impact on flesh pigmentation. However, the regulation of different pathways and their importance for flesh pigmentation and health have not been much studied in Atlantic salmon.

Salmon diets have gone through major changes the last decades from essentially a marine based diet with a protein/fat ratio of 3:2 in the early 90s to a diet with 70% plant ingredients and a protein/fat ratio of approximately 1:1 today. This shift in ingredients has resulted in reduced levels of the omega-3 FAs EPA and DHA, and increased pro-inflammatory omega-6 FAs content in organs and tissues. High fat diets with 70–80% plant ingredients also increase whole body lipid retention and lipid concentrations in the liver of Atlantic salmon when additional marine micronutrients are not added. The question raised in this project is how this potentially may affect the muscle deposition of astaxanthin in salmon.

Methods
The possible effects of such dietary changes on absorption, metabolism and flesh retention of astaxanthin were tested in a controlled experiment in tanks using a 2x2 factorial design with replacement of fish meal (FM) and fish oil (FO) with plant protein (PP) and plant oil (PO). Diets with different content of marine and plant ingredients were fed to Atlantic salmon post-smolts (star weight 197 g) in 1 m^3 tanks supplied with flow-through seawater (6 and 12 °C). The fish were fed a marine-based diet (Diet 1, FM/FO), a diet containing fish meal and plant oil (Diet 3, FM/PO), a diet with plant protein and fish oil (Diet 4, PP/FO) and a low marine diet with plant protein and plant oil (Diet 2, PP/PO). The low marine diet was also supplemented with concentrates of phospholipids, PL (soy lecithin, Diet 5, and a marine phospholipid product, MPL, Diet 6) to study potential effects of phospholipids on astaxanthin absorption and retention. The diet formulation is shown in Table 1. Diet 1,3 and 4 were fed on both 12 and 6°C. A low marine diet without astaxanthin (- Ax) was also fed to the salmon to study effects of astaxanthin on salmon performance and gene expression. Liver cells were isolated from marine and low marine diets and incubated with ^{13}C astaxanthin and cortisol to study effects on astaxanthin metabolism.

Table 1: Diet formulation (%)

	Diet 1 FM/FO	Diet 2 PP/PO	Diet 3 FM/PO	Diet 4 PP/FO	Diet 5 PP/PO + soy lec	Diet 6 PP/PO + MPL
Fish meal	58,7	7,5	58,7	7,5	7,5	7,5
Soy P concentrate	-	26,0	-	26,0	26,0	26,0
Wheat gluten	-	22,5	-	22,5	22,5	22,5
Wheat starch	13,5	10,0	13,5	10,0	10,0	10,0
Fish oil	22,0	6,5	5,5	25,8	6,5	6,5
Rapeseed oil	-	19,4	16,5	-	19,4	19,4
Sum phosfolipid	0,5	0,3	0,6	0,3	0,8	0,6
Sum EPA/DHA	4,1	1,1	1,7	3,5	1,2	1,2

(Continued on next page)

Results

The results from the study show that salmon diets with plant protein (PP) led to reduced feed intake and growth, and low digestibility of lipid and astaxanthin. There was also abnormal lipid accumulation in liver and intestine in salmon fed low FM diets without supplementation with phospholipids. Adding phospholipids increased absorption of astaxanthin and fat, and digestibility of fat and astaxanthin was at the same level in diets 1,3,5 and 6, and lower in diets 2 and 4. The digestibility of astaxanthin and lipid was highly correlated and both parameters were linearly correlated with the concentration of PL in the diet. There was however no significant effect of temperature on astaxanthin and fat digestibility. Astaxanthin retention in the fillet was dependent on both absorptive and post-absorptive processes such as metabolism of astaxanthin in various organs. The concentration of astaxantin metabolites in fillet and liver were affected by both diet composition and temperature. The concentration of the metabolite idoxanthin was higher at 6 than at 12°C. FM and supplementation with PL also increased idoxanthin. There was an interaction between diet and temperature, at 12°C there was no effect of plant oil (PO) on astaxanthin and idoxanthin concentration in muscle, but at 6°C there was a negative effect of PO on astaxanthin concentration in muscle, and an increased concentration of idoxanthin.

Stress, both in cell culture studies with liver cells and *in vivo* seemed to increase the amount of metabolic products of astaxanthin. The results on gene expression suggest different functions in liver, muscle, and intestine in salmon fed a low marin diet. In intestine the main effect of lack of astxanthin was immune suppression, in muscle there were symptoms of mild inflammation, and in liver the main effect was a stimulation of steroid biosynthesis. The largest number of differentially expressed genes were found in muscle.

Conclusion

Diet composition and temperature affect the muscle deposition and metabolism of astaxanthin, and stress seem to increase the turnover of astaxanthin. To fully understand the mechanisms that control the muscle deposition of astaxanthin, the interaction between environment and diet must be considered.

Acknowledgements

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POTENTIAL IMPACT OF WARMING SEA WATER TEMPERATURES ON ATLANTIC SALMON (*Salmo salar*) FARMING IN NORWAY

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Introduction

Climate changes may result in longer and more frequent periods of elevated sea temperatures. Periods with seawater temperatures above the optimum temperatures for salmon has been shown to result in reduced appetite and growth, increased mortality and disease problems in the intensive farming of Atlantic salmon. Changes in temperature may further affect the site production, the spreading, distribution and severity of diseases and outbreaks, and the carrying capacity of the environment. For most aquaculture production areas, climate projections are only available from global or regional climate models that have coarse resolution which does not take into consideration the local conditions. Higher resolution models that focus on the coastal areas used for production would be useful for future planning. Assessment of temperature changes across spatial and temporal scales can provide information to support the industry in evaluating opportunities and risks and enable the development of adaptation, or mitigation, strategies where necessary.

We have modelled the potential impact on Atlantic salmon growth under possible future conditions, including increased sea temperature and extreme temperature events. Sea temperatures have a strong impact on productivity through several mechanisms, including fish growth and welfare. Severe temperature increase may challenge biological thermal thresholds, and thereby ruling out production or requiring costly adaptation measures. We show how observation-based bias-correction of modelled temperatures are crucial for applications at local scale. For long-term sustainability and allowing for adaptation, knowledge of both present-day situation and the potential future characteristics of a farming area are vitally important.

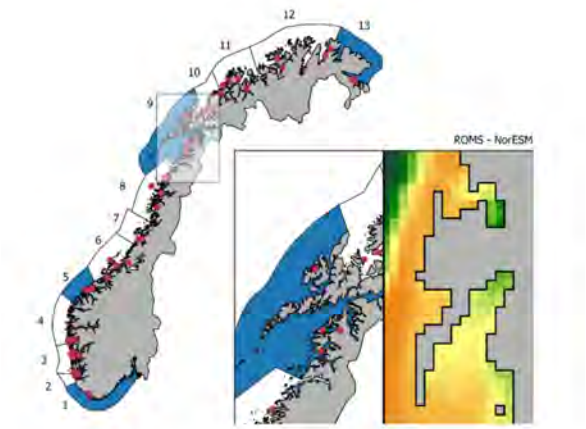

Figure 1: Map of Norway showing the 13 aquaculture production regions, red dots represent farms. Regions 1, 4, 9 and 13 are highlighted in blue and are in focus in this study. Each region had four modelled sites, resulting in a total of 52 sites. Enlarged image of regions 9 shows the ROMS-NorESM grid temperature resolution.

Materials and methods

Stakeholders provided farm data from the 13 Norwegian management regions (Fig. 1) that were used in this study. The dataset covered a wide range of geographical locations, illustrating the different impact scenarios and the need for local adaptation strategies. Four regions, 1, 5, 9 and 13, are in focus in the work presented here.

Salmon growth was modelled using a combination of the Ewos-Cargill EGI model to capture seasonal trends in feed, and a Dynamic Energy Budget model (DEB) for Atlantic salmon from Føre et al (2016).

The temperature projections were extracted from a regional downscaling of the IPCC RCP4.5 scenario simulated by the Norwegian Earth System Model (ROMS-NorESM) (Bentsen et al., 2012; Iversen et al., 2013) covering 2006 – 2070 (Skogen et al., 2019; Sandø et al, in prep). The ROMS-NorESM downscaling is one of the highest resolution regional climate simulations available for the case study area. Since only the moderate IPCC scenario (RCP4.5) at sufficient resolution was available, we wanted to evaluate the potential risk associated with more extreme summer temperatures. Scenarios were constructed based on average farm measurements to indicate how more extreme temperatures, beyond average conditions, could affect growth. Thus, "high" and "very high" summer temperatures were added to illustrate "what-if-scenarios" for more extreme temperatures,

(Continued on next page)

Results

Comparisons between climate model outputs and farm measurements revealed that the model often over- or underestimated the farm temperatures by several degrees. We therefore calibrated the projected temperatures to local farm conditions using a bias correction technique, where the difference between actual observed farm temperatures and simulated temperatures for a recent reference period were calculated and added to future climate projections (Hawkins et al., 2013). This method corrected the temperature models to local conditions and made them relevant for future projections of aquaculture along the coast of Norway.

Modelling growth and comparing temperatures scenarios with salmon thermal thresholds illustrated that farmers in the South will have to prepare for, and adapt to, unfavorable farming conditions, while farmers in the North may experience increased production due to more days closer to the thermal optimum, although there may even be at risk of higher temperatures in these regions. Also, the possible increased growth seen in the northern regions are far smaller than the reduced production observed in the South. In addition, prolonged periods of higher temperatures may further result in health and welfare issues other than increased mortality, that are not simulated in the model, as the model used in this trial only consider increased temperatures.

Conclusions

With the 52 sites considered in this study, we have demonstrated that the regional climate projections are not suitable for local forecasting, thus to generalize possible adaptation strategies directly from uncorrected predictions is impossible. In order to make functional adaptation plans, local environmental conditions and farming strategies. Extensive information on how the environment may change at the productions must be considered and this depends upon the availability of detailed environmental data at farm sites and potential future farm sites and how this may further effect on the biology of the farmed specie will be crucial for the development and implementation of the adaptation strategies.

ClimeFish

The EU financed, H2020 project ClimeFish, aim at estimating the effects of climate change on fisheries and aquaculture in Europe from today until 2050. Follow our results at the ClimeFish website: www.climefish.e

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GROWTH OF PURPLE SEA URCHINS (*Anthocidaris crassispina*) BY FEEDING DIFFERENT SEAWEEDS

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Introduction

Sea urchin is one high price seafood (up to US$100/kg). Recently, the amount of sea urchins that could be captured is decreasing in Taiwan because the market demand is growing. Therefore, much attention has been paid to farming of sea urchins by feeding seaweeds. In this study, the effects of seaweeds on the growth of purple sea urchins (*Anthocidaris crassispina*) were reported.

Materials and methods

Twenty purple sea urchins were bought from Hua-Quao Fish Market in Donggang Twon, Pingtung County on January 6, 2018. Five purple sea urchins were bred in one glass tank with aeration. One-hundred grams (wet weight) of single seaweed were fed every week. The seawater was replaced, the sea urchins were weighted and tank was washed at the same time every week.

Results

According to the results, the body weight of purple sea urchins did not increase after feeding them with seaweeds, and even slightly decreased over time (Table 1).

When fresh seaweeds were used as feed, the seawater remained clean for ten days. However, the seawater became dirty after feeding dried *S. ceylanica* for three days. It is noted that feeding dried *S. ceylanica* to sea urchins would increase seawater turbidity and cause sea urchin death.

Discussion and conclusion

The purple sea urchin (*Anthocidaris crassispina*) was fed with different seaweeds (e.g., *S. ceylanica*, *G. filicina*, *G. lemaneiformis*, and *U. lactuca*). The body weight of the purple sea urchins did not increase with time over the course of the study. It is suggested that the size of seaweeds might influence the feeding efficiency of the seaweeds. Further studies are needed to enhance the growth of the sea urchins.

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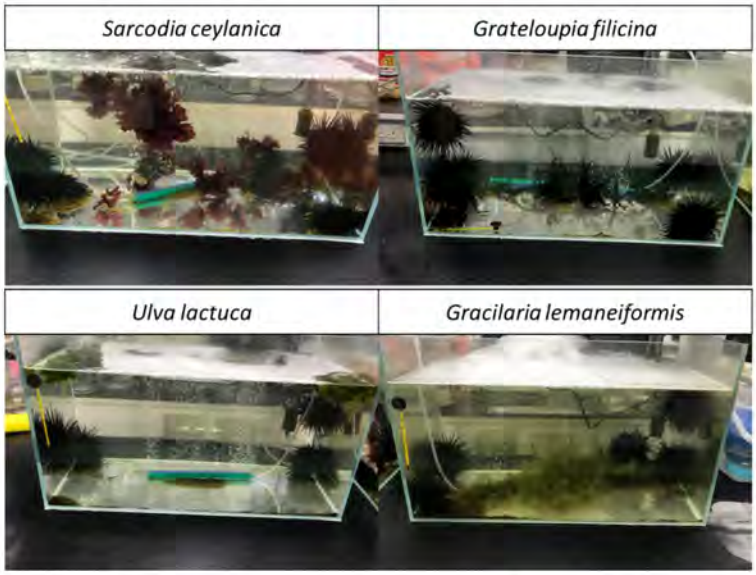

Fig. 1. The cultivation of purple sea urchins in the lab

Tab. 1. The changes in body weight of purple sea urchins feeding different seaweeds

Seaweed feeding / No. of sea urchins / Time	*S. ceylanica*	*U. lactuca*	*G. lemaneiformis*	*G. filicina*
	5	4	5	5
Initial	729	519	788	731
1st wk	733	514	784	702
2nd wk	720	531	782	708
3rd wk	712	525	791	706
4th wk	709	510	766	697
5th wk	716	510	762	695

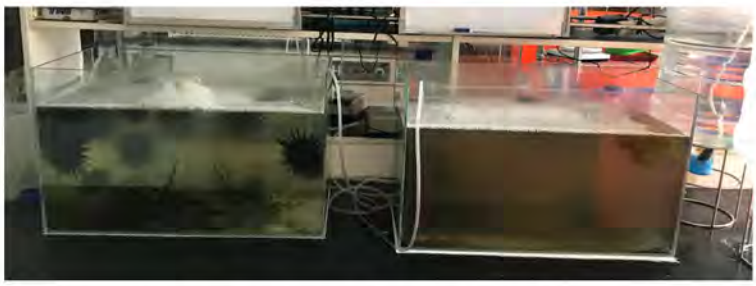

Fig. 2. The appearance of seawater after feeding dried *S. ceylanica* for one week

EXTRACTION OF MYCOSPORINE-LIKE AMINO ACIDS FROM LAVER (*Porphyra dentata*)

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Introduction

Mycosporine-like amino acids (MAAs) are a family of intracellular ultraviolet (UV)-absorbing compounds which protect aquatic organisms against solar UV radiation. Occurrence of MAAs in microalgae and macroalgae are well-known. Due to lack of pure compounds, quantitation of MAAs is still a challenging work. This work explored Pohphyra-334 contents in laver (*Porphyra dentate*) using water flush extraction methods and evaluated extraction conditions on the recovery of MAAs.

Materials and methods

The multiple extraction test (10 times) was carried out to evaluate the Porphyra-334 leachability for dried laver (Fig. 1). First of all, a half grams of dried laver was filled into a syringe. Then, extraction solvent was poured in the syringe. The leachate was collected by a beaker. Next, the leachates were lyophilized under -80^{o}C. Two mL of 0.2% HAc were used for reconstitution and then filled by 0.2 μm membrane filte . Final, high performance liquid chromatography (HPLC) was applied for Poyphyra-334 analysis.

Results

With regard to the extraction conditions (Tab. 1), the total Porphyra-334 contents in Porphyra were 3.76-5.98 mg/g. 20% methanol is best for Porphyra-334 extraction. For S/S ratio of 60 and 90, total Porphyra-334 contents were similar by different solvents. For the extraction times required for 80% efficienc , three times are needed by S/S ratio of 60 and 90. Overall, S/S of 60 and 20%-methanol are the best extraction condition (Fig. 2).

Discussion and conclusion

The effects of extraction conditions on MAAs recovery were investigated in this study. The obtained optimal parameters were S/S of 60 and 20% of methanol. This result is similar to that reported by Cardozo et al. (2011), showing that extraction with 20% methanol was the most efficient and with 50% ethanol was the least efficient among the tested combinations. Similar results were also observed by Zhang et al. (2016) who reported that optimization of extraction of MAAs from *Gloiopeltis furcata*. The conditions optimized were S/S ratio of 67, and methanol concentration 12%.

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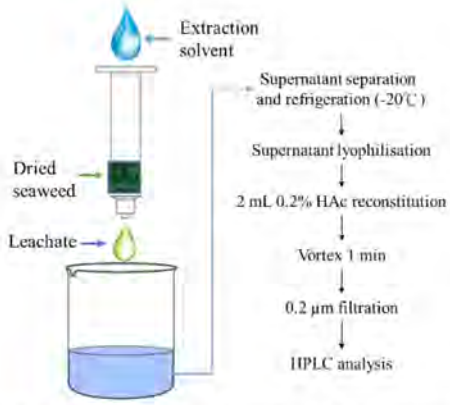
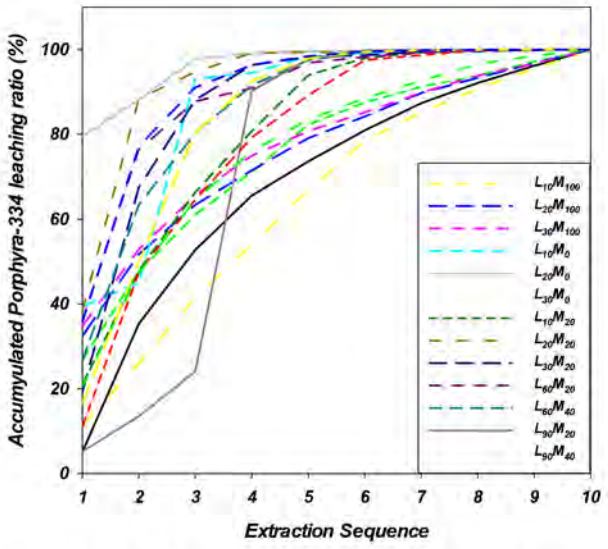


Fig. 1. Processing flowchart for MAAs extraction

Tab. 1. The total Porphyra-334 (mg/g) extracted under variant extraction conditions

Solvent	Methanol			
Solvent ratio (%) / S/S ratio	0	20	40	100
10	2.52	5.37	NA	0.03
20	1.33	3.45	NA	0.22
30	3.06	3.21	NA	0.17
60	NA	5.98	3.90	NA
90	NA	5.96	4.30	NA



$L_{oo}M_{xx}$: L_{oo} for S/S ratio, M for methanol, $_{xx}$ for solvent ratio

Fig. 2. Cumulative Porphyra-334 leaching ratio

TRAINING FOR AQUACULTURE PERSONNEL BY MOBILE PLATFORMS

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Introduction
In aquaculture sector, the information flow between the producers and relevant authorities is vital in order to sustain technology updating and access to information (Subasinghe et al., 2010). In today's word thanks to variety of media, learning activities are easily shared and knowledge is spread instantly. The app HEALTHY_FISH is an ERASMUS+ project led by the Spanish Marine Fish Farming Association (APROMAR) in collaboration with four partners: SGS Tecnos (Spain), the Italian Aquaculture Association API (Associazione Piscicoltori Italiani), (Dokuz Eylül University, Institute of Marine Sciences and Technology (DEU-IMST) and the Croatian Chamber of Economy (CCE).The objective throughout this project is to establish a "Training Program standardized at the European level for the aquaculture industry". The program will consist of training modules. The modules will compensate the needs of the professionals by e-learning tools. The second face of the project is to study the possibility of further education materials of the farm and apply e-learning method on these subject and adapt additional and up-to-date e-mobile training modules.

Materials and Methods
The first step was to consider the current training programs for the sector. Later to specify the need and evaluate extra training necessities. Moreover, for training materials, the needs of four different countries were gathered to qualify professionals in their own countries Spain, Italy, Turkey, Croatia. By the time the assessed and clarified training needs are addressed the "Standardized Aquaculture Training Program" is also made reachable on an "Open Access Moodle Learning Platform".

Results
It is inevitable that mobile devices are extremely handy as educational tools which will be used by farm workers who usually work in distant locations. The programme will be transported into an App that the content can be reviewed by users, they also can evaluate and obtain a certificate at the end. It serves in five languages English, Turkish, Italian, Croatian, and Spanish and 11 modules. The first impression of the farm workers show that they find "e-learning" user friendly, easily accessible and inspiring. The Standardized Aquaculture Training Program is available at http://www.apromar.es/healthyfish.

Discussion and Conclusion
There is a growing demand on aquaculture in accordance with that worldwide aquaculture production should be increase (FAO, 2011). The basis of this growth depends on education and training methods with regards to the need for human resources. If the aim is to increase productivity aquaculture it is fundamental that the skills of farmers and employees should be enhanced (Seixaz et al., 2015). New approaches for professional education are providing platforms that evolves Information and Communication Technologies (ICT). Many different digital learning resources see the light of day created by different types of organizations. Currently there is a fast paced production of learning and training materials on line that helps users easily access the huge potential of the digital resources (Moises et al., 2010). This training programme will also help to identify weaknesses and resolve them. Another advantage of e-learning is that subjects and matters can easily and rapidly be renewed. These are some of the reasons why e-learning is often promoted. This education program, which was developed with the aid of ICT, can function as a good example of digital learning within the aquaculture sector for each country.

The overall impacts of the project are:

During the application at farms, provision of an innovative training tool to the professionals of aquaculture is observed. Innovative training materials are created. Approaches of the sector to e-learning evaluated positively. Practice of vocational training activities for professionals was gained. Linguistic diversity for the programme which in English, Spanish, Italian, Croatian and Turkish was enabled.

In the future, this study will provide European level training to professionals and personnel of the aquaculture sector. This will also help standardization of vocational programmes.

By means of communication and debating among professionals the quality and sustainability standards will be endorsed.

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TESTING THE EFFECT OF INCLUDING EXOGENOUS CARBOHYDRASES IN PLANT-BASED FEEDS FOR GILTHEAD SEABREAM

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Introduction

Non-starch polysaccharides (NSPs), carbohydrates present in plant ingredients, are formed by cellulose and other heteropolymers of more complex sugars such as mannose, xylose, and arabinose, among others. They give important differences in solubility, water retention capacity, interaction with other food ingredients, and in the intestinal microbiota. In many cases, NSPs form a matrix that hinders the access of digestive enzymes to the protein and starch present in seeds of cereals and legumes. Therefore, the use of enzymatic complexes capable of partially degrading NSPs, has shown positive effects in the nutritive use of feeds with high amounts of plant ingredients. Additionally, exogenous carbohydrases can promote the development of a beneficial microbiota and consequently results in a better health status. The inclusion of these enzymes has been evaluated in feed for freshwater species such as tilapia, catfish, rainbow trout and carp, but not in marine fish. Consequently, the present study aimed to assess the effect of including a commercial mixture of carbohydrases in a feed containing high amounts of plant ingredients on growth, digestion, metabolism, and gut microbiota in the gilthead seabream (*Sparus aurata*).

Materials and methods

Gilthead seabream juveniles (mass 250 ± 4 g) were randomly distributed into four 250-L tanks (for each tank N = 24), with flow-through water system at 19.5 ± 1.0 °C, and a photoperiod of 11L/13D. In two tanks the fish was fed a standard plant-based diet for this species (C-diet; including 66% of vegetable meal and 17% of animal meal), and the other two tanks the same diet was supplemented with endo-beta-glucanase (E-diet) that hydrolyses (1,3)- or (1,4)-bonds in beta-D-glucans. All tanks were fed a daily ration of 2.5 % body mass distributed in three meals. At the end of the experiment, after 3 months, fish were sampled for growth determination, and biochemical and molecular analyses. Gut content was collected for microbiome analysis. The 16S-rDNA amplicon sequencing was done by Illumina. Relative expression of genes related to growth and digestion were quantified in the whole gastrointestinal tract. Potential differences were tested by one-way ANOVA ($p < 0.05$).

Results

No significant influence of the tested diets was obtained on the studied variables, except for length, plasma triacylglycerol, and *igf* expression (Table I). Weight and other analysed parameters were generally higher (except for the alkaline proteases expression) in the E-diet group, although these differences were not statistically significant

Gut microbiota was dominated by firmicutes, followed by proteobacteria and actinobacteria. No statistical differences were detected in the taxa composition between treatments (PERMANOVA analysis), but the amount of proteobacteria was higher in fish fed the E-diet. The presence of *Photobacterium* reached at significantly higher levels in fish with E-diet. However, this difference was mainly driven by the individuals from only one tank, probably due to a tank effect rather than a diet.

Discussion and conclusion

Inclusion of endo-beta-glucanase in the diet seems to induce some beneficial changes. However, they are not evident enough, but a longer experimental on-growing period could reveal significant biological e fects.

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Table I. Growth, metabolites, and gene expression (mean ± SEM) in gilthead seabream with the two diets. Different letters denote significant differences between treatments.

	Control diet	Enzyme diet	N	p value
Length	27.28 ± 0.18 [b]	27.85 ± 0.17 [a]	24	**0.0231**
Weight	422.7 ± 9.16	448.1 ± 10.46	24	0.0740
Condition Factor	2.08 ± 0.03	2.07 ± 0.04	24	0.8613
Metabolites				
Plasma Glucose	7.41 ± 0.51	8.70 ± 0.62	18	0.1157
Plasma Lactate	5.87 ± 0.41	5.84 ± 0.30	18	0.9426
Plasma TAG	3.94 ± 0.26 [b]	5.60 ± 0.57 [a]	18	**0.0127**
Plasma Protein	35.44 ± 2.72	36.72 ± 2.79	18	0.7450
Liver Glucose	0.32 ± 0.02	0.35 ± 0.03	18	0.4940
Liver Glycogen	2.75 ± 0.07	2.75 ± 0.09	18	0.9740
Liver TAG	1.32 ± 0.08	1.44 ± 0.09	18	0.3297
Gene expression				
Growth factor ***igf***	0.64 ± 0.04 [b]	0.97 ± 0.08 [a]	11 to 12	**0.0010**
Amylase ***amy2a***	0.51 ± 0.10	0.63 ± 0.18	11 to 12	0.5932
Chymotrypsin ***ctrb***	1.31 ± 0.31	0.98 ± 0.21	11 to 12	0.3859
Trypsin ***try***	0.83 ± 0.16	0.80 ± 0.19	10 to 11	0.8944
Pepsin ***pga***	1.01 ± 0.15	1.12 ± 0.30	11 to 12	0.7393
Proton pump ***atp4a***	0.99 ± 0.21	1.04 ± 0.30	11 to 12	0.8919

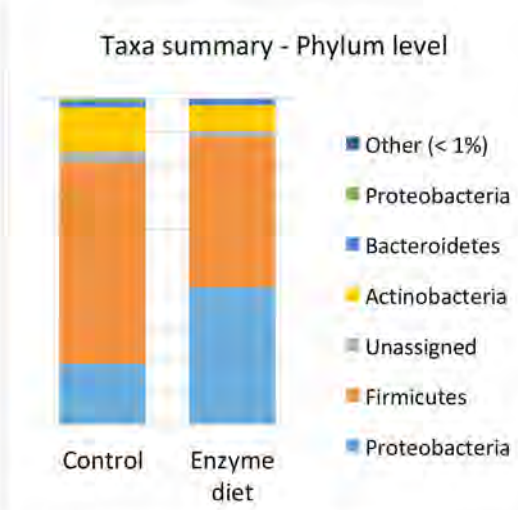

Fig. 1. Frequency distribution in gut microbiota for each treatment at the phylum level.

SIMULATING THE EFFECT OF FEEDING TIME AND FREQUENCY ON GUT TRANSIT AND DIGESTIBILITY IN GILTHEAD SEABREAM AND SENEGALESE SOLE JUVENILES

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Introduction

Understanding the factors that affect the digestion processes in fish is essential to ensure an efficient and sustainable aquaculture, given the large effect that impaired digestion can have on all downstream indicators (growth, conversion efficienc , environmental impact). In this presentation, we discuss the results of simulating the digestive processes of gilthead seabream (*Sparus aurata*) and Senegalese sole (*Solea senegalensis*) under different feeding protocols, using a mathematical model developed for Ballan wrasse [1] and calibrated for these species using ground-truth observations from a recent study by Gilannejad et al. [2].

Methods

The simulations reflected the experimental protocol of Gilannejad et al. [2], where different feeding protocols were tested. we simulate the feeding protocols sequentially: each protocol is simulated for 5 days, for a total of 20 days of simulation. In each 5 days period, the following sequence is applied: on the first two days, fish are fed with unmarked diet (to pre-condition the model state before introducing the marker); on the third day, marked diet is introduced (to evaluate yttrium wash-in dynamics); on the fourth day, the marked diet is again replaced by unmarked diet (to evaluate yttrium wash-out dynamics); on the fifth da , no feed is provided (to ensure all protocols are tested under the same initial conditions).

Results and Discussion

Results of the simulations, in terms of the some of the most important observables parameters (maximum amount of yttrium measured and apparent digestibility coefficients) can be seen in Table I. Plots displaying the time-dependent filling and emptying behavior of the different gastrointestinal tract segments can be seen in Figure 1.

The calibrated model for gilthead seabream generally displays simulated responses that are consistent with measured values in vivo (i.e. are in the correct order of magnitude). Nevertheless, current limitations in the model, particularly as it relates to feeding behavior, restrict its accuracy. When these limitations are addressed, the use of such digestion models can be useful to identify gaps in our knowledge of digestive processes, and eventually be used as predictive tools in digestive physiology and nutrition research.

Table I. Comparison between measured and simulated digestibility (dry matter and protein ADC) and marker-related responses, for the different feeding protocols in both species.

Sparus aurata	measured	simulated	
maximum yttrium (stomach)	250	148	ng/mg tissue
maximum yttrium (intestine)	150	86	ng/mg tissue
ADC protein (Protocol 1) One diurnal meal	91.5	93.4	%
ADC protein (Protocol 2) Three diurnal meals	93.6	93.8	%
ADC protein (Protocol 3) Five diurnal meals	92.1	93.5	%
ADC protein (Protocol 4) Continuous diurnal feeding	94.1	88.9	%
Solea senegalensis	measured	simulated	
maximum yttrium (stomach)	150	117	ng/mg tissue
maximum yttrium (intestine)	100	30	ng/mg tissue
ADC protein (Protocol 1) One diurnal meal	62.7	89.0	%
ADC protein (Protocol 2) Six diurnal meals	82.4	90.2	%
ADC protein (Protocol 3) Six nocturnal meals	71.6	90.2	%
ADC protein (Protocol 4) Continuous noctur. feeding	76.4	90.2	%

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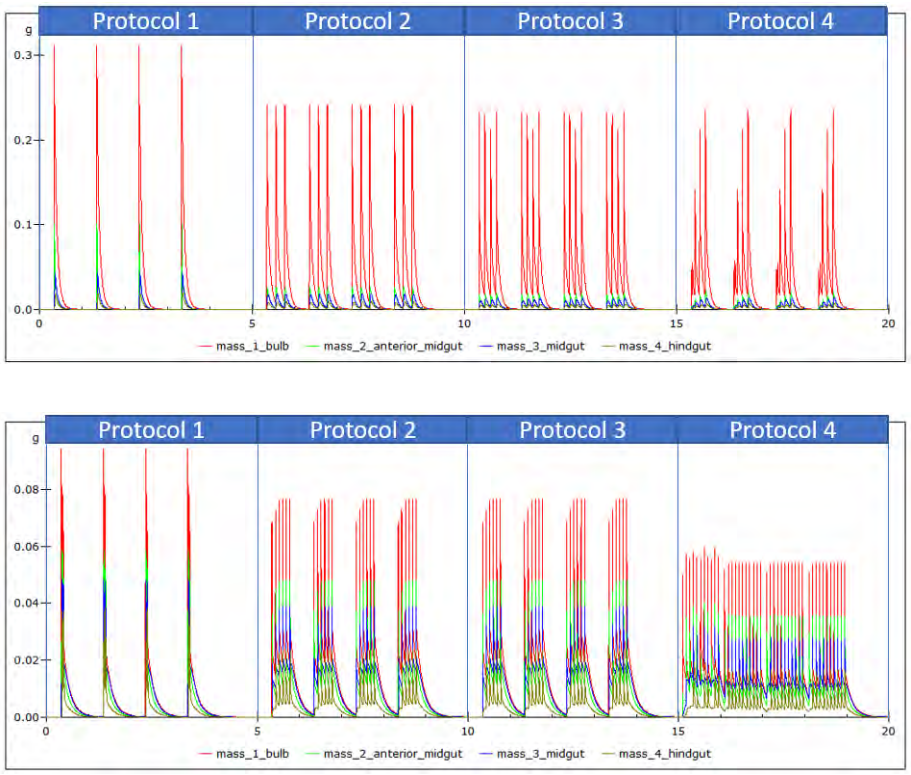

Figure 1. Plot displaying the simulated amount of mass in each GIT segment along time, over the course of the four feeding protocols.

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BIOFLOC TECHNOLOGY – KEY TO WATER QUALITY MANAGEMENT IN FISH/ SHRIMP FARMING

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Introduction

The main limiting factors in aquaculture production are insufficient land and water. Intensified practices may progress an overall aquaculture production. But, indiscriminate intensification may polluting the culture water by accumulating organic materials and nutrient discharge which leads to chances of acute toxic effects and environmental impacts. Hence to sustain the fish/shrimp production as well as to safeguard the environment new technologies like, the Biofloc technology (BFT) showed promising results in rectifying the problem associated with aquaculture intensification. Present chapter encompasses wide knowledge of concept and effects biofloc technology towards the water conservation

Biofloc technology (BFT)

Biofloc technology (BFT) is an eco-friendly and limited water exchange system which is also called active suspension ponds, heterotrophic ponds, green soup and Aerobic Microbial Flocculent Technology. Biofloc is an aggregates of algae, diatoms, bacteria, protozoans, planktons and particulate organic matter such as faeces and uneaten feed. Each floc is held together in a loose matrix of mucus that is secreted by bacteria and bound by filamentous microorganisms. There are different types of biofloc is present based on microbial abundance and coloration. One is green water biofloc system (outdoor open culture) that are open to natural light. In this system, both algae and bacteria which control the water quality. And those that are not exposed to natural system is called brown- water biofloc system (indoor closed system). In this system, water quality is controlled by bacteria.

Effect of biofloc technology in water quality management

High temperature influences floc characteristics and microbial metabolism. But, it can be varies depending on the cultured species. Dissolved oxygen is a crucial parameter in biofloc systems, the oxygen demand will be higher, because of the interaction between the bacteria, algae and fish size. The recommended dissolved oxygen to be maintained is 6–8 mg/l to ensure proper functioning of the system. The pH should be ranges from 6.5 to 9 depending on the culture species. Alkalinity is the total concentration of bases in the water which includes carbonate, bicarbonate and hydroxide ions. Total alkalinity ranges from 70 to 150 mg/l which provide a well buffered environment and suitable for growth of the fish and pond primary productivity. Total alkalinity was stable in normal culture system and oscillates in biofloc system due to buffering action. Hardness is a measure of alkaline ions of Calcium, Magnesium in water with other ions like Aluminium, Zinc and Hydrogen. Acceptable range of hardness in aquaculture is 50-250 ppm. Less than 0.1ppm of NH_3 suitable for fish culture. It can be reduced by adding carbon source.

Higher biological oxygen demand (BOD) specifies the abundance of more heterotrophic bacterial population that assimilate the inorganic nitrogenous compounds. Biofloc system should operate with ranges between 200 to 500 mg/L of suspended solids are appropriate. Floc volume should be maintained within 25 to 50 ml/l to provide good nutrition for tilapia in AMF systems. In lined AMF shrimp ponds, 10 to 15 ml/l is the suitable range to provide good nutrient.

NUTRIENT DISCHARGE FROM SHRIMP POND AND IT'S MANAGEMENT BY ADVANCED TECHNOLOGY

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Introduction

An indiscriminate intensificatio of aquaculture practices may polluting the environment in the form of sludge accumulation and nutrient discharge by over feeding and fertilization, antibiotics usage and poor management which leads to cause negative impacts on ecosystem. Hence, protect the environment as well as reduce the nutrient pollution we have to adopt advanced technologies like, Recirculatory Aquaculture System (RAS) and Biofloc System (BFT). These technologies can rectify the problem. Present review paper comprise the of concept technologies to ecosystem management

Nutrient discharge management by Biofloc technology (BFT)

BFT is an environmental friendly technology which can effectively control the sludge and minimize the nutrient discharge. It recycles nutrients continuously by addition of intensive aeration and carbon source which can be maintained by carbon/ nitrogen (C/N) ratio in the water which stimulates heterotrophic bacterial growth and converts ammonia into microbial biomass. If carbon and nitrogen are well balanced, bacteria briskly utilize ammonium.

The uneaten feed, faecal matter and fertilizers were the reason for sludge accumulation and nutrient discharge. The sludge accumulation can be minimized by addition of intensive aeration and carbon source. Type of aerator and position of aerator have positive impact on sludge recycling process. Low protein feed is sufficient because remaining protein has been balance from biofloc, this could effectively minimize the nutrient discharge problem. Sludge and sediment accumulation in the shrimp pond can be mechanically resuspended or removed by proper location of aerators. Resuspension is a possible solution to enhance the recycling and utilization of the feed residues as nutrient.

Nutrient discharge management by Recirculatory Aquaculture System (RAS)

Recirculatory aquaculture system (RAS) is a sustainable practice to produce safety and quality shrimps throughout the year. The culture waste water has been treated by sludge removal, mechanical filter (removes the larger particle), biofilter (removes microorganism), UV treatment (removes bacteria), aeration (oxygenation of water) which can be reused within same system. This technology effectively used to conserve the water budget and effluent removal. RAS installation investment is high. Hence, this suitable for urban area and developed countries.

Recirculatory Aquaculture System and Biofloc System supports nitrogen removal even biological oxygen demand and organic matter are higher in culture water. Adaptation of these technologies in shrimp pond offers a solution to avoid the ecological impact of high nutrient discharges and to reduce the use of artificial feed and feed cost.

REPEATED HORMONAL INDUCTION OF SPERMIATION AFFECTS STRESS BUT NOT IMMUNE RESPONSE IN PIKEPERCH (*Sander lucioperca*)

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Introduction

Obtaining suitable amount of high quality sperm is a key element of successful controlled reproduction in finfishes In domesticated pikeperch (*Sander lucioperca* L.) lack of synchronization of spawning of males and females as well as low quantity of sperm are among the main bottlenecks in the production of high quality larvae. Hormonal stimulation allows the control over the maturation and spawning process in many commercial fish species, including percids. However, in the case of pikeperch typically applied hormonal preparation (human chorionic gonadotropin [hCG]) was found to cause elevated levels of cortisol (Falahatkar and Poursaeid, 2013) suggesting a stress response following its application. Besides, it is also known that hCG treatment may trigger immune response (Zohar and Mylonas, 2001) which can cause health issues of the broodstock. This has stimulated intensive research on the potential application of gonadoliberine analogs (GnRHa) in controlled reproduction of pikeperch. However, despite the significant progress observed in terms of hormonal manipulations of reproduction in this species the aspects related to the immune response following hormonal treatment was not explored yet.

The highest quantity and quality of sperm in percids were recorded between 4 and 8 days following hormonal treatment (Żarski et al., 2017). At that period typically the whole sperm is collected and used for fertilization purposes. However, very little is known about the possibilities of a second induction of spermiation and sperm collection, as it is practiced in other cultured species. This would allow facilitating the hatchery operations and the potential usage of the sperm of the most valuable individuals for fertilization of eggs coming from more female spawning at a longer time period.

The aim of this study was to evaluate the effects of repeated induction of spermiation on sperm quality and quantity in domesticated pikeperch. A special emphasis was put on the immune response following injection with either hCG or GnRHa, the most commonly used spawning agents.

Materials and methods

Mature pikeperch males (n=21), being reproduced 3 times before the experiment, were reared according to the protocol described by Żarski et al. (2019). At the beginning of the spawning period fish were treated with either NaCl (negative control, 1 ml kg^{-1}), hCG (at a dose of 500 IU kg^{-1}) or salmon GnRHa (50 µg kg^{-1}). Five days following the treatment sperm was collected from all the fish and the sperm motility parameters (using computer assisted sperm analysis, Minitub, Germany) and quantity of total sperm volume possible to be obtained were recorded. Just after this first sperm sampling fish were again treated with the same hormones and doses. After another 5 days a second sperm sampling was performed. Also, blood from each fish was collected for biochemical analysis of stress and immune response indices (cortisol and glucose levels, lysozyme and peroxidase activities as well as haemolytic activity of the alternative complement pathway). Temperature throughout the study was 12°C and photoperiod was 14 h. The data obtained were analyzed with one-way ANOVA test followed by Tukey's post-hoc at $P<0.05$ (Statistica).

Tab. 1. Results recorded during the 2nd sampling point of the experiment (see Material and methods for details). PRG – progressive spermatozoa motility; VCL – curvilinear spermatozoa velocity. Data in columns marked with different letters were significantly different ($P<0.05$).

	PRG [%]	VCL [µm s^{-1}]	Cortisol [ng ml^{-1}]	Glucose [µg ml^{-1}]	Lysozyme [U ml^{-1}]	Peroxidase [U ml^{-1}]	ACH50
NaCl	-	-	148.2 ±45.7^{b}	55.1±12.9^{b}	3130 ±527	76.1 ±20.8	110.1 ±28.0
hCG	77.1 ±19.0	166.6 ±19.0	268.5 ±99.4^{a}	71.1 ±16.6^{a}	3214 ±249	62.3 ±24.7	115.4 ±37.0
GnRH	78.1 ±13.5	152.9 ±18.0	214.4 ±84.0^{ab}	43.4 ±20.9^{b}	3614 ±610	60.4 ±20.2	124.2 ±34.6

(Continued on next page)

Results

It was not possible to strip more than 0.1 ml of sperm from control group at any of the sampling point. From the experimental groups similar sperm volume (1.5-2.6 ml kg^{-1}) was always obtained. There was no difference between the first and second sampling in terms of motility parameters among groups as well as between groups at the same sampling point ($P>0.05$). Stimulation with hCG induced higher cortisol level, when comparing to control group. Glucose level was the highest is hCG treated fish ($P<0.05$) (ab. 1). The immune status parameters were similar among all the groups (Tab. 1).

Discussion

In this study we confirmed the finding of Falahatkar and Poursaeid (2013) that application of hCG induces a stress response in pikeperch, in contrast to GnRH. However, we showed, for the first time, that despite increment of stress indices none of the hormonal preparations affected the immune markers analyzed in our study, which remained at the same level as in control fish. It was already reported, that stress-induced cortisolemia is not clearly related to circulating immune parameters in percids (Milla et al., 2010). However, the reason of elevated cortisol and glucose level following hCG injection as well as its consequences are still to be explored in future studies.

Our results indicate that GnRHa is a safe agent to be used in controlled reproduction of pikeperch from the perspective of its effects on the immune status of the fish. However, despite hCG was found to induce a stress response, the potential negative effect of this phenomenon should not be linked with immune response, as this was not confirmed in our stud .

Acknowledgements

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TRANSCRIPTOMIC PROFILING OF EGGS OF PIKEPERCH (*Sander lucioperca*) REVEALS NOVEL EGG-QUALITY-ASSOCIATED TRANSCRIPTS

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Introduction

Gamete quality is one of the most important element of successful reproduction of aquacultured fish. This especially applies to a newly domesticated fish species, such as pikeperch (*Sander lucioperca*) in which lowered and/or variable egg quality is one the biggest obstacles toward rapid production expansion. Recently published data indicates that egg quality in response to husbandry and hatchery practices in pikeperch may be specimen-specific suggesting heritability of this trait (Żarski et al., 2019). However, mechanisms underlying pikeperch egg developmental competences remain unknown.

Finfish egg quality molecular profiling, having huge potential in understanding processes involved in shaping egg quality, has brought attention of scientists for over a decade now. Typically very few molecules (transcripts or proteins) were found to be related with egg developmental competence (Bobe and Labbé, 2010; Bizuayehu et al., 2019) which slows further progress. Besides, among the few studied fish species most were marine and model species (such as zebrafish, *Danio rerio*). Freshwater commercial fish egg quality transcriptomic profiling was performed only in rainbow trout (*Oncorhynchus mykiss*) (Bonnet et al., 2007) and Eurasian perch (*Perca fluviatilis*) (Almeida et al. – unpublished).

This study compares transcriptomes of high (HQ) and low (LQ) quality domesticated pikeperch eggs.

Materials and methods

Domesticated pikeperch broodstock was subjected to the reproduction protocol described in details by Żarski et al. (2019). From each female (n=85) an egg sample was snap-frozen for molecular analysis. From the remaining eggs other subsamples (n=3 for each female) were fertilized *in vitro* and incubated in controlled environment. Survival rate of embryos (SR) at 72 h post fertilization, hatching (HR) and deformity rates (DR) of hatched larvae were determined (for details see: Żarski et al. 2019).

Transcriptomic analysis was performed with the use of a specifically designed microarray (Agilent, 8x60k). Microarray probes were obtained by mRNA sequencing of 8 different pikeperch tissues (2x250 paired-end, Illumina). Transcriptome assembly was performed according to the pipepline described by Pasquier et al., (2016).

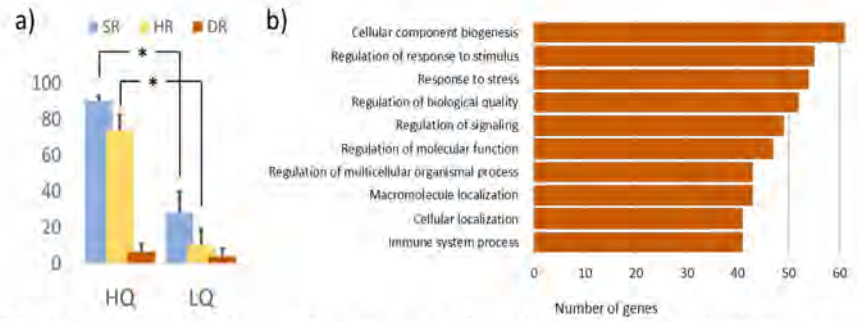

Fig. 2. Results of (a) evaluation of egg quality in pikeperch and (b) high level gene ontology of biological processes (ShinyGO v0.51, top 10 results based on the number of genes taking part in particular process). SR – survival rate at 72h post fertilization, HR – hatching rate, DR – deformity of hatched larvae. Data marked with sterisk were statistically different (ANOVA, $p<0.05$).

(Continued on next page)

Microarray sample hybridization, scanning and statistical analysis were performed according to methods described by Żarski et al., (2017). 16 samples were chosen (8 characterized by either HQ and LQ) following strict preselection procedure, where all the egg samples exhibiting any morphological alteration (lipid droplet fragmentation, signs of overmaturation, etc.) were excluded from further research. Differentially expressed genes (DEGs) between HQ and LQ were identified following analysis made with GeneSpring GX software (Agilent Technologies). Gene ontology was performed with ShinyGO v0.51 (http://bioinformatics.sdstate.edu/go/) .

Results

Samples representing HQ were characterized by higher SR and HR, when compared to LQ group. There were no difference in terms of DR between HQ and LQ (Fig. 1a).

The microarray design contained probes corresponding to 36,348 unique protein-coding genes. From among those genes expression of at least 10000 were detected in each of the sample. Analysis revealed 266 DEGs between HQ and LQ samples, from which only 7 were found to be up-regulated in LQ. All the remaining genes were up regulated in HQ. Gene ontology analysis revealed that DEGs are involved in various important processes including stress response or regulation of biological quality (Fig. 1b).

Discussion

Results obtained in this study following precisely planned operation allowed to obtain for the first time unexpected high number of differentially expressed transcripts between high and low egg quality. Typically, such an analysis allowed to reveal less than 100 egg-quality-associated transcripts (Sullivan et al., 2015). This was also probably possible due to the eggs selection procedure, in which only eggs representing very high and low quality, but not degraded by post-ovulatory ageing, were chosen, what was previously suggested to be a crucial element of such analysis (Żarski et al., 2017).

From among the DEGs we have identified 6 genes (cyclin G1, 39S ribosomal protein L4, ras and rab interactor, cell death-inducing p53-target protein 1, RING finger protein unkempt homolog) being related (but not the same) to the genes already suggested for being indicators of egg quality in other teleosts (Sullivan et al., 2015). However, the remaining 260 genes found to be differentially expressed between HQ and LQ are new candidates for determination of egg quality in teleosts.

The findings of our study bring new knowledge on the processes involved in the determination of developmental competence of pikeperch eggs as well as a new egg-quality-related gene repertoire.

Acknowledgements

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ROTIFERS ENRICHED WITH A MARENNINE PROMOTE SURVIVAL AND GROWTH OF YELLOWTAIL KINGFISH LARVAE, *Seriola lalandi*

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Introduction

As is common for many marine fish species, there is considerable inter- and intra-cohort variation in survival and growth during the early life stages of yellowtail kingfish (Moran et. al., 2011). Different algal species have proved to be beneficial for larval performance, for instance microalgae could be used indirectly, as food for zooplankton such as rotifers which are essential food for some fish larvae. CAMAFAN (Characterisation and Industrial Application of Marennine as Aquaculture Feed Additive and Nutraceutical) is an applied research project focusing on blue-green pigment marennine. Marennine is produced by the marine diatom *Haslea ostrearia* during its life cycle. This water-soluble pigment is found intracellularly and is also released into the culture medium. Apart from the widely known colouring capabilities, recent research has shown that marennine also has antibacterial, antiviral, antiproliferative and antioxidant (Pouvreau et. al., 2018) properties. Given its properties and potential, marennine can be valuable in hatcheries for the production of fish larvae by reducing pathogenicity; have health and growth benefits, when supplied with live feeds. The present study aimed to provide new insights into the effect of enriched rotifers and artemia conditioned in different marennine concentrations (0, 0.5, 1.0 mg/L) on the growth and survival rate of yellowtail king fish larvae during the first feeding perio

Material and methods

Yellowtail kingfish larvae were fed with the live feeds rotifers (3-16 days-post-hatch) and/or Artemia (12-21 days-post-hatch) that have been enriched in 0.1 mg/L, 0.5 mg/L marennine concentration, or without marennine (control diet) from 8 days-post-hatch. Larvae were reared in 1500 L tanks with a stocking density of c.a. 40 larvae L^{-1} with feed delivered up to 6 times during the day. Growth was monitored as total length at 8, 11, 16 and 22 dph. Additionally, swim bladder inflation is observed at 8 dph. At the end of the experiment (22 dph), all remaining larvae from each tank representing each treatment larvae were counted, and total survival rate was calculated per tank as percentage of initial number of larvae.

Results

Survival rates and lengths of the larvae fed with marennine-enriched live feeds performed better than control groups (Figure. 1 and Figure 2). In terms of larval length, there were noticeable separations between control and marennine-treated groups as early as 11 dph. There were no significant differences in terms of swim bladder inflation at 8 dph between the groups.

Discussion and conclusion

The corresponding pattern of survival and growth of larvae is encouraging, which suggests that the inclusion of marennine through live feeds be a useful additive for early larval stages of finfish

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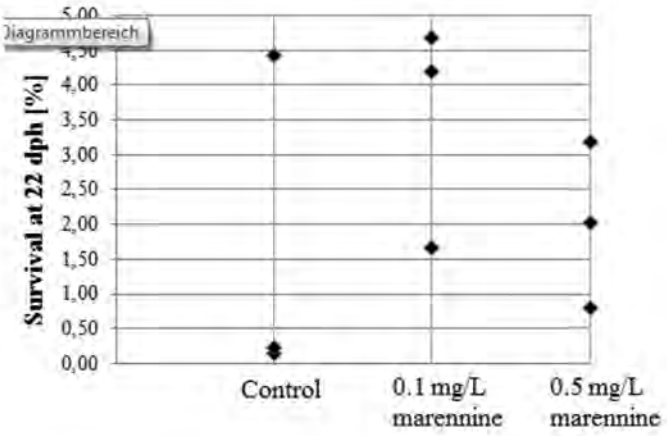

Fig. 1. Survival rate of yellowtail kingfish larvae fed with and without enriched marenine.

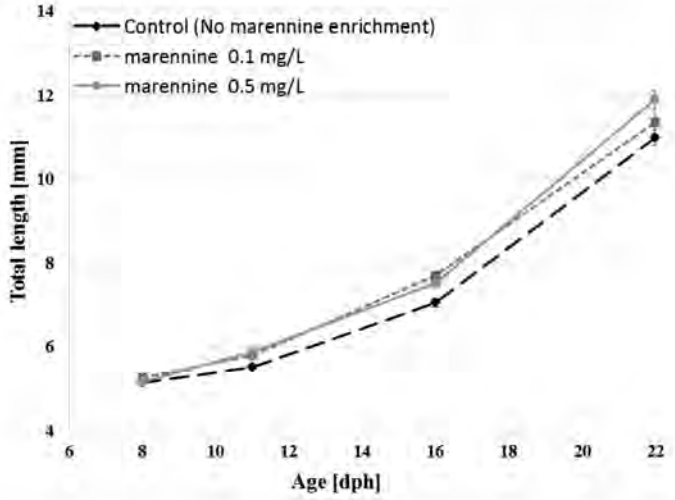

Fig. 2. Total length of sampled yellowtail king fish larvae over the experimental period of 22 days. Data are presented as mean ± S.E. (n = 30 larvae/tank).

EFFECTS OF DIFFERENT LIGHT COLORS ON THE GROWTH PERFORMANCE OF LARGEMOUTH BASS (MICROPTERUS SALMOIDES) IN A RECIRCULATING AQUACULTURE SYSTEM

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Introduction

The reasons for the low survival of largemouth bass larvae include the anti-stress ability, and the infection of viruses, bacteria and parasites. Recirculating aquaculture system has the advantages of enhancing aquaculture temperature control, improving water treatment capacity, and reducing pests and diseases. Light is widely recognized as an important factor in closed industrial farming because it affects the growth and behavior of fish(Espigares, Rocha, Gómez, Carrillo, & Zanuy, 2016; Yoseda et al., 2008). Therefore, this experiment was designed to evaluate the effects of light color on survival rate and growth of largemouth bass larvae.

Materials and methods

The experiment was set up with blue (half-peak bandwidth=409-490nm), green(half-peak bandwidth=493-572nm), white (371<λ<1047) and full spectrum four light environments. The 7200 largemouth bass larvae were randomly divided into 12 culture ponds, the water temperature in the water tank was (23±1) °C, the pH was (7.5 ± 0.2), the dissolved oxygen was (5.6 ± 0.2) mg/L, and the photoperiod was light: dark = 12h: 12h. During the experiment, the growth of the larvae was recorded every 3 days, and the number of fish deaths was recorded dail .

Results

Preliminary results showed that the survival rate of larvae in green light group, full spectrum group and white light group was higher than that in blue light group; the survival rate of larval in the whole spectrum group was the highest (Table 1).

The experimental results also showed that the body length and weight of the larvae in the white light group and the full spectrum group were greater, which indicated that the larvae grew faster in these light environments. However, under blue light conditions, the larvae were the lightest and grew slowly.

Table 1
Effect of light color on the survival and growth performance of largemouth bass larvae at the end of the experiment (16 days). The data were presented by means±S.D. (n=5).

Parameters	Blue Light	Green Light	Full spectrum	White Light
Initial length (mm)	6.52 ±0.23	6.52 ±0.23	6.52 ±0.23	6.52 ±0.23
Final length (mm)	12.1±0.4	12.2±0.1	12.4±0.3	12.3±0.03
Final weight (mg)	16.7±1.0	16.8±1.5	18.4±0.2	18.4±2.7
Survival (%)	11.6±0.7	17.7±2.4	33.5±3.5	20.4±4.6

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Discussion and conclusion

In the long-term evolution process, organisms adapt to changes in light through various aspects such as morphology, behavior, and physiological response (Villamizar, García-Alcazar, & Sánchez-Vázquez, 2009). In this experiment, the survival rate of larvae was lower under blue light, and the growth of larvae was lower than other groups, which fully indicated that blue light may be a stress factor for largemouth bass larvae, the larvae could not self-adjust to adapt to this lighting condition through a series of behavioral and physiological mechanisms, so the mortality rate was higher.

The early growth and development of the largemouth bass were greatly affected by light. Inappropriate lighting conditions (blue light) at this stage may result in slow growth and low survival rates, which can impair the productivity of the fish and should be avoided. In conclusion, full-spectrum light can increase the survival rate of largemouth bass larvae and promote their growth performance in this experiment.

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EFFECTS OF DIETARY *Bacillus subtilis natto* SUPPLEMENTATION ON GROWTH, OXIDATIVE STATUS AND IMMUNE RESPONSE OF RED SEA BREAM (Pagrus major)

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In this study, *Bacillus subtilis natto* was evaluated on the growth, digestive enzyme activity, blood chemistry, oxidative status, immune response and the growth-related genes' expression in the skeletal muscle of red sea bream (Pagrus major). Fish fed five different levels of *B. s. natto* at 0 (BN0), 1×10^5 (BN1), 1×10^7 (BN2), 1×10^9 (BN3) and 1×10^{11} (BN4) CFU kg^{-1} diet for 56 days. The fish of BN3 and BN4 groups displayed better growth performance and feed conversion ratio (FCR) than the BN0 groups ($P < 0.05$). Compared with the BN0 and BN1 groups, there were significant increases ($P < 0.05$) in whole-body protein content in BN3and BN4 groups. *B. s. natto* supplementation significantly improved the specific activities of amylase, protease and lipase enzymes when compared to the control group ($P < 0.05$). Furthermore, the plasma total protein was increased significantly in fish fed BN4 diet when compared with the control diet ($P < 0.05$). Hemoglobin and the nitro blue tetrazolium values up regulated significantly upon *B. s. natto*, especially in case of BN3 and BN4 diets ($P < 0.05$). Serum peroxidase activity enhanced significantly in fish fed BN2 and BN3 diets ($P < 0.05$). Compared with the fish of BN0 growth-related genes' expressions increased ($P < 0.05$), and the myostatin and myocyte enhancer factor C mRNAs were downregulated ($P < 0.05$) in the fish of BN3 and BN4 groups. The specific growth rate analysis and expressional regulation of the growth-related genes stimulated by *B. s. natto* suggest the potential application of *B. s. natto* in improving the growth performance on the red sea bream. Additionally, the supplementation of *B. s. natto* in the diet of red sea bream at 1×10^9 and 1×10^{11} CFU kg^{-1} diet could improve the growth, feed utilization, health condition and immune response.

DEEP LEARNING-BASED MONITORING OF THE LOCAL UNUSUAL BEHAVIORS FOR FISH SCHOOL IN INTENSIVE AQUACULTURE

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Introduction

Behavior change is an effective reference index of fish welfare in aquaculture. Compared to the global unusual behaviors, most unusual behaviors occur within the relatively small areas (i.e., local unusual behaviors), such as aggressive behaviors, abnormal swimming behaviors associated with clinical symptoms of poor health or parasitic infections (Ashley, 2007). For the monitoring of the local unusual behaviors, computer vision based-tracking, apparently, is the most accurate measure. However, this type of technology is difficult to be applied in production, especially in practical intensive aquaculture (Delcourt *et al*., 2013). Therefore, a practical method, mainly based on Graph Networks and Recurrent Neural Networks (RNN), was developed to detect, localize and recognize the local unusual behaviors of fish school on the basis of the algorithm proposed in Zhao, *et al*., (2018).

Materials and methods

Tilapia (*Oreochromis niloticus*) were used in this study. Before the experiment, all tilapia have been kept in the laboratory RAS for about three months. To obtain enough samples of the local unusual behaviors, experiment in this study was carried out based on the emptying of the gastrointestinal contents. And the behavior dataset was made manually following All Occurrences Sampling. Totally 1000 verified video clips of 4 clusters are contained in this behavior dataset, and each cluster is presented by a representative behavior.

Results

According to the results, the proposed method demonstrates better performance than many other state-of-art methods (Zhao, *et al*., 2018), and reaches the accuracies of 98.91%, 91.67% and 94.16% of the detection, localization and recognition of the local unusual behaviors, respectively.

Discussion and conclusion

Benefiting from the properties of Graph Networks and RNN, the proposed method in this study shows a lot of improvement in recognition in contrast to that in Zhao, *et al*., (2018). And how to enhance performance of the front-end would be the focus of further research. In conclusion, although challenges still exist, method presented in this study has revealed its potential in practical monitoring of the local unusual behaviors for fish school in intensive aquaculture, and could be applied to assess the status of fish school.

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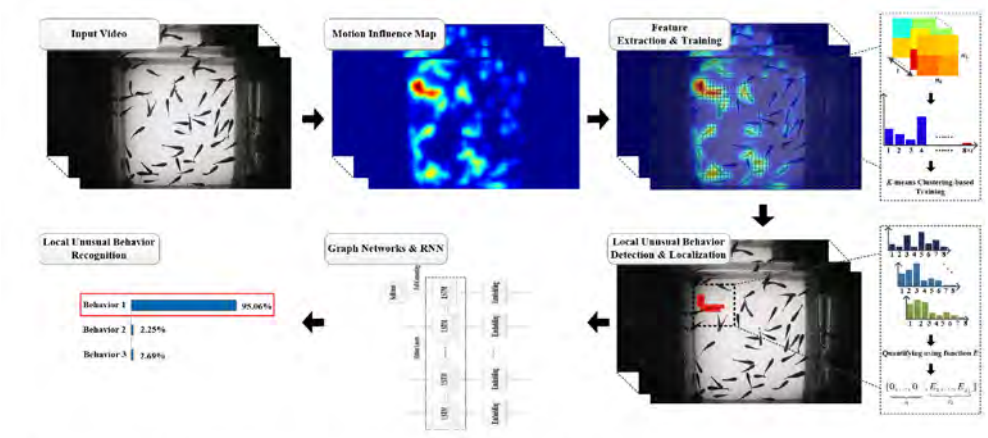

Fig. 1. Flow chart of the proposed monitoring method.

THE RELATIONSHIP BETWEEN KOI HERPESVIRUS DISEASE RESISTANCE AND OTHER PRODUCTION TRAITS INFERRED FROM SIBLING PERFORMANCE IN AMUR MIRROR CARP

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Introduction

Koi herpesvirus disease (KHVD) can cause severe mortality in farmed carp populations (Novotny et al., 2010). In Europe, KHVD is on the list of notifiable diseases. Prevention is a sensible strategy for tackling this disease and improved resistance of carp strains is a desirable goal. Previous study showed KHVD resistance to be heritable trait that can be improved by selection (Palaiokostas et al., 2018). In selective breeding, selection for a single trait can lead to unwanted genetic changes in other traits. Hence, the main aim of this study was to investigate the genetic relationship of KHVD resistance to other production traits in Amur mirror carp.

Materials and methods

A population of Amur Mirror Carp was created from four factorial crosses of five dams and ten sires. One-year fish (n=1500) of Amur Mirror Carp were pit-tagged and finclipped. They were challenged to KHV, alongside with a small (n = 215) sample of Koi carp. Koi carp were a positive control, since they are highly susceptible to KHV. Mortality of individual fish was recorded twice a day for a period of 35 days post infection until mortalities were negligible. Resistance was measured as 0 = died and 1 = survived. Presence of KHV on a sample of dead fish was confirmed by PCR. Before the challenge, juvenile growth-related traits (body weight and Fulton's condition factor, FC) were measured in the same individuals. Traits like FC, visceral index, hepatosomatic index, muscle fat, and fat, glycogen and protein analysed in hepatopancreas, as well as energy content in hepatopancreas measured in the first spring were recorded in siblings of the challenged fish. An additional set of the same families were phenotyped before and after second wintering and at third summer (market size) and measured for absolute and relative muscle fat change, weight change during winter expressed as specific growth rate, survival, condition factor, muscle fat content, body weight, log-log residuals of headless carcass yield and fillet yield. The genetic correlations between KHVD resistance and production traits were estimated using DMU statistical software. All traits were recorded from the same families and had a common pedigree (family structure) but KHVD resistance and other performance traits were recorded from different individuals. As a result, residual covariance was set to zero between these traits. Moreover, mating design (for KHVD resistance and energetic reserves) and sex (for production traits) were set as fixed e fects in the statistical model.

Results

Almost all genetic correlations between KHVD resistance and other production traits were insignificant. Genetic correlations between KHVD resistance and energetic reserves were also weak and insignificant. The genetic correlation between FC after the second overwintering and KHVD resistance was significantly different from zero and negative (r_g = -0.32 ± 0.14). Likewise, intermediate genetic correlations were found between KHVD resistance and slaughter yields at market size (log-log residuals of headless carcass yield and fillet yield, respectively) r_g = 0.37 ± 0.14 and 0.44 ± 0.13, respectively).

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Discussion and conclusion

Almost all genetic associations between KHVD resistance and production traits as well as energetic reserves were insignificant. In conclusion, selection for improved production efficiency would not increase the susceptibility of fish to KHV and vice versa. Only genetic correlation between FC after overwintering and KHVD resistance was significantly different from zero and negative. So, KHV resistant families had genetically lower FC after the second overwintering. In previous studies (Prchal et al., 2018) it was shown that FC is in Amur mirror carp genetically related to the body shape (e.g. relative body height). So, such negative correlation might be related to typically more prolonged body shape of Amur carp, one of the parental lines of Amur mirror carp, which is more resistant to KHV than other farmed carp breeds (Piackova et al., 2013). Intermediate genetic correlations between KHVD resistance and slaughter yields suggest that selection for KHVD resistance might lead to slightly higher muscling of fish and vice versa.

Acknowledgements

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EVALUATION OF THE ANTI-*VIBRIO* ACTIVITY OF ESSENTIAL OILS AND THEIR COMPONENTS IN GNOTOBIOTIC *ARTEMIA* TEST SYSTEM

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Vibrio harveyi, a marine Gram-negative luminous bacterium, has become recognized as a serious pathogen of the aquatic animals. Once its outbreaks, it causes significant losses in the aquaculture industry, particularly in economically important penaeid shrimp (Austin and Zhang 2006). Plant-based extracts, namely essential oils (EOs), concentrated hydrophobic liquid containing volatile chemical compounds, are complex mixtures of secondary plant metabolites. EOs contain many EO components (EOCs), which are pure and single chemical compositions (Randrianarivelo, Sarter et al. 2009, Manju, Malaikozhundan et al. 2016). Several EOs and EOCs have been known for exerting a multitude of biological effects, such as bactericidal and fungicidal, which have documented *in vitro* studies (Feyaerts, Mathé et al. 2017, Feyaerts, Mathe et al. 2018). However, some studies have concentrated exclusively on one oil. While these data are useful, the results are not directly comparable due to different methodologies such as the selection of plant extract(s), the extraction parts of the plant and antimicrobial test methods. The aim of this study is to determine a large number of essential oils and their components against *V. harveyi* and to investigate the putative effect of EO(c)s in *vivo* using the highly controlled gnotobiotic *Artemia* model system.

In the present study, we evaluated the vapour-phase-mediated antimicrobial activity (VMAA), different concentration of antimicrobial activity and specific quorum sensing-inhibitory activity (AQSI) of 22 essential oils and 12 essential oil components, against V. harveyi. And then, we tested the toxicity of the selected essential oils and determine the challenge test in Artemia. Results showed that the VMAA of a volatile spread symmetrically across a microtiter plate and one-quarter of the tested essential oils and their components showed growth-inhibitory VMAA at 24h. Then, six essential oils and three essential oil components inhibited 50% of the growth at 0.0001%, compared to the control. Moreover, just the four most active essential oils inhibited quorum sensing at 0.001%, with A_{QSI} higher than 2. We selected the three best candidates and then using them to control *V. harveyi* in Artemia system. The selected essential oils can improve the survival rate of brine shrimp at certain doses.

In conclusion, the anti-*Vibrio* activity of essential oils and their components both in broth cultures and via their vapour-phase. Not all the essential oils and their components are able to inhibit quorum sensing in *V. harveyi*. And these three essential oils were considered to be highly promising to control *V. harveyi* in aquaculture.

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EFFECTS OF VARIATION IN DIET MACRONUTRIENT COMPOSITION ON GUT FUNCTION IN LUMPFISH (*Cyclopterus lumpus*)

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Background
The parasitic problem in Atlantic salmon (*Salmo salar* L) caused by sea lice infestation is still a major challenge for the salmon industry in Norway. To use cleaner fish for delousing is an alternative to medicine treatment. One of these species is lumpfish which is useful particularly at the low temperatures in winter (Powell, et al., 2018). Accordingly, as harvesting from the wild stocks is not sustainable, cultivation of lumpfish in Norway has increased in recent years. The number of lumpfish used in salmon production in Norway reached 3.8 million in 2018. However, in the build-up of capacity for cultivation several knowledge gaps must be filled, not at least regarding nutrient requirement. The results to be presented are part of an experiment conducted to estimate the macronutrient requirement of lumpfish. Effects of diet composition on intestinal function and health were evaluated, including digestive enzyme activity, expression of functional genes in the gut mucosa as well as histomorphology of the gut wall.

Material and methods
Twelve diets were designed according to a three-component mixture design. The proportion of protein, lipid and carbohydrate varied between all diets, ranging from 43% to 68%, 4% to 17% and 6% to 18%, respectively. After a 41-day feeding trial, six fish from each tank were sacrificed, opened and the whole intestine was sampled. The intestine was divided into three sections, i.e. proximal intestine (PI), mid intestine (MI) and distal intestine (DI), according to the morphological structure. For each section, the intestine was opened to collect digesta for the measurement of trypsin activity and bile salts concentration, but only MI contained sufficient digesta for analysis. Tissue was taken from the remaining intestine for measurement of digestive enzyme activity and gene expression, and for evaluation of histology.

Tissue samples from all sampled fish were processed for analyses of leucine aminopeptidase (LAP) and maltase activity. Gut tissue samples from fish given five selected diets, i.e. the diet of medium macronutrient composition and four other diets of extreme macronutrient composition, were processed to observe expression of genes involved in the immune and osmoregulatory apparatus, including proliferating cell nuclear antigen (pcna), immunogobulin M (IgM), inhibitor of nuclear factor kappa B kinase subunit beta (IKBKB), nuclear factor kappa B p65 subunit (RelA), cyclooxygenase-2 (cox-2) and aquaporin 1 (aqp1). Four out of the five selected diets groups were evaluated regarding histological changes. Length and fusion of mucosal folds, width of the lamina propria and submucosa, cellularity of lamina propria and submucosa and enterocyte vacuolization were evaluated for each section. The degree of changes was graded as normal, mild, moderate and severe, depending on the morphological characteristics.

Results
In PI, the specific activity of LAP and maltase showed a significant relationship with feed composition ($p < 0.05$). The highest activity appeared in fish fed with diets of high level of carbohydrate, medium level of protein and low level of lipid. In MI, the same significant trend (Fig.1, $p < 0.05$) was shown for both the specific activity and total capacity of LAP and maltase. In DI, neither the specific activity nor the total capacity of LAP and maltase were affected by the variation in diet composition. Moreover, the trypsin activity and the concentration of bile salts in the collected gut contents in MI did not show significant relationship with diet composition.

Expression of the IKBKB gene in MI, a key element in the immune apparatus, was higher in fish fed the diet with lowest level of lipid and carbohydrate and highest level of protein than in fish fed the diet with lowest level of protein and highest level of lipid and carbohydrate. However, none of the other investigated genes showed differences in expression related to variation in diet composition in any of the gut sections. Enterocyte vacuolization, which is often associated with lipid steatosis (Gu, et al., 2014), increased in PI and MI as the dietary lipid level increased as often observed also in Atlantic salmon. In DI, however, no such effect was apparent.

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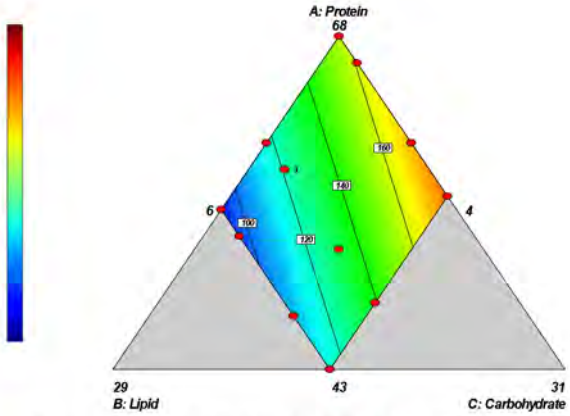

Fig.1. Specific activity of LAP in MI. The colour from blue to red represents an increasing level of activity.

Conclusions

1) Variation in macronutrient composition affected the specific activity of the brush border enzymes LAP and maltase in both PI and MI.
2) Variation in macronutrient composition affected expression of the IKBKB gene, a key element in the immune apparatus but did not change expression of the other observed genes related to intestinal immunity and osmoregulation.
3) Lipid steatosis increased in PI and MI as lipid level increased.

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EVALUATING THE EFFECT OF DIFFERENT FEED PHOSPHATES ON BLOOD PARAMETERS, CORPSE ANALYSIS, INTESTINAL HISTOLOGY AND GROWTH RATE OF KOI CARP *Cyprinus carpio*

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Introduction

A study was undertaken to evaluate the effect of two supplemental phosphorus sources: Windmill® Aquaphos (Aquaphos) and Aliphos® Monocal (MCP) and *Spirulina* on blood chemical factors, corpse analysis, intestinal histology and growth rate on koi carp (*Cyprinus carpio*).

Trial protocol

Triplicate groups of 38 carp with an average weight of 17g, were fed one of the trial diets for 8 weeks. 10 trial diets were formulated to contain increasing inclusion levels of Windmill® Aquaphos and one inclusion of Aliphos® Monocal, with and without *Spirulina*, including two control feeds (with and without *spirulina* without addition of feed phosphates). Carp were fed 2 times daily at a feeding rate of 2% of the body weight. Stagnant water tanks of 220 l were used to enable to study the effects of the different feed phosphates on water quality. At day one, three fish from each tank were randomly selected for determining blood chemical factors and histological analysis of the intestine. Same fish were used for carcass and vertebrae analysis. At day 63 carp were also sampled for determining blood chemical factors, histological, carcass and vertebrae analysis.

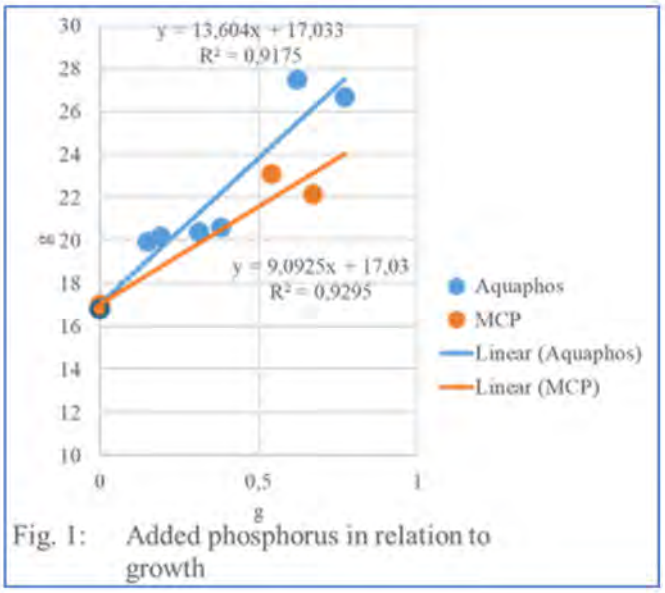

Fig. 1: Added phosphorus in relation to growth

Results

Growth (g) ranged from 17g, for the control diet, up to 27g for the diet including 3% Aquaphos. Increasing levels of Aquaphos showed an increase in both blood and bone phosphorus content.

Other blood parameters showed no significant differences over the groups. However, highest alkaline phosphatase levels were observed in the two control groups, without addition of any feed phosphate. The groups fed diets containing spirulina showed a higher muscle protein content. Histological analysis of the intestine showed that the intestinal *villi* were better developed in the diet with 3% Aquaphos against the diet with 3% MCP.

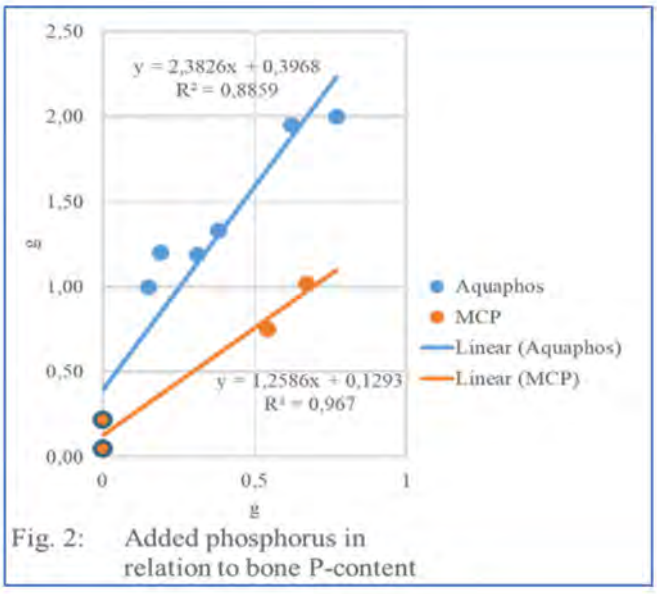

Fig. 2: Added phosphorus in relation to bone P-content

Between the different groups there were no differences in ammonia content of the water, confirming that the inclusion of Aquaphos had no negative effect on the possible increase of ammonia in the water. Lowest phosphorus levels in the water were seen with the control groups. Highest P-level was observed with the diet containing MCP at 3% inclusion with *spirulina*.

Conclusions

These trial results suggest that Windmill® Aquaphos has a significantly higher phosphorus-bioavailability than in case of Aliphos® Monocal for carp.