Application of Transgenic Technology in Aquaculture: Pros and Cons

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Current Constraints in Aquaculture

- Lacking sufficient pristine quality water
- Lacking complete control of reproductive cycle
- Lacking superior genetic traits of broodstocks
- In need methods of efficient detection and protection of diseases
- Lacking complete knowledge of nutrient requirement and appropriate feeds
- Insufficient understanding of development, physiology and environmental impacts
- In need of innovative management skills

Desirable Traits for Aquaculture

- Improving feed conversion efficiency and enhancing somatic growth
- Increasing disease resistant
- Creating value-add on products
- Increasing survival in extreme environment
- Improving control of reproductive cycle
- Producing high commercial value pharmaceutical products

Transgenic Technology

- Transgenic organisms: Organisms with foreign gene integrated in its genome
- Transgenic organisms have been produced for microorganisms, seaweeds, plants. invertebrates, lower vertebrates and lower mammals
- Transgenic organisms have been produced by microinjection, electroporation, infection with pantropic retroviral vectors, particle gum bombardment and lipofection

Strategies of Controlling Fish Diseases

- **Vaccination with inactivated or subunit vaccines** ***(effective but time consuming)***
- **Treatment with antibiotics or other antimicrobial chemicals** ***(selection of antibiotics resistant pathogens and causing environmental contamination)***
- **Eradicating the infected population**
- **Breed disease-resistant strains** ***(low efficiency of genetic selection)***
- **Development of specific pathogen free organisms** ***(maybe effective but need more proof)***

Antimicrobial Peptides

- **Small linear peptide, helical, without cysteine residues, with or without a hinge residue (*e.g.,* cecropins, magainins & melitin)**
- **Small linear peptide, without cysteine residue, with a high proportion of certain residues (*e.g.,* apidaecins & dorsocin)**
- **Small peptide with one intramolecular disulfide bond (*e.g.,* bactenecins & brevinins)**
- **Small peptide with two or more disulfide bonds, β-sheet structure (*e.g.,* α-defensins & β-defensins)**

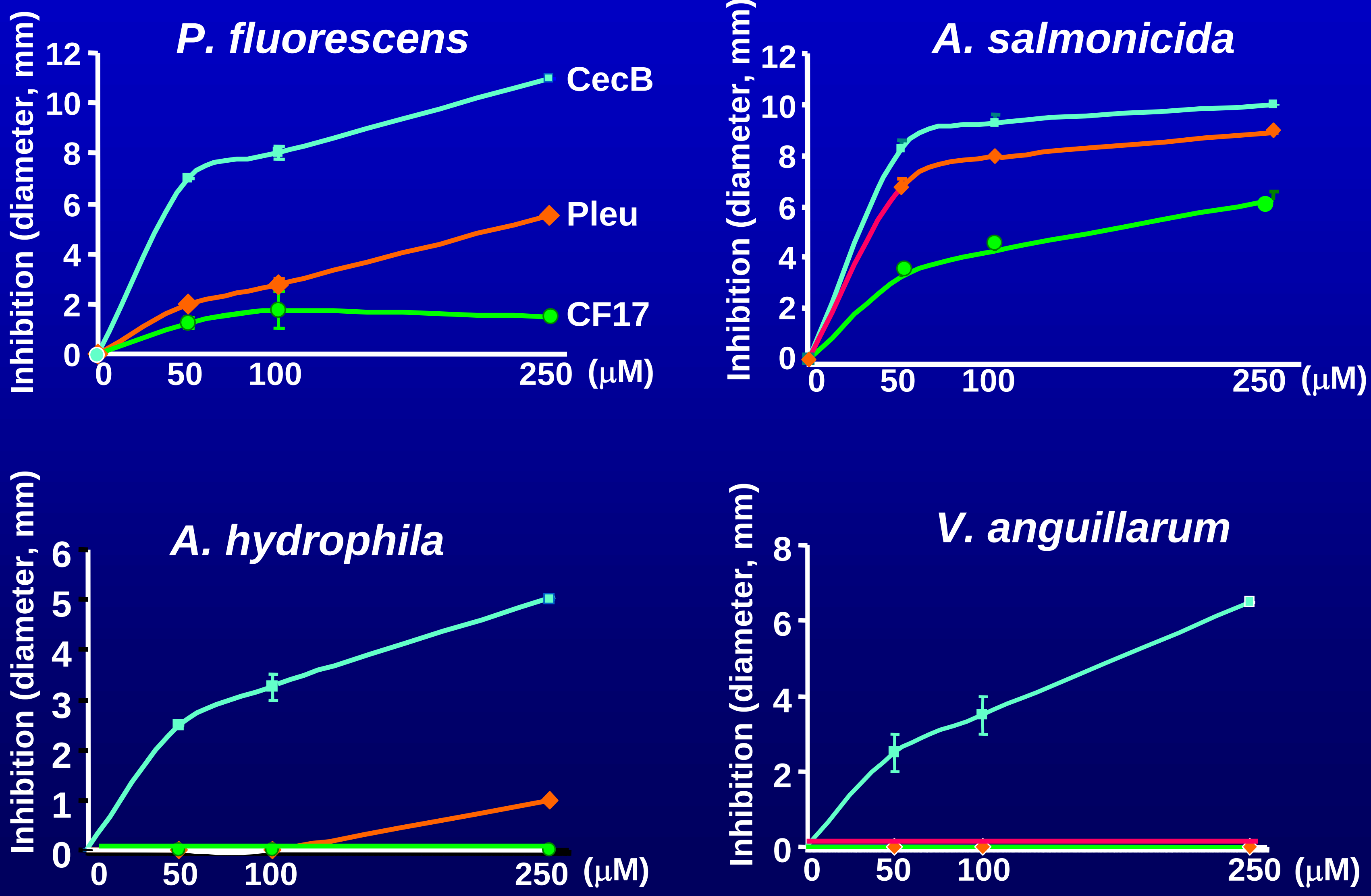
Antimicrobial Peptides of Interest

Cecropin B -- **KWKLF KKIEK VGQNI RDGII KAGPA VAVVG QATQI AK -NH_2**

Pleurocidin – **GWGSF FKKAA HVGKH VGKAA LTHYL -NH_2**

Peptide CF17 -- **a designed CecB analog with varied length and substituted amino acid residues.**

Bactericidal Activities of Cecropin B, Pleurocidin and CF-17

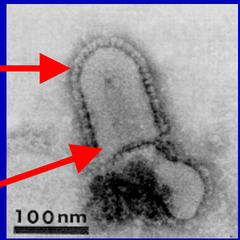


Common Fish and Shrimp Viral Pathogens

Rhabdoviruses

Envelop

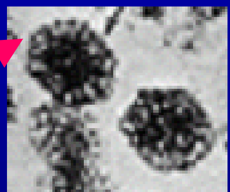
Capsid



infectious hematopoietic necrosis virus (*IHNV*), viral hemorrhagic septicemia virus (*VHSV*), & snakehead rhabdovirus (*SHRV*)

Birnavius

Capsid

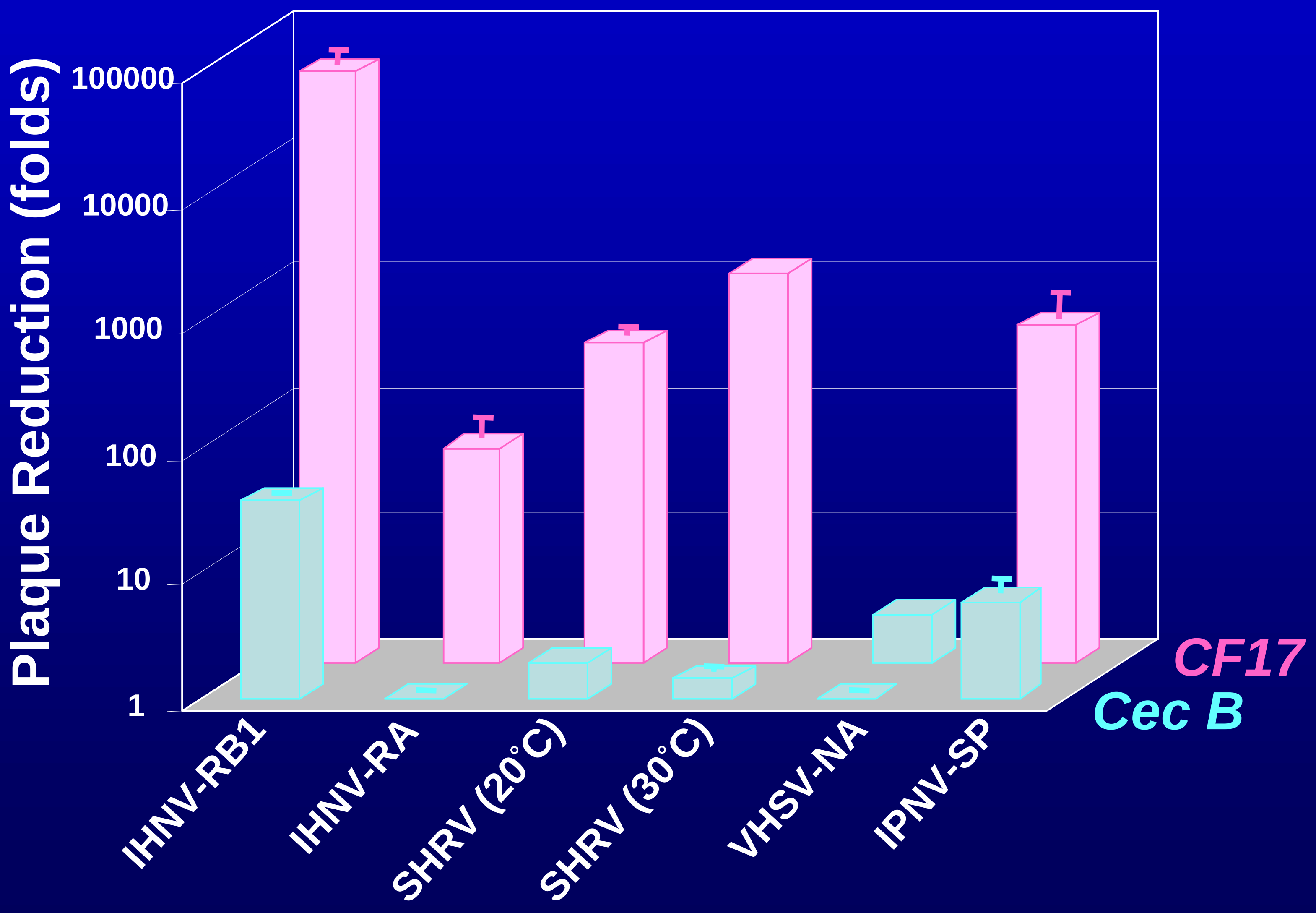


infectious pancreatic necrosis virus (*IPNV*)

Shrimp Viruses

White spot syndrom virus (WSSV) [DNA, envelop], Taura syndrome Virus (TSV) [capsid, single-stranded RNA) & Infectious hypodermal & hematopoietic virus (IHHNV) [capsid, single-stranded DNA)

Antiviral Activity of Cecropin B and CF-17 against Several Important Fish Viruses



Conclusion (I)

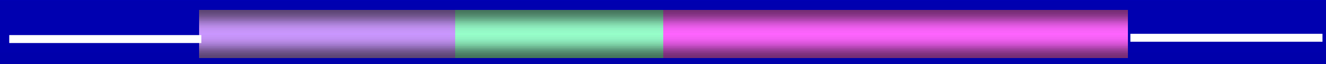
- **While cecropin and pleurocidin have higher antibacterial activity, CF-17 has higher antiviral activity *in vitro***

Hypothesis

- **Transgenic fish or crustacean carrying antimicrobial transgene should exhibit higher resistance to bacterial and viral infection**

- **To prove this hypothesis, cecropin transgene was introduced into medaka and the resulting tragenic F_2 progeny were subjected to challenge studies**

Prototype of Cecropin Transgene

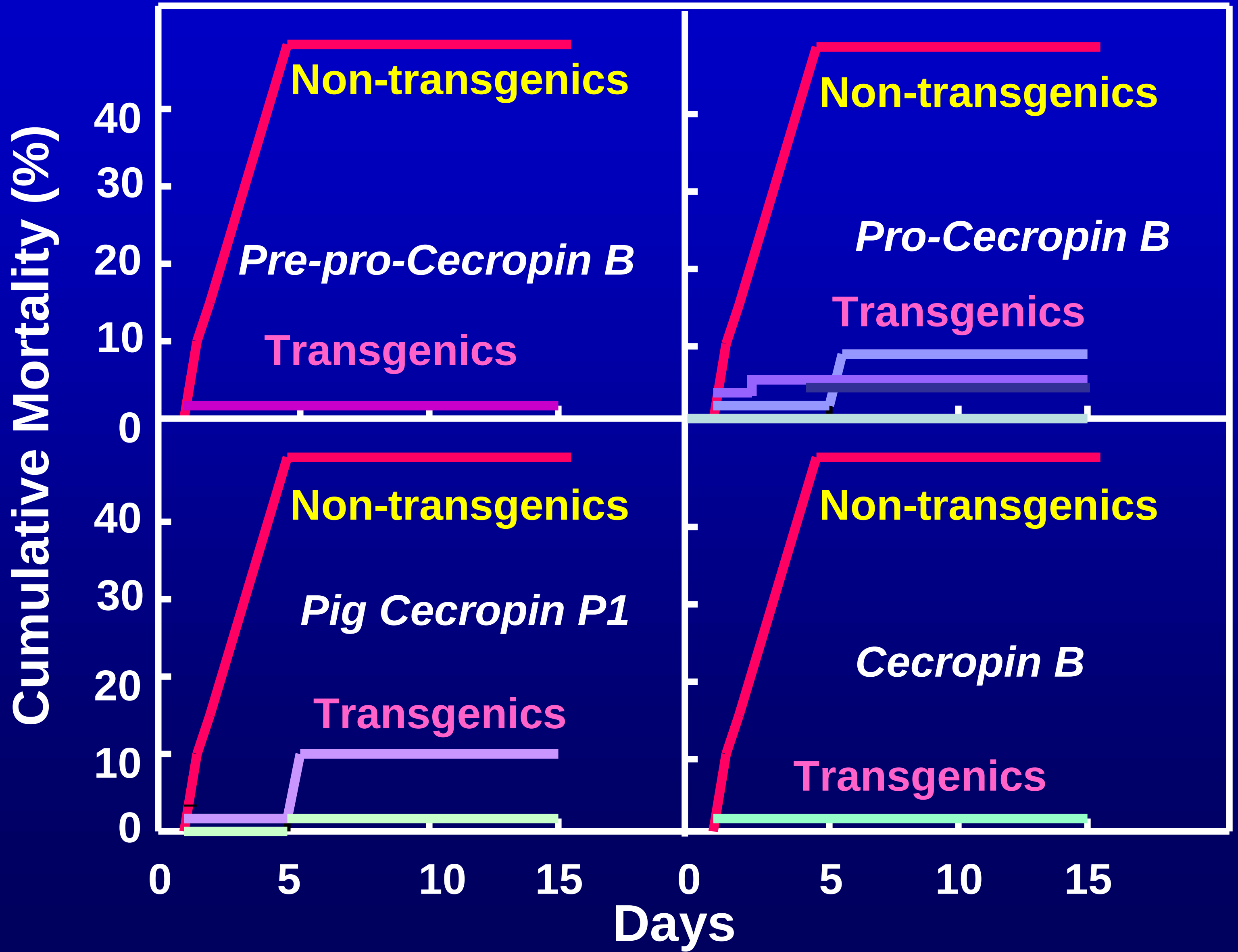


Introducing transgenes into fertilized medaka eggs by electroporation

Summary of Cecropin Transgenic Medaka

Transgene Construct	# F_2 Females	Transgene Expression
Preprocecropin B	2	Expression Detected
Procecropin B	10	Expression Detected
Cecropin B	2	Expression Detected
Porcine P_1 Cecropin	3	Expression Detected

Challenge Against *Pseudomonas fluoresens*



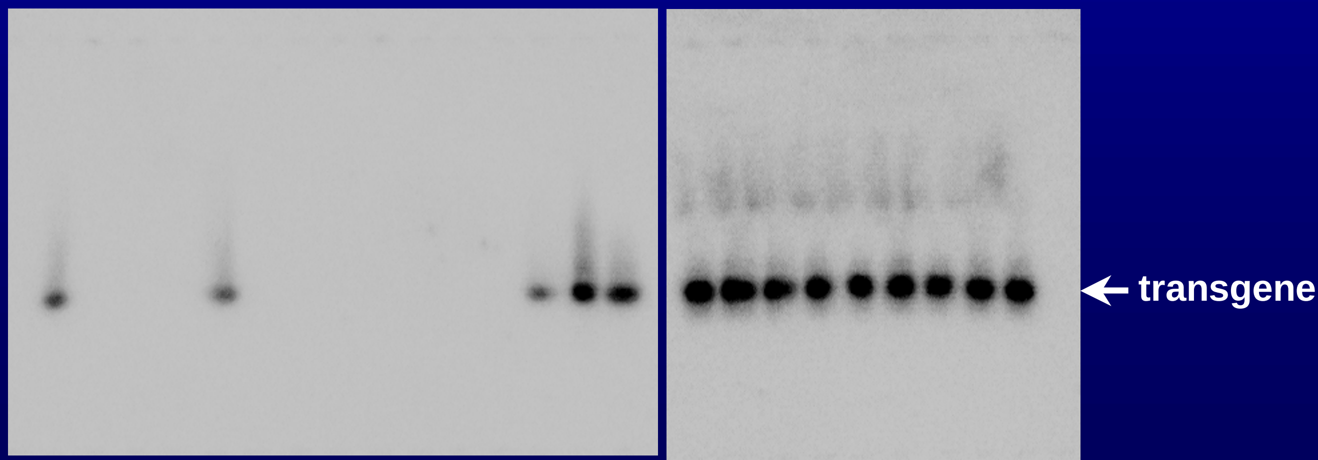
Sperm-Mediated Gene Transfer

Transgene

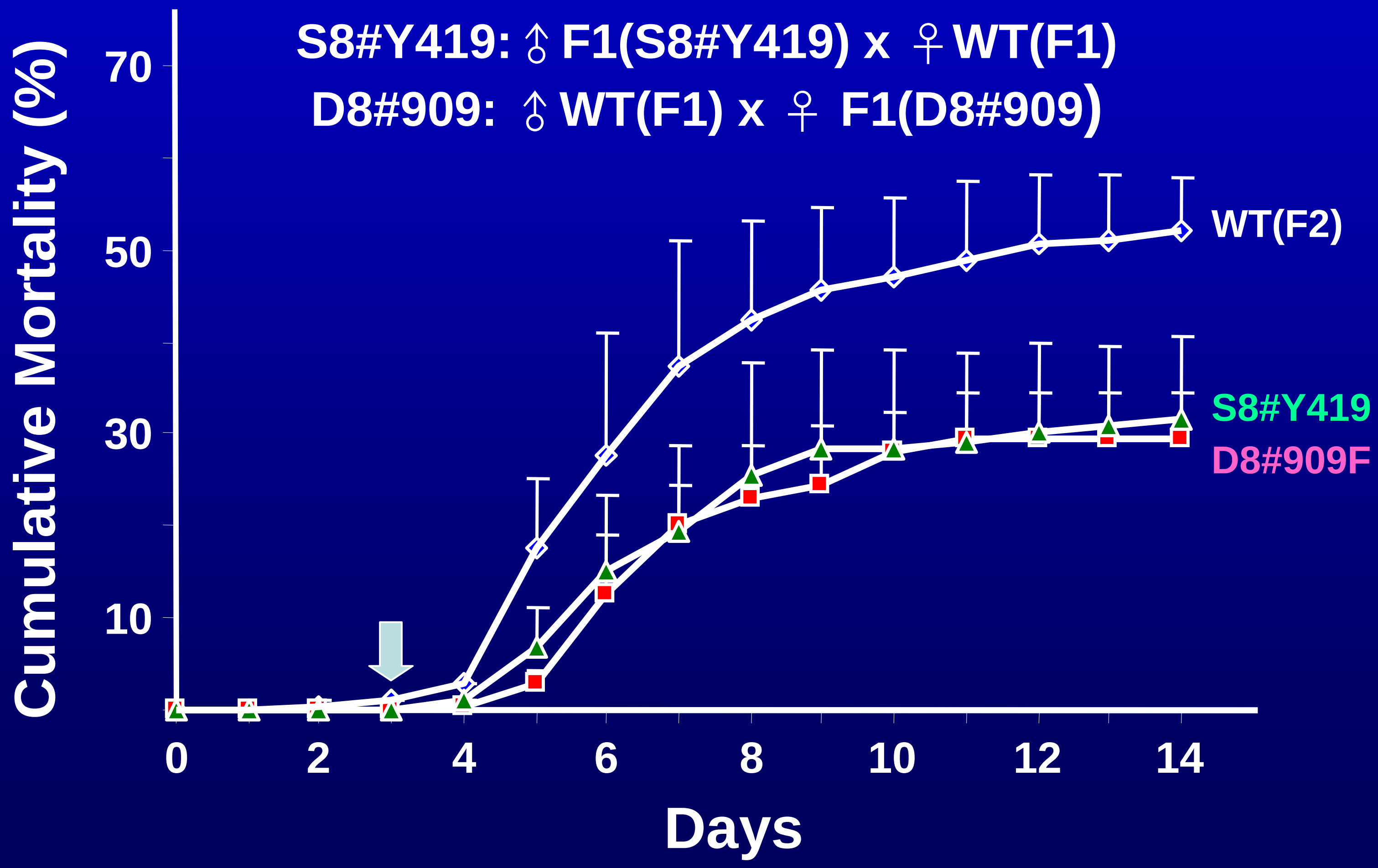
Parameters: Dilution buffer, Dilution factor, DNA/sperm, Voltage, Pulse #

Establishment of Transgenic Trout

Species	Genes	% Transgenics
Rainbow trout *(Oncorhynchus mykiss)*	*cecropin B, cecropin P1*	5-40%
Steelhead trout *(Oncorhynchus mykiss)*	*cecropin B, cecropin P1*	
Rainbow trout *(Oncorhynchus mykiss)*	designed peptide *CF17*	



Mortalities of Cecropin-Transgenic F2 Trout Challenged with *A. salmonicida*



Relative Percent Survival (RPS) of Cecropin-Transgenic F_2 Trout Challenged with *A. salmonicida*

F_2 Line	Transgene	Genetic Background	Cumulative Mortality (%)	Average RPS*
WT (F2)	none	♂WT(F1) x ♀WT(F1)	52.0 ± 5.7	-
D8#909	cec P	♂WT(F1) x ♀ F1(D8#909)	29.5 ± 4.9	43%
S8#Y419	cec P	♂F1(S8#Y419) x ♀WT(F1)	31.5 ± 9.2	39%
WT (TL)	none	♂WT(TL) x ♀WT(TL)	27.0 ± 14.1	-
S7#375	cec P	♂F1(S7#375) x ♀WT(TL)	13.5 ± 2.1	50%
S9#659	cec P	♂F1(S9#659) x ♀WT(TL)	13.5 ± 0.7	50%
S9#747	cec P	♂F1(S9#747) x ♀WT(TL)	19.0 ± 4.2	30%
S9#746	cec P	♂F1(S9#746) x ♀WT(TL)	27.0 ± 12.7	N.P.
U7#949	cec B	♂F1(U7#949) x ♀WT(TL)	31.5 ± 4.9	N.P.

*RPS = [1 - %mortality of tested F_2 fish / %mortality of control fish] x 100

External Signs of Morbid Fish Infected with *A. salmonicida*

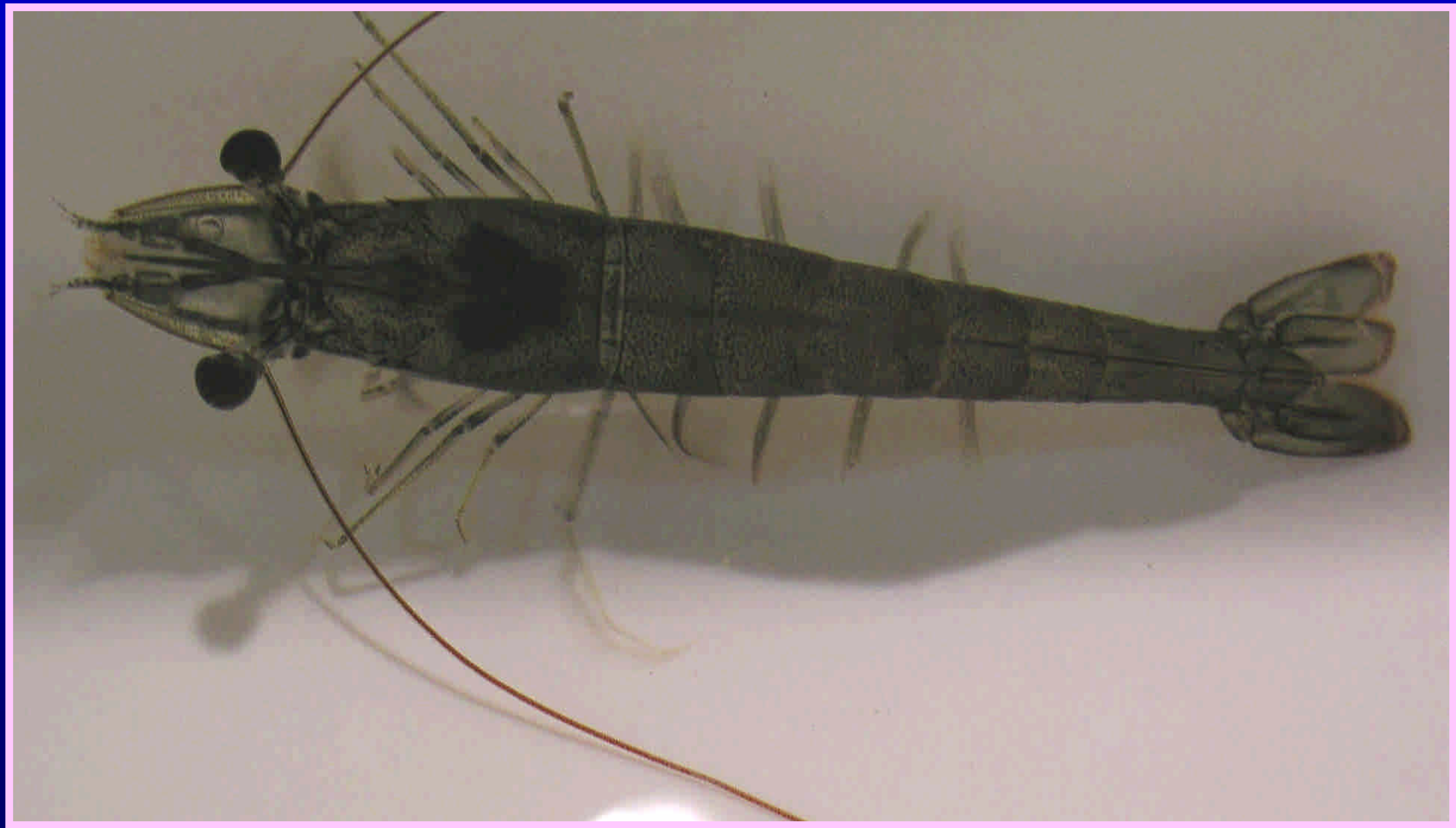
Frunculosis

External Signs of Morbid Fish Infected with IHNV-RB1

Mortalities of Rbt99 F_2 Challenged with IHNV

F2 Line	Transgene	Genetic Background	Weight (g)	Cumulative Mortality (%)
1 x 10^5 pfu/L				
WT (F2)	none	♂WT(F1) x ♀WT(F1)	0.58	48.0 ± 5.7
S8#Y419	cec P	♂F1(S8#Y419) x ♀WT(F1)	0.61	30.0 ± 12.7
S7#375	cec P	♂F1(S7#375) x ♀WT(TL)	0.41	22.5 ± 2.1
S9#746	cec P	♂F1(S9#746) x ♀WT(TL)	0.51	4.0 ± 4.2
5 x 10^5 pfu/L				
WT (F2)	none	♂WT(F1) x ♀WT(F1)	0.58	54.5 ± 6.4
S8#Y419	cec P	♂F1(S8#Y419) x ♀WT(F1)	0.61	35.0 ± 11.3
S7#375	cec P	♂F1(S7#375) x ♀WT(TL)	0.41	34.5 ± 2.1
S9#746	cec P	♂F1(S9#746) x ♀WT(TL)	0.51	8.0 ± 1.4

Pacific White Shrimp
(*Litopenaeus vannamei*)



Collaboration with Dr. Shaun Moss of the Oceanic Institute, Hawaii

Methods of Introducing Transgenes into Pacific White Shrimp (*L. vannamei*)

- **Pantropic viral vector mediated gene transfer**

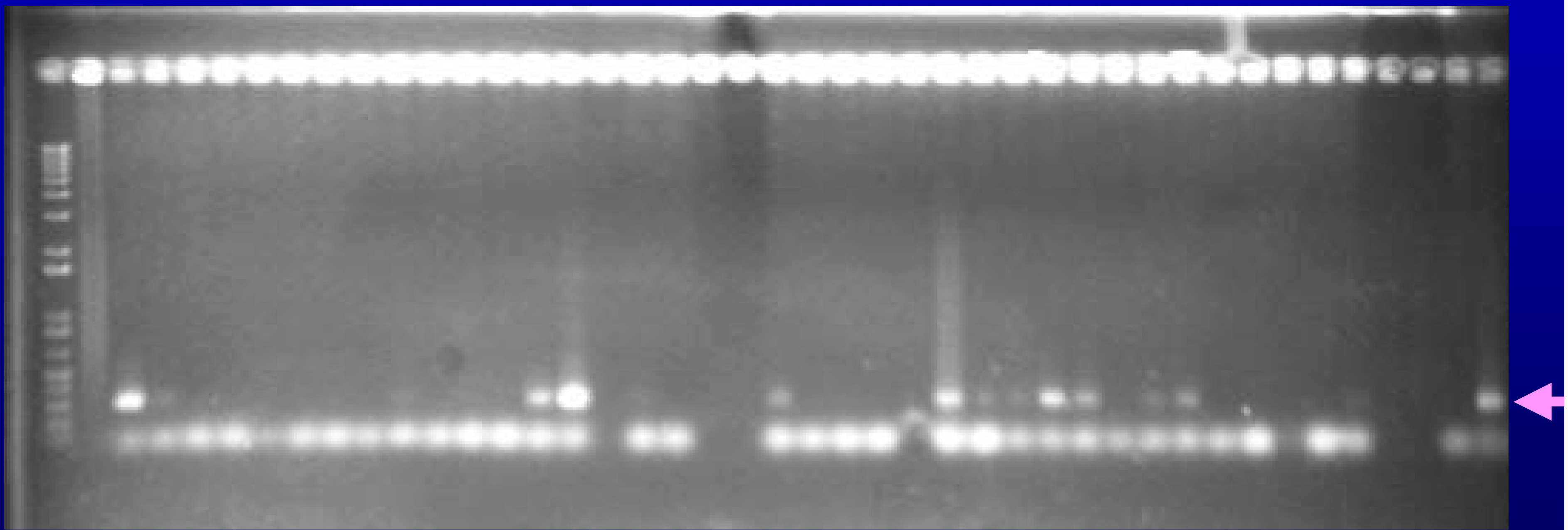
- **Lipid vesicle mediated gene transfer**

- ***In situ* electroporation mediated gene transfer**

In Situ Electroporation Conditions

In situ **Electorator (BTX); Voltage: 600-800 volts**
DNA: 10 μg/20 μl; number of pulses: 2

Detection of CF-17 Transgene by PCR in F_1 Transgneic Shrimps



Percent of Transgenic Shrimp Produced by Different Methods

Method of Production	% Transgenic F_1
In situ Electroporation	25
Pantropic Retroviral Vector	27
Lipofection	22

Application of Transgenic Technology in Aquaculture: Pros

- Simple method for targeted improvement of genetic traits
  - Enhanced growth, disease resistance, muscle mass, and nutrient utilization etc.
  - Value add-on characteristics: carotenoids, unsaturated fatty acids and pharmaceutical products
  - Control of reproductive cycle
  - Resistant to extreme environment: low oxygen requirement, low temperature etc.
- Efficient and time saving
- Specific and minimum side effects to animals been modified (*i.e.,* single gene or a small number of genes been modified)

Application of Transgenic Technology in Aquaculture: Concerns

- Impacts of genetic modifications on the health and welfare of the animals
- Impacts of genetic modified animals on environment
- Impacts of human health upon consumption of genetic modified animals

Impacts of Transgenic Animals on Human Health

- **Unexpected effects from new proteins created by the transgene**
  - **Suitable for human consumption? Toxin?**
  - **Biological activity of new protein harmful to human?**
  - **Allergenic to humans**
- **Solutions: Stringent assessment of the product**
  - **Toxicity determination**
  - **Assay for allergincity**

Impacts of Transgenic Animals on Environment (I)

- **Genetic impacts**
  - **Transgene affects fitness: Mating success, juvenile viability etc.**
    - **Empirical observation showed that GH transgenics require higher O_2, display lower critical swimming speed, take higher risk exposure to predator and lower viability of young**
- **Ecological impacts**
  - **Resource competition with target and non-target species**
  - **Habitat impact**
  - **Interbreeding with wild populations**
  - **Predation upon natural populations**

Impacts of Transgenic Animals on Environment (II)

- **Solutions:**
  - **Transgenic fish should be propagated in indoor close re-circulation facilities with high physical and biological containment**
  - **Stringent environmental assessment of transgenic animals**
  - **Developing sterile strains for large scale grow out**

Impact of Gene Transfer on Transgenic Animals

- **Unexpected genetic effects caused by transgene insertion:**
  - **Mutation: disruption of endogenous genes**
  - **Influence of the transgene promoter**
  - **Both events will influence the fitness of the animals**
- **Unexpected effects from new proteins created by transgenes**
  - **Toxic effect?**
  - **Adverse biological activity affecting development, metabolism, reproduction etc.**
- **Solutions:**
  - **Targeting transgene insertion**
  - **Selection**

Acknowledgement