Department of Pathobiology

University of Washington

Genogroup-specificity of DNA vaccines against infectious hematopoietic necrosis virus (IHNV) in rainbow trout (*Oncorhynchus mykiss*)

Ma. Michelle D. Peñaranda*, Scott LaPatra, and Gael Kurath

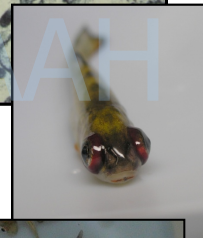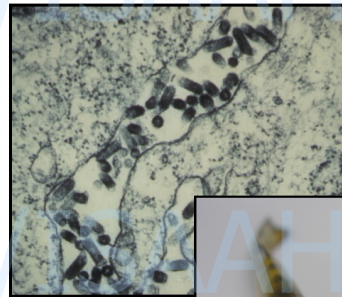Sixth International Symposium on Aquatic Animal Health

Global Strategies for a Changing Environment
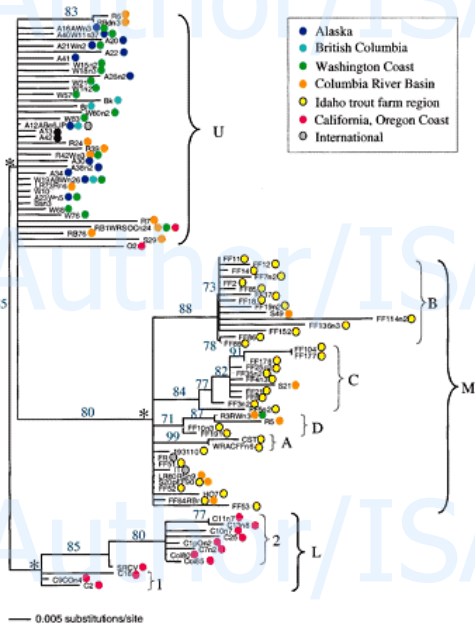
September 5-9, 2010   Tampa, Florida USA

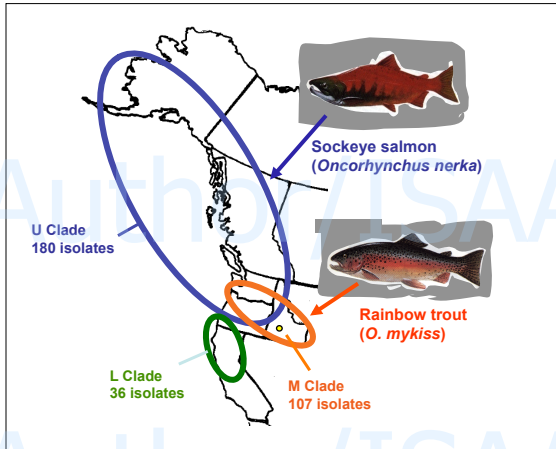
Infectious Hematopoietic Necrosis Virus

- **Fish rhabdovirus**

- **Acute infection**

- **Main target organs: kidney and spleen**

- **Significant mortality in salmon and trout species**

IHNV Genogroups

IHNV Prevalence in the Field

U and M IHNV Virulence in Immersion Challenges

Garver et al. 2006
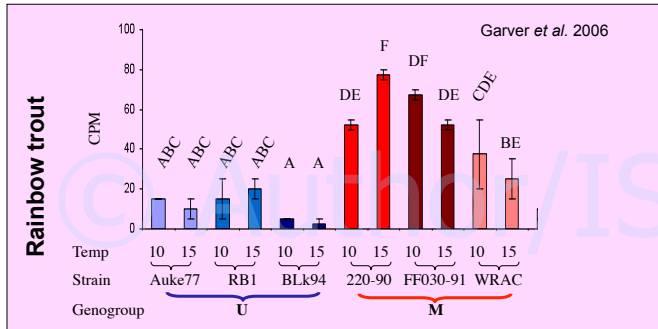
VIRULENCE	U	M
RBT	low	high
Sockeye	high	low

IHNV Glycoprotein

3' N P M G NV L 5'

Nucleocapsid protein (N)
Genome RNA

Matrix protein (M)
Polymerase (L)
Phosphoprotein (P)
Glycoprotein (G)
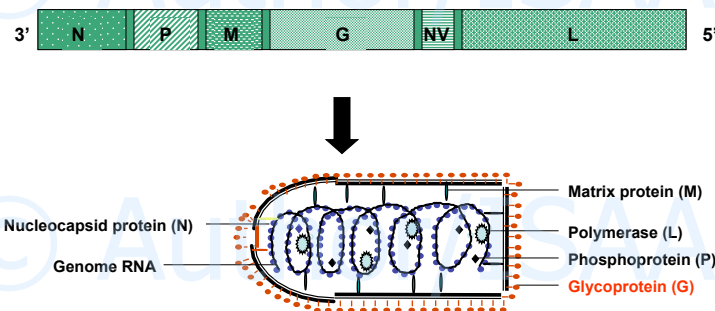
Only surface protein of IHNV

- Attachment to host cell receptors (virus entry)

- Induction of strong protective host immune response

Protection of IHNV DNA vaccine against virulent IHNV challenge

Buffer
Vector
IHNV Vaccine (RPS 80-100%)

% Mortality

Days post-challenge

Anderson *et al*. 1995, Corbeil *et al*. 2000, LaPatra *et al*. 2000, Lorenzen *et al*. 2002, Kurath *et al*. 2001, LaPatra *et al*. 2001, Garver *et al*. 2005

Figure courtesy of G. Kurath

Three Phases of Protection of IHNV DNA Vaccine

IHNV DNA Vaccine

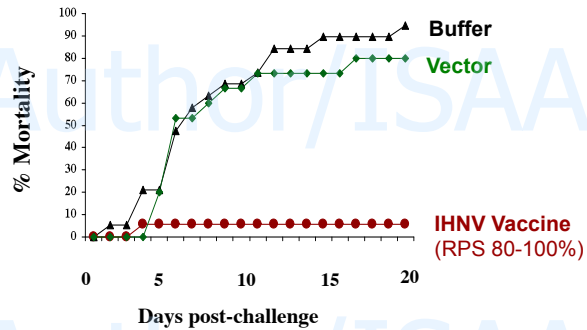**Innate**   **Adaptive**

Early Anti-viral Response (EAVR)
- Cross-protects against unrelated viruses
- High RPS

Specific Anti-viral Response (SAVR)
- Specific to IHNV
- Neutralizing antibodies often present
- High RPS

Long Term Anti-viral Response (LAVR)
- Presumably IHNV specific
- Lack of detectable neutralizing antibodies
- Reduced but significant RPS

0d 3d   7d   10d   15d   28d   6 mo   24 mo

Exact timing of these phases varies with water temperature

LaPatra *et al*. 2001, Lorenzen *et al*. 2002, Sommerset *et al*. 2003, Kurath *et al*. 2005

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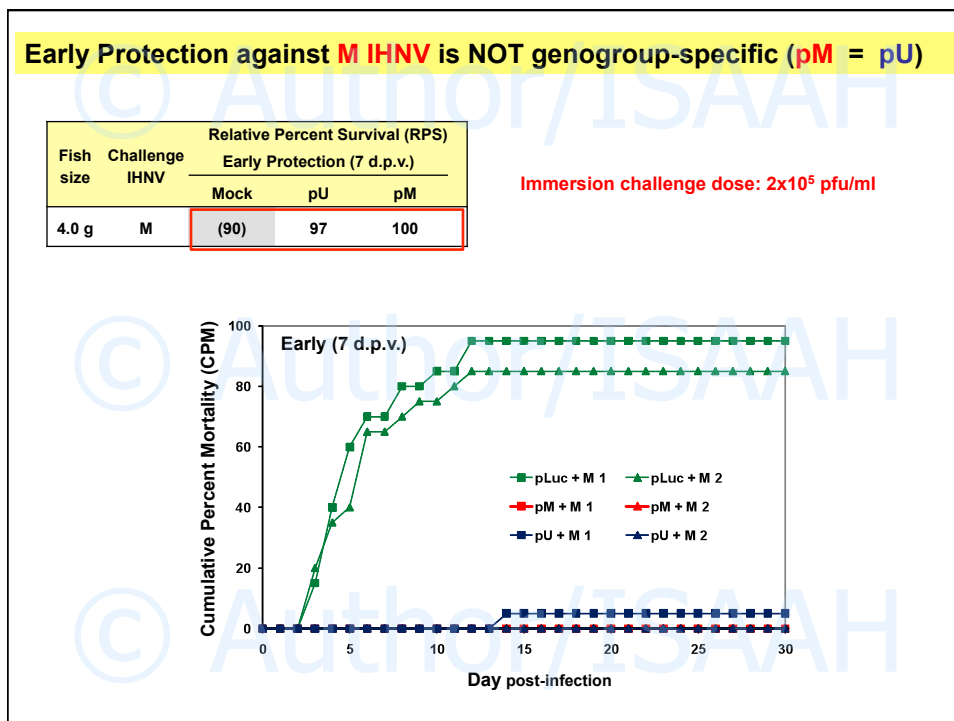## General schematic of IHNV DNA vaccine

Nucleotide diversity = 1.8% (28 nt differences/1527 nt of ORF)

Amino acid diversity = 1.8% (9 aa differences/508 aa)

M IHNV   U IHNV

IHNV genomic RNA, ~11,000 nt

N   P M   G   NV   L

3'   5'

G gene 1610 nt

CMV promoter

G gene

polyadenylation signal

ampicillin resistance

neomycin resistance

ori

pU

pIHNV-rG U

pM

pIHNV-wG M

pLuc

Control
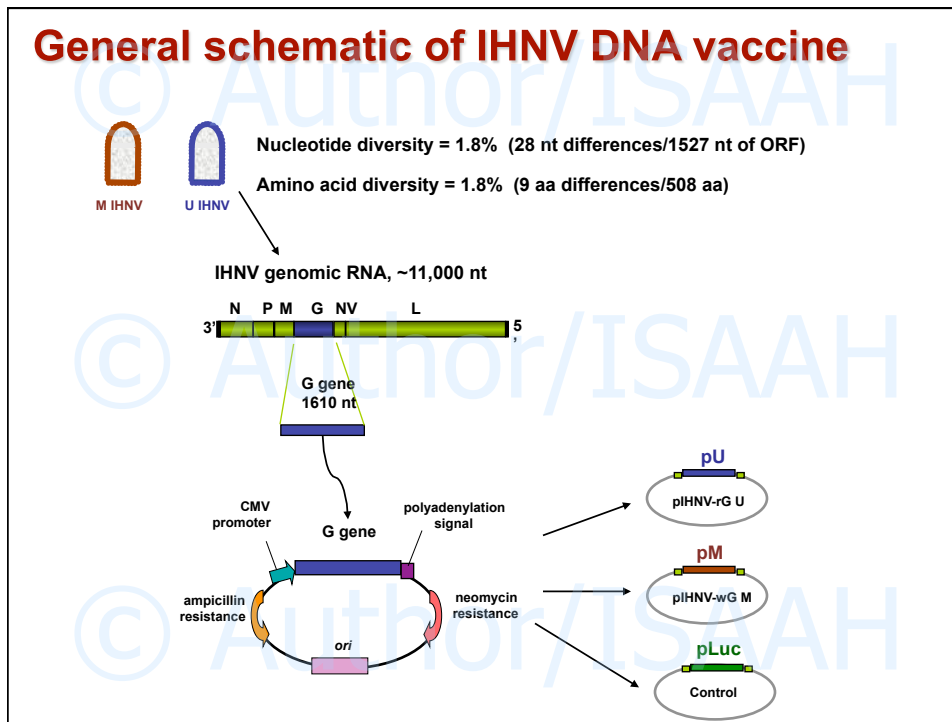
RESEARCH OBJECTIVES:

- **Compare efficacy of pM and pU DNA vaccines against virulent M and U challenges in rainbow trout**

- **Compare host immune responses induced following pM and pU DNA vaccination**

- **Determine the correlation of Mx-1 gene expression and NAb production with protection elicited by the pM and pU vaccines**

Fish Vaccination

Intramuscular (IM) vaccination with pM or pU DNA vaccine
(Dose: 1.0 μg/fish)

Rainbow trout (RBT) held at 15°C

I. Vaccine Efficacy

IHNV challenges (2x20 fish/group):
M IHNV – Immersion Challenge
U IHNV – IP injection Challenge
Control: PBS and/or pLuc vaccinates

Early protection challenge: 7 d.p.v.

Late protection challenge: 28 d.p.v.

Daily monitoring of fish mortality for 30 days

1. Cumulative percent mortality (CPM)
2. Relative Percent Survival (RPS)

Early Protection against M IHNV is NOT genogroup-specific (pM = pU)

Immersion challenge dose: 2×10^5 pfu/ml
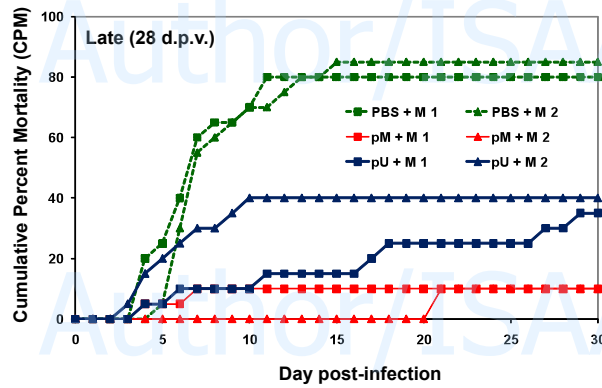
Fish size	Challenge IHNV	Relative Percent Survival (RPS) Early Protection (7 d.p.v.)		
		Mock	pU	pM
4.0 g	M	(90)	97	100

Late Protection against M IHNV is genogroup-specific (pM ≥ pU)

Fish size	Challenge IHNV	Relative Percent Survival (RPS) Late Protection (28 d.p.v.)		
		Mock	pU	pM
1.2 g	M	(82.5)	58	88*
4.0 g	M	(95)	95	100

Immersion challenge dose: 2x10^5 pfu/ml

Late (28 d.p.v.)

Cumulative Percent Mortality (CPM)

PBS + M 1, PBS + M 2
pM + M 1, pM + M 2
pU + M 1, pU + M 2

Day post-infection

Early Protection against U IHNV is NOT genogroup-specific (pM = pU)

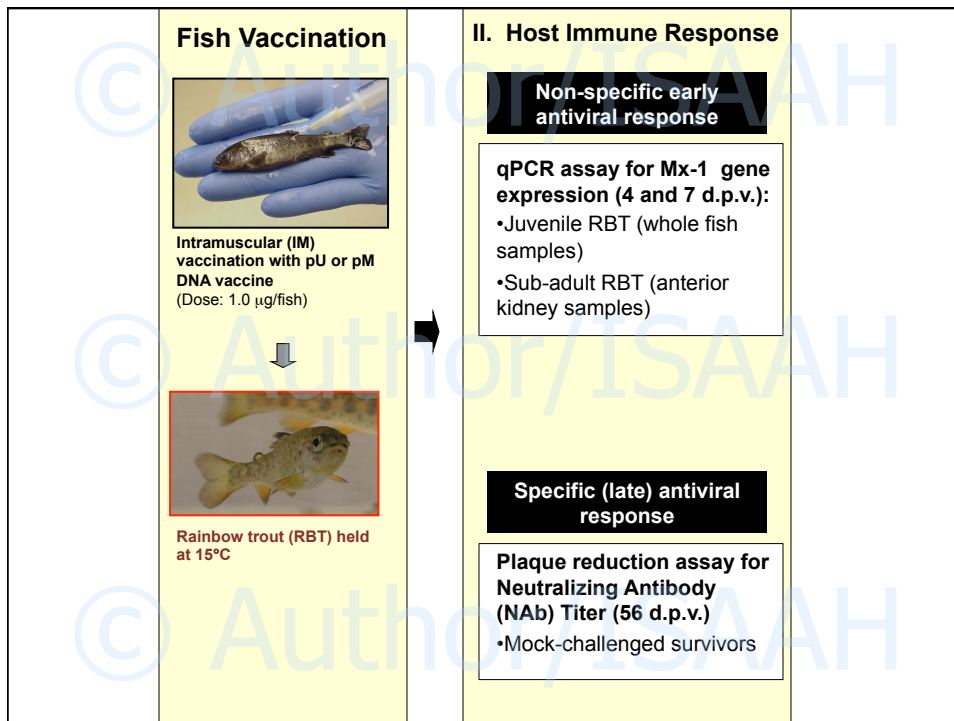| Fish size	Challenge IHNV	Relative Percent Survival (RPS) Early Protection (7 d.p.v.)		
		Mock	pU	pM
1.4 g	U	(79)	94	96

IP injection challenge dose: 5x10^6 pfu per fish

Early (7 d.p.v.)

Cumulative Percent Mortality (CPM)

pLuc+U 1, pLuc+U 2
pM+U 1, pM+U 2
pU+U 1, pU+U 2

Day post-infection

Late Protection against U IHNV is NOT genogroup-specific (pM = pU)

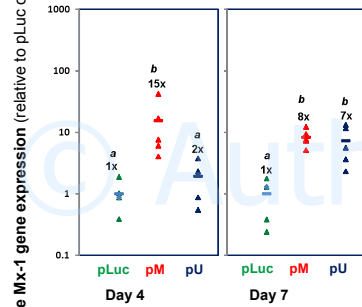| Fish size	Challenge IHNV	Relative Percent Survival (RPS) Late Protection (28 d.p.v.)		
		Mock	pU	pM
1.4 g	U	(86)	96	93

IP injection challenge dose: 5×10^6 pfu per fish

Cumulative Percent Mortality (CPM) — Late (28 d.p.v.)

Legend: pLuc+U 1, pLuc+U 2, pM+U 1, pM+U 2, pU+U 1, pU+U 2

Day post-infection

Fish Vaccination

Intramuscular (IM) vaccination with pU or pM DNA vaccine
(Dose: 1.0 μg/fish)

Rainbow trout (RBT) held at 15°C

II. Host Immune Response

Non-specific early antiviral response

qPCR assay for Mx-1 gene expression (4 and 7 d.p.v.):
• Juvenile RBT (whole fish samples)
• Sub-adult RBT (anterior kidney samples)

Specific (late) antiviral response

Plaque reduction assay for Neutralizing Antibody (NAb) Titer (56 d.p.v.)
• Mock-challenged survivors

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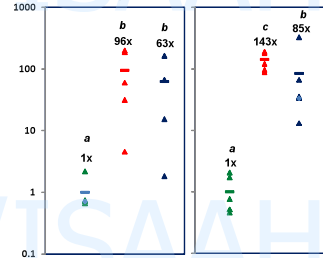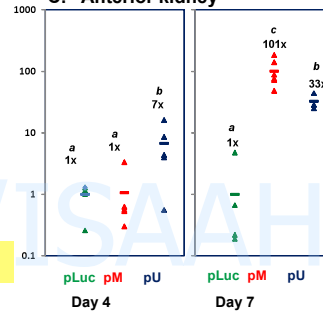**Mx-1 Gene Expression in pM and pU vaccinates**
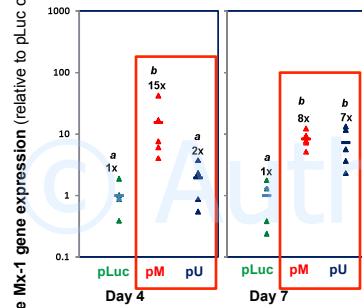
A. Whole fish samples

B. IM injection site (muscle)

C. Anterior kidney

Mx-1 gene expression in RBT: pM ≥ pU

Mx-1 Gene Expression in pM and pU vaccinates

A. Whole fish samples

Vaccine group	RPS	Mx-1 fold change	
		4 d.p.v.	7 d.p.v.
M Challenge			
pM	100	15x*	8x
pU	97	2x	7x

Innate immune response following pM and pU DNA vaccination appear to be sufficient to confer high protection at early challenge (7 d.p.v.)

Neutralizing antibody of pM and pU vaccinates

Vaccine group	RPS	No. positive sera/ total no. sera pools		Neutralizing antibody titers[a]	
		Anti-M IHNV	Anti-U IHNV	Anti-M IHNV	Anti-U IHNV
Expt. 1 (pool of 2-3 fish)					
pM	88*	5/6	4/6	< 20, 2(20), 2(40), 80	2(< 20), 20, 2(40), 80
pU	58	4/6	6/6	2(< 20), 20, 2(40), 80	6(≥160)
Expt. 2 (pool of 2-3 fish)					
pM	100	12/14	11/14	2(< 20), 20, 5(80), 6 (≥160)	3(< 20), 3(20), 3 (40), 80, 4(≥160)
pU	95	3/12	11/12	9(< 20), 2(20), 80	< 20, 80, 10(≥160)
Expt. 3 (pool of 3-4 fish)					
pM	93	10/11	8/11	< 20, 20, 80, 8(≥160)	3(< 20), 20, 2(40), 3(80), 2(≥160)
pU	96	9/11	10/11	2(< 20), 40, 8(≥160)	40, 80, 9(≥160)

NO clear correlation between NAb production and observed protection

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SUMMARY: DNA VACCINE EFFICACY

RAINBOW TROUT	M IHNV	U IHNV
Early protection (7 d.p.v.)	pM = pU	pM = pU
Late protection (28 d.p.v.)	pM ≥ pU	pM = pU

➢ **In rainbow trout, both pM and pU vaccines elicit significant protection against U and M IHNV**

➢ **Early Protection: Comparable high non-specific protection against both M and U IHNV)**

➢ **Late Protection:**
- **Apparent genogroup-specific protection against M IHNV**
- **No genogroup-specificity against U IHNV**

SUMMARY: HOST IMMUNE RESPONSE

RAINBOW TROUT	M IHNV
EARLY: Mx-1 gene expression	pM ≥ pU
LATE: Neutralizing antibody titer against M IHNV	pM ≥ pU
LATE: Neutralizing antibody titer against U IHNV	pM ≤ pU

➤ **In rainbow trout, pM induces higher Mx-1 gene expression than pU vaccine**
- **Correlates with pM ≥ pU LATE protection**

➤ **Neutralizing antibody production appears to be higher in intra- than cross-genogroup target virus BUT NO CLEAR CORRELATION WITH OBSERVED PROTECTION**
- **Role of other components of adaptive immunity (*i.e.* CTLs) in specific antiviral response (late protection)?**

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