Use of Diagnostic Assays to Screen Rainbow Trout (*Oncorhynchus mykiss*) Broodstock for *Flavobacterium psychrophilum*

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Flavobacterium psychrophilum

- Causative agent of Bacterial Coldwater Disease (BCWD)
- Primarily affects salmonids
- No commercial vaccine is available and oxytetracycline resistance is widespread
- Pathogen of worldwide concern

Madetoja et al. 2005

Transmission of *F. psychrophilum*

- Horizontally transmitted
- Strong evidence for true (intraovum) vertical transmission
  - Isolated from the surface of eggs, ovarian fluid, and the contents of unfertilized eggs
  - Capable of surviving within an egg

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Standard Detection Methods

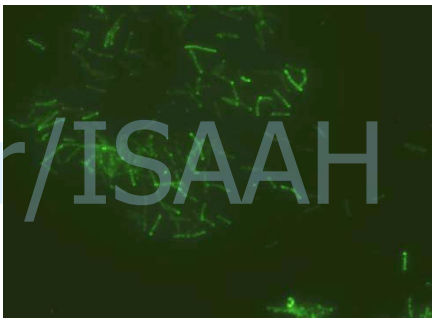
- Culture
  - Samples are plated and monitored for yellow-pigmented bacteria (YPB) growth
  - Confirmed as *F. psychrophilum* by biochemical tests
- Polyclonal antibody tests
- Nested PCR (nPCR)
  - Universal 16S primers and primers specific to *F. psychrophilum* (Taylor, 2004)
  - Detected in brain tissue, ovarian fluid, and fluid from within eggs

Inconsistent Results

- Results do not always agree
- *F. psychrophilum* grows slowly and is often overgrown by other bacteria
- Not all YPB are *F. psychrophilum* and not all YPB can be re-isolated for confirmation

Monoclonal Antibody FL43

- Reactive to an antigen on the outer membrane of *F. psychrophilum* (Lindstrom et al. 2009)
- Assays
  - Capture ELISA for kidney and spleen tissue
  - Membrane filtration FAT (MF-FAT) for ovarian fluid



Initial Studies

- MF-FAT
  - 74% of coho salmon (*Oncorhynchus kisutch*) and 42% of rainbow trout (*O. mykiss*) OF samples were positive
- ELISA detection limit
  - 1.6 x 10^3 CFU ml^{-1} in spiked kidney tissue
- Detection limit is now 10^4 CFU ml^{-1}

Current Studies

- Use ELISA to relate disease to infection levels in fish challenged with *F. psychrophilum*
- Rainbow trout were injected with either PBS or *F. psychrophilum* (2.7 x 10^8 CFU ml^{-1})
  - Random samples taken prior to challenge and again at 7, 10, 14 and 21 days post-injection
  - Kidney and spleen streaked on TYES plates
  - Kidney and spleen tissues used in ELISA

Results

- No mortalities
- O.D. values ranged from 0.102 – 0.209 corresponding to $7.86 \times 10^4 - 2.16 \times 10^5$ CFU ml^{-1}
- Samples from both Day 7 and Day 10 were positive by ELISA
- Positive culture results for Day 7 samples only
- ELISA can detect subclinical infections

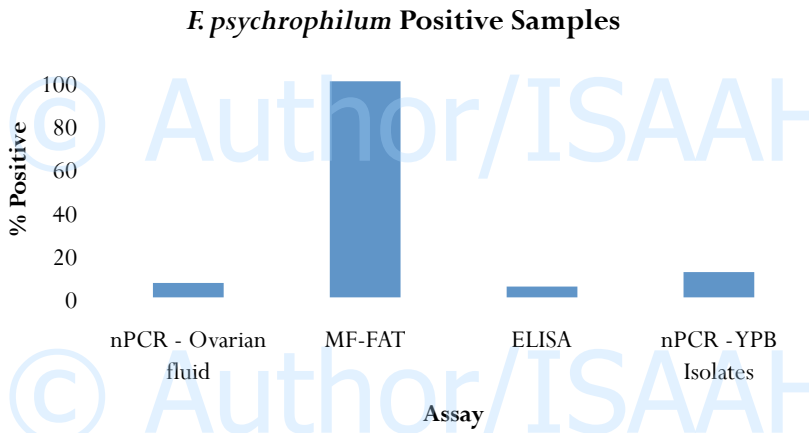
Objectives

- Compare four assays (nPCR, MF-FAT, culture, and capture ELISA) for screening broodstock for *F. psychrophilum*
- Evaluate risk of BCWD outbreaks in progeny from broodstock with varying infection levels as determined by assays

Broodstock Sampling

- Sixty rainbow trout broodstock were sampled at a private hatchery in February 2010
  - Kidney, spleen, and ovarian fluid
- All samples were plated on TYES and monitored for YPB growth
- Ovarian fluid used in nPCR and MF-FAT
- Kidney and spleen tissues used in ELISA

Broodstock Results



Results

Assay	Ovarian Fluid	Tissue	CFU ml^{-1}	YPB growth
nPCR	4/60 (6.67%) ¤	N.A.	N.A.	8/29 (27.6%)
MF-FAT	60/60 (100%)	N.A.	N.A.	N.A.
Culture	4/60 (6.67%) ¤	3/60 (5%) §	N.A.	N.A.
ELISA	N.A.	3/60 (5%) §	1.21×10^5 – 1.79×10^6	N.A.

¤Positive samples are not from the same fish

§2 fish were positive by both assays

Conclusions

- Broodstock were carrying *F. psychrophilum* but not at high levels
- Little agreement between the different assays
  - Detection limitation of assays is the likely reason for the discrepancies
- ELISA is the only effective assay to quantify infection levels

Objectives

- Compare five assays (nested PCR, MF-FAT, culture, and capture ELISA) for evaluating presence of *F. psychrophilum* in broodstock
- Evaluate risk of BCWD outbreaks in progeny from broodstock with varying infection levels as determined by assays
  - Rear under stressful conditions to induce outbreak

Selected Families

	ELISA		Ovarian Fluid		Culture
Family	**Tissue**	**CFU ml^{-1}**	**nPCR**	**MF-FAT**	**nPCR confirmed**
F54	-	.	-	+	.
F61	+	1.21E+05	-	+	+
F70	+	1.79E+06	-	+	-
F74	+	1.41E+06	-	+	.
F87	-	.	-	+	+

Progeny Monitoring

- Eyed eggs were shipped to University of Idaho
  - Surface disinfected in 100 ppm Ovadine® for 10 minutes upon arrival
- Weekly sampling
  - Day 0: Fluid extracted from disinfected eyed eggs
  - Day 9 – 51: Five fry were disinfected, homogenized, and homogenate used in nPCR
  - Day 57 – 72: Kidney and spleen from five fish were plated on culture media

F. psychrophilum in Progeny

Sampling Days

Family	0	9	16	23	26	36	44	51	57	64	72
F54	+	-	+	+	+	-	-	+	-	-	-
F61	+	+	+	+	-	-	-	-	-	-	-
F70	+	-	+	+	+	-	-	-	-	-	-
F74	+	+	-	+	+	-	-	-	-	-	-
F87	+	+	-	+	-	-	-	-	-	-	-

Culture

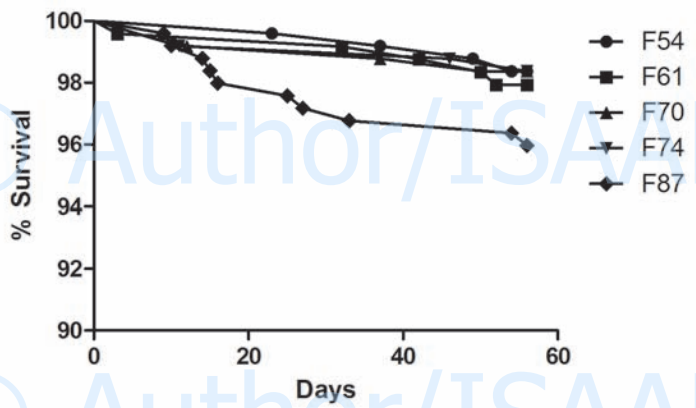
Experimental Design

- Fish transported from wetlab to Coldwater Research Laboratory at Aquaculture Research Institute (UI)
- Six treatment tanks per family
- All tanks exposed to supersaturated water (chronic)
  - N_2 gas levels ranged from 101-111%
- Half of the tanks subjected to handling stress (acute) in addition to chronic stress
  - 20 seconds twice a day for 56 days
- Sampled once a week

Results

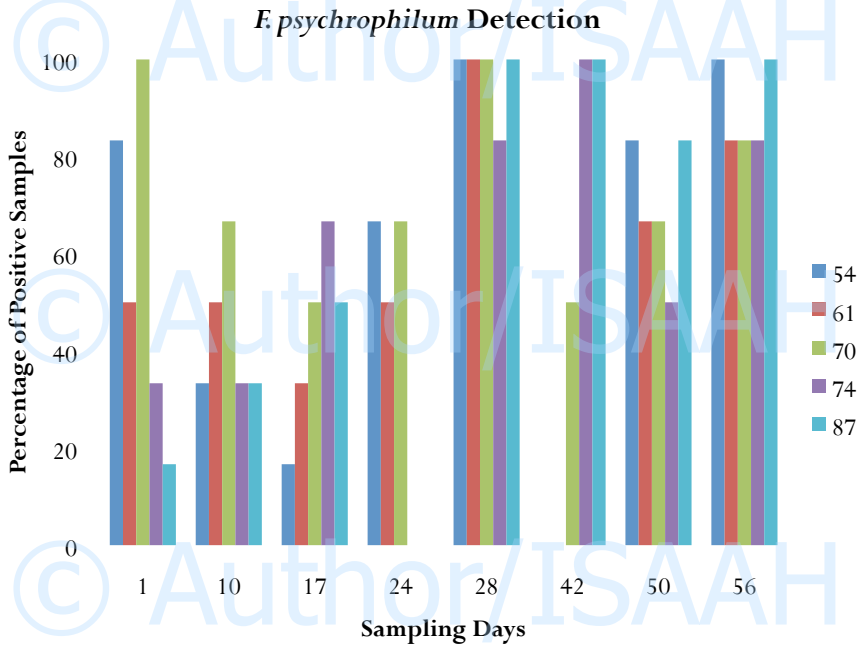
- Exhibited signs of chronic stress including frayed fins, petechial hemorrhaging, scale loss, and overinflated swim bladders
- No difference between chronic and acute stress
- 27 mortalities
  - Majority of mortalities were in F87
- 29.6% of mortalities were positive by nPCR
  - Positives were in F61, F74, and F87

Family Survival



Weekly Sampling

- No bacterial growth on any plates from weekly samples
- Increase in percentage of samples positive for *F. psychrophilum* by nPCR on tissue samples



Conclusions

- *F. psychrophilum* was detected in disinfected eggs suggesting that vertical transmission had occurred
- Infection levels decreased to below detectable levels but stress resulted in an increase in percentage of fish positive for *F. psychrophilum*
- Broodstock had low infection levels but preliminary trial indicates there may be a relationship between infection levels in broodstock and outbreaks in progeny

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