GREAT LAKES FISH HEALTH COMMITTEE

2014 Winter Meeting
State College, Pennsylvania
February 4-5, 2014

Minutes
(with attachments)

Submitted By:

Christina Haska
Great Lakes Fishery Commission

GREAT LAKES FISHERY COMMISSION
2100 Commonwealth Blvd, Suite 100
Ann Arbor, Michigan 48105
Great Lakes Fish Health Committee

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List of Attendees

John Coll	U.S. Fish and Wildlife Service- Pennsylvania
John Dettmers	Great Lakes Fishery Commission
Mohamed Faisal	Michigan State University
Christina Haska	Great Lakes Fishery Commission
Nick Jamison	Ohio Department of Natural Resources
Sunita Khatkar	Fisheries and Oceans Canada
Kevin Loftus	Ontario Ministry of Natural Resources
Sue Marcquenski	Wisconsin Department of Natural Resources
Dave Meuninck	Indiana Department of Natural Resources
Brian Niewinski	Pennsylvania Fish and Boat Commission
Ken Phillips	U.S. Fish and Wildlife Service- Wisconsin
Ling Shen	Minnesota Department of Natural Resources
Gary Whelan	Michigan Department of Natural Resources
Coja Yamashita	Pennsylvania Fish and Boat Commission

Other attendees included:

Dale Honeyfield	U.S. Geological Survey
Jan Lovy	New Jersey Department of Environmental Protection
Gavin Glenney	U.S. Fish and Wildlife Service

Great Lakes Fish Health Committee Meeting

February 4-5, 2014

Days Inn
240 South Pugh Street
State College, PA

Draft Agenda

Tuesday, February 4

8:30 am – 8:40 am	Welcome & Introductions (Shen)
8:40 am – 8:45 am	Approval of Meeting Minutes (Shen)
8:45 am – 8:50 am	CLC update (Dettmers)
8:50 am – 9:05 am	Model Program/Risk Assessment Update (Shen/Dettmers)
9:05 am – 9:45 am	APHIS update (Dr. Lee Ann Thomas)
9:45 am – 10:00 am	PA Dept of Agriculture on Aquaculture (Thomas Alexander/Nan Korn)
1:00 am – 10:30 am	Update on Cutthroat trout virus research (Sue M/ Tom)
10:30 am – 10:50 am	Break
10:50 am – 11:30 am	Research updates on fish tumor prevalence, its risk factors and emerging contaminant/endocrine disruption in the Great Lakes (Dr. Vicki Blazer)
11:30 am – 12:00 pm	Update on thiamine deficiency research (Dale Honeyfield)
12:00 pm – 1:30 pm	Lunch (on your own)
1:30 pm – 2:00 pm	Lamar USFWS Fish health center update (Gavin Glenney)
2:00 pm – 3:00 pm	Flavobacterium and MI State University research updates (Dr. Faisal)
3:00 pm – 3:15 pm	Break
3:15 pm- 3:45 pm	Flavobacterium and MI State University research updates (Dr. Faisal)
3:45 pm – 4:30 pm	Roundtable Discussion on Unusual Fish Disease Cases
4:30 pm – 5:00 pm	Furunculosis outbreak at a NJ state Hatchery (Jan Lovy)

Wednesday, February 5

8:30 am – 9:45 am	Strategic vision (Dettmers and Subcommittee)
9:45 am – 10:30 am	Technical advisors (Dettmers and Shen)
10:30 am – 10:45 am	Break
10:45 am – 11:15 am	Risk Assessment (Shen and subcommittee)
10:45 am – 11:05 am	Fish Health pre-proposal (Shen)
11:05am – 11:20 am	EEDV history and current status at Marquette SFH (Whelan)
11:20 am – 12:00 am	Agency Updates— (All)
12:00 am – 1:00 pm	Lunch
1:00 pm – 3:30 pm	Tour Lamar Fish Health Center
3:30 pm – 4:40 pm	Agency Updates— (Continued, All)
4:40 pm – 4:45 pm	Future meetings (Shen)
	-Dates/location for winter 2015 Meeting
4:45 pm – 5:15 pm	Meeting Wrap-up/Parking Lot (Shen)
5:15 pm	Adjourn

Tuesday, February 04, 2014

1. Welcome and introductions

2. Agenda changes

Due to a forecasted snowstorm, the visit to Lamar was moved to Tuesday and individual talks and other agenda items were moved as appropriate.

3. Approval of meeting minutes

Whelan motioned to approve the minutes, Faisal seconded. Minutes were approved pending changes.

4. CLC update (Dettmers)

Corey Puzach and Gary Jagodzinski presented to the CLC in October 2013, so members are aware of the risk of pathogens being transported with bait fish.

There has been a change in personnel at the commission: Chuck Krueger moved to a research position at Michigan State University, and the acting Science Director is Andrew Muir. The position will be advertised and filled permanently, hopefully by the end of the year.

Currently, scientists are questioning the ecological effects of net pen aquaculture in Lake Huron on the Canadian side and the magnitude and effects of escaped fish. There's concern about mixing pen-fish with naturalized steelhead stocks. It's unclear if the fish are marked to distinguish them from the wild fish or their susceptibility to VHS and other diseases.

- A discussion followed about net pen use in the states and Ontario. Wisconsin has a slow-release program for salmon. Anglers are working with the legislature to prolong the holding period in the net pens, requiring someone to feed and care for the fish. Fishing clubs would take control of this, but it may be opening a door for net pens to be used for commercial purposes. Michigan's legislature is pushing to be able to use net pens in Lakes Michigan, Superior, and Huron. No details yet on where exactly, which species, or when. Ontario has a rainbow trout farmer switching a cage to walleye.

5. Model Program/ Risk Assessment update (Shen/Dettmers)

There are some obstacles to get the document published, but this will allow the Model Program and the Risk Assessment to come online at the same time before the summer meeting.

6. APHIS update (Thomas)

There has been some reorganization in the APHIS aquaculture team. Janet Whaley left federal service to work for a consulting company in Washington. A replacement will be made as quickly as possible. Lynn Credmore and Lori Gustufson are on the Aquaculture Team. Lee Ann Thomas, Director of the Avian, Swine, and Aquatic Animal Health Center, gave the update.

The Avian, Swine, and Aquatic Animal Health Center is a new facility with veterinary services. The program was reorganized in November 2013. There are 4 units: Surveillance, Preparedness and Response Service (SPRS); Science, Technology, and Analysis Service; National Import/Export Service; and Program Support Service. SPRS has 3 commodity centers which represent functional congressional budget lines. The unit provides leadership and direction to the commodity industry, directs planning to protect animal health, plans and implements surveillance, manages disease specific programs, prepares emergency response plans, develops regulatory and program standards, maintains SOPs for foreign and emerging diseases, and oversees animal health regulations. There are now 6 animal health districts-midatlantic, northeast, central, plains, western, and southern/southeastern region. SPRS is now within one organizational structure.

The aquaculture budget saw a small increase to $1.9M. To ensure stakeholder engagement, there was a meeting hosted by the APHIS administrator, Kevin Shea, to get feedback from the industry (avian, cattle, and aquaculture). Nine individuals attended the meeting to provide input about the strengths, weaknesses, opportunities, and threats to the industry. There was a strong desire to develop a model animal health plan to use with the NAAHP. It would not be intended to be a regulatory document but would provide guidelines about surveillance, movement, testing requirements, etc. U.S. products are exported and a zoning/certification system is needed to ensure products are safe and do not contain disease. Veterinary services is developing a business plan with staff for stakeholder input, and it should be released in 6-8 weeks. A 5- year business plan would look to the future and which activities to initate/eliminate. It's a high priority to have electronic signatures on the certification. Aquaculture practitioners play a critical role in providing information and serve as an early warning system for the detection of diseases. Wildlife services were at the meeting and support depredation efforts of predators, including cormorants and other birds.

In September 2012, a subcommittee met about aquatic animal health and provided feedback and recommendations about the VHS federal order. They did a risk assessment (RA) to determine the effects if the Federal order was rescinded, and the final decision is going to John Clifford. The results showed the risk of spread of VHS without the federal order would not significantly increase as long as states did not change their current regulations. The RA found there were 5 pathways by which VHS could move: two are mitigated by the federal order, but the other three were unmitigated (i.e., movement through water). An announcement will likely take place next month at the USFWS fish health meeting in Denver.

The list of susceptible species would not be maintained if the federal order is rescinded; however there would be nothing from keeping that information as guidance. If the federal order is rescinded, the order to the states would be to continue doing what they're doing; however, without an official list, some states would have to change their statutes because they refer to the APHIS list. There is concern states would drop species without the list of susceptible species, and there needs to be a one-place reference.

The National Animal Health Laboratory Network (NAHLN) has approved tests and requirements, and there was a request in December 2013 to gather information about creating an aquatic component, training non-NAHLN labs about quality management systems, and bringing in privates labs. Two agents of concern are ISA and VHS. A mechanism will be developed to add other pathogens.

Lee Ann asked about member impressions about VHS in the wild and whether there are fewer or more outbreaks. Mohamed has been surveying NY, WI, and MI and collected approximately 5000 samples. Some of the fish have the virus in the serum, others have it in kidneys and spleen, and some have antibodies (some are short-lived, being exposed in the last 12 months), and others are detected with ELISA. Mortalities are only reported in big outbreaks with major sport fish. It is unknown if younger fish are experiencing mortalities, but the virus is there. VHS is certainly in the environment and will reappear under certain conditions, and therefore needs to be managed around.

7. Update on Cutthroat Trout virus research (Marcquenski)

See Appendix 1 for the presentation.

8. Lamar USFWS fish health center update (Glenney)

See Appendix 2 for the presentation.

- They only sampled along the belly, so it's unknown if it matters what region of the skin is sampled.
- It would be interesting to run a model and see how the viruses changed and if there was independent development of EEDV.
- People could send him samples from the Upper Lakes and see if the probe works for those cases.
- Mohamed thanked him for sharing his expertise and guidance.
- It is now time to do something about EEDv and understand what it is. We have a source of EEDv, fish that survived 3 epizootics, infected fish at varying degrees, and an excellent group of scientists.

9. Michigan State University research update (Faisal)

See Appendix 3 for the presentations.

10. Furunculosis outbreak at a New Jersey state hatchery (Lovy)

See Appendix 4 for the presentation.

- There's probably a fair number of waters where it wouldn't matter if you put these fish in them because they won't survive past June. There wouldn't be any effect. From an administrative perspective, putting them in seasonable non-trout waters would be ok.
- Pequest is in the red area.
- Rainbow trout would also be stocked in some of those waters, but only those which don't have native rainbow trout.
- Northwestern New Jersey is mountainous, whereas the south is more flat. The geography makes only the northwest suitable for trout survival.
- These fish may be potential carriers, and if under stress, could develop furunculosis. That would be negative for public perception, etc.

- If fish go out early, would there be enough time for furuncles to form? The fish don't look bad, and some of them may no longer have the pathogen. Broodstock should be immediately vaccinated after eggs and milt are taken. The eggs should be disinfected. Furunculosis is everywhere and can't be eradicated, so it's important to protect the fish.
- A potential hypothesis about why this is happening now is the hatchery had a horrible osprey and heron problem last year, and these birds likely had fed on fish from the wild. This was the first incident of the pathogen being found in the water. There had never even been a background noise for it, so the fish had never been exposed previously.
- Use this as an opportunity to cover the raceways with pole barns over the water. The fish would convert food better, have less stress, and not be sunburned. This might fix a lot at once. Also, if the fish are not destroyed, put them in carefully chosen put-and-take waters.
- The placement of fish in the raceways could be tweaked. Broodstock are worth more than production fish, so they should be held in the upper raceways. Keep rainbow trout in the lower raceways because they are resistant. Be weary of adding A.sal positive fish to waters that is A.sal negative because other fish can be hosts for this pathogen.
- Since there aren't any good policies to provide guidance about moving forward, referring to the Model Program may be helpful. Running through the risk assessment would be a good exercise for everyone.

Wednesday, February 05, 2014

11. Strategic Vision (All)

Committee members reviewed the draft Strategic Vision (Appendix 5) and made comments for the writing subcommittee to address. Specifically, the strategies (1-5) should be mentioned in the introduction. Also, the vision of the committee should be bolded instead of the statement below it.

12. Technical advisors (All)

The following criteria should be used to elect Technical Advisors to the committee:

- He/she should have scientific accomplishments,
- He/she should be willing to participate, and
- The committee should need his/her expertise.

Mohamed, Sunita, and Ling will use these criteria to create a matrix to rank potential advisors (Appendix 6) and will report back at the summer meeting.

Advisors could have 2 year terms, which would allow the committee to reevaluate every two years what its needs are.

13. Risk Assessment introduction (All)

Edits were made for the Risk Assessment introduction. See Appendix 7.

14. EEDv history and current status at Marquette State Fish Hatchery (Whelan)

See Appendix 8 for the presentation.

- There were some differences between the weather in 2012 vs 2013. First, in 2013 there weren't any big storm events. In 2012, the storms probably produced suspended particles which increased stress levels. It was also warmer in 2012. Right now, because of the cold winter, the raceways are frozen.
- There was an EEDv outbreak in the 1980's at the Marquette SFH, but there were differences between then and now. In the 1980's, the fish had nose deformities in addition to skin lesions and fungal infections. The fish in the recent outbreak did not have those nose issues. Also, the strains were different in each outbreak, and there is a newer broodstock line at the hatchery now.
- The hatchery will manage around this disease because it is possibly endemic and unlikely to be eradicated.

15. Update on thiamine deficiency research (Honeyfield)

See Appendix 9 for the presentation.

- As American Eels leave the St. Lawrence River, a portion of them are probably thiamine deficient.
- It is possible that when the eels move through the ocean on their way to the Sargasso Sea, they could become thiamine-replete again based on diet.
- It's unknown what paths they take and where they spawn. Radio tagged fish are not found after the St. Lawrence River. The fact that hormone injections are needed indicates the eels are not near spawning.
- It would be interesting to see the thiamine levels of eels in the Upper Lakes.
- Phenotypically, the eels are expressing a less heterogenetic population. The environmental impact of thiamine deficiency makes them look less genetically diverse. In the replete group, there are two clear populations, but the deplete group may have a gene turned off which the replete have turned on.
- Fish appear to have similar immune response mechanisms as mammals do. Different fish populations can respond in different ways. The complexity of fish population immunology may be more than mammalian immunology.

16. Fish health research pre-proposals (All)

The committee was given six research pre-proposals to review and provide recommendations. The comments about each were noted and sent to the Fishery Research Program of the GLFC.

17. Agency updates (All)

Pennsylvania FBC: Furunculosis outbreaks occurred at several hatcheries last year. IPN hasn't been found at Benner Spring hatchery for the last four years, so that hatchery's classification changed. This month, there were 3 mortality events which were worse than normal. It's unclear what's going on. VHS has not been detected, but there was one detection of BKD and whirling disease. The two hatcheries which had CTv detections in brown trout last year have no new occurrences. Trout Lodge eggs will be coming in for a disease free egg source, so hopefully that will help with some problems.

Wisconsin DNR: Furunculosis vaccines have been continuing with good success. The coho program has improved since VHS was found in 2007. The coho were previously raised at the Lake Mills hatchery, which is outside of the Great Lakes basin. They were moved to the Wild Rose hatchery, which is similar to Oden in MI. Ever since they were transferred, there have been no renibacterium detections. The Lake Mills hatchery had crowded conditions, low tank space, and BKD in the summer. Now, at Wild Rose, the conditions are improved and the fish are constantly swimming due to fast water velocity and they are fed more. There continue to be Ich infections at Besadny in coho and brown trout--- maybe related to climate change? The fish likely got infected all at once and the pathogen is maturing. As the ichs fall off, holes are left in the skin and the electrolyte balance and kidney functions are impaired. There's likely Ich in Lake Michigan. They monitor fathead minnows that are fed to walleyes and muskies and found lots of viral isolates in 2012. 2013 had less sampling, but none was found.

Indiana DNR: Broodstock are being injected with thiamine. Since the last meeting, there was one mortality out of 688 fish. There's a new source of fish by the sea lamprey barrier at Trail Creek in Michigan City. The late summer had high temperatures, and steelhead migration went well at the end of November with approximately 7300 steelhead being counted. Late August had river mortalities, likely from heat stress and potential dissolved oxygen crashes at night. Webster Lake, a premier muskie lake, is struggling with maintaining trophy size fish--- clubs are blaming the DNR for not raising good enough fish. Trout Lodge eggs don't seem to survive the next year in inland lakes and are susceptible to gill bacteria and Aeromonas. The London strain from previous years seemed to do better.

FWS PA: Allegheny had IPN in 2005, and was subsequently depopulated and cleaned. Everything is now under roofs and back in production, and 2013 was the third inspection. The hatchery obtained Class A status. When Allegheny was down, the program was moved to 3 hatcheries in Vermont and Massachusetts. One is currently down and the others have Class A status. During a wild fish survey, the Service sampled 22 sites and 1250 fish of different species. A cell culture DNA assay was done for various pathogens and no VHS or nucleospora were detected. Currently, no service facility in Region 5 is rearing coldwater fish.

Wisconsin FWS: A UV system was added to the intakes at Pendells Creek in August. Bacterial coldwater disease was found at Jordan River hatchery in the fall, but the fish were EEDv negative. In the spring, *Y. rucckerii* was detected in smallmouth bass and walleye at Genoa, but no outbreaks occurred. It was not found again in the fall. Iron River has been free of *A.sal* since 2011 in brook trout, which has a

vaccination program for broodstock. More Superior-strain klondikes at Genoa should clear and moved to Iron River.

DFO: Non-NAAHP testing is going away slowly as CFIA is taking over the sampling and submitting to DFO labs. Most hatcheries are small mom-and-pop businesses, and it's hard for them to meet the bio-containment standards so it's unclear how many of them will be in the program in the future. The NAAHP is being enacted in phases. There's been limited surveillance for VHS in the Quebec area, and all results were negative. These were PCR-based assays, so they miss out on detecting anything other than what was asked for. IPNv validation is complete for OIE standards. The initial testing done last year indicated that qPCR assay is able to detect IPN in cases without clinical signs. Validation is complete for KHV (qPCR, serological assay, and ELISA).

Michigan DNR: Please refer to the BKD PowerPoint (Appendix 10) for Michigan's update.

Minnesota DNR: There was one hatchery mortality case: fish had lesions and eroded tails, and flavobacteria were isolated so it's likely coldwater disease. The fish were treated with medicated feed and went back to normal. They didn't have VHS, so tests continue. There was a fish kill case last November/early December which generated lots of media/public interest. It affected sunfish, walleye, northern pikes, muskies, and lots of other species in the metro area. Ice was on the lake, but no snow, so fish were seen under the ice. Two muskies were tested with no results, so it might have been gas bubble disease (high oxygen under ice). South Dakota officials have seen this in their area during this time of year.

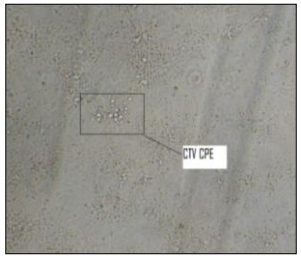
18. Future meetings (All)

The Summer 2014 will be in Winnipeg August 6-7th. The 2015 Winter meeting will be in West Lafayette (Purdue), IN, February 3-4, 2015. Looking ahead, the 2015 Summer meeting will be in New York and the 2016 Winter Meeting will be at Michigan State University, per Mohamed's request.

19. Adjourn

Cutthroat trout virus in Wisconsin

- CTV is a member of the Hepeviridae virus family. It is a small RNA virus that infects fish
- Diagnostic method: cell culture on CHSE-214 cell line
  - CPE is not dramatic and occurs late in the testing period- easily missed



CTV cytopathic effect

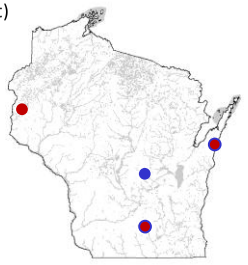
uninfected CHSE-214 cells

CTV in Wisconsin

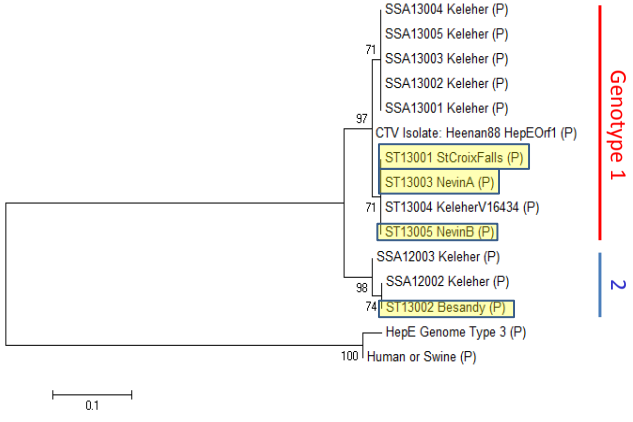
- CTV isolations in Fall 2012:
  - St Croix Falls Hatchery SCF strain BNT ov fl (October)
  - Nevin Hatchery TC strain BNT ov fl (November)
  - Besadny Spawning Facility BNT ov fl and k/s pools (November)
  - St Croix Falls Hatchery yearling TC strain BNT k/s pools (December)

CTV isolations in 2013

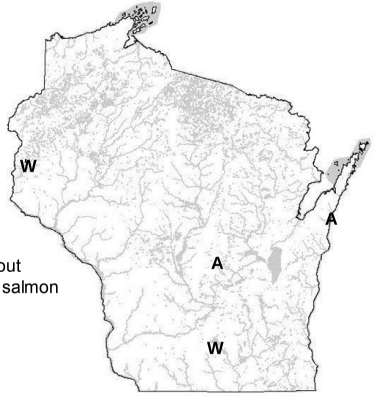
- Wild Rose Hatchery BNT ov fl (July/August)
- Besadny Facility BNT ov fl (December)
- Nevin Hatchery TC BNT ov fl (December)



Genetic relatedness of CTV isolates from WI



Comparison of CTV gene sequences



W = Western US trout
A = East Coast Atl salmon

Other CTV isolations of interest

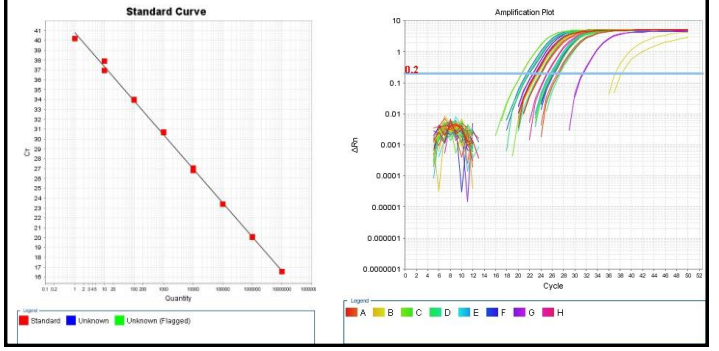
- CTV isolated from a PA hatchery in Fall 2012
  Brown trout fingerlings

  *Potential for additional new isolations in the GL basin and a need to know whether spawning BMPs can be used to manage the virus.

  WDNR contracted with Dr Tom Waltzek U Florida in 2013 to:
  1. Develop a qPCR method for CTV
  2. Collaborate with WDNR to evaluate egg handling BMPs by using the qPCR to test fry produced by the BMPs for CTV

TaqMan Real Time PCR Developed for CTV

- Primers/probes designed to detect CTV Genotype 1
- Consistently detects down to 10 viral copies
- Stnd curve slope = -3.47, Inter=37.098 ,R^2=.998, Eff=94.08%

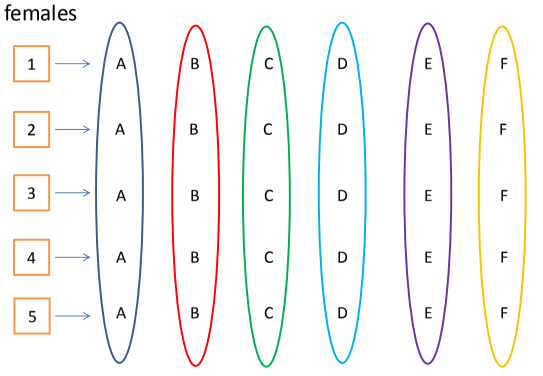


Best Management Practices to minimize CTV transmission during spawning

(with possible application to other egg associated viruses)

	A*	B*	C*	D*	E*	F*
drain ov fl	+	+	+	+	+	-
rinse eggs 0.9% saline	+	-	+	+	-	-
Water harden 100 ppm I_2	+	+	-	-	-	-
incubator type	jar	jar	jar	Heath tray	jar	jar

* In addition to these treatments, all eggs were surface disinfected with 100 ppm iodophor after water hardening, before placement in incubators.



Five brown trout females at each hatchery contributed eggs for the six egg treatment groups.

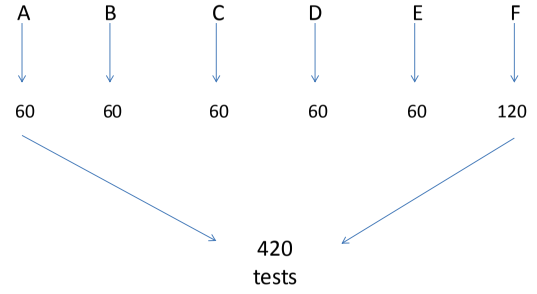
St Croix Falls= B,D,E,F Nevin = A,C,F

Appendix 1

Selection of females for the experiments

Females anesthetized and assessed for ovulation

- Ripe females were floy tagged with unique numbers
- Small amount of eggs expressed into a sterile cup and ovarian fluids drawn off
- Ovarian fluids transferred to labelled cryovials and snap frozen on dry ice
- Samples shipped next day delivery to U Florida for qPCR assay
- UFL provided the qPCR results ASAP
- Females with the highest copy number were spawned one week after samples were collected.
- Milt from three males was pooled and used to fertilize the eggs for all treatment groups for a single female.
- After the eggs were water hardened, eggs of the same treatment were pooled, surface disinfected and placed in the appropriate incubators.



Within a few days of 100% hatch, individual fry from egg treatment groups A, B, C, D, E, F will be tested using the new CTV qPCR method to evaluate treatment efficacy (reduction of CTV RNA).

Selection of females based on ovarian fluid qPCR values

St Croix Falls range of qPCR values

Range = 3700 to 78,500 X=23,214

Five highest females

78,500

40,400

40,000

35,000

26,000

Nevin range of qPCR values

Range = 5 to 22,500 X=2827

Five highest females

22,500*

7500

2900

2800

2675

2100

*egg bound at spawning

Comparison of CTV qPCR values at the Nevin Hatchery 2013 and 2014

2013 Nevin range of qPCR values

Range = 8 to 850,000 X=123,100

Five highest females

850,000

660,000

300,000

295,000

178,000

~ two orders of magnitude reduction in CTV RNA from BNT ovarian fluid from 2012 to 2013 at the Nevin hatchery.

2014 Nevin range of qPCR values

Range = 5 to 22,500 X=2827

Five highest females

22,500*

7500

2900

2800

2675

2100

*egg bound at spawning

Comparison of CTV isolation on CHSE-214 cells and CTV qPCR from the same individual ovarian fluids

Hatchery	2012/2013		2013/2014	
	Cell Culture	qPCR copy number range	Cell Culture	qPCR copy number range
St Croix Falls	+	ND	-	3700-78,500
Nevin	-*	8-850,000	+?	5-22,500

- No CPE observed for the paired samples, however CPE was observed in samples collected 2 months earlier from the same cohort of fish (the first CTV detection at Nevin).
- How does qPCR copy number relate to the number of viable virus particles?

CTV research ideas

- Susceptibility of native cool and warm water fish species and amphibians to CTV
- Long term population effects for stocked trout and cohabiting non-salmonids
- Surveillance to track the spread in WI
- Possible link between CTV infection and enlarged hearts in brown trout
- Others?

Development and Implementation of a real-time PCR assay for Epizootic Epitheliotropic Disease Virus (EEDV/SalHV3)

Gavin Glenney[1], Patricia Barbash[1], Rick Cordes[1], Christina Cappelli[1], Tom Loch[2], Mohamed Faisal[2], and John Coll[1].

[1]USFWS, Lamar Fish Health Center, Lamar, PA 16848
[2]Michigan State University, E. Lansing, Michigan 48824

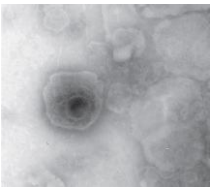
Epizootic epitheliotropic disease virus (EEDV/ SalHV3).

- A serious disease of yearling lake trout, *Salvelinus namaycush*, in the Great Lakes Region of USA

Sue Marcquenski/Wisconsin DNR

Epizootic epitheliotropic disease virus (SalHV 3/EEDV).

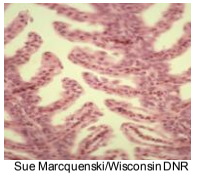
- *Alloherpesviridae*- fish and amphibians
- Enveloped icosahedral capsid
- Double stranded DNA genome



Ron Hedrick/University of California--Davis

Epizootic epitheliotropic disease virus (SalHV 3/EEDV).

- Clinical signs
  - -proliferative epithelial lesions, hyperplasia, hypertrophy
  - -rapid increase in mortalities, spiral swimming, ataxia
  - -hemorrhaging of the eyes
  - -lethargy with periods of hyperexcitability.(Bradely et al. 1988, Bradely et al. 1989, McAllister and Herman 1989)



Sue Marcquenski/Wisconsin DNR

Appendix 2

Problems with EEDV diagnosis.

- 1. Can not culture EEDV on current cell lines.
- 2. Diagnosis by PCR. How do you confirm positives?
  - Terminase gene, polymerase, and glycoprotein genes (Waltzek et al. 2009)
  - sequence?
- 3. Histology- Screening wild pops.- costs $
- 4. At least in our hands, the current published PCR appears inconsistent with carrier or latent infections. (Kurobe et al. 2009)

What we decided to do:

- To increase sensitivity, we decided to develop real-time PCR assay.
- Selected terminase gene
- EPA Grant, Great Lakes Restoration Initiative- Project- Screen fish for emerging pathogens in Great Lakes.
- To get a better understanding of EEDV prevalence in wild fish we initially screen with real-time assay, confirm by (nested PCR) sequencing or now with developing multi-probe assay.

Computer programs used:

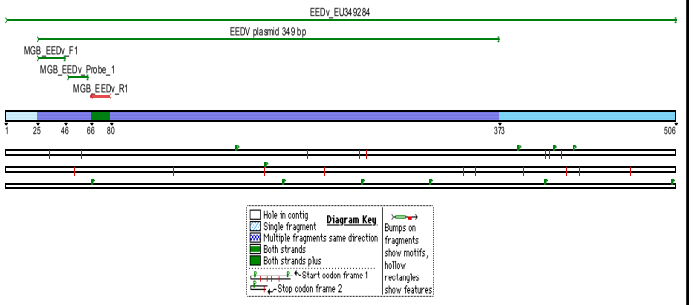
- Real-time assay-Primer Express 3.0
- EEDV_EU349284

ATTTCATCCTCGTCGACGAGGCCGCCTTTGTGAACCTCACCTCCATCACTAGTCTGATCC
CCCTCATGCTGGTCGCCGGGCGAAAGCAGATCCACATTTCTTCCCACGTGGCCAAATC
TTGGATTAACAACGTGGGCGACATTATCGACGAAACAACGGGGGAGCCGGCGTTTCA
TGTTATCTCTCAGAAGTTTAAATGCGGTGCGCACATGCACCTACCAGGTCTGACGTGTC
CCTGTGAAGCAGTCTACTGCCCCAGTCACATAGATATGAACCCCGCTACGCAGGCCCTG
CTCAGCTGTGTGGCCCCCGGGGGAGAAATGGAGATCACAGGTGGCACCGGTGACTT
GGGTAATCTGGTGTCGGACTCGACCTTCCCCTTCCCAGATGAGACGGTGCACAAGATA
ATGAACGATGTGATTGATATCAATGACCCGGGCGCCGAAGTTTCGGCTTTCTACATTGC
CATTGACCCCACCTATTCTTCCGGCAGCCAATCGTCAATG

Forward primer	5'-CCTTTGTGAACCTCACCTCCAT-3'	56 bp
Reverse primer	5'-CCCGGCGACCAGCAT-3'	
Hydrolysis probe	6FAMACTAGTCTGATCCCCCMGBNFQ	

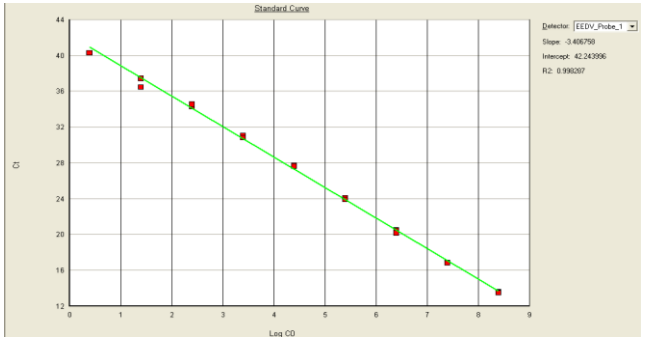
Positive Control

- Scott Weber's Lab, UC Davis, CA. –Kirsten Malm sent us positive control EEDV exposed fish skin.
- A plasmid was made containing 349bp EEDV insert.



Appendix 2

Analytical sensitivity- limit of detection (LOD)



-Plasmid standard curve is linear over 8 logs of plasmid dilution.

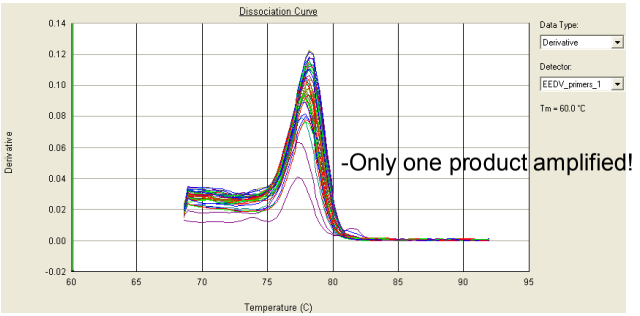
-Consistent detection at the estimated 24.3 copy number dilution.

-Sporadic detection at the estimated 2.43 copy number dilution.

Specificity?

Syber Green assay- primers only

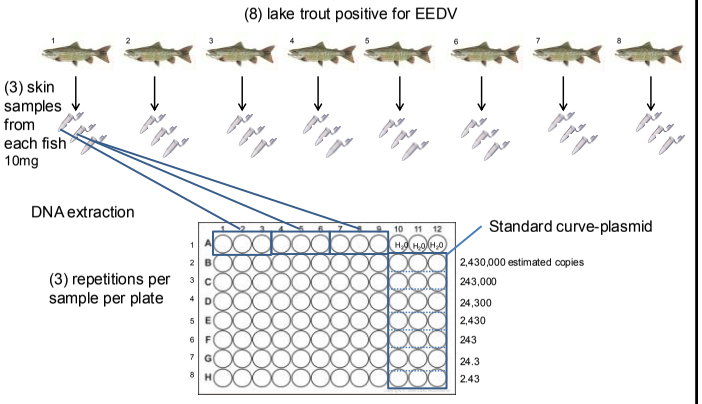
Eight positive lake trout, (5) ten fold dilutions for a total of 40 samples (natural hatchery infection-samples from MSU)



Specificity?

Pathogen	Source	SalHV3 Real-time PCR
SalHV1 (plasmid)	**S. Weber, UCDavis**	-
SalHV1	**ATCC, cell culture**	-
SalHV2 (plasmid)	**S. Weber, UCDavis**	-
SalHV4 (DNA, plasmid)	**A. Doszpoly, Hungarian Acad. of Sciences**	+
SalHV5 (DNA, plasmid)	**Lamar FHC, USFWS**	+
CCV isolate 1	**Lacrosse FHC, USFWS**	-
CCV isolate 2	**Lacrosse FHC, USFWS**	-
R. salmoninarum	Lamar FHC, USFWS	-
F. psychrophilum	Lamar FHC, USFWS	-
LMBV	Lamar FHC, USFWS	-
M. cerebralis	Lamar FHC, USFWS	-
N. salmonis	Lamar FHC, USFWS	-
ISAVcDNA	Lamar FHC, USFWS	-
IPNV (seg.A plasmid)	Lamar FHC, USFWS	-

Test for variance when using EEDV real-time assay-



Total experiment was (5) assays on (5) separate days, multiple operators

Repeatability (intraassay variance)

Fish	Sample	Estimated mean copies/rxn	SD	CV (%)
1	1	32660.5	2535.8	7.7
1	2	17189.7	2247.9	13.2
1	3	20299.4	2053.2	10.2
2	1	5947.2	452.0	7.6
2	2	4342.2	249.7	5.4
2	3	22315.6	1312.3	6.1
3	1	110879.3	5342.1	4.8
3	2	11726.2	955.5	8.3
3	3	10309.9	909.1	8.8
4	1	10670.8	1005.6	9.4
4	2	9840.5	750.7	8.0
4	3	13090.4	467.2	3.7
5	1	28042.3	2064.1	7.7
5	2	37087.0	3446.3	9.3
5	3	57439.5	3707.1	7.0
6	1	55826.1	5181.1	9.2
6	2	24919.5	2269.1	9.2
6	3	46211.3	3453.7	7.8
7	1	77252.2	8761.5	11.3
7	2	18761.3	1642.9	9.0
7	3	30675.1	3930.4	9.6
8	1	21588.3	1894.4	8.7
8	2	29005.3	2051.9	7.0
8	3	26893.3	4546.3	16.9

Reproducibility (interassay variance)

Fish	Estimated mean copies/rxn	SD	CV (%)
1	23383.2	1804.6	7.7
2	10868.4	812.4	7.5
3	44305.1	2172.4	4.9
4	11000.6	725.7	6.6
5	40856.3	4545.2	11.1
6	42318.9	3122.7	7.4
7	42229.5	3954.4	9.4
8	25829.0	3552.5	13.8

CV- Shows the extent of variability in relation to mean of the population.

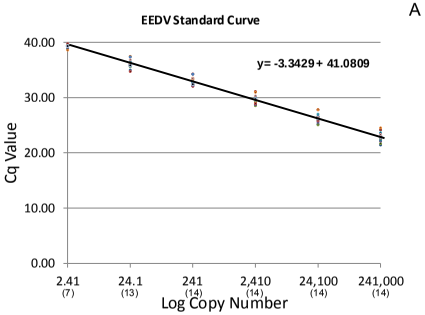
Samples (inter-tissue variance)

	Tissues within Fish		
Fish	Estimated mean copies/rxn	SD	CV (%)
1	23383.2	8198.2	35.1
2	10868.4	9949.9	91.5
3	44305.1	57659.8	130.1
4	11000.6	1407.3	12.8
5	40856.3	15389.0	37.7
6	42318.9	15836.9	37.4
7	42229.5	30923.1	73.2
8	25829.0	3901.2	15.1

Highest SD and CV % observed, could be due to error in tissue collection, and/or DNA extraction between tissues, or due to localization of virus in skin samples.

Plasmid dilutions and PCR efficiencies.

A total of fourteen experiments.

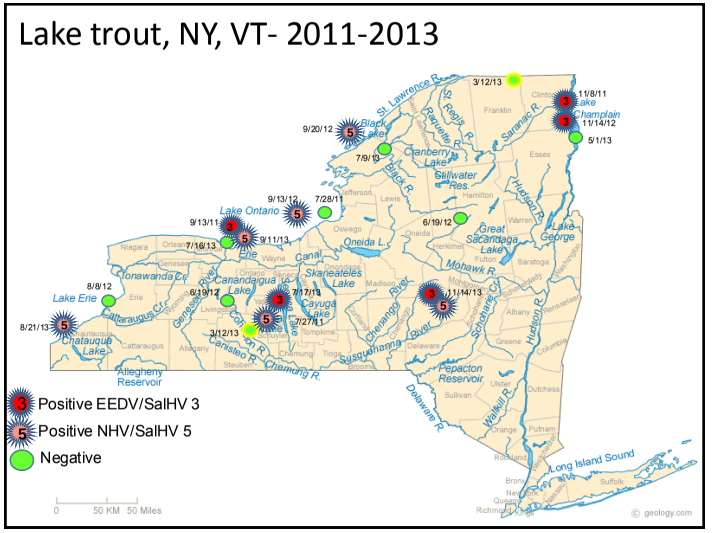


For a total of 14 assays-

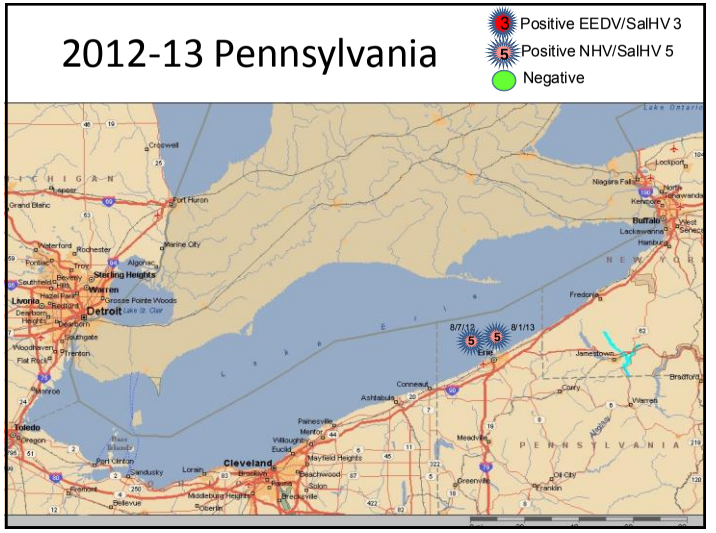
Mean slope-	-3.343
Mean efficiency	0.99
Efficiency SD	0.06
Efficiency 95% CI	0.0296

Efficiency = $-1 + 10^{(-1/\text{slope})}$

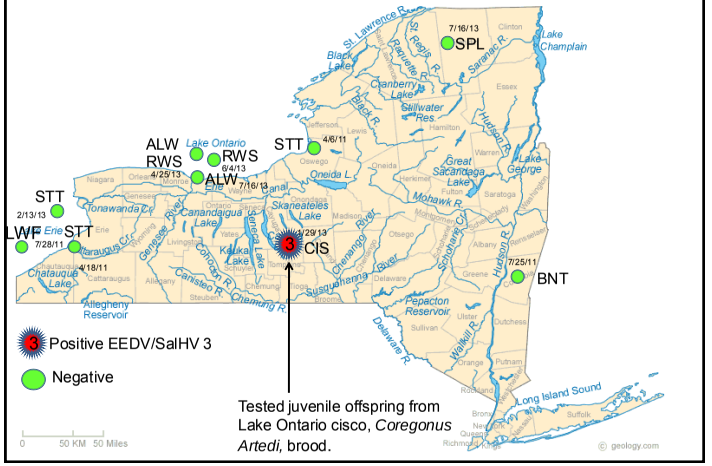
Lake trout, NY, VT- 2011-2013



2012-13 Pennsylvania

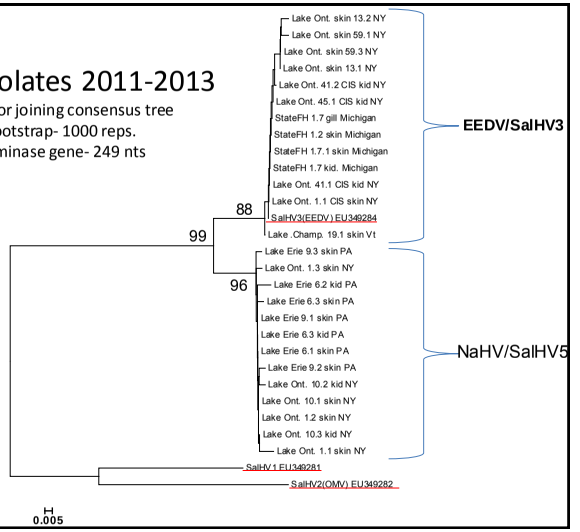


Other species- 2011-2013



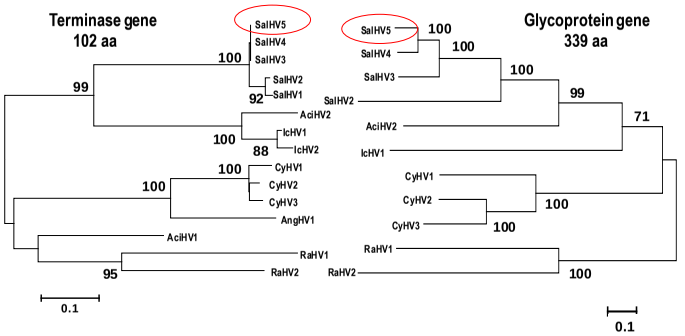
EEDV Isolates 2011-2013

Neighbor joining consensus tree
Bootstrap- 1000 reps.
terminase gene- 249 nts



Appendix 2

Namaycush Herpesvirus/SalHV5



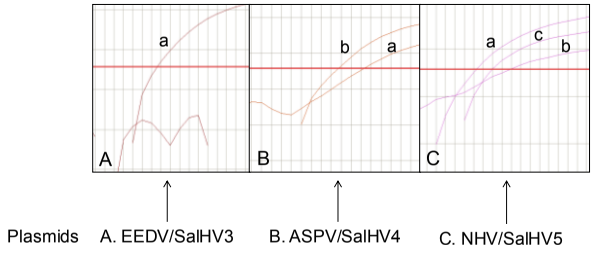
SalHV Multi-probe Assay Design

EEDV	SalHV3_EU349284	CCTTTGTGAACCTCACCTCCATCACTAGTCTGATCCCCCTCATGCTGGTCGCCGGG
ASPV	SalHV4_JX886026	CCTTTGTCAACCTCACCTCCATCACCAGTCTGATTCCCCTCATGCTGGTCGCCGGG
NHV	SalHV5	CACAAGTCTGATCCCCCTCATGCTGGTCGCCGGG
	SalHV3_F1	CCTTTGTGAACCTCACCTCCAT
	SalHV3_Probe	ACTAGTCTGATCCCCC
	SalHV4_Probe	CAGTCTGATTCCCC
	SalHV5_Probe	CACAAGTCTGATCCC
	SalHV3_R1	ATGCTGGTCGCCGGG

a- SalHV3_Probe-FAM
b- SalHV4_Probe-VIC
c- SalHV5_Probe-NED

SalHV Multi-probe Results

a- SalHV3_Probe
b- SalHV4_Probe
c- SalHV5_Probe



Conclusions/Questions:

- EEDV seems more prevalent in wild fish than we first thought.
- Skin samples appear to me more sensitive for SalHV3 and 5.
- Is the EEDV virus (low copy numbers) we are detecting latent? What is required for recrudescence?
- Discovered new *Alloherpesviridae* member- Namaycush Herpesvirus/SalHV5. More prevalent than EEDV. Is this virus benign or pathogenic? Does it afford protection against EEDV?
- Found EEDV in domesticated *Coregonus artedi* (a.k.a. ciscos, or lake herring). Vertical transmission?
- EEDV real-time assay is a sensitive and precise assay. It does cross-react with SalHV4 and SalHV5. When used with the SalHV multi-probe assay, it detects and differentiates between SalHV3, SalHV4, and SalHV5.

Appendix 2

Questions?

Many thanks,

Scott Weber's Lab, UC Davis- SalHV1 and SalHV2 plasmid
Tom Waltzek's Lab, Univ. of Florida- SalHV4 sample
Mohamed Faisel's lab, MSU- SalHV3 infected tissues
USFWS- Lacrosse Fish Health Center- CCV DNA

Photos from: Fish Get Herpes, Too
Battling EED virus in lake trout.
Ken Phillips, USFWS
Eddies, Winter 2010/2011.

Namayacush herpesvirus percent identity with known members of *Alloherpeviridae*

A

	SalHV2	SalHV3	SalHV4	SalHV5
SalHV1	98.0(85.3)	96.1(83.4)	96.1(83.4)	96.1(82.4)
SalHV2		96.1(80.8)	96.1(82.1)	96.1(81.8)
SalHV3			100(93.8)	**100(94.5)**
SalHV4				**100(97.1)**
AciHV1				40.8(NA)
AciHV2				53.8(NA)
IcHV1				55.8(NA)
IcHV2				48.0(NA)
AngHV1				38.9(46.4)
CyHV1				42.9(54.1)
CyHV2				43.9(52.8)
CyHV3				43.8(55.3)
RaHV1				39.8(NA)
RaHV2				41.9(NA)

B

	SalHV3	SalHV4	SalHV5
SalHV2	44.3(55.8)	43.3(51.3)	40.3(52.1)
SalHV3		75.4(75.0)	74.3(78.5)
SalHV4			**86.1(88.5)**
AciHV2			32.2(NA)
CyHV1			19.0(NA)
CyHV2			20.0(NA)
CyHV3			19.1(NA)
IchHV1			18.4(NA)

Table 2. Identified *Alloherpesviridae* members listed by percent identity with *Namaycush herpesvirus*/SalHV5 detected in lake trout from Lake Ontario, N.Y., U.S.A. Percent identity is the percentage of identical matches between the two sequences over the reported aligned region, and is listed by amino acid and (nucleotide). Two genes were analyzed: (A.) terminase gene- 102 amino acids and 306 nucleotides (B.) glycoprotein gene- 339 amino acids and 1,011 nucleotides. Abbreviations: AciHV= acipenserid herpesvirus, AngHV= anguillid herpesvirus, CyHV= cyprinid herpesvirus, IcHV= ictalurid herpesvirus, SalHV= salmonid herpesvirus, RaHV= ranid herpesvirus, and NA= not attempted. Genbank accession numbers: A. terminase- SalHV1, EU349281; SalHV2, EU349282; SalHV3, EU349284; SalHV4, JX886026; AciHV1, ABQ10595; AciHV2, ABQ10594; IcHV1, NP041153; IcHV2, ACD84542; AngHV1, YP003058149(EU349279); CyHV1, ACD86452(EU349288); CyHV2, ACD84549(EU349285); CyHV3, YP001096069(DQ657948); RaHV1, YP656697; RaHV2, YP656576. B. Glycoprotein gene- SalHV2, CAA49846(X70396); SalHV3, AGB07607(JX886027); SalHV4, AGB07608(JX886028); AciHV2, AEF97704; IcHV1, AAA88149; CyHV1, YP007003762; CyHV2, YP007003918; CyHV3, ABC55145.

2011 EEDV Testing-Lake Trout

Sample Date	Water Body	State	Tissue sampled	Fish sampled	Positive via real-time PCR assay	Consistently positive via real-time PCR assay[a]	Estimated range of virus copies detected per reaction <40 Ct	Ct range of real-time positives	Positive via semi-nested PCR assay	PCR amplicons cloned and confirmed by sequencing	Multi-probe PCR confirmed	Case #
7/27/2011	Keuka Lake	NY	kidney	28	8	3	N/A	36-40	2[b]	N/A	3-SalHV5	11-327
7/28/2011	Lake Ontario	NY	kidney	15	0	N/A	N/A	N/A	N/A	N/A		11-328
			skin	15	0	N/A	N/A	N/A	N/A	N/A		
9/13/2011	Lake Ontario	NY	kidney	20	8	0	1.0-14.0	34-40	0	N/A		11-394
			skin	60	19	9	0.7-10.5	35-40	2[c]	2-SalHV3		
11/8/2011	Lake Champlain	VT	ovarian fluid	4(5fp)[e]	2(5fp)	2(5fp)	43.0 - 6,791.0	30-37	2(5fp)[b]	2(5fp)-SalHV3		12-049

Notes:
a. Samples positive by two repetitions in a single assay, and positive in two or more separate real-time assays upon re-extraction.
b. Semi-nested PCR primers for first round (40_F and 249_R), and second (150_F and 249_R).
c. Semi-nested PCR primers for first round (223_F and 224_R), and second (40_F and 224_R).
d. Semi-nested PCR primers for first round (194_F and 249_R), and second (214_F and 249_R).
e. 4(5fp) = A total of twenty fish pooled into four pools of five fish each.

N/A= not attempted

2012 EEDV Testing- Lake Trout

Sample Date	Water Body	State	Tissue sampled	Fish sampled	Positive via real-time PCR assay	Consistently positive via real-time PCR assay[a]	Estimated range of virus copies detected per reaction <40 Ct	Ct range of real-time positives	Positive via semi-nested PCR assay	PCR amplicons cloned and confirmed by sequencing	Multi-probe PCR confirmed	Case #
6/19/2012	Fourth Lake, Fulton Chain	NY	kidney	5	0	N/A	N/A	N/A	N/A	N/A		12-286
			skin	5	0	N/A	N/A	N/A	N/A	N/A		
			gill	5	0	N/A	N/A	N/A	N/A	N/A		
6/19/2012	Hemlock Lake	NY	kidney	20	0	N/A	N/A	N/A	N/A	N/A		12-287
			skin	20	0	N/A	N/A	N/A	N/A	N/A		
8/7/2012	Lake Erie	PA	kidney	14	1	1	3.4	37-38	1[d]	1-SalHV5		12-364
			skin	14	2	2	.38 - 3.9	36-41	2[d]	2-SalHV5		
8/8/2012	Lake Erie	NY	kidney	30	0	N/A	N/A	N/A	N/A	N/A		12-365
			skin	30	0	N/A	N/A	N/A	N/A	N/A		
9/13/2012	Lake Ontario	NY	kidney	12	1	0	-	43	N/A	N/A		12-398
			skin	12	4	2	-	41-45	0	N/A	2-SalHV5	
9/20/2012	Lake Ontario	NY	kidney	20	3	2	.38 -3.1	35-42	1[d]	1-SalHV5		12-406
			skin	20	15	7	.21 - 4.6	35-44	2[d]	2-SalHV5	4-SalHV5	
11/8/2012	Marquette State Fish Hatchery	MI	kidney	16	16	16	10.4 - 53,764.8	22-41	16[d]	1-SalHV3		13-44
			skin	16	16	16	272.0 - 952,923.0	18-29	16[d]	2-SalHV3	6-SalHV3	
			gill	16	16	16	22.6 - 92,899.2	21-39	14[d]	1-SalHV3		
11/14/2012	Lake Champlain	VT	kidney	18	6	4	.9 - 7.2	35-40	2[d]	-		13-52
			skin	18	15	11	.96 - 413.8	31-38	8[d]	1-SalHV3	6-SalHV3	
			ovarian	4	1	0	0.5	39	0	N/A		

Notes:
a. Samples positive by two repetitions in a single assay, and positive in two or more separate real-time assays upon re-extraction.
b. Semi-nested PCR primers for first round (40_F and 249_R), and second (150_F and 249_R).
c. Semi-nested PCR primers for first round (223_F and 224_R), and second (40_F and 224_R).
d. Semi-nested PCR primers for first round (194_F and 249_R), and second (214_F and 249_R).

2013 EEDV Testing- Lake Trout

Sample Date	Water Body	State	Tissue sampled	Fish sampled	Positive via real-time PCR assay	Consistently positive via real-time PCR assay[a]	Estimated range of virus copies detected per reaction <40 Ct	Ct range of real-time positives	Positive via semi-nested PCR assay	PCR amplicons cloned and confirmed by sequencing	Multi-probe PCR confirmed	Case #
3/12/2013	Bath State Fish Hatchery	NY	kidney	64	0	N/A	N/A	N/A	N/A	N/A		13-122
3/12/2013	Chateaugay State Fish Hatchery	NY	kidney	60	0	N/A	N/A	N/A	N/A	N/A		13-123
7/9/2013	Sixberry Lake	NY	skin	10	0	N/A	N/A	N/A	N/A	N/A		13-279
7/16/2013	Lake Ontario	NY	skin	2	0	N/A	N/A	N/A	N/A	N/A		13-285
7/17/2013	Seneca Lake	NY	skin	42	4	3	N/A	N/A	N/A	N/A	3-SalHV3	13-294
8/1/2013	Lake Erie	PA	skin	41	3	3	N/A	N/A	N/A	N/A	3-SalHV5	13-316
8/21/2013	Lake Erie	NY	skin	30	18	9	N/A	N/A	N/A	N/A	9-SalHV5	13-328
9/11/2013	Lake Ontario	NY	skin	20	5	3	N/A	N/A	N/A	N/A	3-SalHV5	13-350
11/14/2013	Otsego Lake	NY	skin	10	3	3	N/A	N/A	N/A	N/A	2-SalHV3 1-SalHV5	14-28
5/1/2013	Lake Champlain	VT	skin	15-fry	0	N/A	N/A	N/A	N/A	N/A		14-33

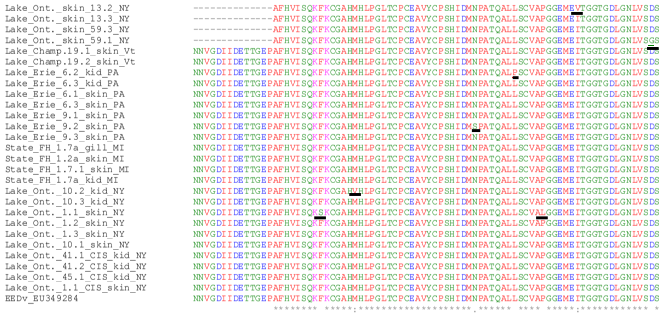
Notes:
a. Samples positive by two repetitions in a single assay, and positive in two or more separate real-time assays upon re-extraction.
b. Semi-nested PCR primers for first round (40_F and 249_R), and second (150_F and 249_R).
c. Semi-nested PCR primers for first round (223_F and 224_R), and second (40_F and 224_R).
d. Semi-nested PCR primers for first round (194_F and 249_R), and second (214_F and 249_R).
e. 4(5fp) = A total of twenty fish pooled into four pools of five fish each.

N/A= not attempted

2011-13 EEDV Testing- other species

Sample Date	Water Body	State	Species	Tissue sampled	Fish sampled	Positive via real-time PCR assay	Consistently positive via real-time PCR assay[a]	Estimated range of virus copies detected per reaction <40 Ct	Ct range of real-time positives	Positive via semi-nested PCR assay	PCR amplicons cloned and confirmed by sequencing
4/6/2011	Salmon River	New York	steelhead trout	kidney	60	0	N/A	N/A	N/A	N/A	N/A
				gill	60	0	N/A	N/A	N/A	N/A	N/A
				ovarian fluid	30	0	N/A	N/A	N/A	N/A	N/A
4/18/2011	Cattaraugus Creek	New York	steelhead trout	kidney	30	0	N/A	N/A	N/A	N/A	N/A
7/25/2011	Roeliff Jansen Kill	New York	brown trout	kidney	3(5fp)[e]	0	N/A	N/A	N/A	N/A	N/A
7/28/2011	Lake Ontario	New York	lake white fish	kidney	25	0	N/A	N/A	N/A	N/A	N/A
1/29/2013	Tunison Laboratory of Aquatic Science, USGS	New York	cisco	skin	60	3	2	4.6 - 125.4	38-41	1	1
				kidney	60	5	3	5.5 - 37.2	37-42	2	2
2/13/2013	Lake Erie	Penn	steelhead trout	kidney	60	0	N/A	N/A	N/A	N/A	N/A
4/25/2013	Lake Ontario	New York	rainbow smelt	kidney/ spleen	4(5fp)	0	N/A	N/A	N/A	N/A	N/A
			alwife	kidney/ spleen	6(5fp)	0	N/A	N/A	N/A	N/A	N/A
6/4/2013	Lake Ontario	New York	rainbow smelt	skin	20	0	N/A	N/A	N/A	N/A	N/A
7/16/2013	Lake Ontario	New York	alwife	skin	15	0	N/A	N/A	N/A	N/A	N/A
7/16/2013	Lake Ozonia	New York	splake	skin	4	0	N/A	N/A	N/A	N/A	N/A

Deduced amino acid sequences from 249 bp terminase PCR products



All but two of the AA changes appear to be conserved

Plasmid dilutions and PCR efficiencies.

A total of fourteen experiments over 1 year period.

No experiments	Estimated template copies	Mean Cq	SD	Interrun CV (%)
14	241000	22.92	0.881	3.845
14	24100	26.36	0.817	3.098
14	2410	29.63	0.812	2.74
14	241	32.92	0.379	2.245
13	24.1	36.09	0.803	2.223
7	2.41	39.34	0.48	1.219
			Mean	2.561667

The slope of the PCR should be (-3.1 to -3.6) yielding an (Eff. from 110% to 90%)

For a total of 14 assays-		
	Mean slope-	-3.343
	Mean efficiency	0.99
	Efficiency SD	0.06
	Efficiency 95%CI	0.0296

Efficiency = $-1 + 10^{(-1/\text{slope})}$

Appendix 2

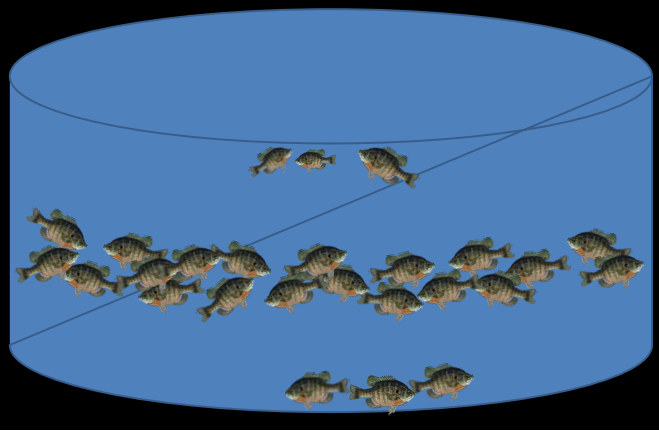
Real-Time Quantitative PCR
Assay Data Analysis,
Evaluation
and
Optimization
A Tutorial on
Data Analysis and Evaluation and
Trouble-shooting for Optimal Real-Time
QPCR Experimentsby
Rainer B. Lanz,
M.S., PH.D.
February 14, 2008

- Implications
- – The calculations of precision given above have been questioned in some peer-reviewed publications.
- – Replicate standard curves may produce potentially large inter-curve variations.
- – In general, the intra-assay variation of 10-20% and a mean inter-assay variation of 15-30% on molecule basis is realistic over the wide dynamic range (of over a billion fold range).
- – Variability is highest at >10 7 and <10 2 template copy ranges
- • Cut-off value: cycle 35, i.e. disregard CT values for cycle numbers 36 and higher.
- – For the threshold methods, the precision is dependent on the proper setting of the threshold, which itself is dependent on proper base line settings.

Clinical Diagnosis

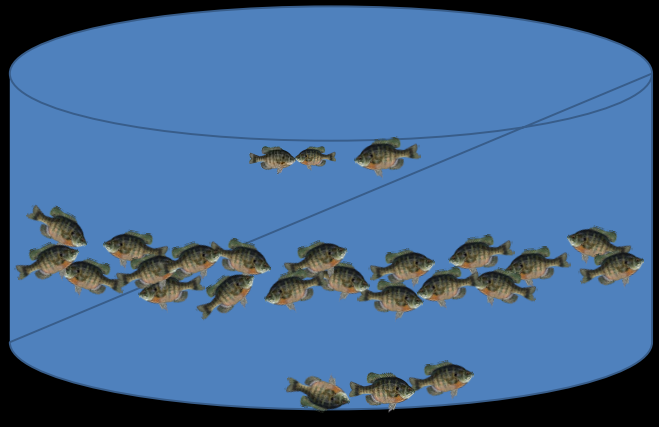
1. Case History
2. Watching Behavior

Position in the water column: High



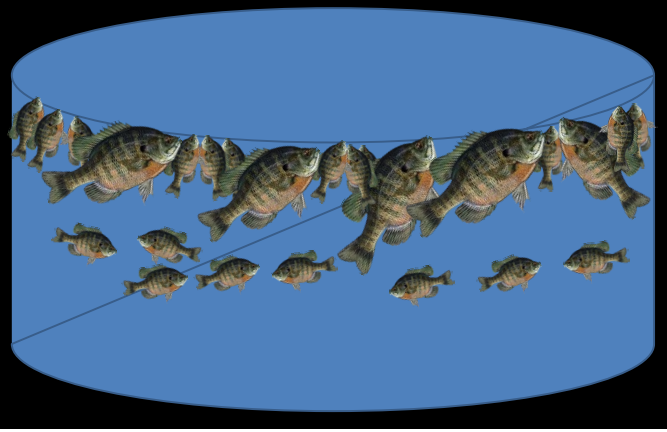
- **High in water column (unpropelled motion):**
  - systemic disease (lethargic)
  - Blindness or cataract
  - Poor intestinal motility
  - General weakness
  - Hypoxia
  - Neurologic disorder

Position in the water column: Low



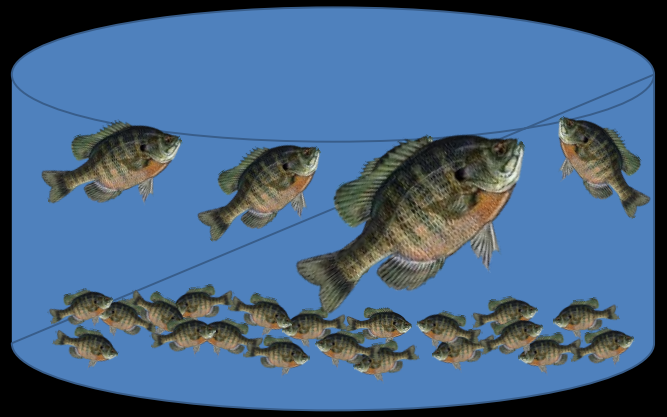
- **Sluggish at the bottom**
  - Problem in buoyancy
  - Severe digestive problems
  - Swimbladder diseases

Piping: gulping air at the surface: Large and small



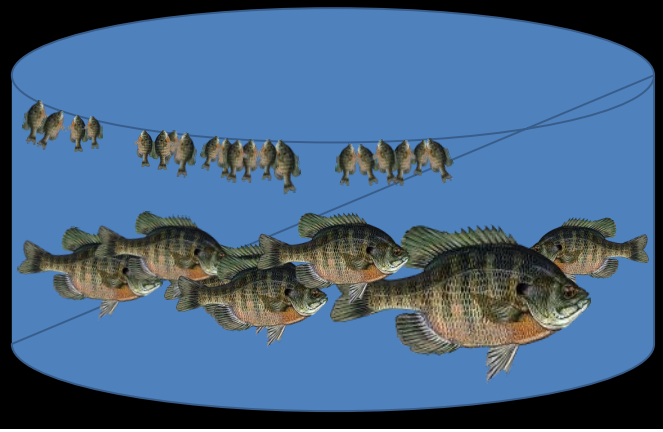
- Temperature is too high
- Ammonia poisoning
- Nitrite poisoning
- Too much organic material in the water
- Severe gill problems

Larger fish only



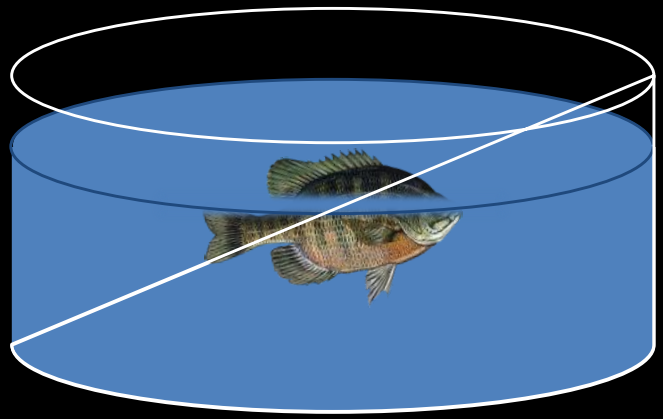
- Hypoxia

Smaller but not larger fish



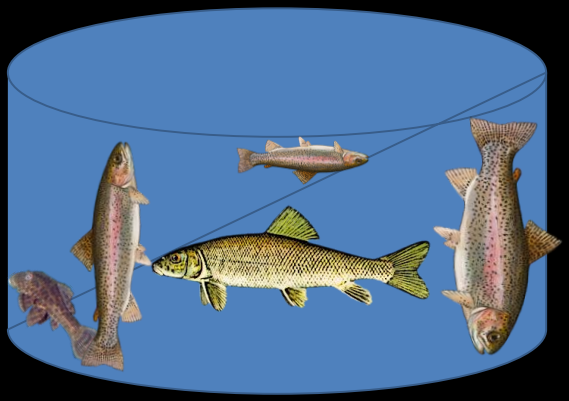
- Microbial infection (bacteria or virus)
- Gill parasites

Portion of the body is outside the water



- High density
- Not optimal environmental conditions

Abnormal posture



Possible causes

- Subordination
- Neurologic disorder
- Swim bladder disorder
- Following long transportation
- Lethargy due infectious diseases

Swimming pattern

No swimming

Rubbing against objects:

- Ectoparasites
- Presence of toxic chemicals
- Skin irritation in general

Erratic swimming

Erratic swimming:

- Ectoparasites
- Neurologic disorder

Side swimming

- Lethargy
- Sign of toxicity
- Damaged fins
- Late stages of microbial infections

Whirling/Circling

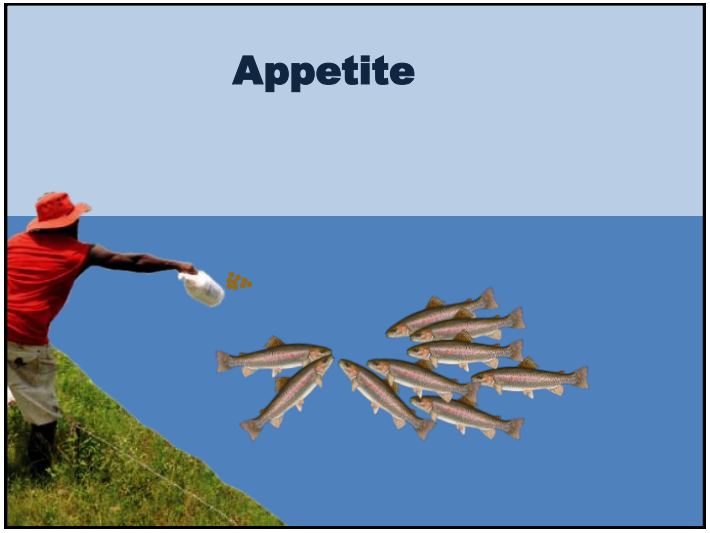
Circling (control):

- Blindness
- Neurologic disease
- Restlessness in public aquaria

Whirling/Circling

Whirling (uncontrolled):
- ***Myxobolus cerebralis***
- ***Flavobacterial infections***

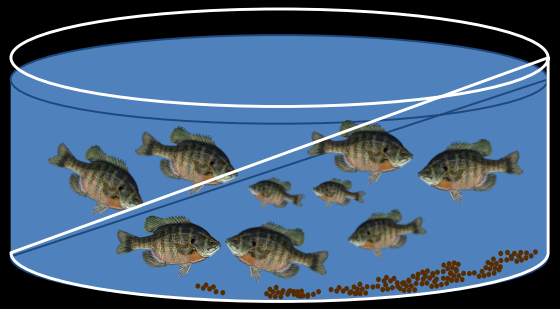
Appetite



Decreased appetite

- **Disease**
- **Anxiety**
- **High temperature**
- **Too much food**

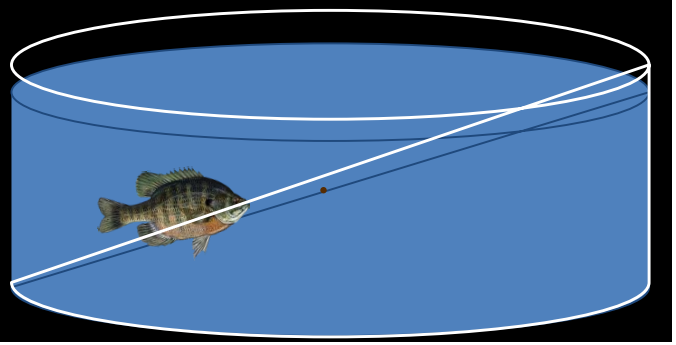
Food leftover



Decreased appetite

- Sign of an ongoing disease
- Pellets are of improper size
- Unpalatable
- You are adding too much food

Food spitting



Food spitting

- Stress
- Infection
- Incorrect pellet size

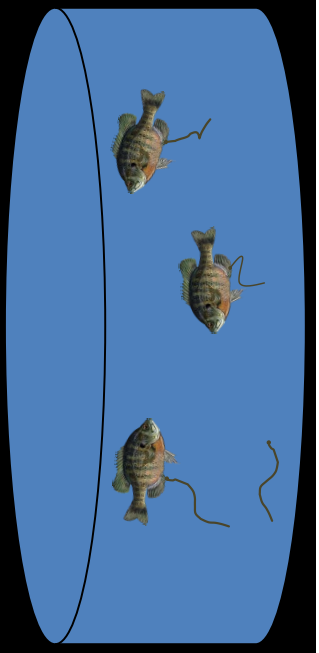
Feces Disorders

Feces is mixed with blood

- Severe intestinal inflammation
- Exposure to toxic chemicals
- Food has sharp objects
- Intestinal perforation by larval worms

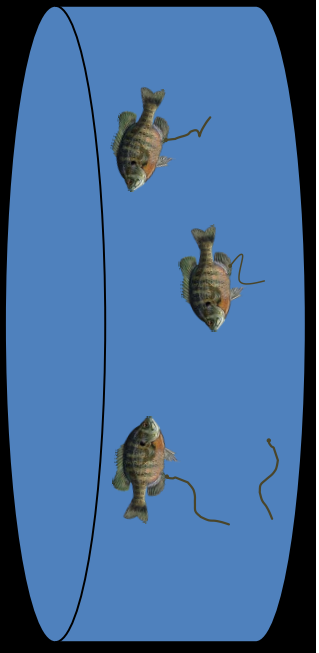
Pseudofeces

- Intestinal parasitism
- Improper nutrition



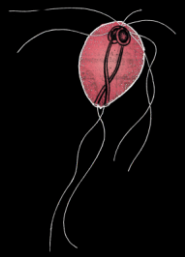
White transparent feces

Pseudofeces



- **Slimy feces with strings:**
  - Intestinal helminth
  - Flagellates
  - Bacterial inflammation

Hexamita

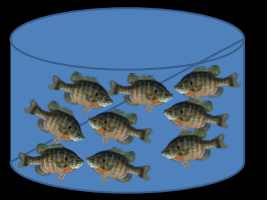


- **Worms coming out:**

- **Worms coming out:**
  - **Red: *Camallanus* spp**
  - **Whiteish: Tape Worm & *Capillaria* sp.**

Opercular movements: #/min

High:



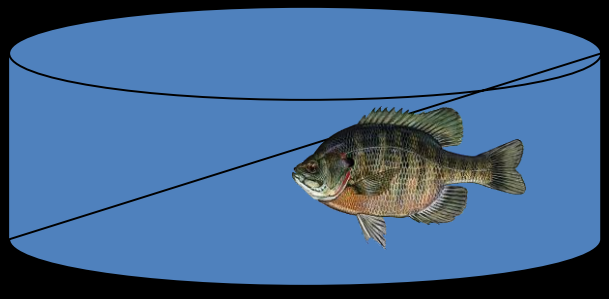
Opercular movements: #/min

High:
- Stress
- Water temperature too high
- High density
- Infection
- Gill disease/parasite
- Hypoxia
- Anemia
- Poor water quality
- Incorrect light cycle

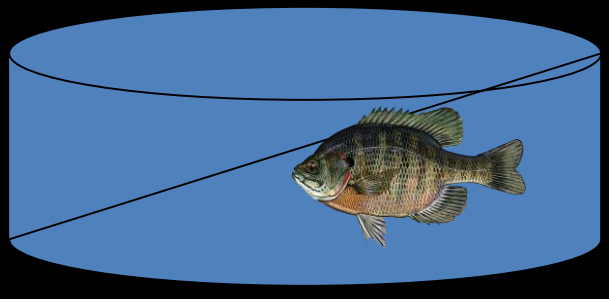
Low:
- Starvation
- Infection
- Lethargy

Coughing

Flaring of the operculum
followed by rapid closure



- Gill disease
- Heavy parasitism



- Starvation
- Stress
- Social conflicts

Cannibalism

Inter-GLB cases

Ken Phillips & Ryan Katona

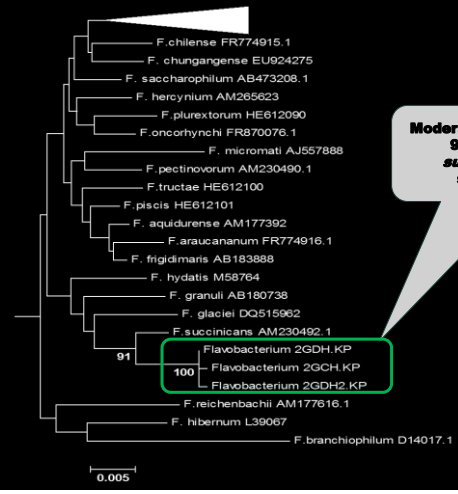
- **Ken Phillips Case- Mortality event in walleye**

- **Three necropsied walleye sent to MSU-AAHL**
- **Autolysis of tissues (see Ken/Ryan's findings)**

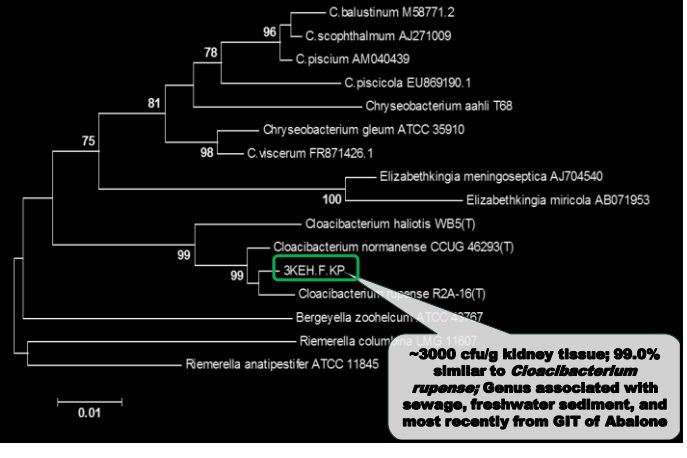
- **Bacterial cultures on TSA, Hsu-Shotts, & Cytophaga agar (Skin/muscle, gills, kidney, brain)**
- **Large numbers of filamentous gliding rods in kidney, skin, and gills preparations (more numerous in kidney); also numerous flagellated motile rods**

- **Multiple morphologies consistent with motile *Aeromonas* spp. as found by Ryan**
- **Multiple YPB recovered from gills and skin on HSM; One YPB recovered from #3 kidney**

Another YPB Morphology Recovered from Gills (3/3 fish)



One YPB Recovered from Kidney & Skin (1/3 fish)



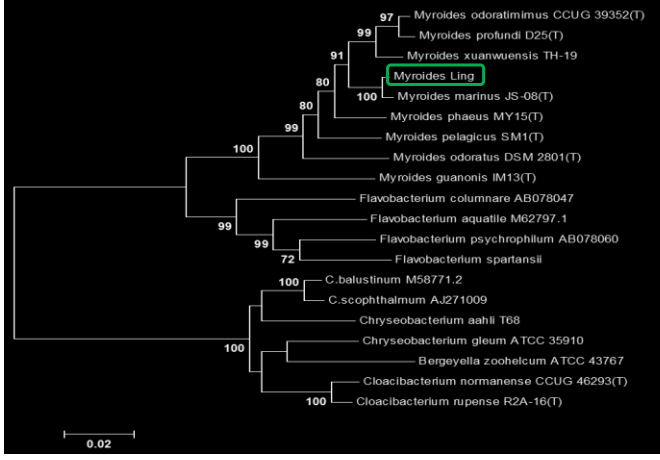
Inter-GLB cases

Ling Shen

Ling Case 1

- Ling Shen- isolates a member of the family Flavobacteriaceae from hatchery fish with typical signs of BCWD
- Sensitive to florfenicol, treatment clears all lesions
- Isolate sent to MSU-AAHL for further analysis

Another Genus within Flavobacteriaceae



- ***Myroides marinus* sp. nov., a member of the family *Flavobacteriaceae*, isolated from seawater**

Sung-Heun Cho[1], Song-Hee Chae[1], Wan-Taek Im[2] and Seung Bum Kim[1]

- *[1]Department of Microbiology and Molecular Biology, School of Bioscience and Biotechnology, Chungnam National University, 220 Gung-dong, Yuseong, Daejeon 305-764, Republic of Korea*
- *[2]Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea*

Ling Case 2
Chryseobacterium chaponense (API)

- First described in Chilean farmed Atlantic salmon that were co-infected with *F. psychrophilum* and from skin ulcers in rainbow trout in France
- MSU-AAHL isolates recovered from the kidneys of systemically infected feral Chinook salmon broodstock (SRW) & rainbow trout fingerlings (OSFH)
- Ling Shen recovered this bacterium from hatchery mortality event (Tx w/ Teramycin effective)

Chryseobacterium chaponense

API 20E	MSU	MN-DNR (Ling)?
ONPG	-	-
ADH	-	-
LDC	-	-
ODC	-	+
CIT	+	-
H_2S	-	+
URE	-	-
TDA	-	+
IND	-	+
GLU	-	-
MAN	-	+
INO	-	+
SOR	-	-
RHA	-	-
SAC	-	-
MEL	-	-
AMY	-	-
ARA	-	-
Terramycin	Resistant	Sensitive

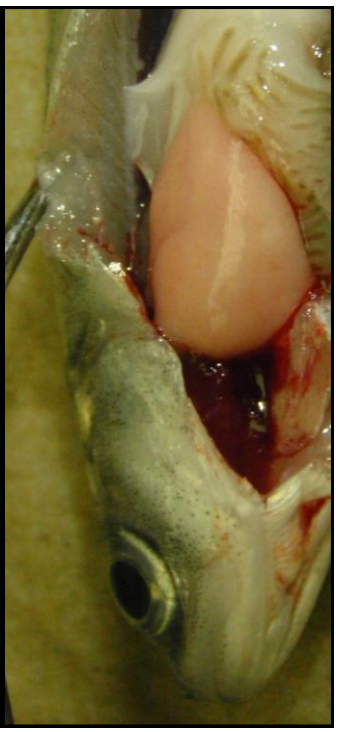
Unique Aquatic Clinical Cases

Mohamed Faisal, DVM, PhD, Dr. Honoris Causa, Cert-AqVet
SF Sniezsko Endowed Sholar and Professor of Aquatic Animal Medicine
Michigan State University
State College, PA
February 4, 2013

Case 1

CHINOOK SALMON (6 m)

Pale Liver



Chronic BKD

Hemorrhages

?

Case 2

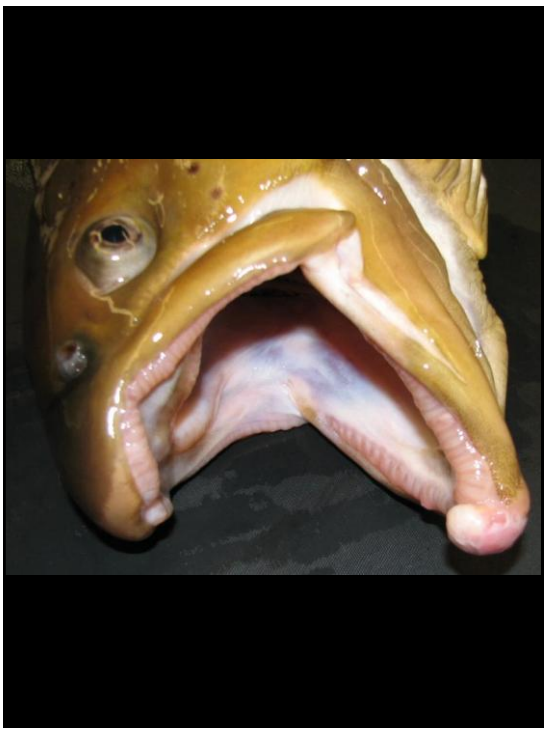
Dropping Jaw
Case History

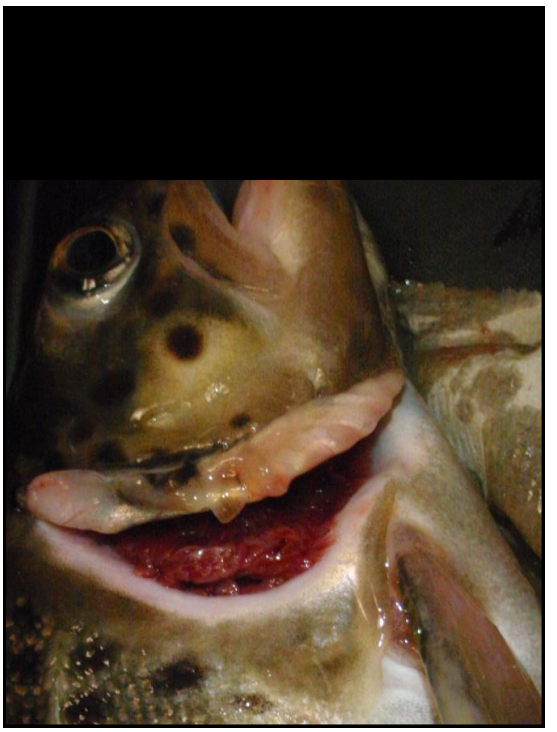
Case 2: Dropping Jaw

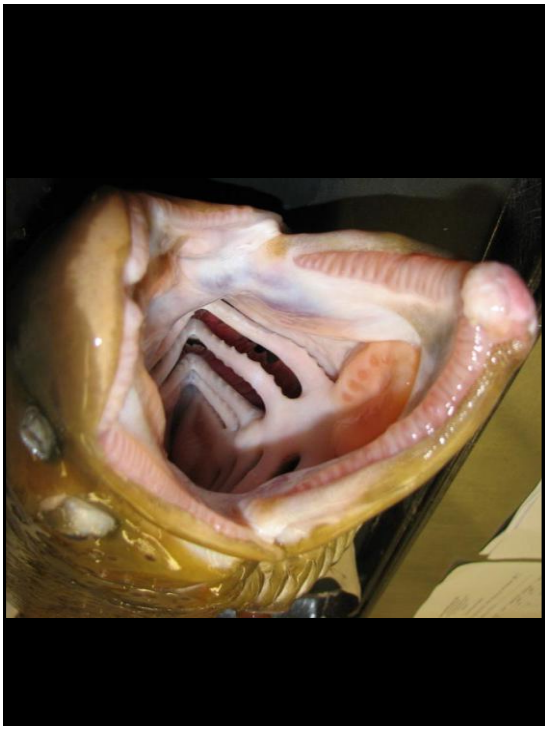
- Fall 2004- Hatchery personnel notice sparse jaw & opercular abnormalities in spawning brown trout
  - Also noted in immature future BNT broodstock

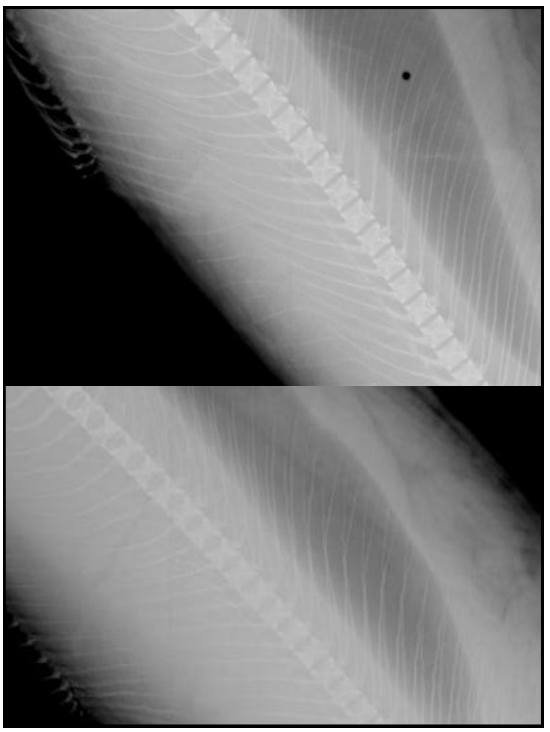
- Spring 2005- 20% of production BNT w/ similar signs

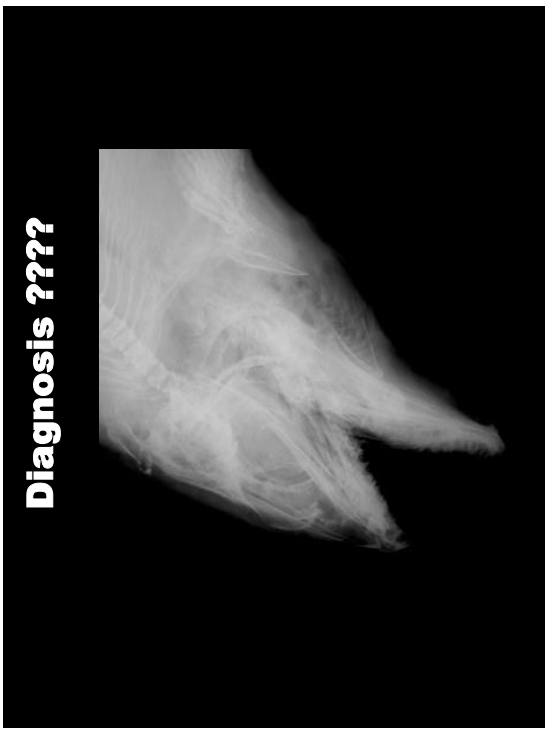
- Spring 2006- Higher prevalence & severity in production & brood BNT
  - Elevated mortalities when handled or crowded for stocking

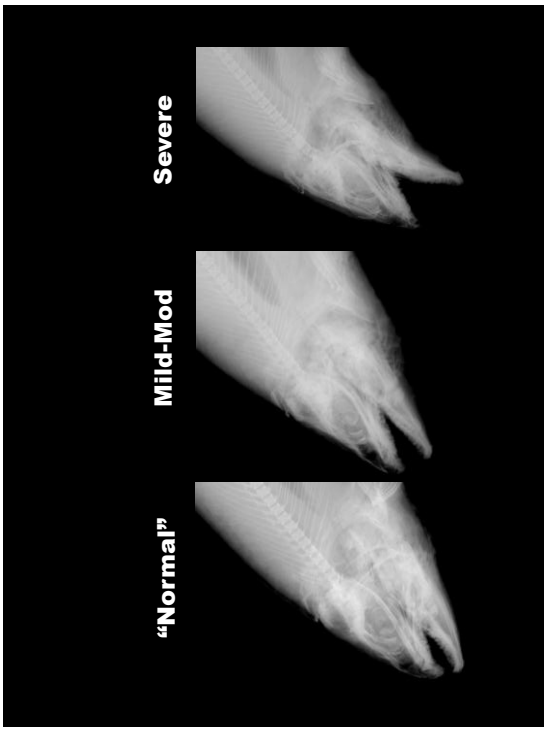


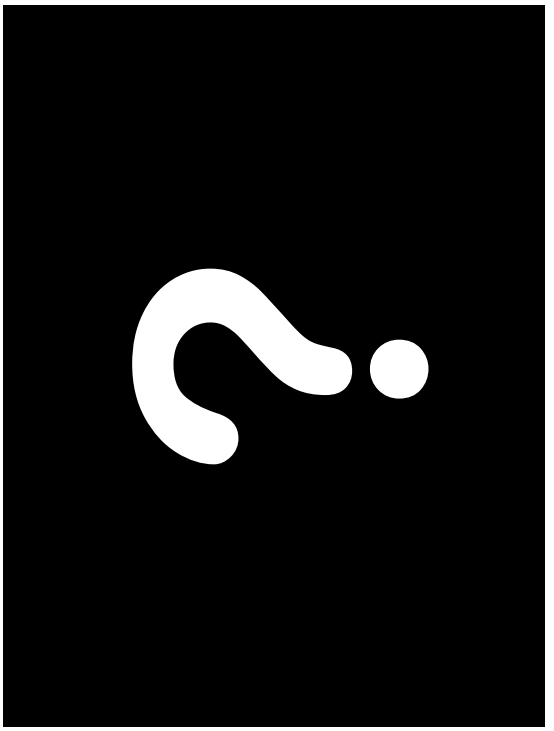







Diagnosis ????


"Normal"
Mild-Mod
Severe


?

CASE 3

Case History

Lateral Line Disease

?

CASE 4

Production Atlantic salmon

- Inappetance
- Lethargy
- Melanosis
- Tachybranchia
- Mortality within 2-3 days

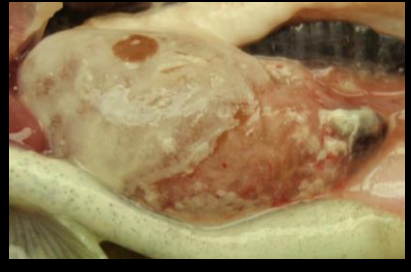
?

Aeromonas salmonicida subsp. salmonicida

Case 5

- **Chinook salmon**
- **20 m**
- **Mortalities**

False membrane covering organs

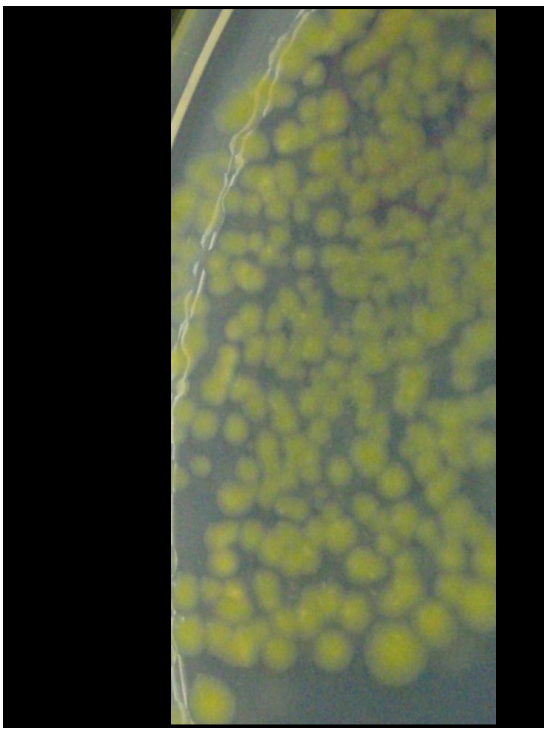


CASE 6

Steelhead trout (12 m)

Acute BKD

?

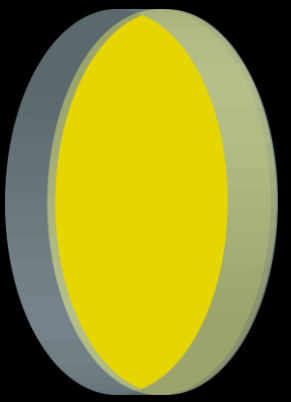


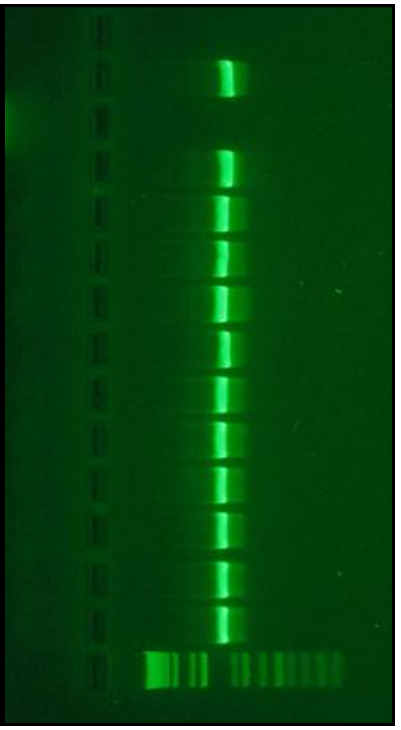
F. psychrophilum

?

CASE 7

Production Brown trout (5 m)





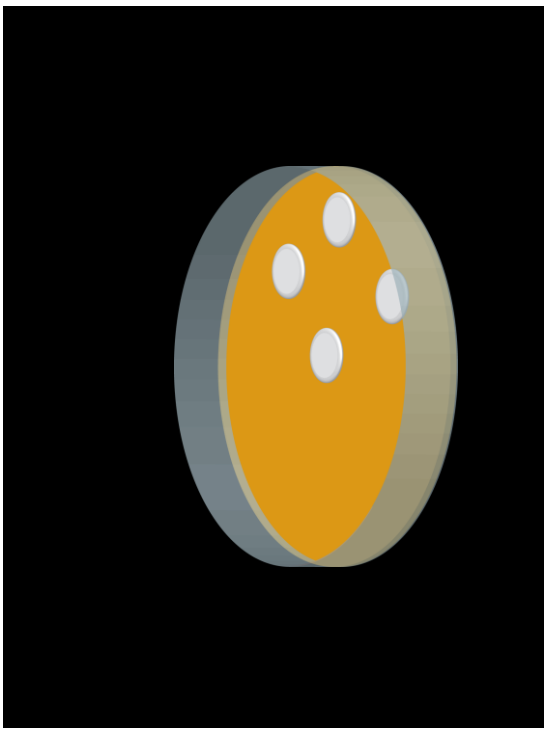
?

Chryseobacterium piscium

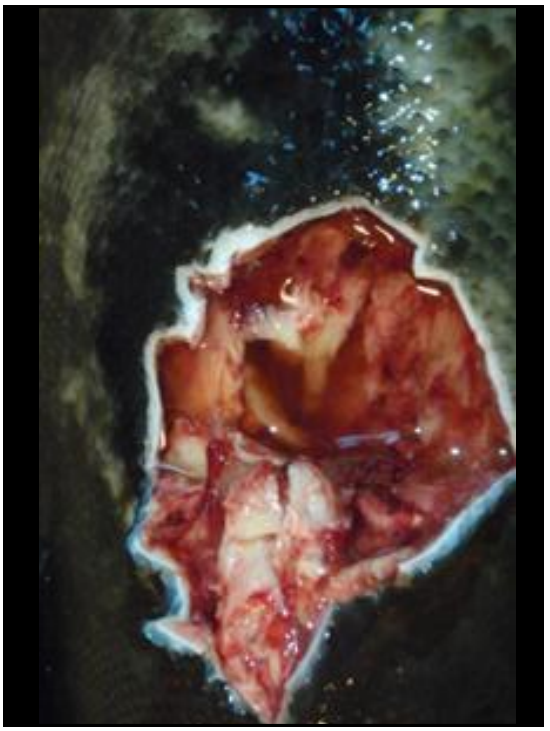
- Originally isolated from marine fish in South Africa
- Never before reported in North America

CASE 8

Chinook salmon returning spawners



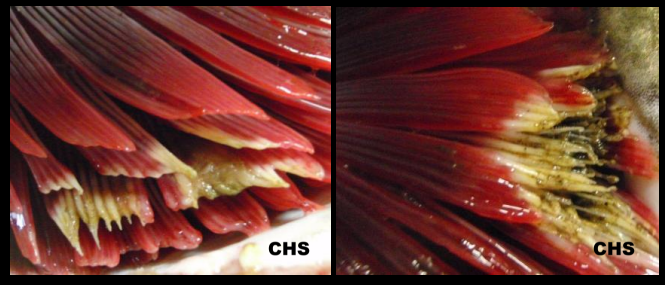
A. salmonicia subspecies salmonicida



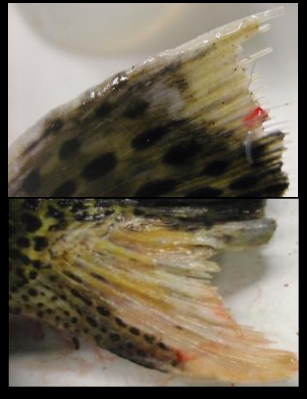
?

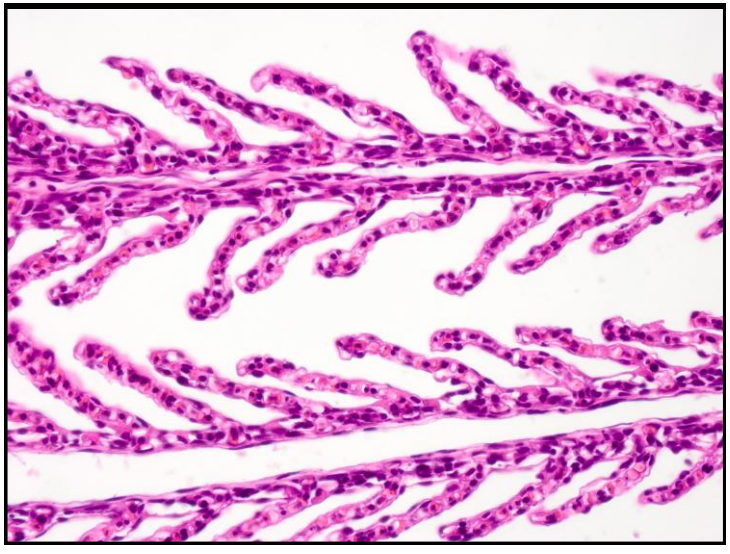
Case 9

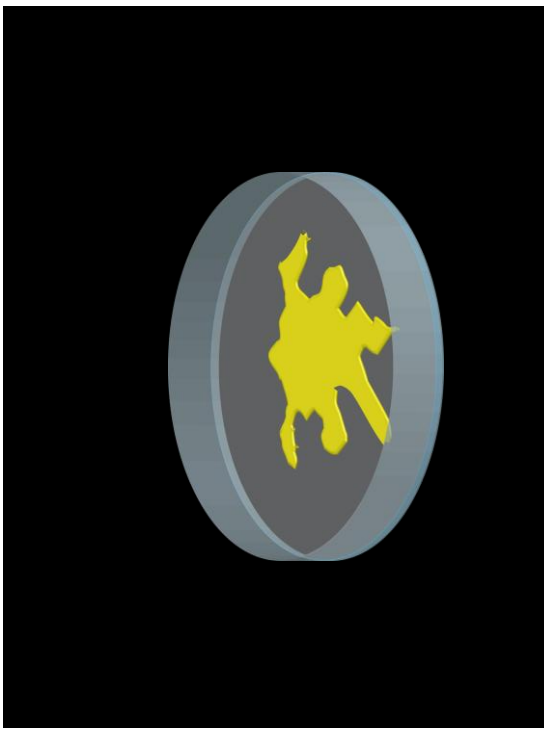
Chinook salmon at Little Manistee River Weir



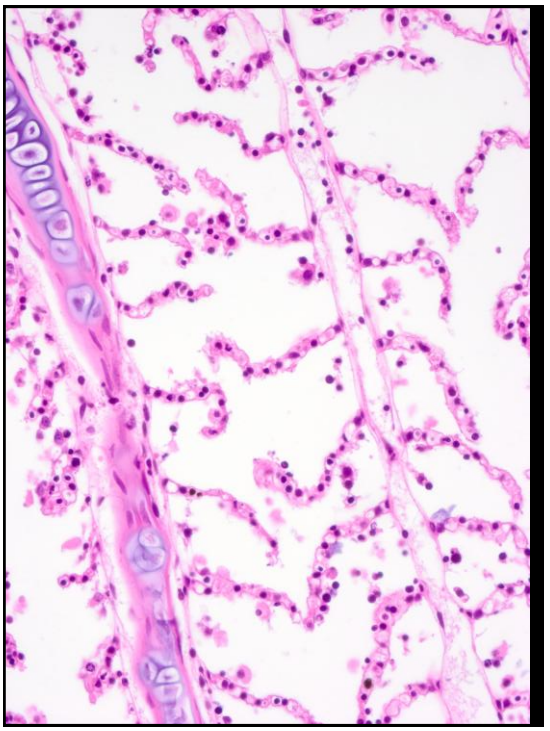
CHS







F. columnare



?

Case 10

Production rainbow trout (12 m)
Lingering mortalities

?

No bacterial or viral growth on most media and cell lines

CASE 11

Production rainbow trout (7 m)

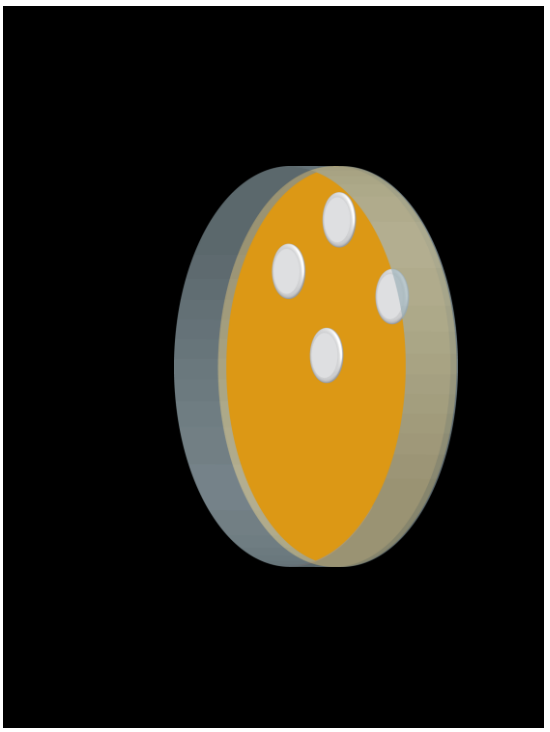
Subacute BKD

?

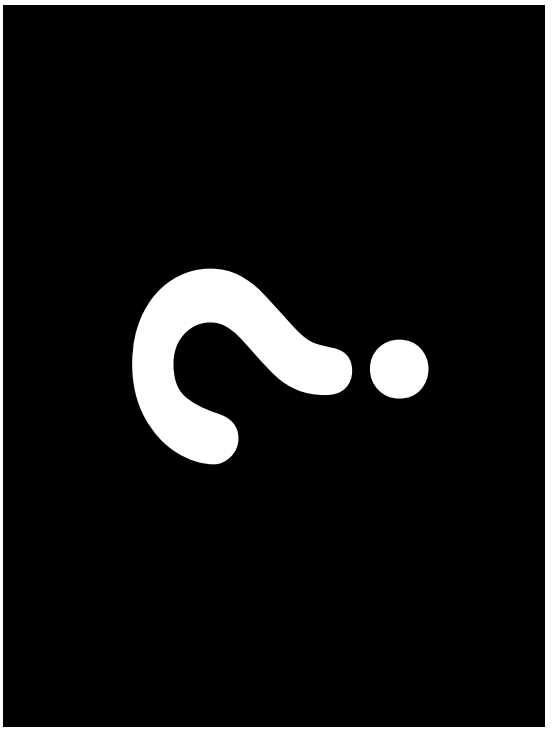
Case 12

Broadstock BNT

Flavobacterium columnare



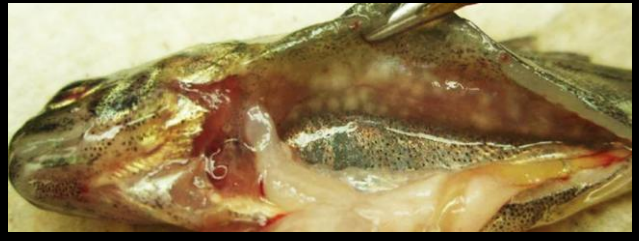

Aeromonas salmonicida
subsp. salmonicida


?

Case 13

Production brown trout (12 m)

Granular lesions in kidney

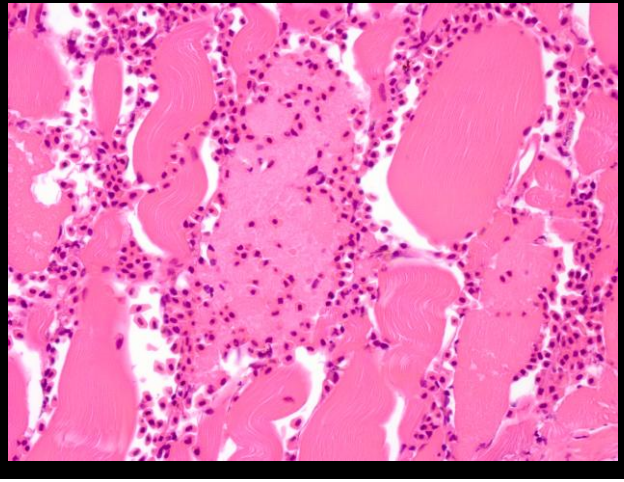


No bacteria or viruses on conventional media and cell lines

Chronic BKD

Case 14

Channel catfish from Ohio



Case 15

Hatchery rainbow trout
(future broodstock)

?

Columnaris disease

No bacteria on conventional media and no viruses isolated on most cell lines

BKD

Blebs or ulcers on skin

?

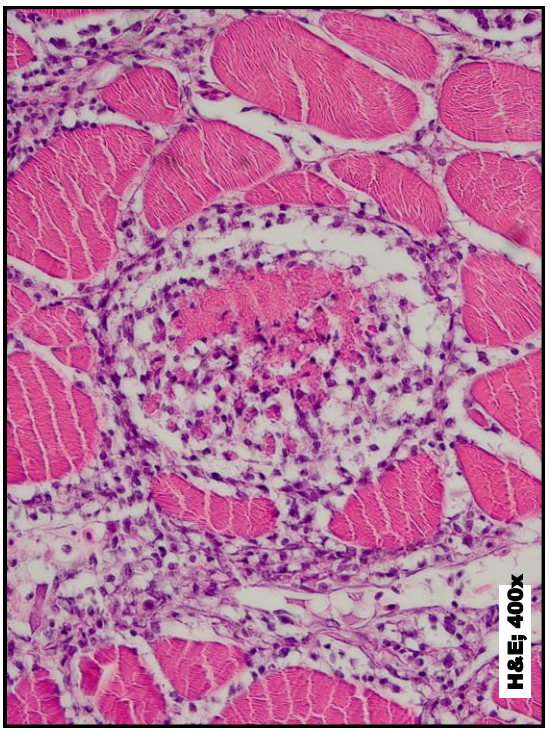
Case 16

Chinook salmon spawners

Aeromonas salmonicida subsp. salmonicida

Case 17

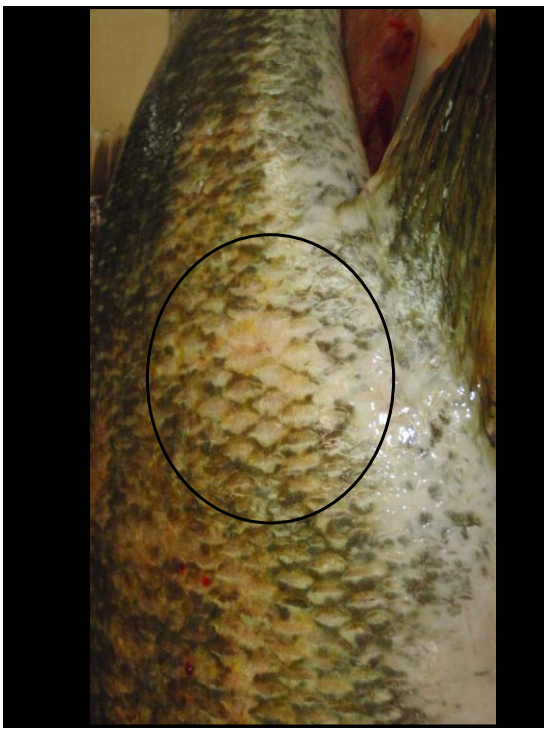
- **Mortalities noted in private indoor tilapia farm**
- **16,000 fish in 28,000 gallon recirculating system**

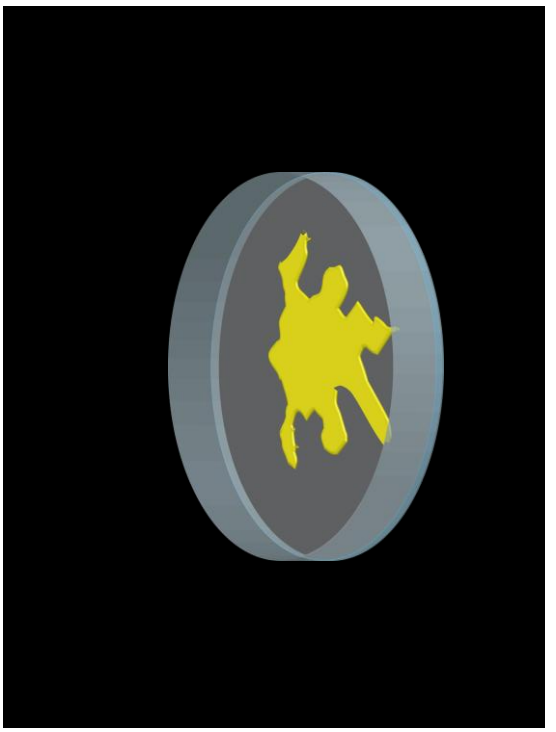

H&E; 400x

Penassay agar

Edwardsiella tarda

?

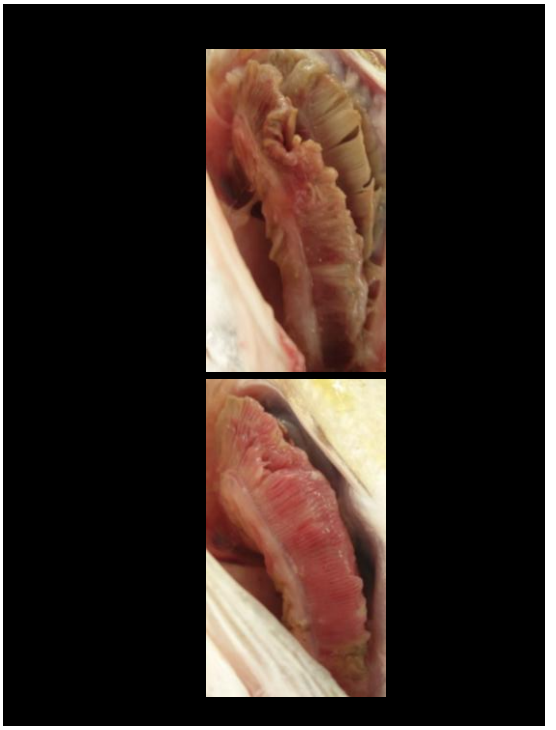




Case 18

Smallmouth bass

Large mortality event at LSC

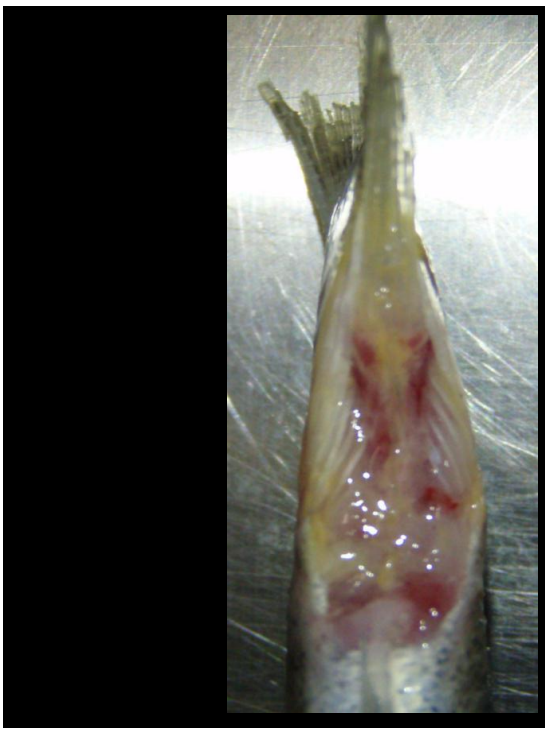


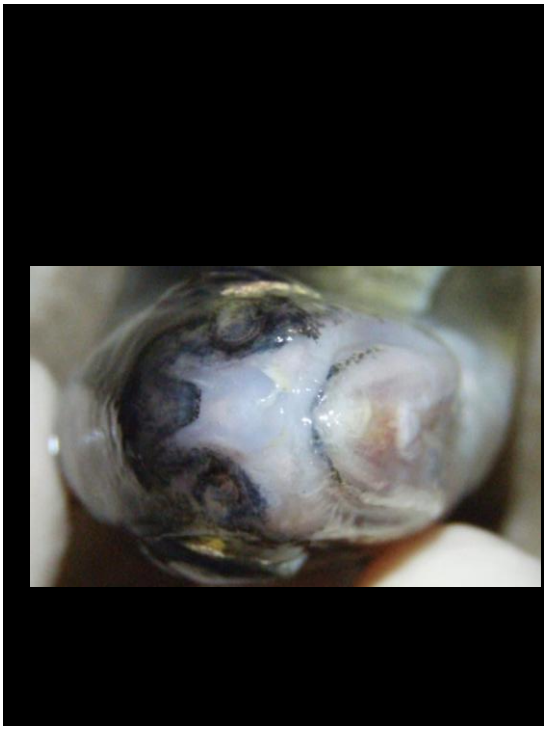
F. columnare

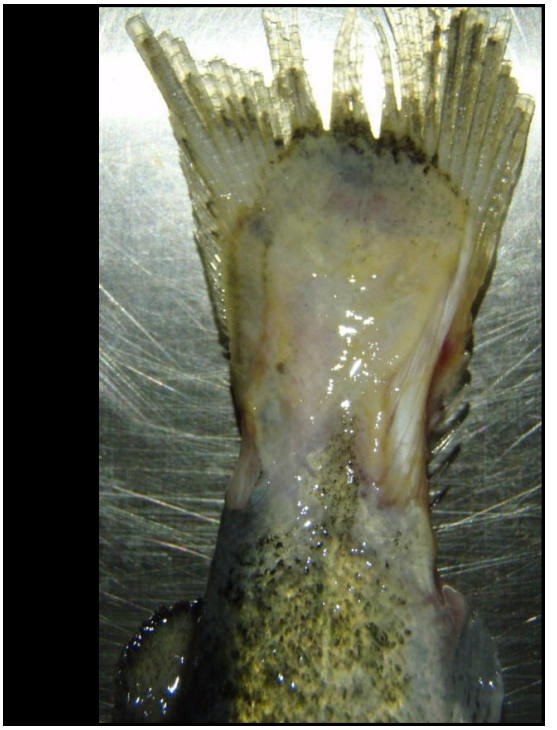
Case 19

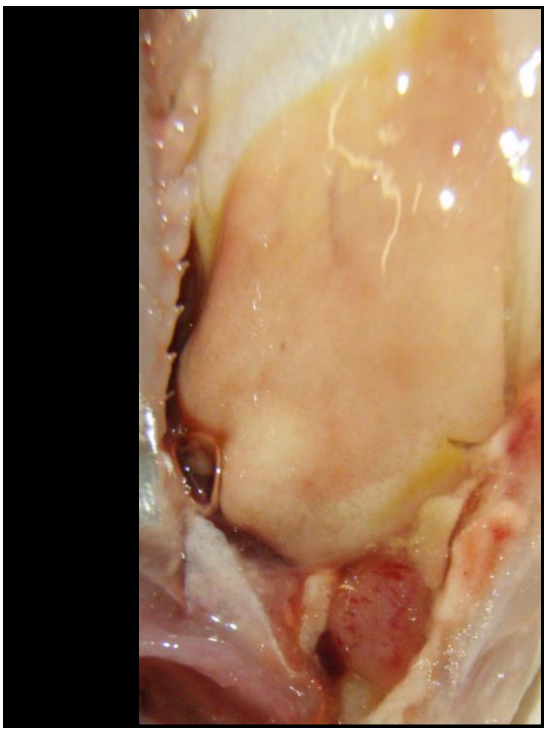
Chinook salmon (12 m)
Mortalities

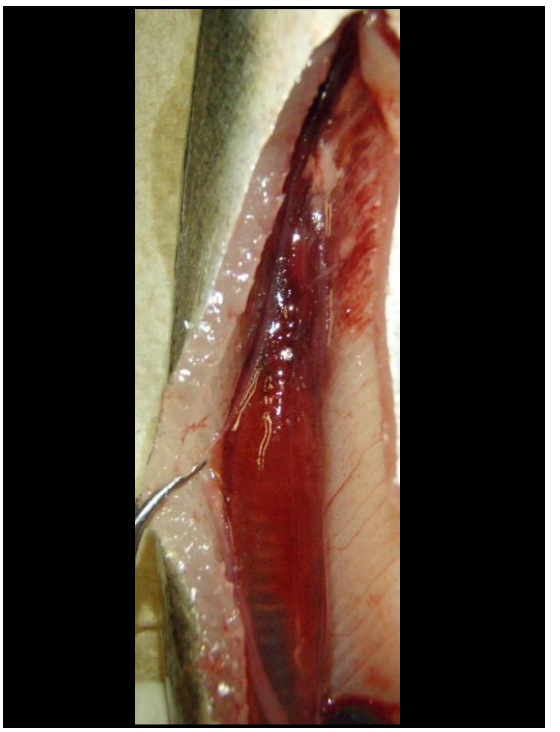
?

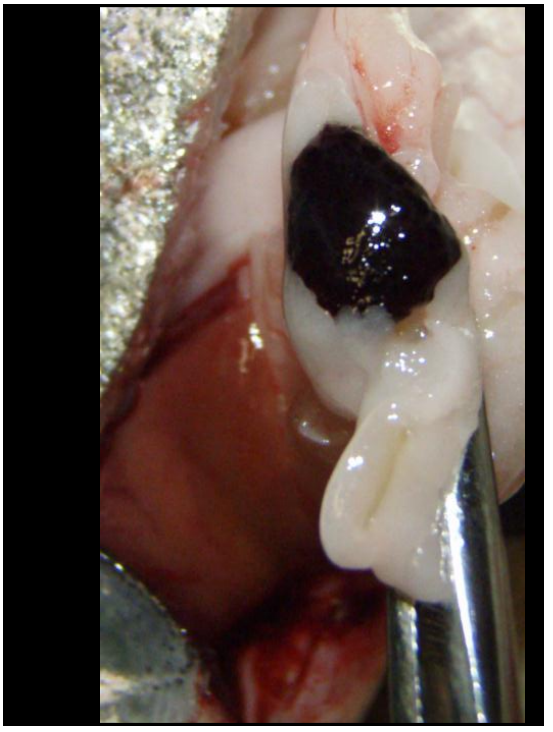


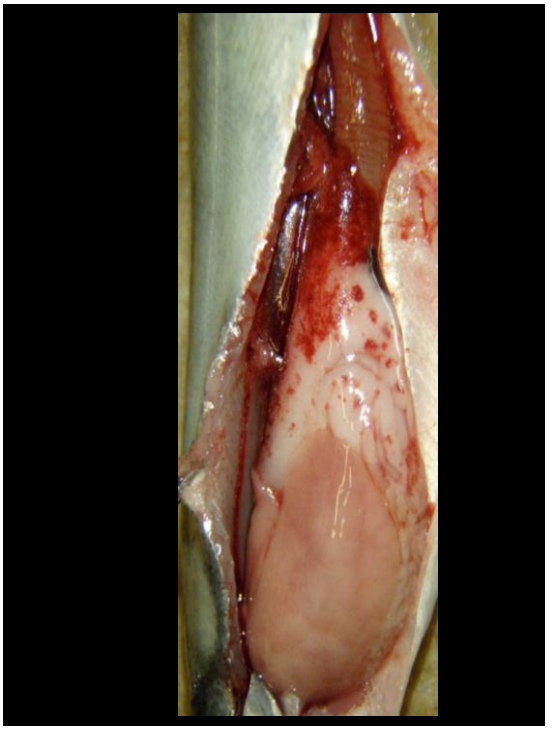


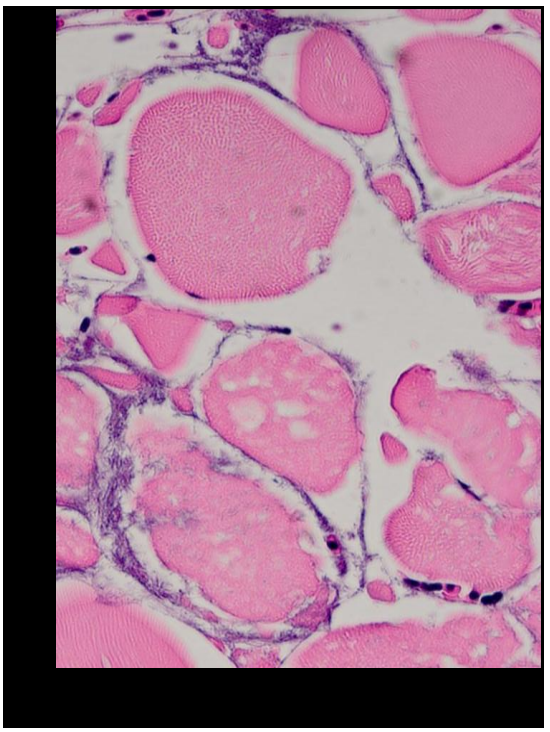


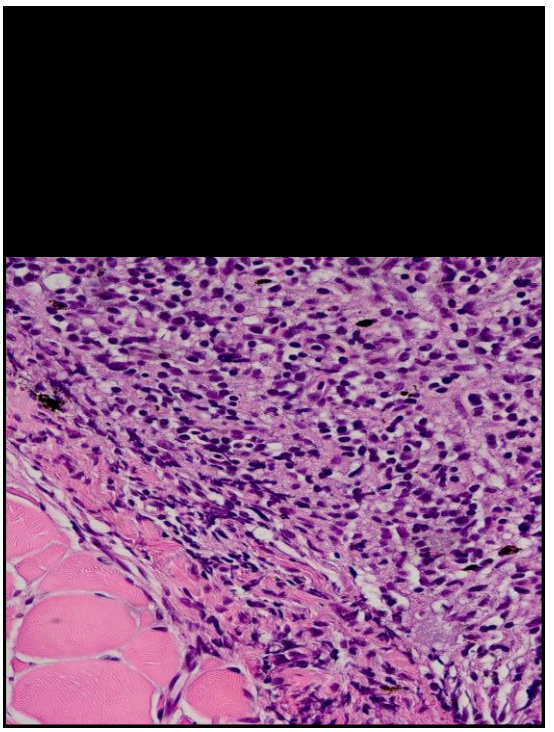


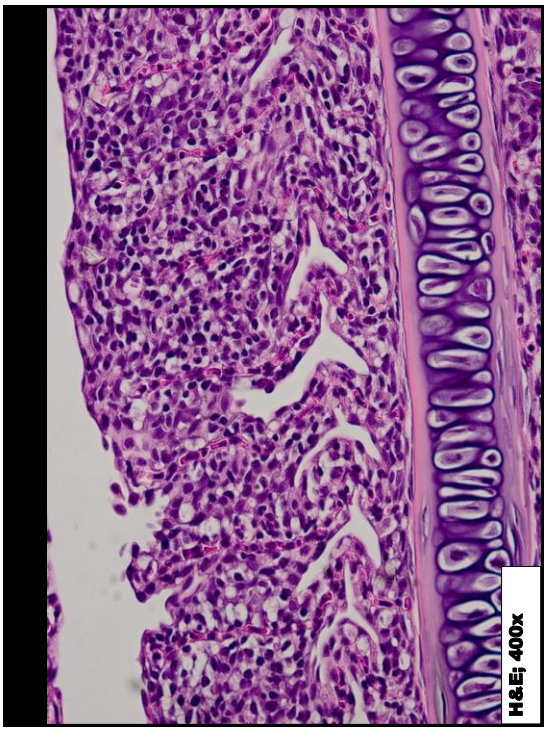


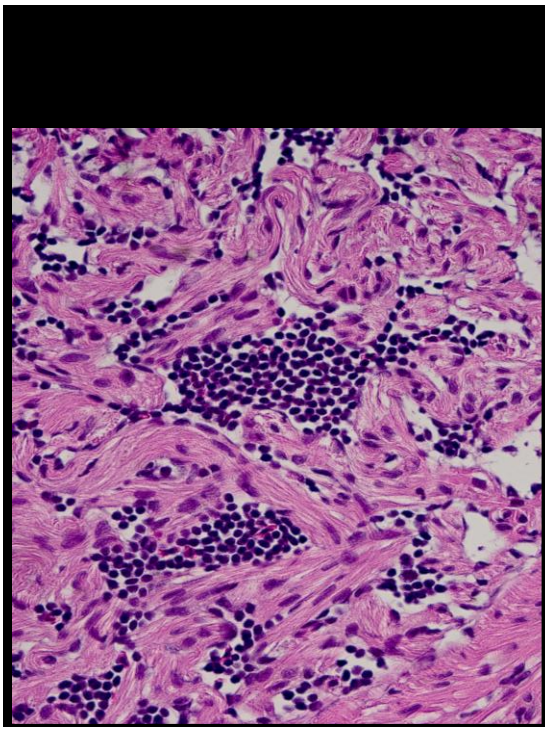


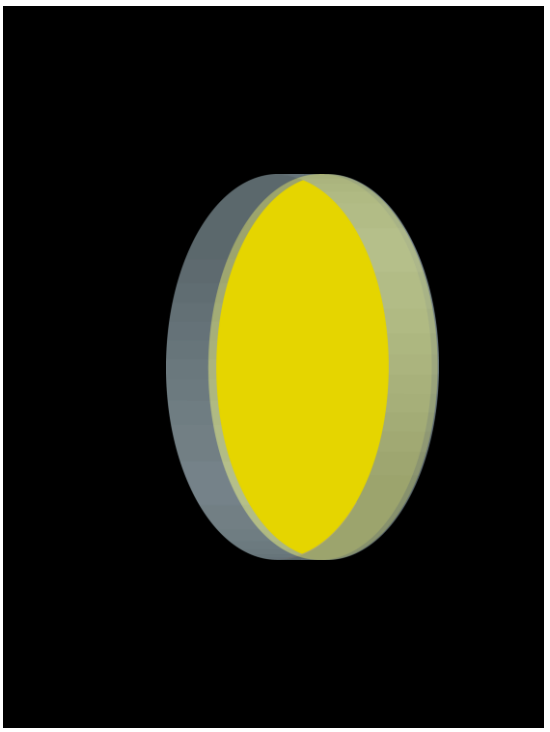




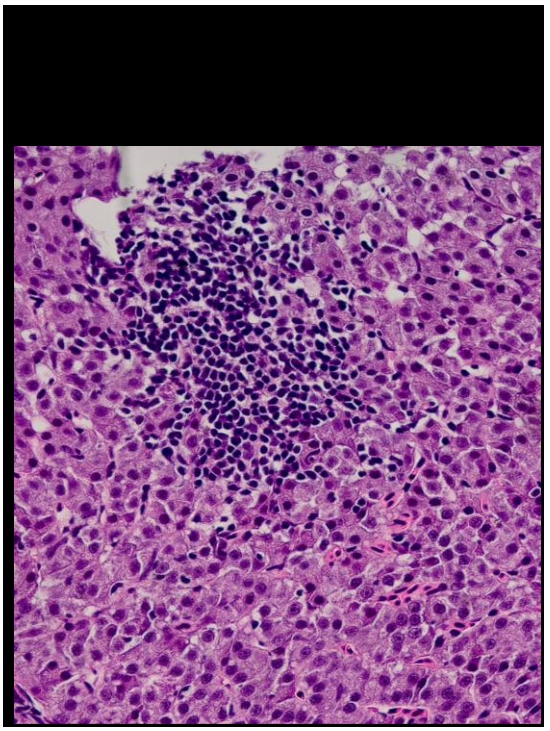



H&E; 400x





Flavobacterium spartansi sp. Nov.



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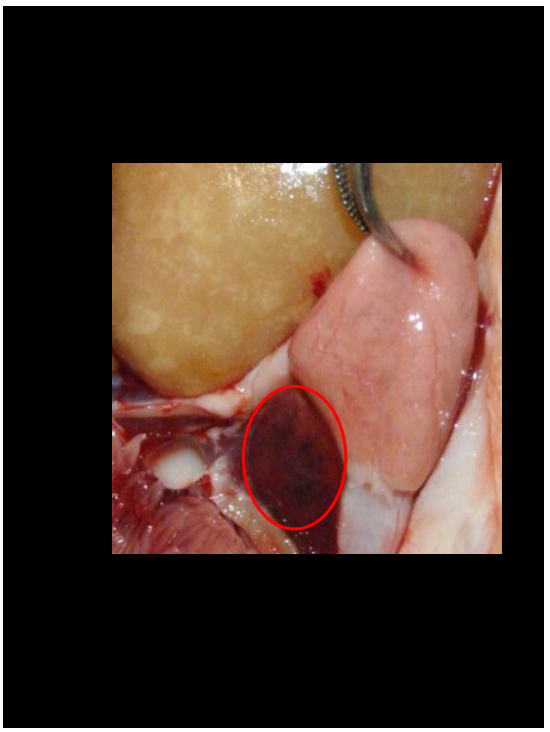
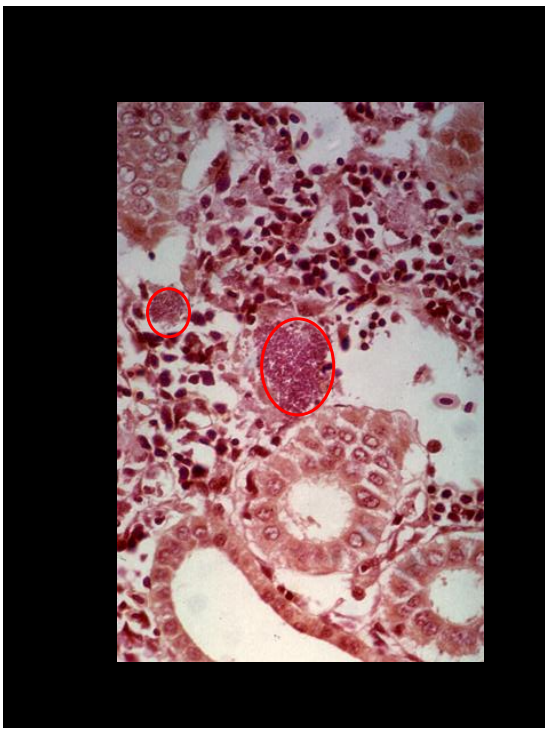
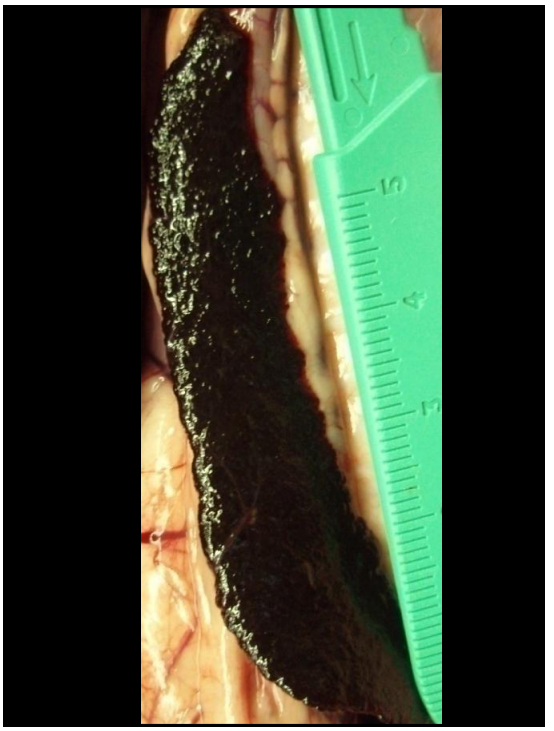




Case 20

Lake White Fish

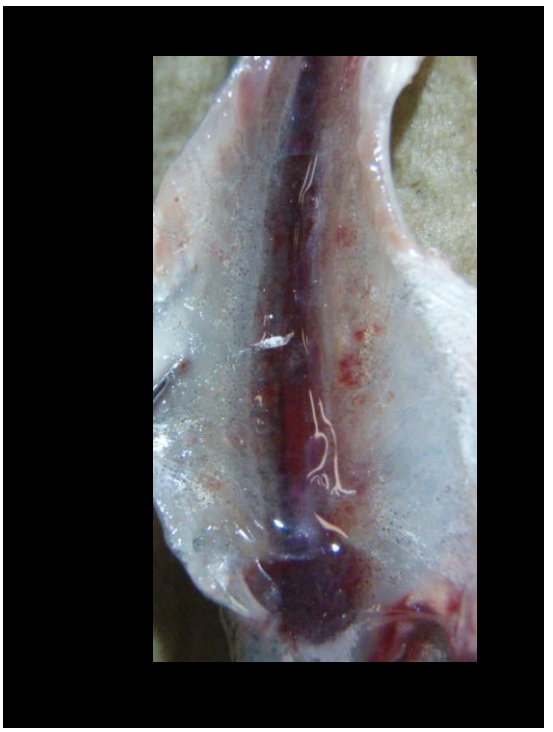


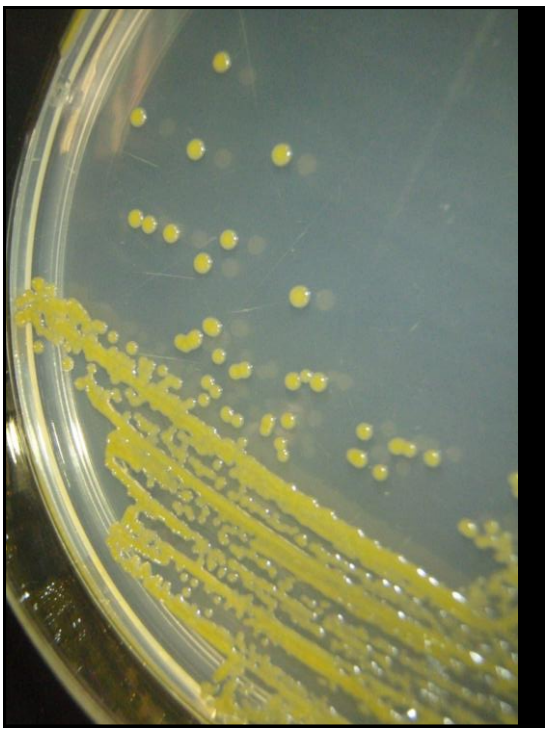
?

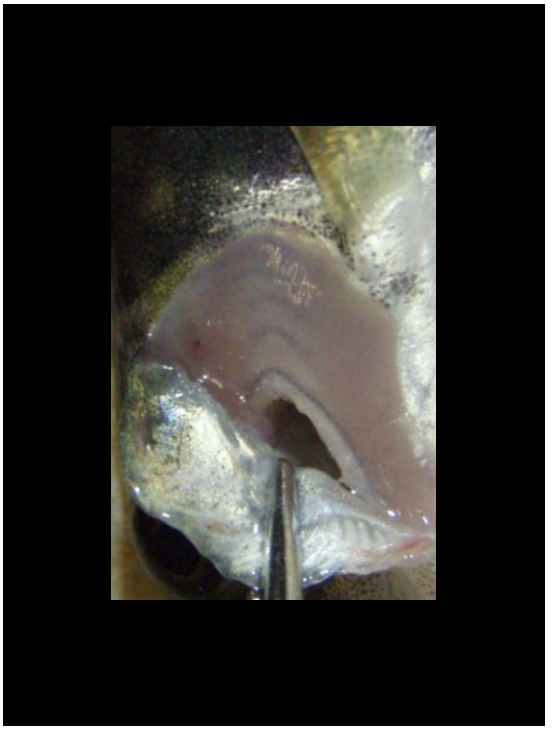
CASE 21

Chinook salmon (12 m)

Aeromonas salmonicida subsp. salmonicida







?

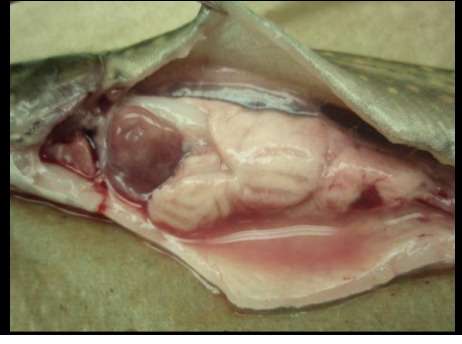
Chryseobacterium piscium

- Originally isolated from marine fish in South Africa
- Never before reported in North America

Case 22

Brook trout (12 m)

Internal Lesions



- Ascites

?

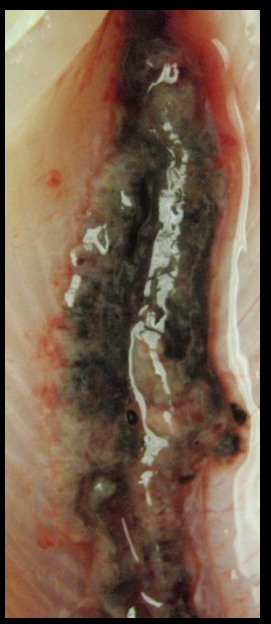
Case 23

Chinook salmon (12 m)

No bacteria or viruses isolated

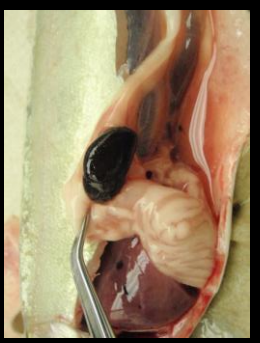
BKD

Grayish kidney

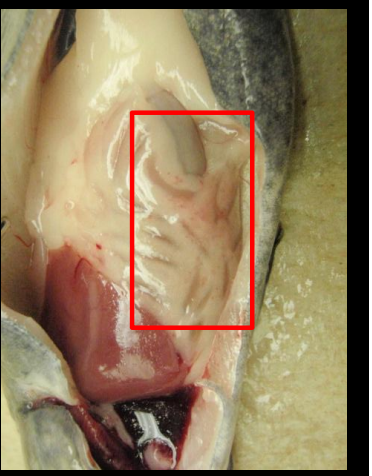


No bacteria or viruses

Enlarged spleen



Petechial hemorrhages

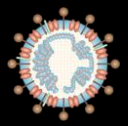


BKD

Order Nidovirales

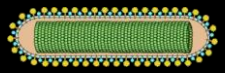
- Enveloped
- Positive sense
- Single stranded RNA genome

Coronavirus

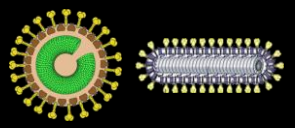


Arterivirus

Ronivirus

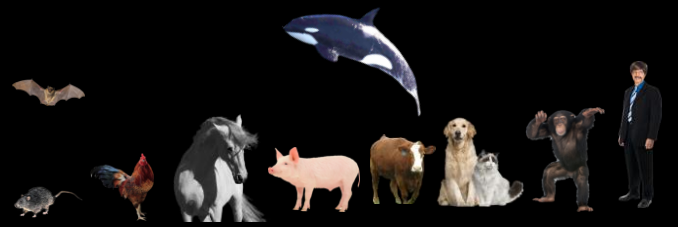


Bafinivirus



Host Range

- Wide variety of nidoviruses
- Wide range of hosts



Examples

- SARS (Severe Acute Respiratory Syndrome)
- EVA (Equine viral arteritis)

Disease Outbreak is a Real Concern to Horse Industry

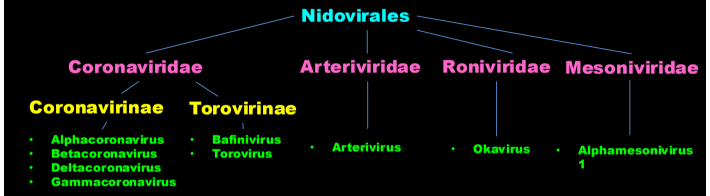
In fish and shellfish

- Yellow head virus
- Gill-associated virus

- White bream virus
- Fathead minnow nidovirus

- The first report of a nidovirus in fish.
- Recovered from white bream in Germany collected during a routine examination of wild fish.
- Novel genus: Bafinivirus
  - Subfamily: Torovirinae
  - Family: Coronaviridae
  - Order: Nidovirales

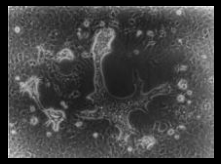
Taxonomy



- Order
- Family
- Subfamily
- Genus

FHMNV

- Isolated from fathead minnow 2001
- Baitfish farm in Arkansas
- Identified as Rhabdovirus
- Formed syncytia in cell culture



- Later analysis by Batts et al. changed from rhabdovirus to nidoviruses
- Order: *Nidovirales* Genus: *Bafinivirus*
- Closely related to WBV

FHMNV in Muskellunge

- FHMNV was isolated from muskellunge that were brought to AAHL from Wisconsin (Sue Marcquenski) in 2011
- It was found in more muskellunge from Wolf Lake
- It is referred to as muskellunge nidovirus

USFWS

- Gary, Sue, and Corey

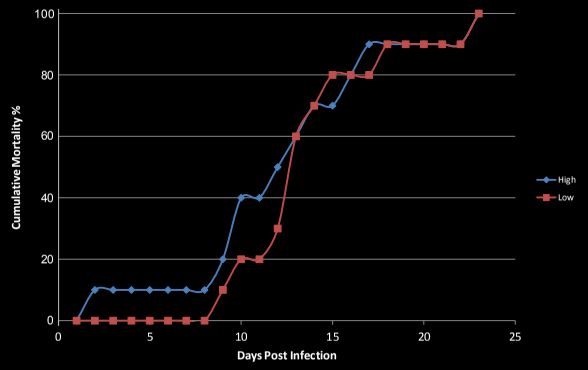
Experimental Infection

- Fathead minnows
- Muskellunge
- Largemouth bass
- Rainbow trout
- Creek chub
- Blacknose dace
- Golden shiner
- Walleye

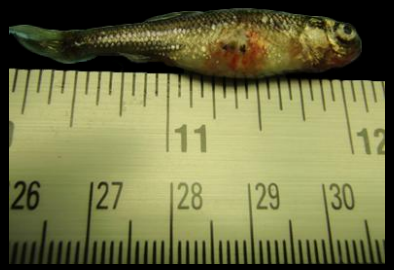
Experimental Infection

- IP-injection
- Two doses (high and low)
- 28 Day Observation
- Collected K/S from moribund/mortalities
- So far: Fathead minnows & muskellunge have been tested.

Fathead Minnow

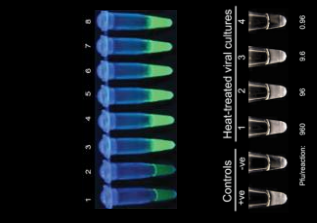


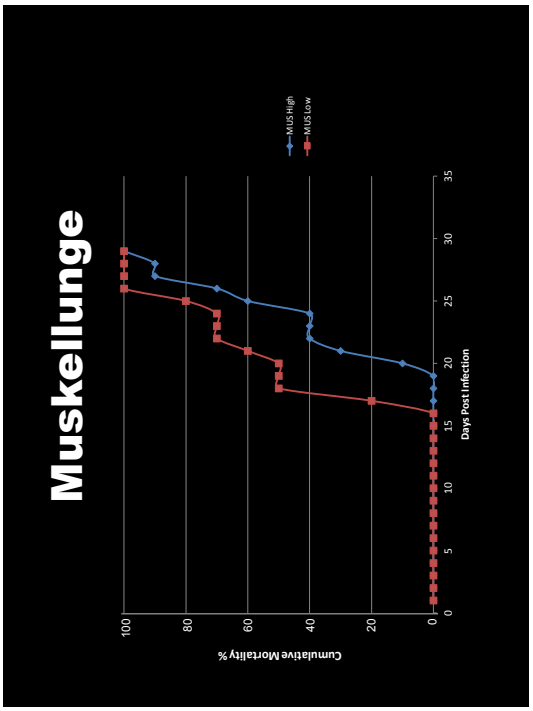
Clinical Signs

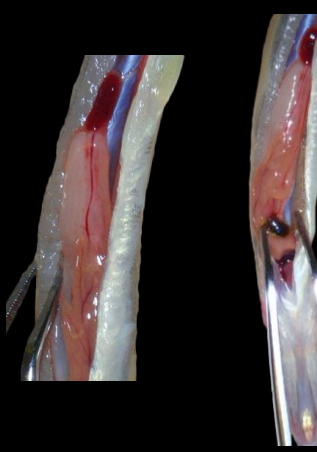


Loop-mediated Isothermal Amplification (LAMP)

- A simple, rapid, specific and cost-effective nucleic acid amplification method.
- 4 different primers specifically designed to recognize 6 distinct regions on the target gene
- Reaction proceeds at a constant temperature.
- Amplification and detection of gene can be completed in a single step.
- It provides high amplification efficiency, with DNA being amplified 109-1010 times in 15-60 minutes.







FHMN Lamp & qLAMP

- Has not reacted with the white bream virus or other nidoviruses
- Highly specific and sensitive

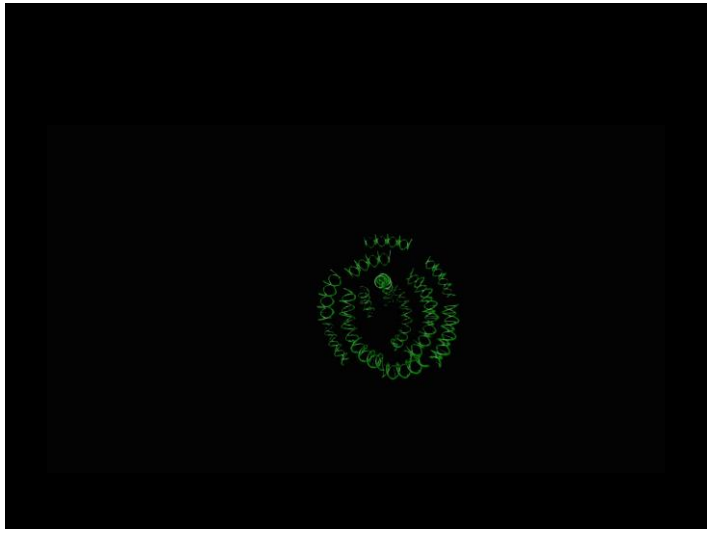
- Virus re-isolation/quantitation
- Virus replicated in FHM and to lesser extent in MUS, yet mortalities are the same
- Mediam LD is being determined

Family: Reoviridae
Subfamily: Spinareovirinae
- Aquareovirus
- Coltivirus
- Cypovirus
- Dinovernavirus
- Fijivirus
- Idnoreovirus
- Mycoreovirus
- Orthoreovirus
- Oryzavirus

Subfamily: Sedoreovirinae
- Cardoreovirus
- Mimoreovirus
- Orbivirus
- Phytoreovirus
- Rotavirus

Aquareoviruses

- Family Reoviridae
- Double stranded RNA virus
- Non-enveloped virion
- Two concentric icosahedral capsids



- There have been more than 30 isolations of aquareovirus from fish both freshwater and marine species

- High prevalence in commercial bait facilities
- May be vertically transmitted
- Could be transmitted to predator species through infected bait
- Have been isolated from multiple fish species
- Also affect mollusks, crustaceans, birds, mammals, insects, and plants

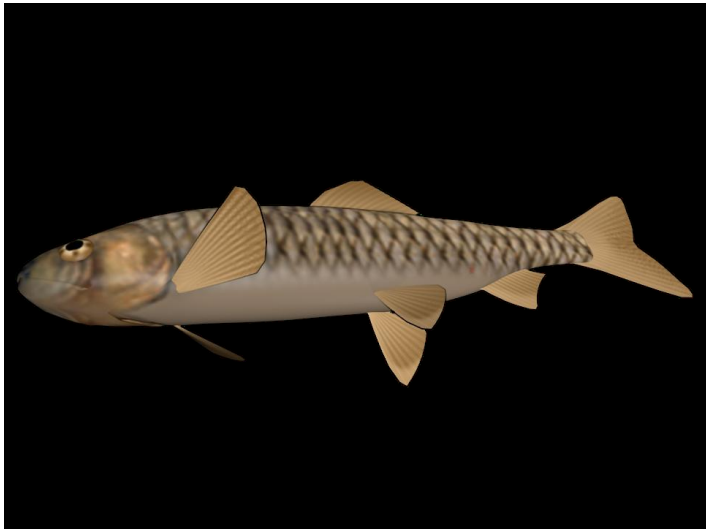
Aquareoviruses with varying pathogenicity

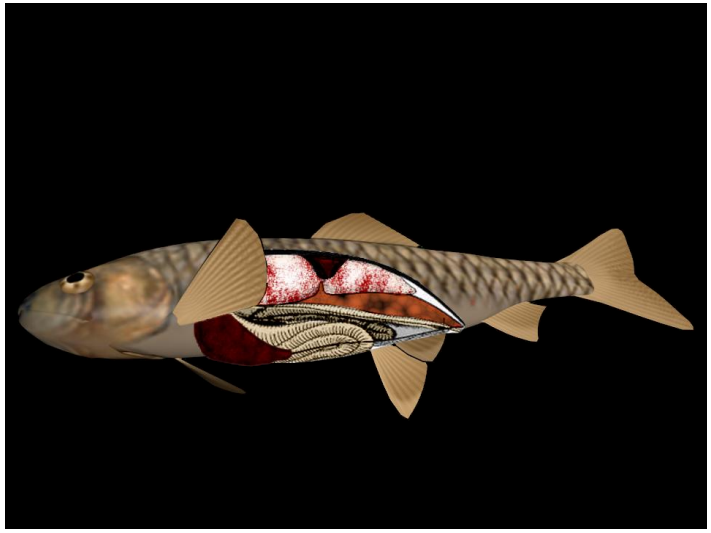
Chum salmon aquareovirus	Apparently healthy
Maso salmon aquareovirus	Heavy mortalities
Common carp aquareovirus	Epidermal hyperplasia
Channel catfish aquareovirus	Chronic mortality
Canadian smelt aquareovirus	Heavy mortalities

- A: Chum salmon reovirus
- B: Chinook salmon reovirus
- C: Golden shiner reovirus
  Grass carp reovirus
- D: Channel catfish reovirus
- E: Turbot reovirus
- F: Coho salmon reovirus
- G: American grass carp reovirus

Grass carp hemorrhagic disease

- Caused by the grass carp aquareovirus.
- Enzootic in grass carp and black carp in China for many years.

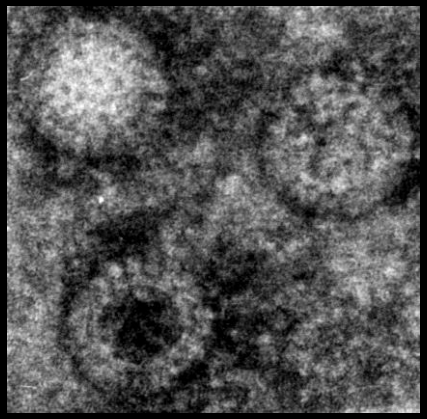




Aquareovirus Type C

- Detected in Great Lakes muskellunge in Michigan state fish hatchery during routine fall production inspection testing, Nov 2012
- Detected in muskellunge in Wisconsin state fish hatchery in 2005

Hatchery-raised muskellunge with Aquareovirus Type C, Nov 2012



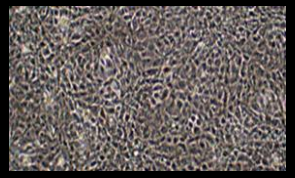
Golden shiner

- First aquareovirus isolated.
- Isolated from cultured golden shiners (***Notemigonus crysoleucas***) in the USA by Plumb et al. (1978)

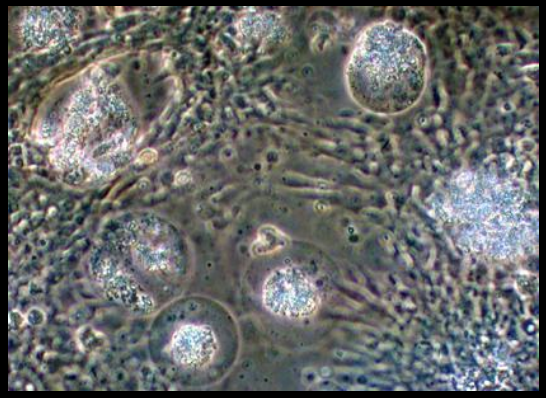
- The disease also affects other cyprinids such as fathead minnow

- Low mortality
- Affected fish are lethargic
- Swim on the surface.
- Signs of septicemia have been described in fish associated with GSRV

- The virus can be isolated on EPC and FHM cell lines at 25-30 °C.
- Its CPE are characteristic: it produces syncytia (giant cells).



48-72 hrs. p.i.



- There are concerns about its pathogenicity to:
  - Other native cyprinids
  - Propagated piscivorous fish

US Fish & Wildlife Service:

Whelan, Marcquenski & Puzach

Current research to determine:

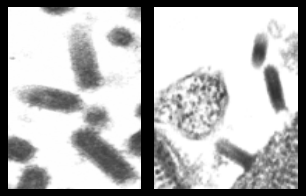
- Susceptibility
- Pathogenicity
- The role of survivors
- Phylogenetic relatedness
- Sources of infection, virus spread, and prey-predator pathogen transmission
- Distribution throughout the GL watershed

Experimental infection

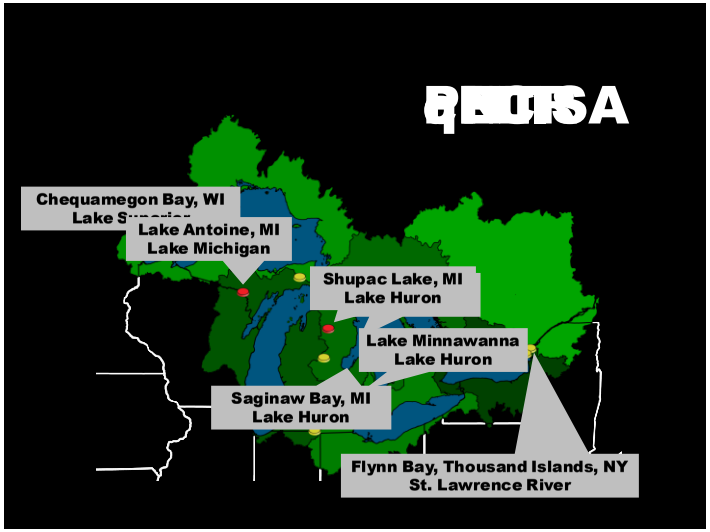
- FHM & MUS
- IP injection
- 10^4 & 10^7/fish
- As of 49d p.i. – no mortalities or apparent signs of disease in either species
- The virus is present in the tissues

LAMP

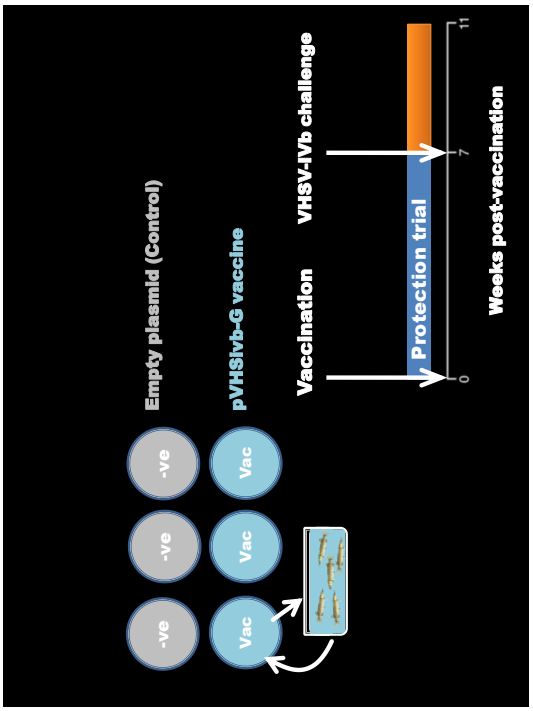
- Has not recognized GCR
- Highly specific and sensitive
- Cost per sample is less than $ 2

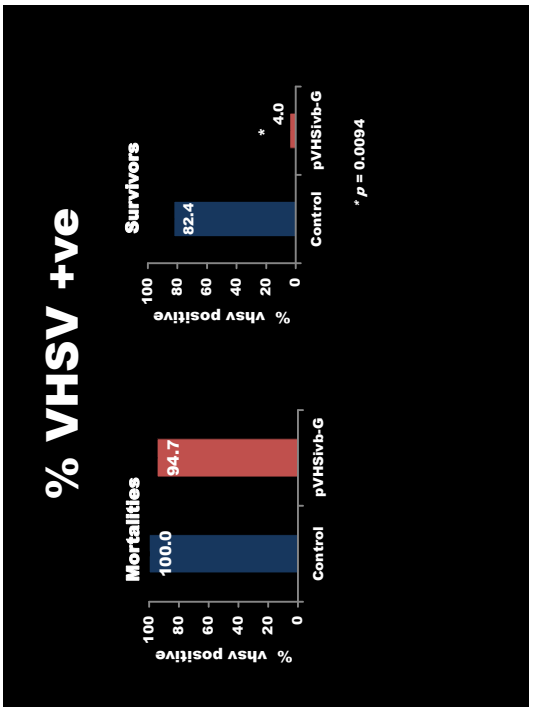


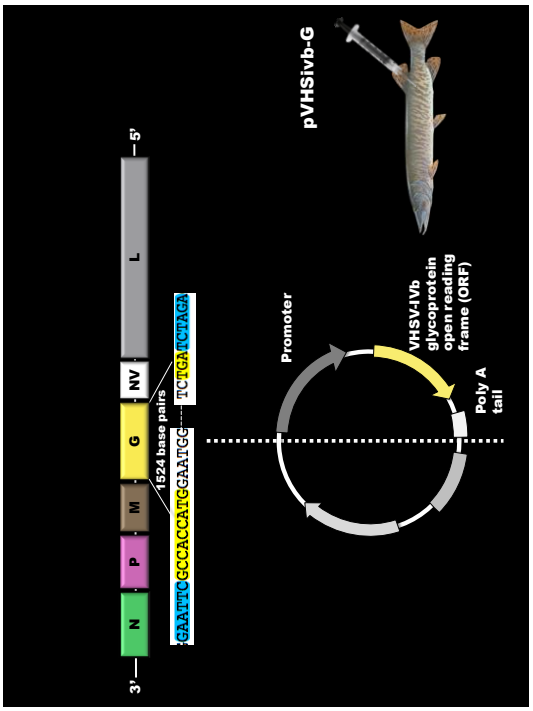
- Order: *Mononegavirales*
- Family: *Rhabodviridae*
- Genus: *Novirhabdovirus*
  - Enveloped
  - Negative single stranded RNA

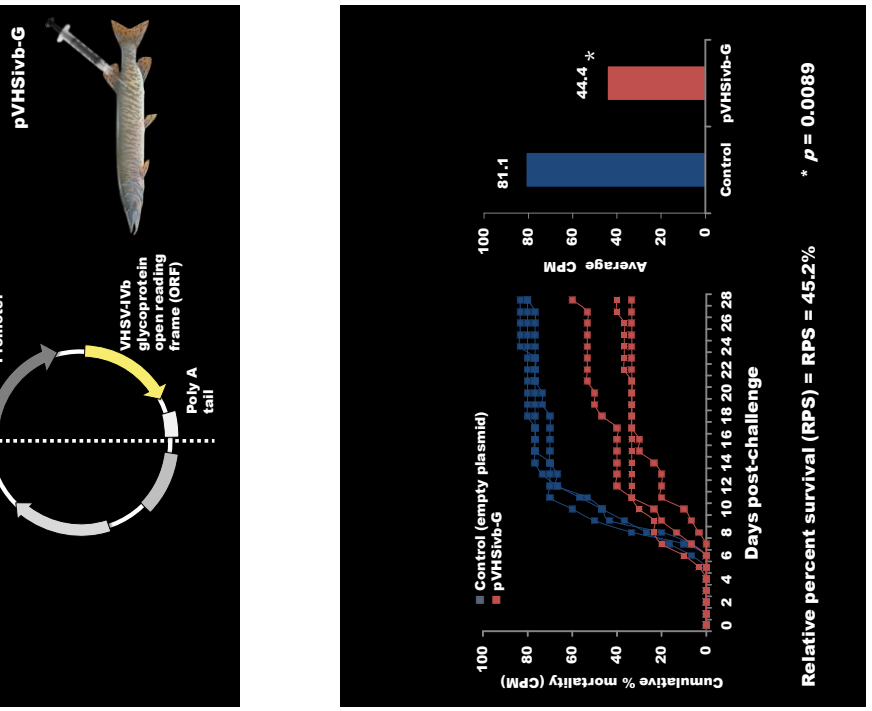


Efficacy of a DNA vaccine encoding the VHSV-IVb glycoprotein


Empty plasmid (Control)
pVHSivb-G vaccine
-ve
Vac
Vaccination
VHSV-IVb challenge
Protection trial
0
7
11
Weeks post-vaccination

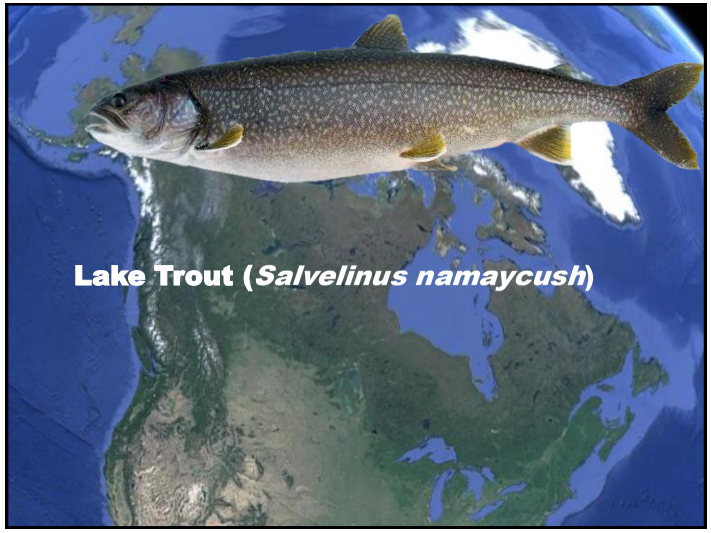

% VHSV +ve
Mortalities
% vhsv positive
100.0
94.7
Control
pVHSivb-G
Survivors
82.4
4.0
* p = 0.0094


3'
N
P
M
G
NV
L
5'
1524 base pairs
GAATTCGCCACCATGGAATGG
TCTGATCTAGA
pVHSivb-G
Promoter
VHSV-IVb glycoprotein open reading frame (ORF)
Poly A tail


Control (empty plasmid)
pVHSivb-G
Cumulative % mortality (CPM)
Days post-challenge
Average CPM
81.1
44.4
*
Control
pVHSivb-G
Relative percent survival (RPS) = RPS = 45.2%
* p = 0.0089

Type III salmonid herpesviruses (Sal HV3)

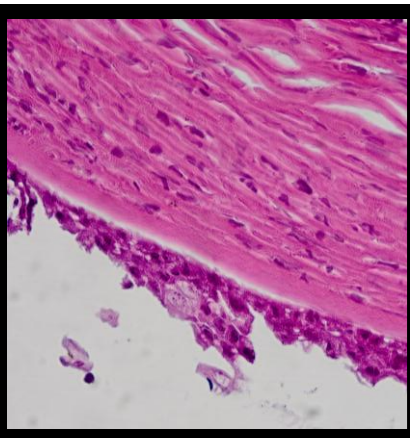
Epizootic epitheliotropic disease virus (EEDV)



- Histological examination showed:
  - Character-istic areas of epithelial hyperplasia with lymphocytic infil-trates, hydropic cells and necrosis

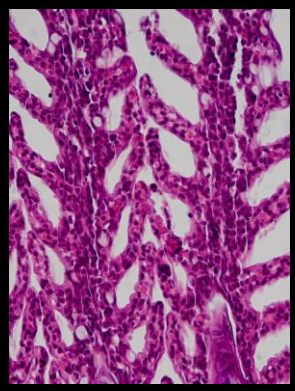
MICHIGAN STATE UNIVERSITY

- Damaged cornea



MICHIGAN STATE UNIVERSITY

- Gills: Epithelial hyperplasia



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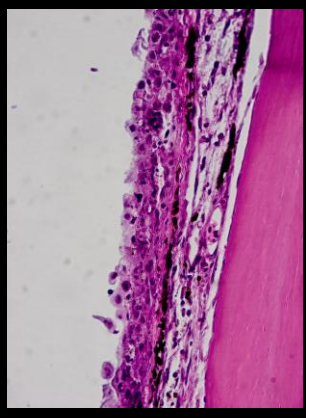
- Epidermal necrosis



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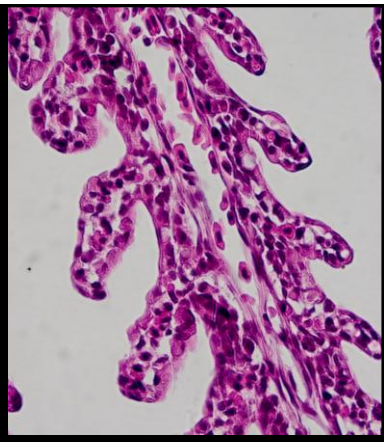
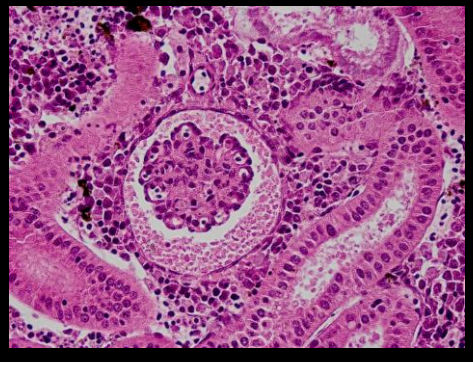
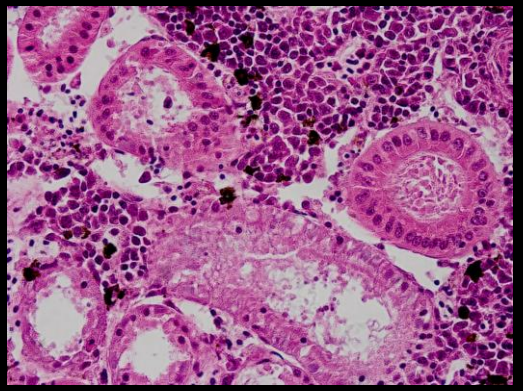
- Gills:
  Lamellar edema



Proteinaceous material in Bowman's space



Renal tubular degeneration



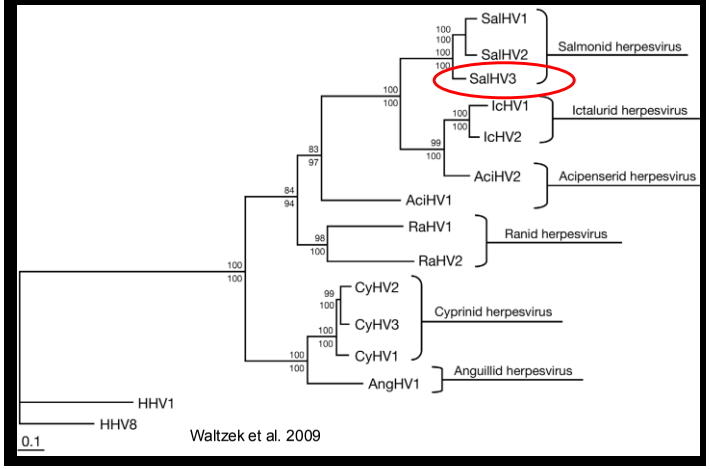
Year Sampled	Purpose of Investigation	Lot ID	Type	Result
2007	diagnostic	B-LAT-LS-W-01-KB-MA	broodstock	Neg
2007	diagnostic	B-LAT-LS-W-04-MS-MA	broodstock	Neg
2011	inspection	Brook trout (Cherry Creek)	wild	Neg
2011	inspection	Brown trout (Cherry Creek)	wild	Neg
2011	inspection	Mottled sculpin (Cherry Creek)	wild	Neg
2011	diagnostic	P-LAT-SE-D-10-HI-MA	production	Neg
2011	diagnostic	B-LAT-LS-D-09-HI-MA	broodstock	Neg
2012	inspection	P-LAT-SE-D-11-HI-MA	production	Neg
2012	inspection	Brook trout (Cherry Creek)	wild	Neg
2012	inspection	Brown trout (Cherry Creek)	wild	Neg
2012	inspection	Mottled sculpin (Cherry Creek)	wild	Neg
2012	inspection	B-LAT-LS-2001-KB-MA	broodstock	Neg
2012	inspection	B-LAT-LS-2003-KB-MA	broodstock	Neg
2012	inspection	B-LAT-LS-2004-MS-MA	broodstock	Neg

Best tissue to sample for monitoring purposes?

Tissue	Average Viral Titer
Spleen	308
Kidney	151
Heart	1371
Caudal fin	2993
Gill	**7633**
Skin	1249

Mottled Sculpin

EEDV is a novel branch of Salmonid Herpesvirus

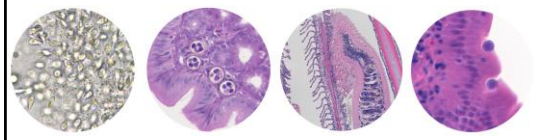


qLAMP

- Highly sensitive
- Primer I. Can discriminate EEDV from 1 but not from 4 and 5 (2 is being tested)
- Primer II. Can recognize EEDV from 1 and 5 but not from 4
- Working on a new primer set

Furunculosis at Pequest trout hatchery: Managing a trout stocking program

Jan Lovy, PhD
Fish Pathologist, Office of Fish & Wildlife Health & Forensics, NJ Fish & Wildlife



NEW JERSEY DIVISION OF Fish and Wildlife

Pequest Pathology Lab

Inter-agency and university collaboration

NEW JERSEY DIVISION OF Fish and Wildlife

THE STATE UNIVERSITY OF NEW JERSEY RUTGERS

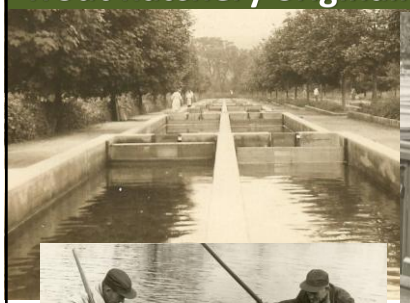
New Jersey AHDL Animal Health DIAGNOSTIC LAB

Topics to be discussed

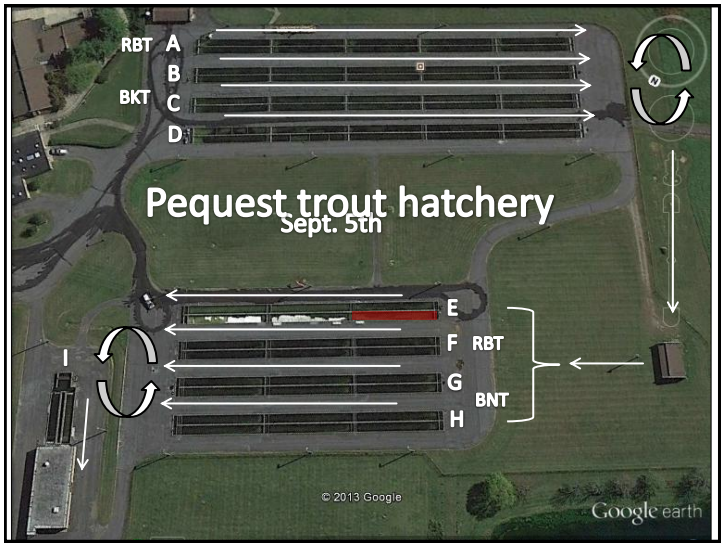
- Furunculosis in Pequest
  - Case presentation
  - Extent of the disease

- Management of the trout stocking program
  - Various strategies have been discussed
  - Divided opinions
  - Discussion/feedback on trout stocking

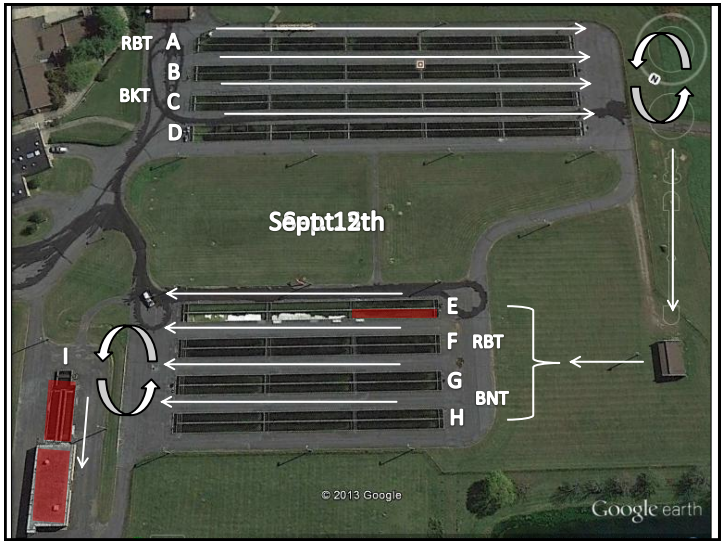
Trout hatchery originally in Hackettstown



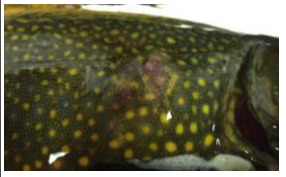
Disease problems drove the opening of the Pequest trout Hatchery in the 80's

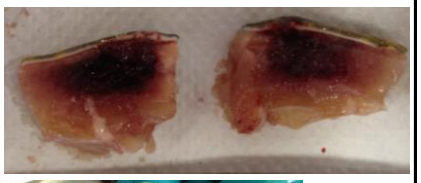


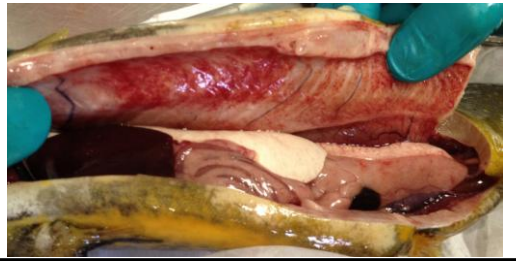
Two-year old brown trout



Broodstock brook trout



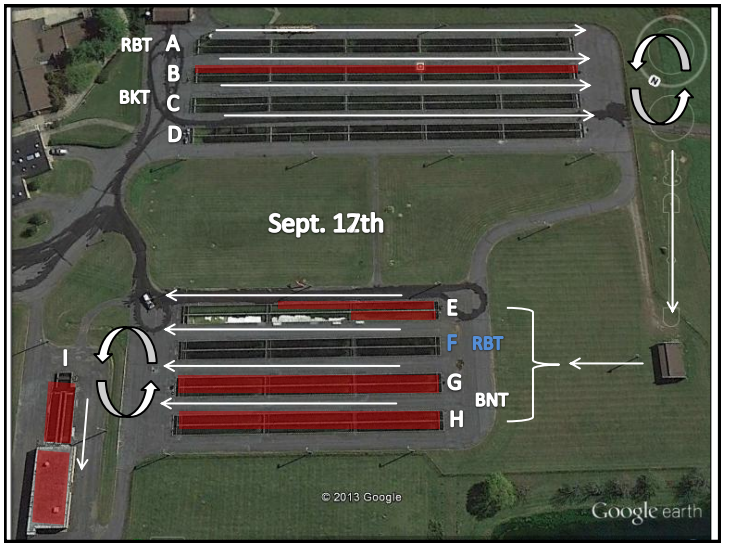




Control of the disease

Immediate action

- Two-year old fish that were scheduled to be stocked in the fall
- Eliminate the source of the bacterium to protect the other hatchery stock
- In best interest of hatchery and environment, eradication of infected fish
  - Humanely euthanized by CO_2



Furunculosis in production trout

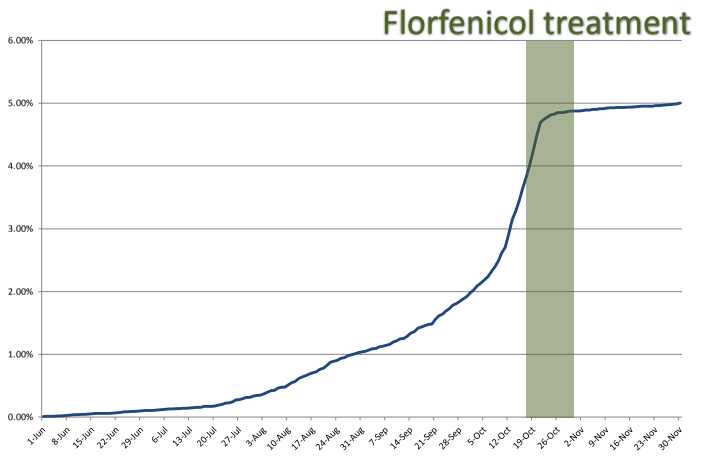
- High mortality through October with losses of over 5,000 brown trout
- Rainbow trout are resistant to disease, but can be carriers of bacterium

Control/treatment for furunculosis

Eliminating the disease from production fish

Treatment	Re-testing
Antibiotic treatment: (reduce bacteria, although will not eliminate carriers) Provide ideal environmental conditions, limit stress 6-month period of monitoring	Fish are retested 1W prior to stocking Goal to get fish clear of clinical disease signs and active infection Carriers will exist in the population

Cumulative mortality in production brown trout

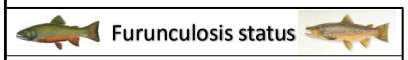


Preventative actions

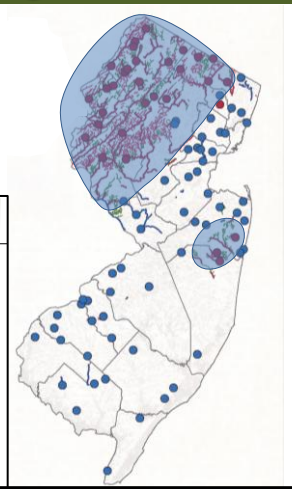
- Disinfection of hatchery system
- Practice strict biosecurity measures at the hatchery
- Increase rainbow trout production starting this year
- Vaccination of this years brook and brown trout
- Acquire furunculosis-resistant strains of brook and brown trout for future years

Trout stocking program in N.J.

- Trout production
- Trout maintenance
- Non-trout waters

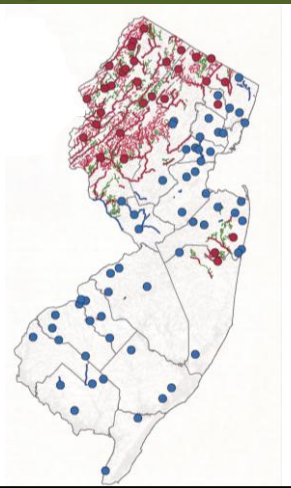


Negative	Fish that do not test positive with standard kidney culture
Positive	Fish testing positive with standard kidney culture
Carrier	Population that has tested positive, subsequently tests negative and no disease signs



Trout stocking program in N.J.

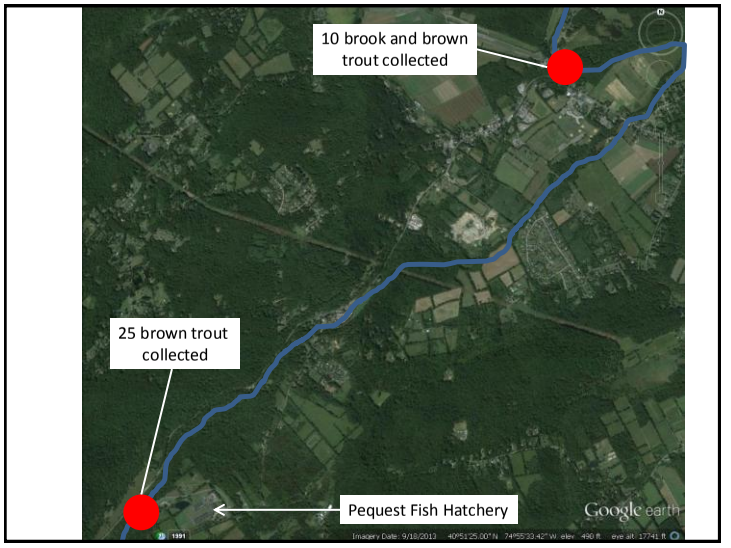
- Negative fish may be stocked throughout the state
- No positive fish will be stocked in any state waters
- Carriers will not be stocked in trout waters
- Carriers will be stocked in non-trout waters (put and take fishery)
  - Early stocking for cool water temperatures



Pequest River trout study

1) Determine what pathogens/diseases are present in the Pequest River, which may threaten the hatchery

2) Determine if active furunculosis is in the river or if fish downstream of hatchery have been affected by the disease



Fish subjected to full health analysis

Results

- All fish tested negative for furunculosis and other bacterial pathogens of concern
- One brook trout sampled upstream of the hatchery had poor condition and was positive for **Infectious pancreatic necrosis virus (IPNV)**

The Great Lakes Fish Health Committee: A Strategy to Help Achieve the Great Lakes Fishery Commission's Strategic Vision

Draft November 2013

The mission of the Great Lakes Fish Health Committee (GLFHC) is to unify and coordinate fish health management efforts of the agencies signatory to the Joint Strategic Plan for Management of the Great Lakes. The GLFHC carries out these efforts in part through the Model Program for Fish Health Management in the Great Lakes, identifying research needs, providing education, and forming partnerships with agencies and organizations outside of the committee.

The vision of the GLFHC is for Great Lakes fish to be free from epizootic pathogens and transmissible diseases that result from human actions.

The Great Lakes Fish Health Committee shall work to protect and improve the health of fish in the Great Lakes basin.

Strategies the Committee will implement to achieve its mission include:

1. DEFINING and MEASURING FISH HEALTH

STRATEGY: To define fish health and quality, develop and recommend monitoring techniques, and establish fish health standards by:

- Promoting the importance of monitoring fish populations for the presence of infection disease agents and encouraging cooperative frameworks across the agencies, as a tool for making management decisions.

Committee's Actions:

- Develop and recommend a framework for monitoring fish health in wild fish populations, including the development and use of fish health index sites and establishment of a standard for an indicator of relative health of a fish community;

- Review processes and format of fish health data collection methodology around the Great Lakes basin and recommend a consistent approach;

- Investigate quality assurance and quality control (QA/QC) programs for fish health monitoring and diagnostic laboratories and ensure consistency with national and international QA/QC initiatives.

2. DISEASE AVOIDANCE, CONTROL AND ERADICATION

STRATEGY: Coordinate fish health management efforts to avoid, control and eradicate serious fish diseases by:

- ❖ Updating the online version of the GLFHC model program as needed to ensure it contains current model policies, procedures and protocols for fish health management and remains applicable to supporting fisheries managers in making rational decisions based on sound fish health management (e.g., disposition of diseased fish). Including:
  - The GLFHC Risk Assessment tool
  - Recommendations for the safe transfer of eggs and fish into and within the Great Lakes basin.

- ❖ Providing a forum for inter-agency peer review and input to fish health issues including deviations from established guidelines.

Committee's Actions:

- ➢ Review and update the online version of the model program annually or as new relevant information becomes available;

- ➢ Assign committee members to coordinate with other national and international fish health committees to determine important issues in other jurisdictions and report back to the committee at its annual meeting;

- ➢ Request annual reports using a standard format by all member agencies reviewing their implementation of the Model Program over the past year.

3. RESEARCH

STRATEGY: Develop a long-term research strategy for aquatic animal health by:

- ❖ Identifying priority research focus areas including detection, prevention, transmission, treatment, causes, and ecological effects of key and emerging aquatic animal diseases in both the wild and in public hatchery systems;

- ❖ Promoting multi-disciplinary approaches to ecological research which clearly include aquatic animal pathogens and their influence on Great Lakes fish populations as part of all research efforts, where applicable;

- ❖ Developing close working relationships with natural resource, research and other agencies and entities with fisheries responsibilities and interests to ensure aquatic animal health research priorities are included in all Great Lakes research programs;

- ❖ Focus attention on needed research to ensure the timely development of treatment drugs and chemicals to effectively reduce or eliminate key and emerging pathogens in public hatchery systems;

- ❖ Aggregating fish health data basinwide by developing systems and decision support tools to facilitate aquatic resource management efforts and to support intensive and extensive Great Lakes research;

- ❖ Developing long-term funding resources and opportunities for aquatic animal health research and management in the Great Lakes Basin.

Committee's Actions:

- ➢ Annually revise existing and develop new GLFHC research priorities that drive aquatic animal health research agendas for the GLFC member agencies and other interested partners;

- ➢ Continue to communicate and incorporate refined and updated GLFHC research priorities into the research priorities of all natural resource, research and other agencies and entities with fisheries responsibilities or interest including, but not limited to: the GLFC Board of Technical Experts, Landscape Conservation Cooperatives, Great Lakes Basin Fish Habitat Partnership, USFWS/AFWA Multi-state Grant Program, NOAA, U.S. Department of Agriculture, Great Lakes Fishery Trust, US Forest Service and U.S. Geological Survey (HIGH PRIORITY);

- ➢ Develop factually accurate and consistent aquatic animal communication tools and outlets on new research concerning Great Lakes pathogens and their affects on aquatic communities to inform a broad range of audiences from resource agency decision makers to field staffs to the general public (Medium Priority);

- ➢ Facilitate research on priority fish health issues to include: the development and implementation of a statistically valid wild fish surveillance system that provides early warnings of new or emerging pathogens; improved understanding of how pathogens affect aquatic communities; new tools and methods to detect and control pathogens in wild fish populations; new treatment drugs and chemicals to reduce or eliminate key or emerging pathogens in public hatchery systems; and focused research on key and emerging fish pathogens to include but not limit to: bacterial kidney disease, Flavobacteria and Aeromonas spp., and viral hemorrhagic septicemia (HIGH PRIORITY);

- ➢ Develop and implement a basinwide aquatic animal health data system and associated decision support tools to facilitate fisheries management and fish health research efforts (Medium Priority);

- ➢ Develop and implement a strategy to secure long-term funding support and opportunities for aquatic animal health research and management in the Great Lakes Basin (Medium Priority).

4. EXTENSION and EDUCATION EFFORTS

STRATEGY: Increase awareness and understanding of fish health issues by:

- ❖ Providing workshops (e.g., new technology development) for member agencies, members of the lake committees and other GLFC boards and committees, and government and private fish culture personnel;

- ❖ Identifying extension needs and supporting extension services provided to other stakeholders by member agencies (e.g., private aquaculture sector);

- ❖ Preparing publications, scientific information, and other information relative to fish health protection;

- ❖ Providing information exchange on Investigational New Aquaculture Drugs (INAD), chemicals, and therapeutants;

- ❖ Supporting good fish culture practices.

Committee's Actions:

- ➢ Assign working groups to organize workshops on a topic chosen by the members to be held in conjunctions with GLFHC committee meetings;

- ➢ Meet with the lake committees to communicate the role of the GLFHC, and to identify and resolve fish health protection issues within each lake;

- ➢ Periodically review and update the model program;

- ➢ Ensure cooperation and coordination on INAD activities;

- ➢ Develop publications related to significant fish health issues in the Great Lakes;

- ➢ Develop publications related to improved hatchery sanitation and disinfection practices.

5. PARTNERSHIPS

STRATEGY: Work with natural resource agencies and other entities with fisheries responsibilities or interests to develop legislative authority and regulations to enable the eradication of fish pathogens or minimization of their spread, minimizing the rearing and release of infected fish, preventing the release of clinically diseased fish, preventing the importation of fish infected with key or emerging pathogens into the basin, limiting the transfer of fish infected with key pathogens within the Great Lakes basin, and developing response plans as needed and appropriate. Potential partners include, but are not limited to state and Federal agriculture departments, fish health committees from outside the basin, and tribal organizations.

- ❖ Encourage the consistent application of GLFHC policies, procedures and protocols in stocking and commercial aquaculture regulations and practices, First Nation/Native American agreements and local hatchery management practices;

- ❖ Encourage research institutions to consider GLFHC research priorities in developing their programs.

Committee's Actions:

- ➢ Review past and current activities to identify further potentials;
- ➢ Develop a list of stakeholders/partners;
- ➢ Encourage participation of APHIS, Department of Agriculture, and other agencies;
- ➢ Create stronger linkage between universities and research institutes/centers;
- ➢ Develop shared goals to prevent spread of fish disease;
- ➢ Define how the GLFHC provides products or services, how the committee and stakeholders will interact, and stakeholder/partnership involvement.

PROPOSED TECHNICAL ADVISORS

Bacteriology: Diane Elliot (USGS), Rocco Cipriano (National Fish Health Laboratory), Thomas Loch (MSU)

Virology: James Winton (USGS), Rod Getchell (Cornell), Sharon Cloutheir (DFO)

Molecular: Nick Phelps (University of Minnesota), Sharon Clouthier (DFO)

Nutrition: Stephen Riley (USGS), Ann Gannam (Abernathy Fish Technology Center), Dominique Bureau (University of Guelph), Jacques Rinchard (State University of New York)

Risk Analysis: Dominic Travis (University of Minnesota)

Modeling: Stephen Riley (USGS), Travis Brenden (Michigan State University)

Epidemiology: Lori Gustafson (USDA)

Parasitology: Patrick Muzzall (Michigan State University)

Risk Assessment for the Introduction or Transfer of Fish and Associated Pathogens into the Great Lakes Basin

Draft December 2013
(supersedes Horner and Eshenroder 1993)

Movement of fish and their gametes has been, and continues to be, the cornerstone of many fishery conservation and restoration programs within the Laurentian Great Lakes. Often, pathogens have invaded new geographic ranges as a result of fish importation or stocking, resulting in negative consequences for fish populations in those systems. Numerous examples can be found in the literature such as the incidence of whirling disease in the intermountain west (Bartholomew and Reno 2002). Recognizing this, the Great Lakes Fish Health Committee (GLFHC) developed and adopted a protocol to assess and minimize the risk of introducing emerging disease agents with the importation of salmonid fishes from enzootic areas (Horner and Eshenroder 1993). Outbreaks of emerging diseases in wild and cultured fishes within the basin (such as *Heterosporis* sp., largemouth bass virus, *Piscirickettsia* sp., *Nucleospora salmonis*, and viral hemorrhagic septicemia virus) have indicated a more quantifiable protocol is needed when assessing the pathogen risk of potential introductions or transfers of fish and their gametes.

National and international agencies have developed a standard, science-based process to accurately assess pathogen introduction risks associated with fish movement, collectively called Import Risk Analysis (IRA) (Amos 2004; Bondad-Reantaso 2004; Hine 2004; Kanchanakhan and Chinabut 2004; Olivier 2004; Perera 2004). Guided by this widely accepted process of IRA for fish importation and movements, the GLFHC adopted a revised Risk Assessment (RA) process in compliance with the World Animal Health Organization Aquatic Code (OIE 2013), the International Council for the Exploration of the Sea Code (ICES 2004), the Food and Agriculture Organization of the United Nations (Bartley et al., 2006), and the U.S. Fish and Wildlife Service Handbook of Aquatic Animal Health Procedures and Protocols. Specifically, the GLFHC sought to

- Develop a general Risk Assessment framework the Committee will follow to reach recommendations regarding introductions or transfers for which no standard procedures are established, or which fall outside of or in conflict with the Model Program.
- Archive each Risk Assessment for review and evaluation when similar cases arise in the future.

The GLFHC's Risk Assessment is designed to determine the likelihood of pathogen introduction into or spread within the Great Lake Basin associated with fisheries management actions such as fish and aquatic organism transfers. The Risk Assessment will also document likely risks of such actions and provide Great Lakes fisheries managers GLFHC recommendations about how to minimize any identified risks using the best available information at the time the Risk Assessment is performed.

The GLFHC Risk Assessment will not address any issues outside of the aquatic animal health considerations of any proposed introduction. The determination of the benefits of fisheries management actions along with the potential ecological or genetic effects, if any, must be part of the decision record and are the responsibility of the proponent fisheries agency(ies), appropriate Great Lakes Committee(s), and the Council of Lake Committees (CLC).

The GLFHC strongly recommends that a Risk Assessment be conducted well prior to the planned importation or transfer of fish or other aquatic organisms, particularly when the Model Fish Health Program does not provide clear guidance to fisheries managers on minimizing potential aquatic animal health risks in receiving facilities and waters. This assessment is designed to support and assist in the decision record for the proposed fisheries management action. Based on all available information, the

GLFHC will review, evaluate and provide recommendations on the proposed introduction exclusively focused on the potential aquatic animal health risks to the receiving facility or water body from the proposed management action. The term "introduction" is defined in this document to include any action in which fish and aquatic organisms and their associated gametes are being moved. These actions include fish or aquatic organism transfers, stocking, or importation.

Risk Assessment Objectives

a. Identify pathogen(s) of concern that may be introduced or transferred into the basin as a result of the proposed introduction of fish or aquatic organism, including their gametes.
b. Document potential aquatic organism disease issues to include epizootic risk associated with the proposed action.
c. Determine the most likely aquatic organism disease risks, to include the likelihood of such risks, associated with the proposed transfer or introduction of fish or aquatic organism and their gametes into the new Great Lakes waters or facilities.
d. Develop and provide Great Lakes basin fisheries managers with the GLFHC recommendation as to whether or not the proposed action to import or transfer fish or other aquatic organisms should proceed from a fish health standpoint.
e. Develop and provide Great Lakes basin fisheries managers with risk management options to eliminate or reduce the effects of the proposed action.
f. Facilitate responses to fish and aquatic organism disease questions from CLC members and other entities to the GLFHC on the proposed fish management action including the Risk Assessment process, supporting documentation, and recommendations.

Risk Assessment Procedure

The Risk Assessment is to be used in the following situations:

- A Level 1 Restricted Pathogen is detected at a member-operated facility,
- The Model Program does not provide clear guidance, or
- A proposed action is in direct conflict with the Model Program.

When one of these situations arises, the GLFHC Chairperson should be contacted by the affected agency's representative on the GLFHC to begin the Risk Assessment process. Once contacted, the GLFHC Chairperson will work with the requesting member to select the appropriate RA form (RA-1 or RA-2) and to complete a preliminary Risk Assessment. The GLFHC Chairperson will share the preliminary Risk Assessment with the entire GLFHC and solicit input from members to develop a final RA report.

Final Assessment of the Pathogen Risk Potential

The process results in a numerical score, which is placed into one of three categories of risk: low, moderate, or high. The GLFHC will provide a summary report (Form RA-3) which will focus and summarize only the most critical information that was used in the process, including its recommendation, documentation of fish health risks to naturally occurring populations of native or naturalized species, important fisheries or aquaculture resources, biological communities and habitats which may be impacted by a proposed action, and potential options for mitigation (if applicable). The summary report will be provided to all member agencies, the appropriate lake committee(s), and the CLC.

Risk Communication

Risk communication represents the interactive exchange of information on risk among risk assessors, risk managers, and other interested parties. It begins when a risk analysis is requested and continues on after the implementation of a recommendation regarding the possible translocation of a pathogen of concern.

The communication of risk should be open, interactive, and involve transparent exchange of information that may continue after the decision on translocation is made. The uncertainty in the model, model inputs, and the risk estimates in the risk assessment should be communicated between the involved parties. The entire risk assessment process should include an evaluation of uncertainty and data sources.

Instructions for Risk Assessment Forms RA-1 and RA-2

Each of the RA forms should be scored as follows:
1. Choose the appropriate option for each situation and place its associated numerical value in the small box immediately to the right of that option.
2. Multiply the numerical value by the weighting factor (in parentheses) for the situational statement and place this value in the larger box on the far right.
3. Total all of the large box scores and place this value in the **Total Risk Score** box at the bottom of the worksheet.

Example for Form RA-1

In an instance where the prevalence of a pathogen in the source population is Medium and its pathogen transmission is vertical, the first part of Form RA-1 would be filled in as follows:

Prevalence of pathogen in the source population (5)		
High (67-100) – 5		15
Medium (33-66) – 3	3	
Low (1-32) – 1		
None – 0		
Pathogen transmission through fish or their gametes (10)		
Vertical (and assumed horizontal) – 5	5	50
Horizontal – 1		
Unknown – 5		

Final Scoring

Form RA-1: For pathogen movements into a facility, the following risk potential and general recommendations apply.

Risk Score	Risk Potential	~~General~~ Recommendation
387 and below	Low	*Place fish or eggs into a standard facility; apply mitigation for pathogens as necessary. The movement must not result in a reduction of the health status of the facility. If the movement would result in a reduction of health status, the fish or eggs should be placed into isolation or quarantine.*
388 - 646	Moderate	*Place fish or eggs into isolation/quarantine. The fish should be tested a minimum of 3 times in 2 years with at*

		least 4 months between tests without the detection of a pathogen listed in the Model Program before transfer or release. Sampling should be done at the 2% prevalence level (95% confidence).
647 and above	High	*Place into quarantine. Fish may only be transferred or released based on recommendations made by the GLFHC in the Risk Assessment Summary document.*

Form RA-2: For pathogen movements out of a facility, the following risk potential and general recommendations apply.

Risk Score	Risk Potential	~~General~~ Recommendation
463 and below	Low	*Allow unrestricted movement of the fish and their gametes.*
464-743	Moderate	*Allow fish and their gametes to only be transferred to facilities or released into waters that are positive for the pathogen(s) of concern.*
744 and above	High	*Stocking and transfers are not recommended. Potential exceptions would ~~A~~allow fish and their gametes to only be stocked into the waters of origin or held in isolation/quarantine for further testing as suggested by the GLFHC.*

Recommendations to Decision-Makers

A risk assessment can result in one of three outcomes:

1. The request is recommended for approval without conditions.
2. The request is recommended for approval with conditions such that specific preventive or mitigating measures are to be followed before the proposed translocation of a potential pathogen takes place.
3. The request is not recommended for approval owing to a level of risk estimated to be unacceptable.

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MI DNR MSFH EEDV Update

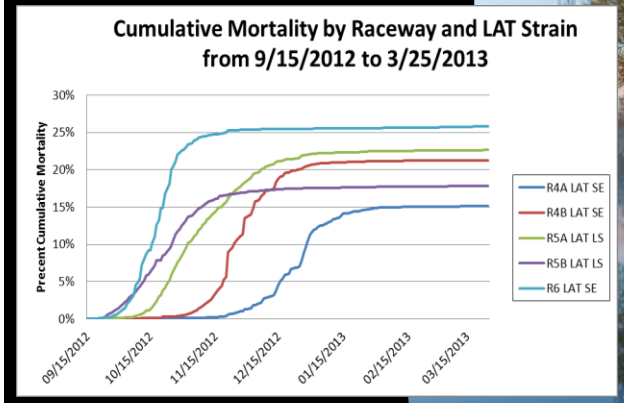
Gary E. Whelan
Program Manager
February 5, 2014

Marquette State Fish Hatchery

Site Details

- Broodstock – BKT and LAT
- Production
  - 300K SPL
  - 300K LAT
  - 50K BKT

Mortality Course at MSFH



LAT Stocking

- Essentially no mortality after January
- Fish were tested on March 15
  - All negative
- Fish were stocked in locations were fish were stocked previously

Traceback and Testing Steps

- Determine Source of Infection
  - Analyzed archived MSFH back to 2005 and Cherry Creek samples back to 2009
    - All negative – K/S/H/G
  - Source Water - Cherry Creek BNT, BKT and MOS
    - Fish collected 8/27/2013
    - BNT (60) and BKT (60) negative – K/S/H/G
    - MOS positive – PCR and gene sequencing
      - 4 of 12 gill samples positive
      - 2 of 12 K/S/H/G samples positive

2014 LAT Rearing and Stocking

- Stocking Location Options
  - Lakes Michigan and Superior – Positive
  - Lake Huron sampled 5/10/2013 (120)
    - Negative
- Rearing – Reducing Stress
  - Increase cleaning and biosecurity
  - Reduced rearing by 25%

2014 LAT Rearing and Stocking

- Increased Tested on Inventory
  - Individual production samples – 5/5/2013
    - 40 fish – All negative with gill tissue
  - Broodstock fish – 8/20/2013
    - 2001, 2003, 2004 – 10 fish each – K/S/G
      - All negative
    - 2012 – 60 fish – K/S/G – All negative
  - Production fish – 8/20/2013 – All negative
    - LAT-SE – 60 fish – K/S/G – 60 fish
    - LAT-LS – 60 fish – K/S/G – 60 fish

2014 LAT Rearing and Stocking

- Increased Tested on Inventory
  - Final fish health sampling done in January
  - 60 fish from each production raceway
    - 240 fish
- Stocking will depend on results

Update on Thiamine Deficiency Research

Great Lakes Fishery Commission Fish Health Committee
State College, PA, February 4, 2014

Dale Honeyfield
Northern Appalachian Research Lab
Wellsboro, PA

Three topical areas will be presented

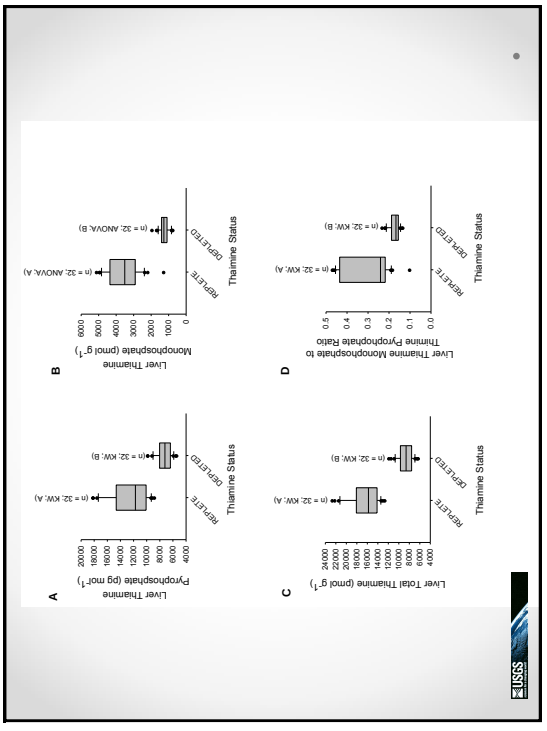
Immune function – fish health
Gene expression
American eel decline

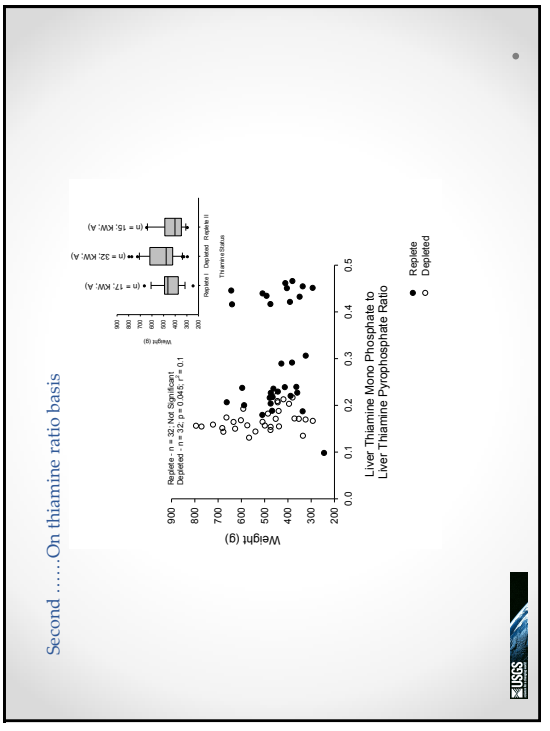
In vitro immune function in thiamine-replete and -depleted Lake trout (*Salvelinus namaycush*)

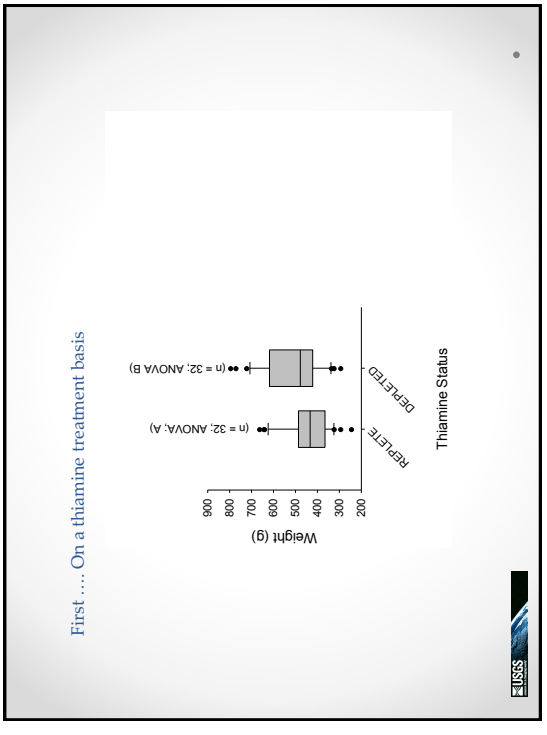
Christopher A. Ottinger[1], Dale C. Honeyfield[2], Christine L. Densmore[1], and Luke R. Iwanowicz[1]

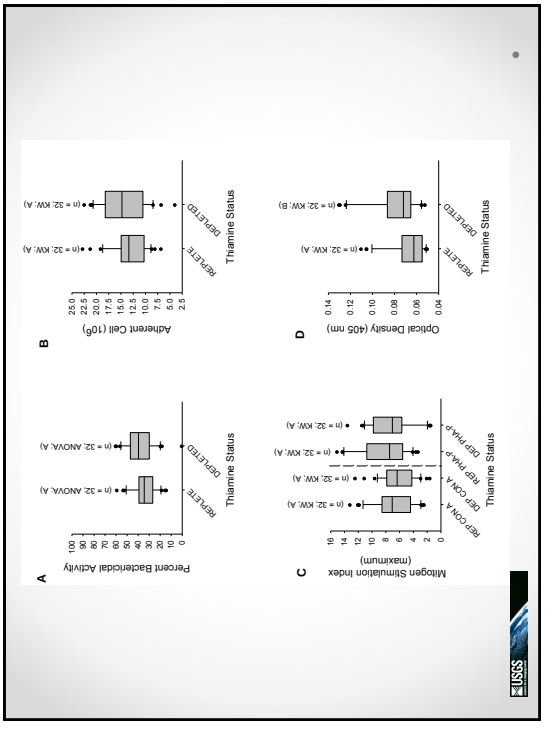
In Vitro Study - Purpose

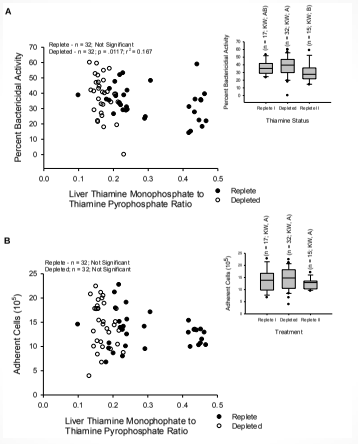
- Leukocyte mitogenesis and macrophage bactericidal activity as determined *in vitro*
- Functional relationships with tissue thiamine concentrations in test environment
  1) thiamine replete and
  2) removed from immediate neuroendocrine regulation

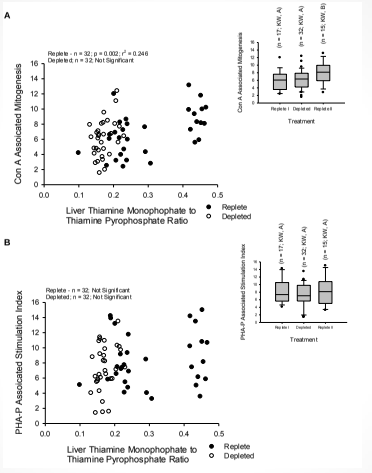

A
Liver Thiamine Pyrophosphate (pg mol^{-1})
B
Liver Thiamine Monophosphate (pmol g^{-1})
C
Liver Total Thiamine (pmol g^{-1})
D
Liver Thiamine Monophosphate to Thiamine Pyrophosphate Ratio
REPLETE
DEPLETED
Thiamine Status
(n = 32; KW: A)
(n = 32; KW: B)
(n = 32; ANOVA: A)
(n = 32; ANOVA: B)


Second ……On thiamine ratio basis
Weight (g)
Liver Thiamine Mono Phosphate to
Liver Thiamine Pyrophosphate Ratio
Replete
Depleted
Replete = n = 32; Not Significant
Depleted = n = 32; p = 0.043; r^2 = 0.1
Replete
Depleted
Thiamine Status
(n = 32; KW: A)
(n = 32; KW: A)
(n = 15; KW: A)

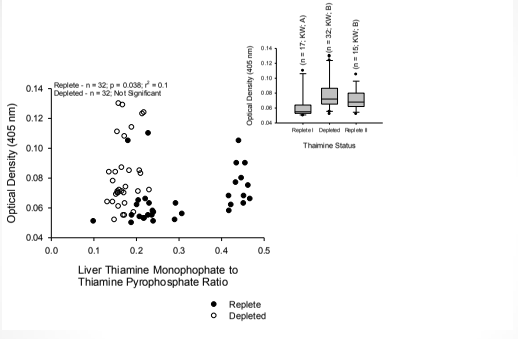

First …. On a thiamine treatment basis
Weight (g)
REPLETE
DEPLETED
Thiamine Status
(n = 32; ANOVA: A)
(n = 32; ANOVA B)


A
Percent Bactericidal Activity
B
Adherent Cell (10^6)
C
Mitogen Stimulation Index
(maximum)
D
Optical Density (405 nm)
REPLETE
DEPLETED
Thiamine Status
REF_CON_A
DEP_CON_A
REF_PHA-P
DEP_PHA-P
(n = 32; KW: A)
(n = 32; KW: B)
(n = 32; ANOVA: A)





Cell Proliferation



Thiamine-Limited Condition and Lake Trout Leucocyte Function

- Oxidative stress
- Altered cellular redox condition
- Cell-type specific sensitivity

- Favors inflammatory response
- Favoring inflammatory response may occur at the expense of immunologic memory development
- May alter neuroendocrine response thus immunoregulatory environment

Thiamine-Limited Condition and Lake Trout Leucocyte Function: Potential Population Consequences

- Reduced development of immunologic memory
- Increased percentage of population susceptible to disease associated morbidity and mortality by increasing relative percentage of "naïve" fish
- Dysfunction aids in the maintenance of disease within population thus increasing risk to population over time
- May alter perceived vs. actual disease susceptibility (e.g.. Do thiamine-replete and thiamine-limited lake trout have the same susceptibility to diseases like VHS?)

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In-Vivo challenge to Lake trout from thiamine replete and deficient eggs

F1 *Aeromonas salmonicida* Exposures - Questions

- Does parental thiamine status impact immunocompetence and thus disease resistance in the F1 generation when the F1 generation is thiamine replete?
- Does parental thiamine status impact the embryonic and early post-hatch development of a fully functional immune system?

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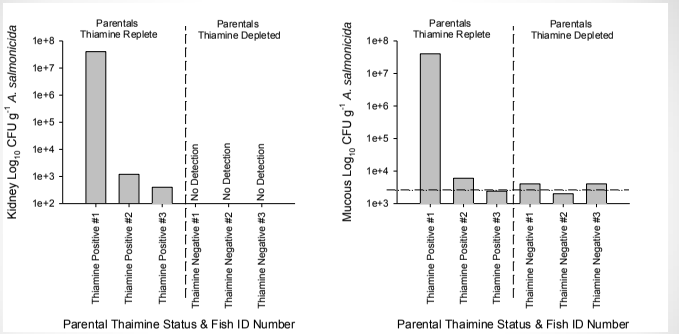
Primary Challenge

F1 Generation from both Thiamine-Replete and –depleted Parental stocks were on the same Thiamine-Replete Diet



Parentals Thiamine-Replete: F1 30% Post Exposure Mortality
Parentals Thiamine-Depleted: F1 0% Post Exposure Mortality

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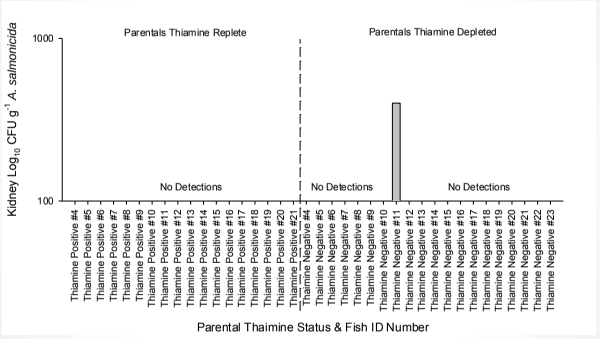
Secondary Challenge

Kidney



Parentals Thiamine-Replete: F1 0% Post Exposure Mortality
Parentals Thiamine-Depleted: F1 0% Post Exposure Mortality

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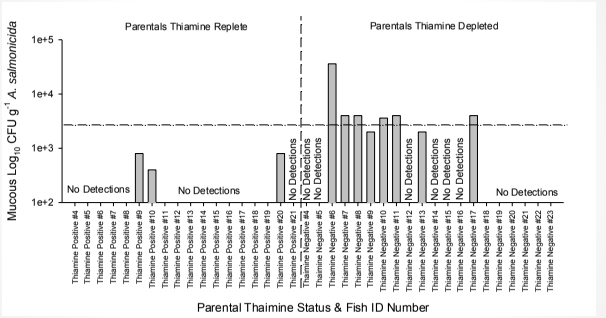
Secondary Challenge

Mucous



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F1 *Aeromonas salmonicida* Exposures –Thoughts

- The replete parental F1 group is responding as expected.
- Some of the thiamine-depleted parental F1 group are responding as expected.
- However some of the thiamine-depleted parental F1 group are responding like they would when faced with a high or low dose IHNV (viral) exposure. This is a very atypical response to a virulent *A. salmonicida* exposure

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F1 *Aeromonas salmonicida* Exposures – Immediate Questions

- Are the F1s from the depleted parental stock mounting a hyper innate-immune response like some virus exposed fish?
- What were the anti – *A. salmonicida* specific antibody concentrations in the two groups.
- What did the cytokine environment look like?
- Is this repeatable?
- Do Pacific salmon respond in the same way?

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F1 *Aeromonas salmonicida* Exposures and Beyond – What could this mean at the population level

Thiamine replete group retained genetic heterogeneity

In terms of disease susceptibility, heterogeneity in immune function means
1) Some fish clear the infection without morbidity;
2) some fish die;
3) some fish recover from the infection but fail to clear the pathogen – become carriers.

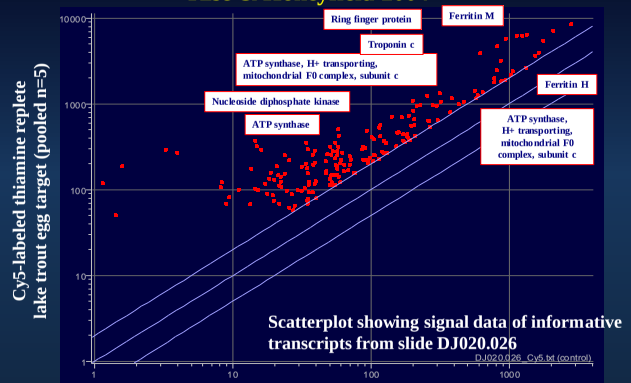
Elevated # carriers from thiamine-associated immunodulation cause shifts the population disease dynamics
- naïve individuals within the population have a greater risk of exposure and disease development.
- Resulting in a ripple effect that alters population age class structure and eventually population reproductive potential

Expression of three *thiamine transporter*-like genes and *thiamine pyrophosphokinase* gene in tissues of rainbow trout

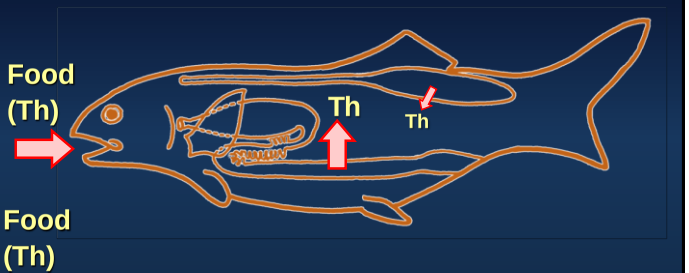
S. Yuge[1], D. C. Honeyfield[2], and W. Li[1]

[1] Dept. of Fisheries & Wildlife, Michigan State University, USA
[2] USGS, Leetown Science Center, USA

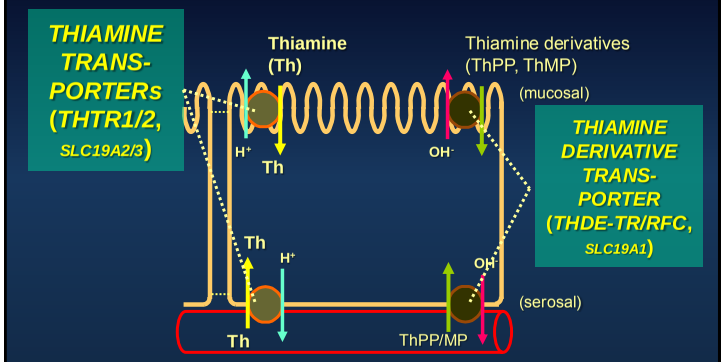
211 genes are reproducibly > 2-fold down-regulated in thiamine deficient lake trout eggs relative to thiamine replete eggs. Rise & Honeyfield 2004



Thiamine absorption ⇒ Intestine, kidney



Genes in Thiamine Transport



{Ganapathy et al. 2004; Lonsdale 2006; Bettendorff and Wins, 2009; Jurgenson et al., 2009}

Transketolase (tpk) in pentose-phosphate pathway requires thiamine (Th)

Glucose

↓

Glucose-6-phosphate

↓

Pentose Phosphate pathway

Transketolase-thiamine pyrophosphate

↓

Ribose-5-Phosphate

↓

Nucleic acids

Rainbow trout study

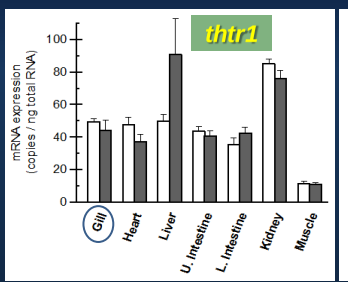
1. Thiamine replete controls
2. Thiamine deficient

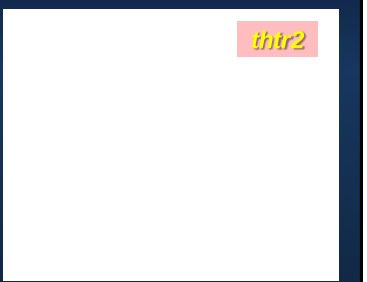
Fed diets containing bacterial thiaminase

mRNA expression in normal ( ) and TD ( ) tissues

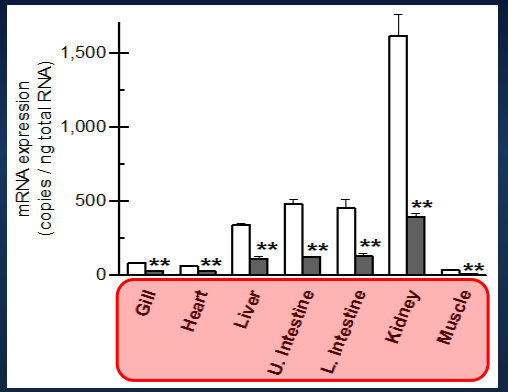
thtr1 : no change in TD

thtr2 : reduced in TD in intestine

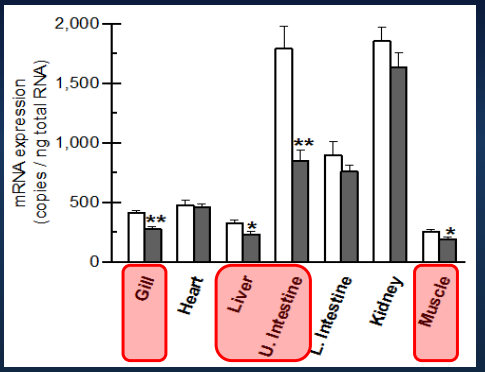




thde-tr1 **: reduced in TD in all tissues examined**



tpk_all tv **: reduced in response to TD in gill, liver, upper intestine and muscle**



Summary:

1. Thiamine deficiency reduces thiamine transport in 2 of 3 carriers

2. Thiamine transport was found in gill

3. Kidney damage may reduce reabsorption of thiamine

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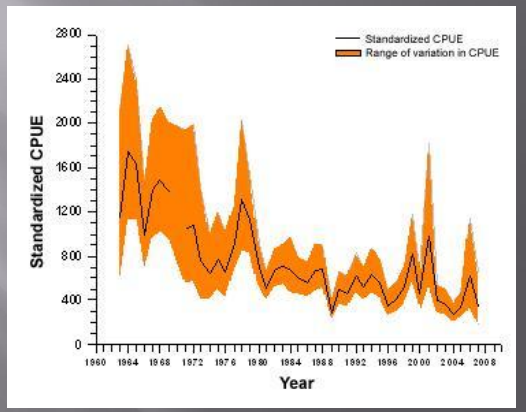
Swimming performance of American eel

Dale Honeyfield[1], Steve McCormick[1] and Ken Oliveira[2]

[1]US Geological Survey, Leetown Science Center
[2]University of Massachusetts Dartmouth

USGS

Problem: Decline in eel population



Yves de Lafontaine, Environment Canada

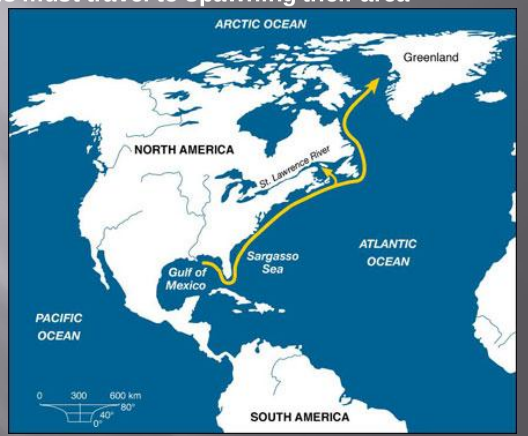
USGS

Dams are an impediment

but Dams are only one of many impediments

USGS

Eels must travel to spawning their area



Yves de Lafontaine, Environment Canada

USGS

Ketola et al. 2005. Thiamine status of Cayuga Lake rainbow trout and its influence on spawning migration. N. Am. J. Fish. Man. 25:1281-1287.

Fitzsimons et al. 2005. The Effect of Thiamine Injection on Upstream Migration, Survival, and Thiamine Status of Putative Thiamine-Deficient Coho Salmon. J. Aquat. Anim. Health 17:48-58.

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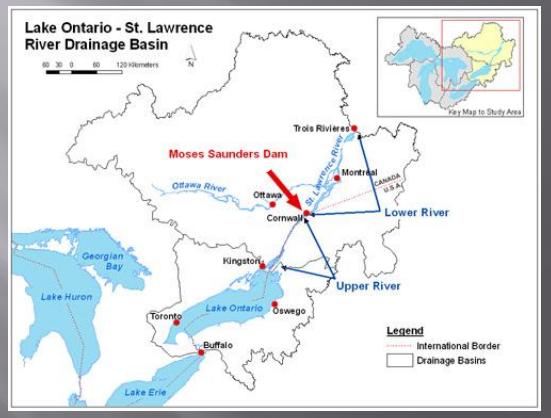
Eel samples ● collected by Fitzsimons



Fitzsimons et al.

USGS

Eel Thiamine, nmol/g

- Fitzsimons et al. 2013

Site	Year	Mean	Range	
Port Weller	2003	1.73	.55 - 4.14	
Dam	2005	1.67	.72 - 3.11	
La Pocateire	2005	1.59	.62 - 2.69	High Mirex
	2005	3.65	.75 - 15.87	Low Mirex

USGS

Based on stable isotope data Lake Ontario eels feed on alewife containing thiaminase

Alewife also has other nutritional considerations

USGS

Swimming Eel Experiment

Glass eels were reared in lab for 1- 2 years

Then eels were
Placed on fish meal based diets
Thiamine 10 mg/kg
Thiamine 0 mg/kg + bacterial thiaminase
0.4 mg/kg thiamine (Honeyfield et al. 2005)

And challenged to swimming test

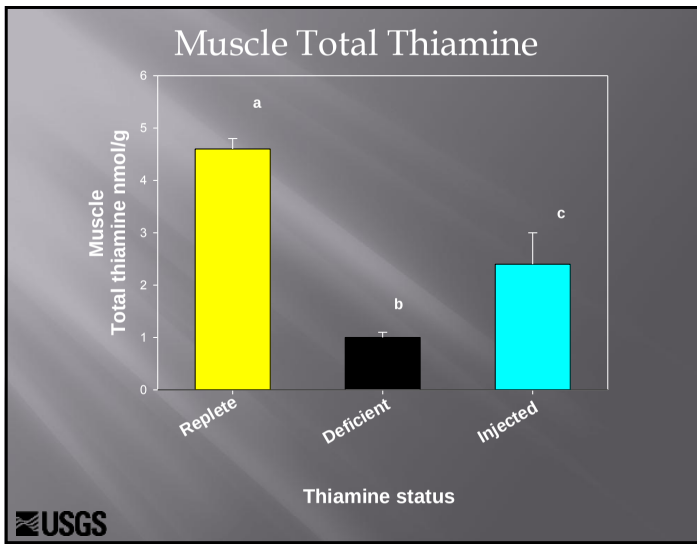
USGS

Critical swimming performance

Start @ 10cm/sec for 1 hour

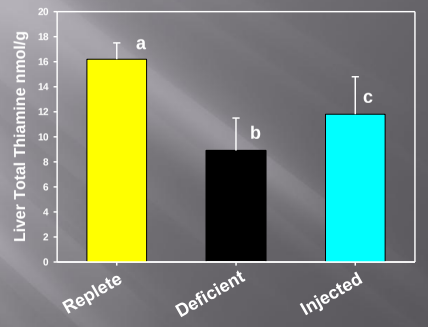
then every 20 min
increased flow by 10 cm/sec
until exhaustion

USGS

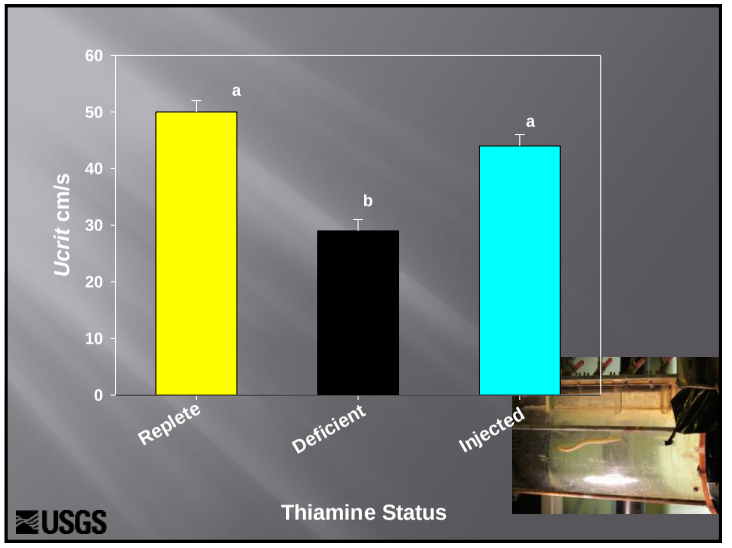


USGS

Liver Total Thiamine



USGS



USGS

How does swimming eel thiamine relate to data of Fitzsimons et al.?

USGS

Eel Thiamine, nmol/g - Fitzsimons et al.

Site	Mean	Range	%< 1.3 nmol	
Port Weller	1.73	.55 - 4.14	(53%)	
Dam	1.67	.72 - 3.11	(44%)	
La Pocateire	1.59	.62 - 2.69	(50%)	High Mirex
	3.65	.75 - 15.87	(15%)	Low Mirex

USGS

What about
Eel egg thiamine and reproduction?

No published data

USGS

If you think working on the Great Lakes is a challenge getting samples……

USGS

Ken Oliveira

Captured Saint Lawrence River eels
&
Spawned in the laboratory

USGS

Male American Eels

Male Maturation

Weekly HCG Injections

Female Maturation

Weekly SPE Injections

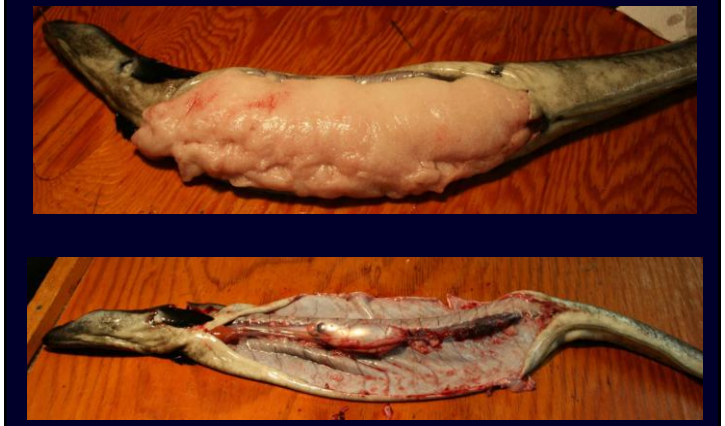
Female American Eels

Induced Ovulation with

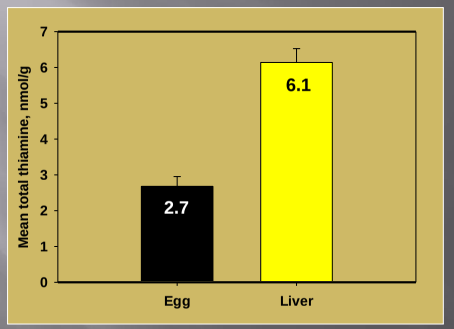
17α,20β-Dihydroxy-4-pregnen-3-one (DHP)

Stripping Eggs Prior to Fertilization

14-20 hrs after DHP injection



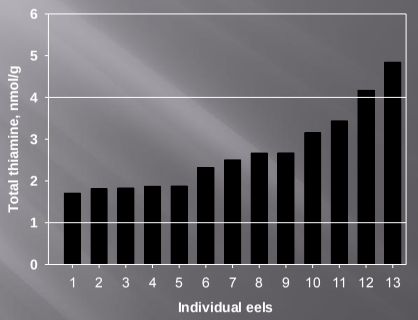
Eel Total Thiamine, nmol/g



Liver thiamine = 8.9 nmol/g in low B_1 group in swim test

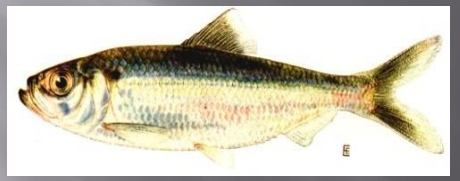
USGS

Egg Eel Total Thiamine, nmol/g



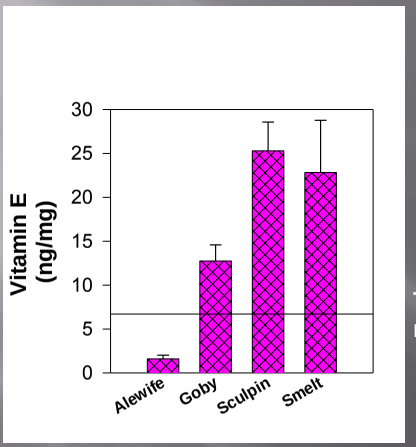
USGS

Eels feed on Lake Ontario Alewife resulting in thiamine deficiency



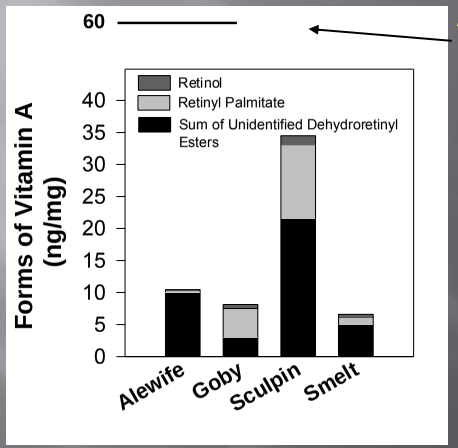
Other nutritional considerations

USGS



Trout and salmon requirement ~6.7 ng/mg

USGS



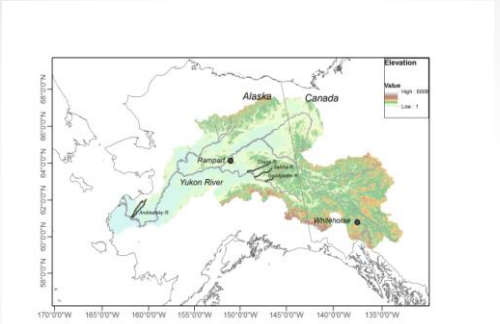
Trout & salmon requirement ~60 ng/mg

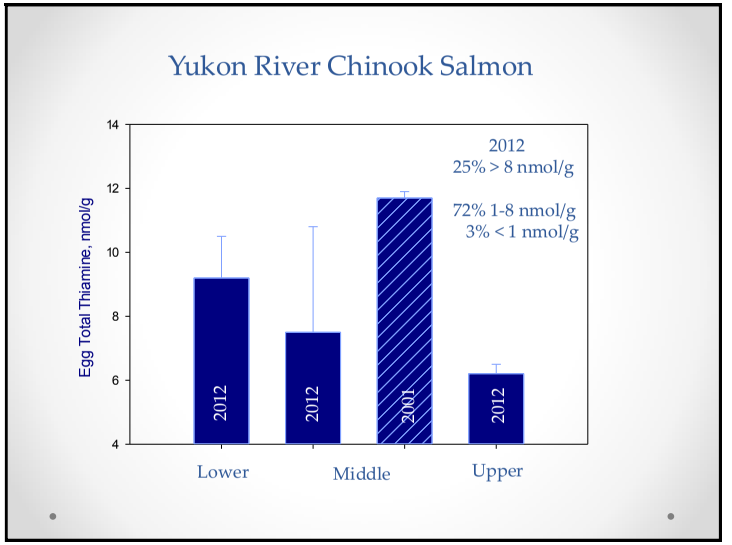
USGS

Lake Ontario Alewife have low concentration of vitamin E & A

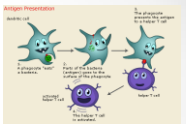
Increased risk of oxidative stress vitamin E , A and B_1 are Antioxidants

USGS





USGS
science for a changing world



Questions ?

Immune function – fish health
Gene expression
American eel thiamine study

BKD at Marquette State Fish Hatchery

History

- A Chronic level of BKD was present as early as 1922 to 1960 and discontinued with the end of the brook trout program at MSFH
- Also present was Furunculosis
- Brook trout program returned to MSFH in the mid 1980's
- Temiscame BKT tested positive for Furunculosis in 1993 and the strain was eliminated in 1994
- Assinica BKT tested positive for Furunculosis the following year

History continued

- Some early attempts of furunculosis vaccination occurred, with some positive results.
- In 2001, BKD was detected in our Iron River strain brook trout
- 2002 BKD and furunculosis were prevalent in all our cultured species at some level
- Concussion was both pathogens are in the water supply and fish of Cherry Creek

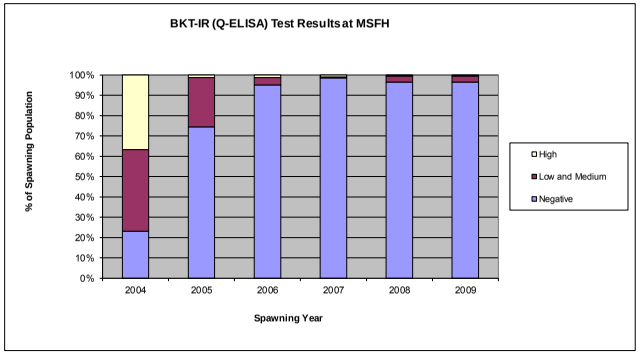
Fish On-site prior to BKD treatments

- **Broodstock fish species and strains**
  1. Lake trout Marquette strain
  2. Brook trout Assinica strain (1 and 2 year olds plus breeding 3 and 4 year olds)
  3. Brook trout Iron River strain (1 and 2 year olds plus breeding 3 and 4 year olds)
  4. Brook trout Nipigon strain (not breed)
- **Production fish species and strains**
  1. Lake trout Marquette strain
  2. Brook trout Iron River strain
  3. Brook trout Assinica strain
  4. Brown trout Wild Rose strain
  5. Splake

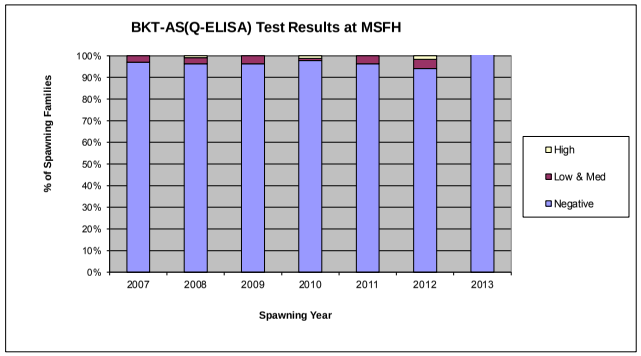
Top 13 Treatments

1. Reduced broodstock numbers and strains (which increased water flow)
2. Reduced production fish numbers and strains (which increased water flow)
3. QELISA testing of individual breeding fish to obtain negative families
4. Furunculosis vaccinated all broodstock and production fish
5. BKD vaccinated all broodstock and production fish
6. Erythromycin injections on all broodstock fish
7. Erythromycin water hardening of all eggs
8. Juvenile broodstock on well water for two years
9. UV disinfection of Cherry Creek water supply for adult broodstock
10. Breeding younger aged broodstock
11. New Lake Superior Strain Lake trout
12. Stopped importations of production brown trout
13. External parasite treatments on all fish

QELISA results Iron River Strain



QELISA results Assinica Strain



Fish On-site after BKD treatments

- **Broodstock fish species and strains**
  1. Lake trout Lake Superior strain (No positive QELISA tests in 13 years)
  2. Brook trout Assinica strain (1 plus breeding 2 and 3 year olds)
- **Production fish species and strains**
  1. Lake trout Lake Superior strain
  2. Brook trout Assinica strain
  3. Splake

Conclusion

- Reduced stress on all fish species
- Increase vaccinations and QELISA testing