

GREAT LAKES FISH HEALTH COMMITTEE

2013 Summer Meeting
Duluth, Minnesota
August 7-8, 2013

Minutes
(with attachments)

Submitted By:

Christina Haska
Great Lakes Fishery Commission

The data, results, and discussion herein are considered provisional; permission to cite the contents of this report must be requested from the authors or their agency.

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

Table of Contents

List of Attendees	3
Meeting Agenda.....	4
Minutes	
Welcome and Introductions	6
Approval of Meeting Minutes.....	6
GLFC Update	6
Model Program Update	6
APHIS Update.....	6
Fish Health Program at the Great Lakes Aquarium	7
Wisconsin Department of Agriculture Trade and Consumer Protection.....	7
Roundtable Discussion on Unusual Fish Disease Cases	8
Detection of Cutthroat Trout Virus in the Mountain Prairie Region	9
University of Minnesota Aquatic Health Research Update	10
Research Priorities	11
Risk Assessment.....	11
EEDv	11
Technical Advisors.....	12
Future Meetings.....	12
Agency Updates	12
Appendices.....	
1: USDA APHIS Aquaculture Update	15
2: Wisconsin Fish Regulatory Issues	23
3: Unique Aquatic Clinical Cases	32
4: Fish Disease Cases on Fish Farms.....	48
5: Detection of CTV- Where Are We Now?.....	59
6: Newly Described Microbial Agents of Fish	66
7: Research Priorities of the GLFHC.....	74
8: Risk Assessment Forms	76
9: Cutthroat Trout Virus in Wisconsin.....	81
10: My Summer: Atlantic Salmon Virus and More	83
11: Mortality Event at Crystal Springs State Fish Hatchery	85

List of Attendees

John Dettmers	Great Lakes Fishery Commission
Christina Haska	Great Lakes Fishery Commission
Sunita Khatkar	Fisheries and Oceans Canada
Sue Marcquenski	Wisconsin Department of Natural Resources
Dave Meuninck	Indiana Department of Natural Resources
Paula Phelps	Minnesota Department of Natural Resources
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:

Nick Phelps	University of Minnesota
Myron Kibus	Wisconsin Department of Agriculture
Janet Whaley	USDA- APHIS
Lacey Hopper	U.S. Fish and Wildlife Service- Bozeman Fish Health Center
Tom Monahan	Minnesota Department of Natural Resources
Jay Walker	Great Lakes Aquarium

Great Lakes Fish Health Committee Meeting

August 7-8, 2013

Inn of Lake Superior
350 Canal Park Drive
Duluth, MN 55802

Agenda

Wednesday, August 7

- 8:30 am – 8:45 am Welcome & Introductions (Shen)
- 8:45 am – 8:50 am Approval of Meeting Minutes (Shen)
- 8:50 am – 8:55 am CLC update (Dettmers)
- 8:55 am – 9:05 am Model Program Update (Shen/Dettmers)
- 9:05 am – 9:45 am APHIS update (Whaley)
- 9:45 am – 10:00 am Fish Health Program at the Great Lakes Aquarium (Walker)
- 10:00 am – 10:30 am Roundtable Discussion on Unusual Fish Disease Cases
- 10:30 am – 10:50 am Break
- 10:50 am – 12:00 pm Roundtable Discussion, cont.
- 12:00 pm – 1:30 pm Lunch (on your own)
- 1:30 pm – 2:00 pm WI Dept. of Agriculture Trade & Consumer Protection (Kebus)
- 2:00 pm – 2:30 pm Detection of Cutthroat Trout Virus (CTV) in the Mountain-Prairie Region: Where are we now? (Hopper)
- 2:30 pm – 3:00 pm University of Minnesota Aquatic Health Research Update (Phelps)
- 3:00 pm – 5:00 pm Visit French River SFH

Thursday, August 8

- 8:30 am – 9:00 am GLFHC Webpage (Christina and Subcommittee)
- 9:00 am – 9:45 am Review & Discussion of Research Priorities (Dettmers/Phillips)
- 9:45 am – 10:30 am Risk Assessment (Shen, Subcommittee)
- 10:30 am – 10:45 am Break
- 10:45 am – 11:10 am Risk Assessment, cont.
- 11:10 am – 11:30 am EEDv @ Marquette SFH update (Whelan/Phillips)
- 11:30 am – 12:00 am Agency Updates— (All)
- 12:00 am – 1:30 pm Lunch
- 1:30 pm – 3:00 pm Agency Updates— (Continued, All)
- 3:00 pm – 3:15 pm Technical Advisors-should we add new members? (Shen/Dettmers)
- 3:15 pm – 3:30 pm Future meetings (Shen)
- Dates/location for summer 2014 Meeting
- 3:30 pm – 4:00 pm Meeting Wrap-up/Parking Lot (Shen)
- 4:00 pm Tour Great Lakes Aquarium

Wednesday, August 7, 2013

1. Welcome and Introductions (Shen)

There was some confusion with the draft agenda sent via email, and Ling apologized for the miscommunication. A new agenda was created and emailed to the committee.

2. Approval of Meeting Minutes (Shen)

Minutes were approved pending changes.

3. CLC Update (Dettmers)

Ken gave the council a nice overview about the current thinking of VHS rules and regulations at the federal level. This helped with the preparation of the APHIS letter.

4. Model Program Update (Shen)

The final edited copies are in the hands of the commission for copyedits. The document will be online before being published, which will happen by the end of the year. The risk assessment should be completed around the same time.

5. APHIS Update (Whaley)

See Appendix 1 for the presentation.

- After January, when National Animal Health Laboratory Network (NAHLN) labs will be invited to be fish health, it's unlikely there will be a rush of labs joining. There is limited surveillance money. It will likely be a voluntary decision, and laboratories will have choices.
- APHIS will not get involved with the interstate transfer of fish in regional zones until the states require it; therefore, there will not be a requirement for a NAHLN lab to test the fish.
- The sequester will not affect the NAHLN program. APHIS will not be asking for additional funds.
- ISAv surveillance is based on molecular techniques (PCR) and cell culture. Labs do not look for antibodies in the serum.
- It was not difficult to get the farm fish community open to testing; however, they wanted to make sure communication and outreach were ready for questions from the press and activists. This involved what tests were being run and how the system was different from that of Canada. The main concern was public perception, so outreach ensured all the states had the same voice. It's been a positive experience.
- There's a push by private aquaculture to change its practices, which may have risky implications for VHS and other diseases. For instance, Michigan is currently having this conversation, and the DEQ is involved due to permitting issues (the plan is on the Michigan SeaGrant website). APHIS has funded a project to assess which aquaculture practices should be the model example. If APHIS rescinds the federal order, there should be best management practices in place that everyone can agree on and do. The certification process also needs to be

reviewed, not just for VHS, but other diseases too. Different pathogens may require different approaches for best management practices depending on their specificities and epidemiology.

- Wisconsin DNR is reviewing its VHS rules. It's unclear who is driving the request or what problems they are trying to overcome. It's counter-intuitive, but now that everyone has followed the federal regulations and VHS isn't a major issue, people are thinking the regulations are not necessary. The APHIS risk assessment will be out this fall, and could be useful to defend the current rules with the Wisconsin DNR.
- Federal orders are for emergency quarantine situations. With VHS, the order either needs to be modified or rescinded, which means APHIS either will keep the regulations or come up with best management practices. The order cannot go in indefinitely, so the question becomes what level of federal oversight is beneficial/necessary.
- Talks have begun about creating pathogen zones. Several health assessments have been done recently, and those involved are questioning what is necessary to be qualified as "pathogen-free."

6. Fish Health Program at the Great Lakes Aquarium (Walker)

The Great Lakes Aquarium educates the public on fish and their health issues. Approximately 127,000 people come through the doors every year, and one mission of the aquarium is to get information out. If the committee would like to use the aquarium as a medium for doing this, let Jay know and he could work with the members. For instance, an invasive species exhibit was installed two years ago using goldfish as the ambassadors/poster children. Kids can relate to putting their goldfish into ponds when they get too big to keep, and this proved to be a great sounding board to get information across. The aquarium is looking for collaboration opportunities with other aquariums and institutes as well.

7. Wisconsin Department of Agriculture Trade and Consumer Protection (Kebus)

See Appendix 2 for the presentation.

- There are similarities between NPIP and aquaculture, but doing an official pilot study on a local or regional level would be difficult because there are many layers involving multiple agencies and states.
- In a sense, we already have a pilot program with the Model Program, which is similar to the NPIP.
- The NAAHP touches on things that should be in a model program. Natural resource agencies and the industry need to come together and agree on the system.
- One difference, however, is that chickens and turkeys are closely related. Lots of different fish, both native and non-native, give the fish system a level of complexity that poultry doesn't have. That said, there may be fewer species of poultry, but the range of pathogens is similar in number.
- *S. palorum* is vertically transmitted. The industry looked at it and realized that taking care of the breeder birds would reduce problems. This definitely helped the program. For fish diseases that

are not vertically transmitted, this wouldn't help, but maybe people could rally behind a vertically transmitted fish disease?

- Would it be worth the committee's time to write a 'Lessons Learned' from the VHS situation? What would we do differently? The same? It may help if another disease becomes a problem. Likely the federal government would not respond the same way.

8. Roundtable Discussion on Unusual Fish Disease Cases (All)

See Appendix 3 for Mohamed's Examples.

Case #1: A freshwater drum from Lake St. Clair was delirious. Upon examination, there was a 1 cm ulcer with a depression in the middle, and smaller ones were present with signs of healing. Looking further, there were more issues, and leeches were found. Sometimes the leeches were tiny, sometimes they were up to 20 grams in weight. In 91 % of the cases, the leeches were on the gills. It was *Actinobdella pediculata*.

Sue has seen issues with leeches in the mouth of walleyes from angler reports. Mohamed has seen this in Michigan and the life cycle of the leach is direct.

Case #2: In the gills of largemouth bass in Lake St. Clair, there were little nodules. They were glochidia--- little mussels. The life cycle is interesting, and they were seeing the larvae. When the mussels grow, they attach to a piece of the gill. After maturing, they drop from the fish to the seafloor bottom and grow into mature mussels.

Case #3: A rock bass from Lake St. Clair had a large strawberry-like shape by the heart. A smear was made and spores were found. It was a *Myxosporidian* cyst.

Case #4: Kent Lake had a common carp fish kill. The gills of the carp were dirty and tissue was gone. Microscopy didn't help much, but electronic microscopy showed it was a virus. It was determined to be koi herpesvirus.

Case #5: In Lake Michigan diporeia, a structure was found lying in areas by the muscle. Spores of different shapes were seen through microscopy, and electron microscopy showed detail of their structures. It was a dinoflagellate in the genus *Haplosporidium*. It is now accepted as a new species: *H. wintersii*.

Case #6: In 2001, Chinook salmon were covered in lesions on fish and the lungs were covered in whitish material. The bacteria were gram positive. Elisa testing showed it was BKD.

Case #7: Atlantic salmon from LSSU would grow for a bit, but then their tails were turn black and they started dying. It looked like *Myxobolus*, but lacked characteristics. PCR testing was negative. It was either another spore-forming organism or it was indeed *Myxobolus* and the infection healed.

Mohamed has starting building an online diagnostic key. Once it's done, he will negotiate with USFWS for people to subscribe to it. It should be done by the end of the year.

See Appendix 4 for Myron's cases.

Case #1: Channel catfish mortalities were investigated. The fish were found at the surface of the water, gasping for air. The problem was diagnosed as a lack of oxygen due to various factors of the pond. A mechanical aerator/agitator was used to increase oxygen absorption.

Case #2: A trout farm was experiencing ~50% mortality rates, and bacterial gill disease was found in the fish. Treatments were not working. There was a lot of uneaten food and fecal matter in the water column, so the suggestion was made to build baffles to quickly rid the tank of suspended solids. This resolved the issue.

Case #3: A yellow perch farmer was experiencing 10% mortality/day of his fingerlings in a brand new tank. The fish had signs of hemorrhages on their fins, tails, and noses. The cause was elevated nitrogen levels in the water. Salt was added and the mortalities decreased. After 3 months, the issue was gone as a bacterial population was established to support the density of fish.

Case #4: Goldfish were shipped from a pond in Missouri (60° water) to a facility in Wisconsin (50° water). Within days, the fish were showing signs of disease and flashing against the sides of the tanks. Some died. A veterinarian determined parasites were activated by the temperature change. An effort was made to increase the temperature of the holding tanks, but all the fish were eventually lost.

Case #5: A clean hatchery (free from pathogens) began to experience problems with trout, where the fish got red patchy lesions on the skin and fins. These fungal infections were due to settled solids in the recirculating system. Once that was cleaned out, the problem went away.

Case #6: Koi were showing signs of disease which appeared to be viral from lab work which was done. It turned out to be the first case of Koi Herpes Virus in the U.S.

Case #7: Farmed rainbow trout had lesions that looked like furunculosis. They couldn't be sold to markets because they looked unhealthy. It was found to be *Aeromonas hydrophila*.

Overall, recirculation systems in Wisconsin have not been successful, even with substantial monetary investment (over \$35 M). It was thought that only a few disease agents were the problem, along with needing to pay more attention to nitrogen/nitrite levels in the water. Diseases kept happening, including *Myxobolus neurophilus*, Ulcerative Dermal Necrosis, Tetrahymena, and White Tail Syndrome. Hydroponic tanks can also be used to raise fish (e.g., tilapia) alongside plants, and this also resulting in disease outbreaks (such as *Streptococcus* infections).

9. Detection of Cutthroat Trout Virus in the Mountain Prairie Region: Where Are We Now? (Hopper)

See Appendix 5 for the presentation.

- There were negative and positive controls with the testing. Three strains were females which they knew were positive. All positives were disinfected.
- Water hardening was done at 50 ppm. The eggs go in a tank with an upwelling system.

- There are theories about how this virus made its way to the Midwest. It appears all the strains are related to the California strains in the 1980s. In 2008, methods in the testing lab were changed and new cell lines were brought in. That may have contributed to finding it throughout the country, in addition to tweaking protocols. Once people saw photos of CTv and its symptoms, many people had seen it already, so it's likely it was just not recognized when it first appeared. New methods are in place to detect it now, and it is being found everywhere.
- It is common the virus will be found during an inspection and then disappear, only to reappear a few years later. This happens a lot at Ennis NFH, where it seems to be found when other issues are going on. Sometimes, it may only be in a couple strains of fish.
- Sue mentioned she has found CTv in moribund fish which had enlarged hearts. The fish appeared to die due to constriction. Lacey has not seen this, but believes it's not exactly a correlation, but rather the fish are weakened by another issue and more prone to infection by CTv.
- CPE may or may not be seen in blind passes. It takes about 10-14 days on average, and usually is seen by Day 14.
- There could be an intermittent host for CTv. If eggs are disinfected and broodstock are not showing signs of disease, but the pathogen is found, it could also be the broodstock are long-term carriers.
- Many different species have been examined for CTv, and it's only been isolated from salmonids. It's unknown if it could mutate and become fatal for other species.

Thursday, August 08, 2013

10. University of Minnesota Aquatic Health Research Update (Phelps)

See Appendix 6 for the presentation.

- It would be nice if there were something in the Blue Book about Heterosporis. Nick is on the committee, and he will talk to the other members about this potentially being added. It's not known to infect/proliferate in human cells, but it may be able to enter them. It has been found in Michigan, and Gary will work with Mohamed to get Nick tissue samples from the Lake Emily cases.
- Sturgeon herpesvirus was found on a fish from the Great Lakes Aquarium. It had lesions over 90% of its body. It was a 17-year old lake sturgeon about 3 feet long. Three other fish had gotten infected but recovered.
- There have not been any laboratory tests for morbidity or mortality with the fathead minnow picornovirus because grant proposals have not been funded. There's no immediate impact from this virus, and so funding is difficult to receive.

- It's important to test for pathogens at different times of the year. Seasons may have different environmental stresses which could inhibit or promote a pathogen. A high percentage of fish kills are often not investigated, and these could provide insight.
- The baitfish trade is secretive and territorial. Nick Mandrak may have data from Ontario that would be useful to combine with Nick's research from Minnesota. Michigan has also done a baitfish spiderweb analysis.
- Baitfish may serve as pathogen dispersers. Indiana anglers have caught crappie with cancerous-like growths and are questioning the treatment process. Weevils may be involved as intermediate hosts to move the pathogen along.

11. Research Priorities (All)

The committee members reviewed and revised its Research Priorities. See Appendix 7 for the revised document.

12. Risk Assessment (All)

The committee members provided input to the latest draft of the risk assessment forms. See Appendix 8 for the final documents.

13. EEDv (Whelan)

There have been no further lake trout mortality events after January. The total loss was approximately 20%, somewhere between 50-60k yearlings. The replacement cost was \$60k. After January's infections, 240 fish were tested again and there was only one low positive. The virus was therefore difficult to find. The fish were stocked in southern Lake Michigan and showed no stress or strain, which is surprising because the drive was about 10 hours. The 2014 fish were tested in June and were negative (Marquette and Seneca strains). Testing is happening now with fall fingerlings and will happen again in January. Last year's weather was similar to that in the 1980's, when Marquette had issues before. There was little rainfall until September with high heat. The broodstock are UV treated but production fish are not. It's likely it's been endemic and has been there all along, but needed trigger events to initiate the infection. This year has been cool and rainy, so they aren't expecting any mortalities. The other measure they took was to reduce the lake trout allocations by 25%. This has increased the cost per fish but now they will have larger lake trout.

In May 2013, 120 fish were collected near Alpena in Lake Huron and tested. All were negative for EEDv, and 60 fish went through a full workup for viruses. The second batch will be tested shortly (another 60 fish). Most fish were wild and of a variety of age classes and sizes.

- The administration is looking into whether or not to replace the broodstock. They have had this strain since 2002. They are expecting to generate a new broodstock between 2015-17 and make changes to how they are handled. Until then, they are dealing with what they have now.
- The Blue Book committee wants to revise old chapters and add new ones. This scenario could be written up and included. Suggestions for authors include: Mohamed and Gavin Glenney for the

PCR section; Sue, Gary, and Mohamed for the main document; and Ron Hedrick as a potential co-author. There should be standards for testing. The deadline for a draft is January 1, 2014.

14. Technical Advisors (Shen/Dettmers)

Initially, the committee had two permanent advisors to provide technical expertise on situations about which the committee members may not know. Both of these advisors retired and were not replaced. Shortly after, BKD and other emerging issues happened, and the committee called on Diane Elliott to report on her BKD research each year. Next, Dale Honeyfield began to report on thiamine deficiency, and eventually the committee had a host of advisors with different expertise.

Committee members feel it is important to keep in touch with a variety of experts and it is time to update the advisor list. There are currently three advisors to the committee (Diane Elliott, Patrick Muzzall, and Jim Winton). Members should consider what the committee's needs are and what is missing in the advisor list.

Specifically, the members are looking for experts in the following: bacteriology, virology (could break up into specialists), nutrition, development of new technologies, modeling, epidemiology, and risk analysis.

Members should consider colleagues for these positions and find out who may be interested in serving these roles. The nominations will then be discussed at the Winter 2014 meeting.

15. Future Meetings (All)

The Summer 2014 meeting will be held August 6-7, 2014 in Winnipeg, Manitoba. Sunita will schedule a tour of her lab.

The upcoming winter meeting is in Pennsylvania from February 4-5, 2014. Committee members should send potential Department of Agriculture invitees to Ling and she will contact them. A Northeastern Fish Health contact will be invited as well. A hot topic right now is flavobacterium, so it might be beneficial to have someone give a research talk on that. The meeting will likely be in State College, and there are five hatcheries within a ½ hour drive.

16. Agency Updates (All)

Wisconsin DNR: See Appendix 9 for the CTv presentation. CTv was first found in adult brown trout in western Wisconsin at the St. Croix Falls hatchery. After spawning occurred at the Nevin hatchery, it was found there as well. Feral brown trout were spawned in eastern Wisconsin at the Besandy facility, and it was found there in ovarian fluids and kidney/spleens. It is unknown if deficient biosecurity issues were the cause or if somehow the fish were exposed in another manner. Isolates were genotyped from each hatchery and they were all different from each other, so somehow there was simultaneous transmission of CTv but the sources were different. Fry have been tested from St. Croix Falls, Nevin, and the Wild Rose hatchery and all were negative, even if the broodstock were positive. A new PCR test is working well to pick up different isolates, and they are looking to create a qPCR. Vertical transmission or egg-associated transmission does occur. They are now planning to test different techniques during spawning

using five different methods in different combinations: drain ovarian fluids; drain and rinse eggs with saline solution; drain, rinse, and harden eggs with iodofor; and vary the type of upwelling incubator with heat trays. The control will be regular hatchery practice. Sixty fry from each group will be tested with molecular methods. Eggs will come from positive CTv broodstock, likely from 5-6 females only.

See Tom's presentation, "What I did on my summer vacation" in Appendix 10.

It would be interesting to compare strains from the Mountain Prairie range to those of the Great Lakes. Bill Battes has sequencing information. Tom (who did the sequencing) said it was classic CTv similar to western strains. It would be helpful to know the epidemiology of the virus. Coja had his CTv strains compared and believes it was slightly different but doesn't have the results available now to look. Lacey would be happy to be involved in ring testing.

Wisconsin is testing at 100 ppm. The lab is not set up as a research facility, so Bill Horns at WI DNR wants Sue to try something extreme to measure the difference. IPN is tested at 100 ppm, so that is why that value was chosen. If 100 ppm works, will it have a detrimental effect on egg survival?

Michigan DNR: It's been a quiet summer with only a below average number of fish kills. There's been a push from private aquaculture in Michigan to take an unused state facility and turn it into an intensive culture facility. The Grayling SFH had lots of problems in the 1970s and was closed. The area is positive for whirling disease. The MI DNR will monitor this situation and expect it will be a pump for pathogens, creating an exponential increase in disease expression. "Before" samples have been taken from a broad range of brown and brook trout in the Au Sable River to scan for pathogens. The aquaculture facility will be raising rainbow trout. The fish were originally going to be raised for market but now the owner says he wants to stock waterbodies. He has approximately 10k pounds of fish now and is looking to go up to 500k lbs, which is significantly higher than anything the original hatchery raised. Gary will report back on this later. The owner will not be able to stock in Michigan's waters without passing the DNR's quality test.

Pennsylvania FBC: It's been a slow summer. A hatchery which tested positive last year for CTv had one new isolation from a different broodstock. No major studies are on the horizon. Last year, Pleasant Gap Hatchery switched feed and the pathogen disappeared. IPN has been removed from the Bennet Spring hatchery. Furunculosis vaccines don't seem to have worked as well this year as last year. The FBC has been dealing with a gill amoeba found in rainbow trout. They have treated it with peroxide, it will go away, and then come back. It's a small, non-ciliated organism found in gill smears. Sue suggested speaking to Myron about this issue.

Fisheries and Oceans Canada: The private hatcheries are clean. One provincial hatchery had massive mortality but testing showed nothing. The brook trout were cleaned up and started to look better. Surveillance is happening on the B.C. coast. Screening is done by qPCR. Last year, they did 80k tests, which is a lot of work for a small lab. Currently, there are two research projects: an IPN validation study tested 4k tissue samples and is in the process of being published; and they compared 3 methods of KHv validation. In February, they found an irrovirus and are looking to genome sequence it. ISA virus is keeping the East Coast labs very busy.

Wisconsin FWS: In the spring, they detected *Y. ruckerii* at Genoa NFH. The highest prevalence was in smallmouth bass but it was also found in largemouth bass and walleye. There was a small outbreak of bacterial coldwater disease at Jordan River NFH but it was treated and cleared up. They tested for EEDv because of clinical signs but results were negative. Becky Lasee retired as project leader and will be missed. Teri Yotts is acting project leader. The Service is working on updating Title 50 regulations, and Ken will let the members know when it comes out.

Indiana DNR: Things are going well. It was a cool spring with normal to high precipitation levels. There were no fish kills of note. There was a report about tumors on crappie and bluegill, which was similar to an incident from two years ago. The DNR will be investigating it. Hatcheries are doing well. Sometimes there's an *A. sal* positive without clinical signs of disease but that's typical. Curtiss Creek trout rearing station had multiple issues last year but is doing better. There were low stocking numbers in the spring because of that. The Trout Lodge strain doesn't perform as well as the old Ohio London strain, and so the DNR will be stocking Skamanias again. The Skamania broodstock were caught in July at an alternate trapping source from a sea lamprey barrier in Michigan City. The broodstock underwent thiamine injections and there were no signs of EMS in the progeny 21 days post-injection, so they probably won't do thiamine treatments in the future. VHS surveillance is ongoing in warmwater hatcheries and walleye and muskie brood lakes.

Minnesota DNR: It's been pretty quiet. See Appendix 11 for a special fish health case. The mouth dropping stopped after the terramycin. The tail drop is typical of flavobacterium. It's possible the bacteria could eat through the slender connection, but gram staining but didn't show anything conclusive. Nick recommended trying other stains to see what is found.

17. Adjourn



USDA APHIS Aquaculture Update



Aquatic Animal Health Program

Janet E. Whaley, DVM
U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services
August 7, 2013

APHIS
Safeguarding Animal Health

USDA
Veterinary Services
1

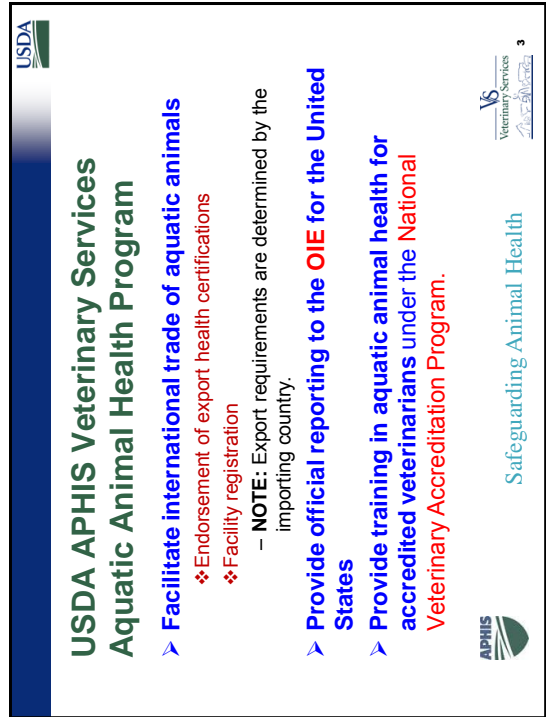


Aquatic Animal Health Program

- Provide coordination with other International, Federal, State, and Tribal officials on aquatic animal health issues in the U.S. in accordance with the **National Aquatic Animal Health Plan**.
- Provide policy and program management - Set program goals and policy for APHIS related to **farmed** aquatic animal health.
 - ❖ Policy on **Viral Hemorrhagic Septicemia (VHS)**, **Spring Viremia of Carp (SVC)**, and **Infectious Salmon Anemia (ISA)**

APHIS
Safeguarding Animal Health

USDA
Veterinary Services
2

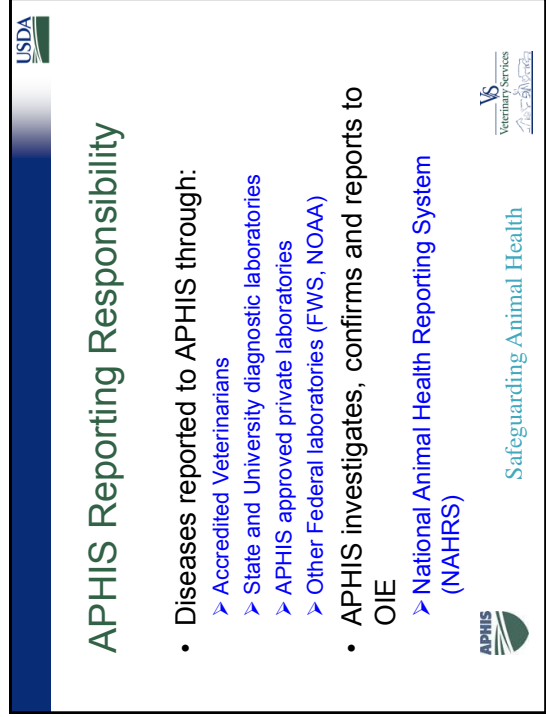


USDA APHIS Veterinary Services Aquatic Animal Health Program

- Facilitate international trade of aquatic animals
 - ❖ Endorsement of export health certifications
 - ❖ Facility registration
 - NOTE: Export requirements are determined by the importing country.
- Provide official reporting to the **OIE** for the United States
- Provide training in aquatic animal health for accredited veterinarians under the **National Veterinary Accreditation Program**.

APHIS
Safeguarding Animal Health

USDA
Veterinary Services
3

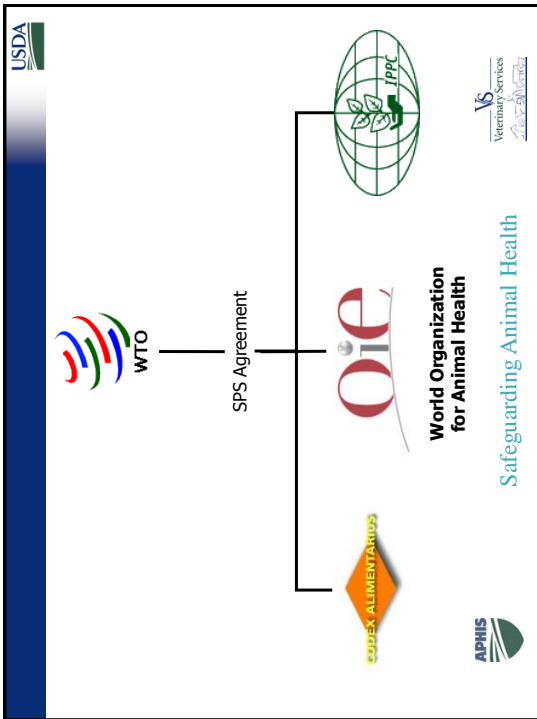


APHIS Reporting Responsibility

- Diseases reported to APHIS through:
 - Accredited Veterinarians
 - State and University diagnostic laboratories
 - APHIS approved private laboratories
 - Other Federal laboratories (FWS, NOAA)
- APHIS investigates, confirms and reports to OIE
 - National Animal Health Reporting System (NAHRS)

APHIS
Safeguarding Animal Health

USDA
Veterinary Services



What is the OIE?

- Voluntary international organization
- **Goal: Prevent spread of animal diseases**
- "Voluntary" member guidelines, not regulations
- Track disease ranges
- Guidelines for testing
- List of important diseases

U.S. National Aquatic Animal Health Plan

The diagram shows four nested circles representing the hierarchy of the U.S. National Aquatic Animal Health Plan. The outermost circle is labeled "Aquaculture". Inside it is a circle labeled "JSA". Inside that is a circle labeled "NAAHP". The innermost circle contains the logos for "APHIS", "NOAA", and "USFWS".

Logos for APHIS, NOAA, USFWS, and the Department of the Interior are present at the bottom of the slide.

Goals of the NAAHP "Our Guide Book"

- Recommendations:
 - Identify pathogens of national concern
 - Prevent and manage diseases they cause
 - Describe and implement surveillance zones
 - Identify key areas for research
 - Develop an outreach strategy
 - Identify education & training needs
 - Implementation strategy/plan


Logos for APHIS, Veterinary Services, and Safeguarding Animal Health are present at the bottom of the slide.

USDA

NAAHP Progress Update

Establish Advisory Committee on Aquatic Animal Health



- ✓ **Subcommittee of Aquatic Animal Health**
 - Membership follows recommendations of Senator Snowe's Amendment to the Farm Bill
- ✓ **Establish Laboratory Testing Network** - Include labs outside APHIS with QA/QC standards
 - National Animal Health Laboratory Network approach
 - Start with tests for ISA and VHS
- ✓ **Develop an IT Infrastructure to support national animal health surveillance database**
 - APHIS Surveillance Collaboration Services (CORE One)

APHIS  Safeguarding Animal Health 

USDA

Objective #1 Establish Advisory Committee on Aquatic Animal Health

- Subcommittee on Aquatic Animal Health (SAAH) appointed in July 2011
- Operates under a FACA **Parent committee**
 - Secretary of Agriculture Committee on Animal Health (SACAH)
- **18 Members:**
 - **2008 Farm Bill** – Senator Snowe recommendations
 - Industry (7)
 - State (5)
 - Tribal (1)
 - Federal (4)



APHIS  Safeguarding Animal Health 

USDA

NAAHP - Next Priorities

NEW

- Add Aquatic testing to the National Animal Health Laboratory Network
 - Start with VHS and ISA
 - In Phases beginning in late 2013
- Pursue harmonizing state requirements for aquatic animal health
 - VHS might be a good start
- Pursue zonation and regionalization concepts – regions of disease freedom and like-to-like status



APHIS  Safeguarding Animal Health 

USDA

Aquatic Animal Testing Added to National Animal Health Lab Network

NEW

- Need to standardize testing – use same protocols
 - VHS and ISA examples
- Demand by international trading partners
 - EU, Chile, and Canada
- The time is right - NAHLN is changing structure
 - Allow private labs and other state and federal labs

APHIS  Safeguarding Animal Health 

NEW

Implementation of NAHLN Aquatics

- Phases
 1. 2013 – offer SOPs to existing NAHLN labs
 - ❖ Start with two protocols (VHS and ISA)
 2. 2014 – extend invitation to non NAHLN labs
 - ❖ Training and partnering with NAHLN labs
 - ❖ Review current system of APHIS approval of labs for export
 3. 2015 and beyond – consider adding other aquatic diseases
 - ❖ Input from SAAH, NCIE, and NVSL




 Safeguarding Animal Health

Surveillance for Infectious Salmon Anemia Virus PNW

- Unconfirmed report of ISA during investigation of fluctuating returns of sockeye salmon in Fraser River watershed in BC, Canada
- Canadian officials (Canadian Food Inspection Agency – lead aquatic animal health issues) investigated claims and found **no** ISAV. CFIA immediately notified and coordinated with U.S.
- **U.S. Congress requested further information from Federal Agencies**
 - ❖ U.S. capacity to detect and respond to ISAV in Pacific Northwest and the potential impact on wild salmon




 Safeguarding Animal Health

Infectious Salmon Anemia

- **First reported in 1984 in Norway.**
- OIE recognized the disease in 1990 & named it ISA.
- ISA virus was characterized by Falk *et al.*, in 1997 (now classified in virus family *Orthomyxoviridae*, genus *Isavirus*).
- First reported outbreak outside Norway was in Canada (New Brunswick) in 1996.
- **First reported outbreak in USA was in Maine in 2001.**
- First reported outbreak in the Southern Hemisphere was in Chile in 2007.
- In 2012, and 2013 Norway, Canada (Nova Scotia) and Chile have reported ISA outbreaks.
- **Primarily a disease of “farmed” Atlantic salmon**
- **No ISAV confirmed cases in “wild” Pacific salmon**




 Safeguarding Animal Health

ISA - Typical clinical signs

- **ISA clinical signs**
 - Anorexia
 - Lethargy
 - Anemia
 - Mortality (slow increase; variable)
- **Gross lesions**
 - Pale gills
 - Ascites
 - Exophthalmia
 - Petechial hemorrhages on abdomen, visceral adipose tissue & eyes
 - Congestion and enlargement of liver & spleen








 Safeguarding Animal Health

Godoy et al., 2008



Pacific NW ISAV Project Strategy “A National Approach”

- **Enhanced Surveillance**
 - ❖ The **strategy** builds on existing State, Tribal, Federal and industry health infrastructures and activities
 - ❖ Current focus is Alaska and Washington due to proximity to BC, Canada
 - ❖ Geographically-distributed biannual sampling (Summer/Fall and Spring) of Pacific salmonids native to the Pacific Northwest for **2 years**
- **Coordinated Screening and Testing**
 - ❖ Screening and testing tools (molecular and culture) are being evaluated and calibrated
- **Parallel Research Efforts**
 - ❖ genetics of “ISA-like” virus
 - ❖ Develop proper diagnostic tools
 - ❖ Risk assessment



Safeguarding Animal Health




Project Partners


Investigators <ul style="list-style-type: none"> • Alaska State Dept. of Fish and Game • Northwest Indian Fisheries Commission • Washington State Department of Fish and Wildlife • USDA APHIS Veterinary Services • DOC NOAA National Marine Fisheries Service • DOI USFWS and U.S. Geological Survey 	Laboratories <ul style="list-style-type: none"> • Diagnostic/Screening Testing: • Idaho Fish Health Center Laboratory • USFWS, the Fish Pathology Laboratory • Alaska Dept. of Fish and Game • Washington Animal Disease Diagnostic Laboratory, Washington State University • Confirmation testing: • USDA APHIS National Veterinary Services Laboratories 
--	--

Safeguarding Animal Health




ISAV Surveillance in Pacific Northwest - Update

- Wild Salmon (returning adults – all 5 salmon species and steelhead trout)
 - **Field Season One completed**
 - ❖ Washington – 3 regions
 - ❖ Alaska – 4 regions
 - **Testing underway (WA all negative)**
- Farmed Atlantic Salmon (WA)
 - **Sampling season One underway**
- Gearing up for Field Season Two




Safeguarding Animal Health





VHS Proposed Rule Status


On Hold



- Given that **NO** reports of VHS IVb on aquatic animal facilities (farms or hatcheries), we are:
- 2012-2013 Stakeholder discussions to explore additional options
 - Is there a need to continue Federal regulation?
 - Should management be by States?
 - ❖ Individual states
 - ❖ As a region
 - Other Options
- **Risk Assessment Now** – decision in Fall 2013.



Safeguarding Animal Health



Risk Assessment Objective

- To assess the impact of Federal regulations in reducing the risk of exposure of live fresh water fish species in the United States to Viral Hemorrhagic Septicemia Virus (VHSV)
- Intent:** results will be used to influence decisions regarding the extent of Federal regulations necessary to mitigate the perceived risk of VHSV

USDA
Veterinary Services
Safeguarding Animal Health

Risk Assessment Process (per OIE)

ENTRY (PATHWAYS) ASSESSMENT describes the biological pathway(s) necessary for an activity to introduce pathogenic agents into a particular environment and estimates the probability of that occurring.

For the purposes of this assessment, the entry assessment will consider the 5 highest-risk pathways of introduction of VHSV from a VHSV-affected area to a VHSV-free area.

EXPOSURE ASSESSMENT describes the biological pathway(s) necessary for exposure of animals at risk to the hazards released from an infected population.

For the purposes of this assessment, the exposure assessment will consider the factors necessary for exposure of live fish in VHSV-free areas, given that VHSV has been introduced into that area.

CONSEQUENCE ASSESSMENT describes the potential consequences of a given exposure and estimates the probability of those consequences occurring.

For the purpose of this assessment, the consequence assessment considers the extent of regulatory or policy framework governing fish health and the sufficiency of oversight to ensure the timely reporting and investigation of VHSV outbreaks.

RISK ESTIMATION summarizes the overall risk, aggregating the results of the entry assessment, exposure assessment, and consequence assessment.

USDA
Veterinary Services
Safeguarding Animal Health

Methods

- Using State-HUC-4 Combinations as Risk Areas of Interest (RAIs)
- Focus on relative risk in each RAI
 - How overall risk is affected by Federal regs
 - Which RAIs present highest overall risk
- Estimate relative risk for each RAI (using RA process)
- Aggregate risk estimates by state
- Re-compute overall risk estimates for different levels of Federal regs

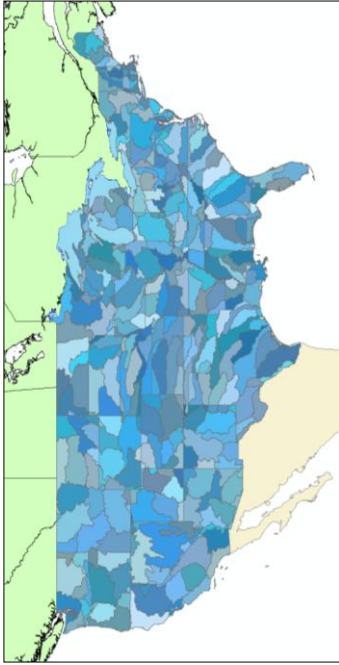
USDA
Veterinary Services
Safeguarding Animal Health

HUC-4s (222)

USDA
Veterinary Services
Safeguarding Animal Health

USDA

Risk Areas of Interest (RAIs) (499)



APHIS

Safeguarding Animal Health

USDA
Veterinary Services
Center for Disease Prevention and Epidemiology

USDA

Data

- Publicly Available or Government Data
 - Hydrologic connectivity
 - Linear distance
 - Water temperature
 - Native range of susceptible species
 - Sufficiency of regulations
- Results of VHSV state survey conducted in 2009
 - Preventive Veterinary Medicine 94 (2010) 128–139; *Viral hemorrhagic septicemia virus (VHSV /Vb) risk factors and association measures derived by expert panel.*

APHIS

Safeguarding Animal Health

USDA
Veterinary Services
Center for Disease Prevention and Epidemiology

USDA

Switch focus to infrastructure where risks remain



- Public education
- Continuing education for fish health practitioners
- Laboratory capacity
- Basic biosecurity
- Database development
- Passive surveillance

APHIS

Safeguarding Animal Health

USDA
Veterinary Services
Center for Disease Prevention and Epidemiology

USDA

Design sustainable approaches for Great Lakes industries

- Mitigation through implementation of biosecurity and/or regulatory practices
 - Testing to include history and risks
 - Program Standards?
 - Best Management Practices?
 - Facility Certification Program?
 - Intra-State zones?

APHIS

Safeguarding Animal Health

USDA
Veterinary Services
Center for Disease Prevention and Epidemiology

**Questions, comments,
suggestions?**

janet.e.whaley@aphis.usda.gov
o. (301) 651-3460
c. (301) 346-4740
<http://www.aphis.usda.gov/focusonfish/>



USDA

APHIS

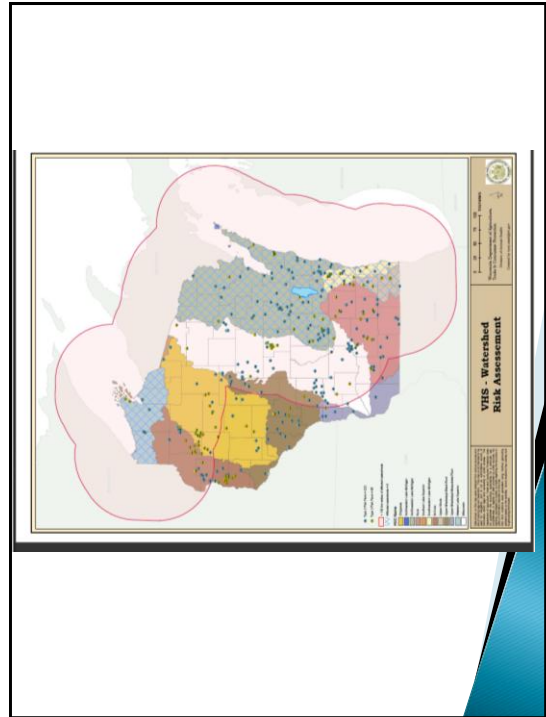
USDA
Veterinary Services
Safeguarding Animal Health

29

Wisconsin Fish Regulatory Issues

Myron Kebus, M.S., DVM
 Wisconsin Department of Agriculture, Trade & Consumer
 Protection (WDATCP)

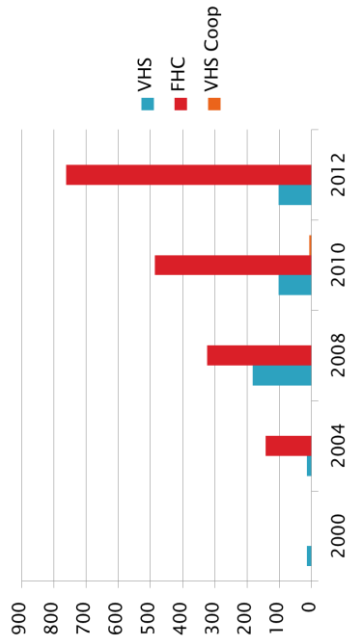
Federal Funding
 Cooperative Agreement Award for VHS
 2008 to 2012



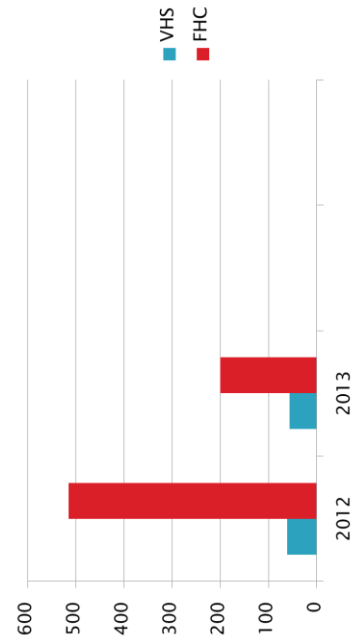
Surveillance

- ▶ Six farms
- ▶ 70 fish per group
- ▶ 560 fish total
- ▶ All VHS negative

Lots Tested



Trend for First 6 Months



VHS

- ▶ VHS testing has greatly increased
- ▶ VHS has not been found on fish farms
- ▶ Compliance with VHS testing requirements is good
- ▶ Demand for funds for VHS testing is low



**Fish Health for Producers
Certificate Program**
<http://vetmedce.vetmed.wisc.edu/fhm>

Fish Farmer Certificate Program

Myron Kebus
WI Department of Agriculture, Trade & Consumer Protection

Chris Hartleb
University of Wisconsin-Stevens Point
Northern Aquaculture Demonstration Facility

Jeannette McDonald
University of Wisconsin-Madison, School of Veterinary Medicine
March 1, 2012

Road Map

- ❖ Module 1 – Introduction
- ❖ Module 2 – Risk Management and Biosecurity
- ❖ Module 3 – Water Quality
- ❖ Module 4 – Fish Health Inspections
- ❖ Module 5 – Veterinary Health Assessments

Veterinary Workshop on Fish Regulatory Medicine



- April 26 -27, 2010
- September 19-20, 2011
- July 31, 2012
- Interstate regulations
- OIE testing requirements
- AFS Bluebook Standards
- VHS testing and surveillance result
- Epidemiology

Chickens of the Sea?



Myron Kebus, MS, DVM
Wisconsin Department of Agriculture,
Trade & Consumer Protection

What Shape Should Fish Health Regulations Take?

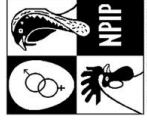
- ▶ Questions???
- ▶ National Aquatic Animal Health Plan
- ▶ Veterinary-based
- ▶ Non-veterinary based
- ▶ Are there other examples in other animal industries?



Wisconsin Poultry & Egg

National Poultry Improvement Plan (NPIP)Poultry

The National Poultry Improvement Plan was established in the early 1930's to provide a cooperative industry, state, and federal program through which new diagnostic technology can be effectively applied to the improvement of poultry and poultry products throughout the country.



Welcome to
MacFarlane Pheasants
your #1 Source
for Game Birds



National Poultry Improvement Plan (NPIP)Poultry

The development of the NPIP was initiated to eliminate Pullorum Disease caused by *Salmonella pullorum* which was rampant in poultry and could cause upwards of 80% mortality in baby poultry.

National Poultry Improvement Plan (NPIP)Poultry

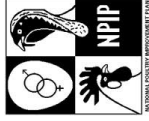
The program was later extended and refined to include testing and monitoring for *Salmonella typhoid*, *Salmonella enteritidis*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Mycoplasma meleagridis*, and *Avian Influenza*. In addition, the NPIP currently includes commercial poultry, turkeys, waterfowl, exhibition poultry, backyard poultry, and game birds.

National Poultry Improvement Plan (NPIP) Poultry

The technical and management provisions of the NPIP have been developed jointly by Industry members and State and Federal officials. These criteria have established standards for the evaluation of poultry with respect to freedom from NPIP diseases.

National Poultry Improvement Plan (NPIP) Poultry

- ▶ Exploratory visit by Aquaculture Industries to Georgia in 1996 to learn about NPIP
- ▶ What is NPIP?
- ▶ Why Poultry?
- ▶ How do poultry producers like NPIP?



Is Poultry Like Aquaculture?

- ▶ In many ways yes
- ▶ In other ways a bit different

Large numbers of small animals raised in high density facilities



Fish Farmers Are Generally Unhappy with Fish Health Regulations

- ▶ Poultry farmers are generally happy with NPIP!
- ▶ How could this be? Farmers that like regulations???
- ▶ Why do they like NPIP?

Influence on Regulations

- ▶ Poultry Farmer have **Great** influence on NPIP rules, and how they are interpreted.
- ▶ Fish Farmers have much lower influence on fish health rules.

Cost

- ▶ Fish farmers complain about high cost
- ▶ Poultry farmers don't seem to nearly as much



Interaction with Resource Agency Regulations

- ▶ Poultry – low but some with game birds
- ▶ Fish farmers – High especially for those who harvest from the wild and stock into the wild

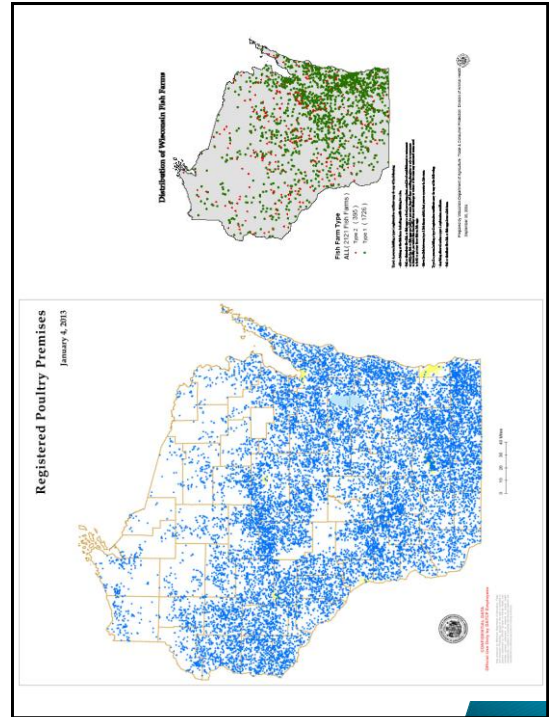
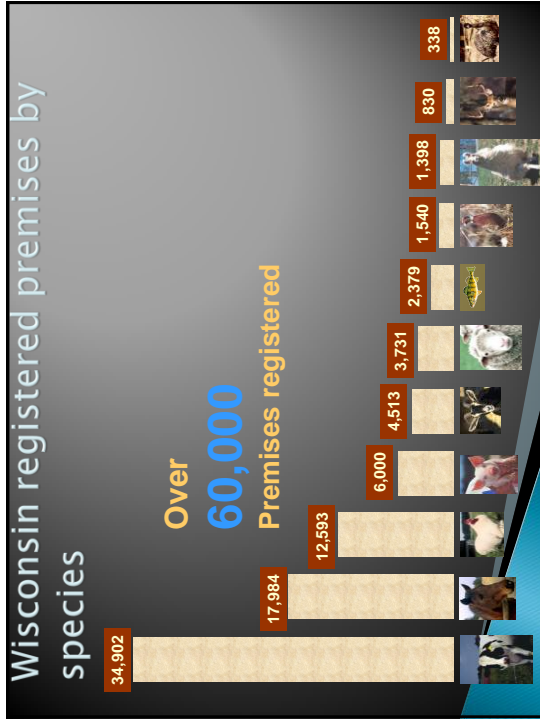


U.S. Aquaculture

- ▶ value of aquaculture products (domestic farm raised) sold at **\$1.4 billion** or about 1 percent of the total \$153.6 billion sales for all livestock, poultry, and their products in the United States.

Poultry

- ▶ **\$35.6 billion** poultry industries' production in 2011, or about 25% total \$153.6 billion sales
- ...



Farm Size

Both industries have very large farms and very small farms but the spread is greater in poultry

Can NPIP Be A Model For Fish?

- ▶ Maybe
- ▶ Does fish have the equivalent of Salmonella pullorum?
- ▶ “Fish NPIP” depends much on aquaculture industries influence

Unique Aquatic Clinical Cases

By
Mohamed Faisal, DVM, PhD, Dr. Honoris Causa

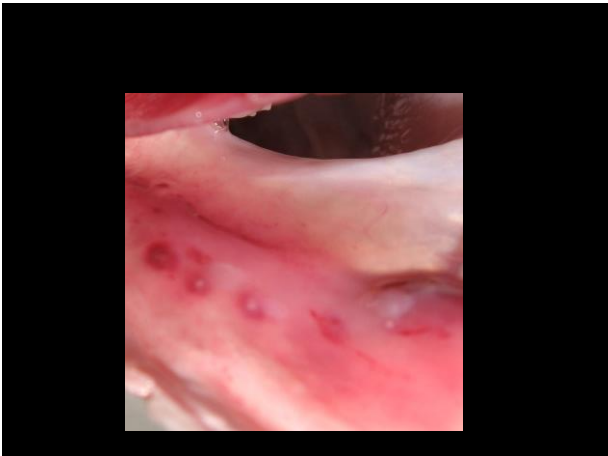
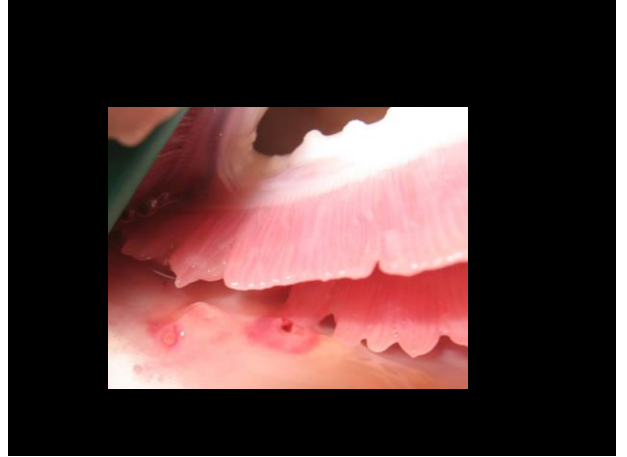
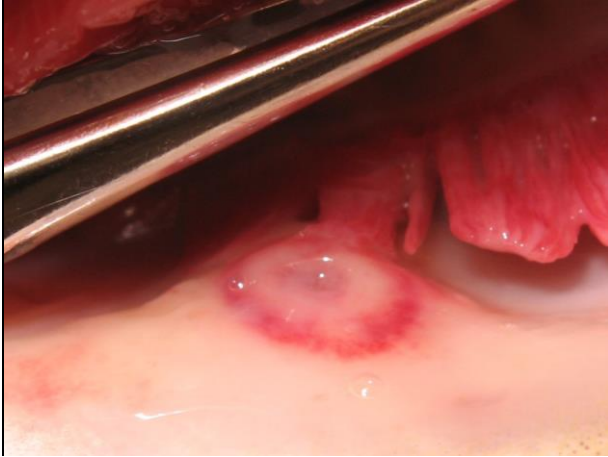
Michigan State University

August 7, 2013

Case 1

- Freshwater drum in Lake St. Clair

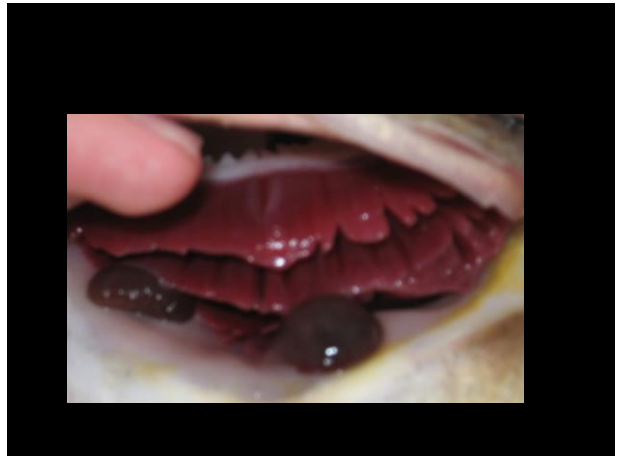


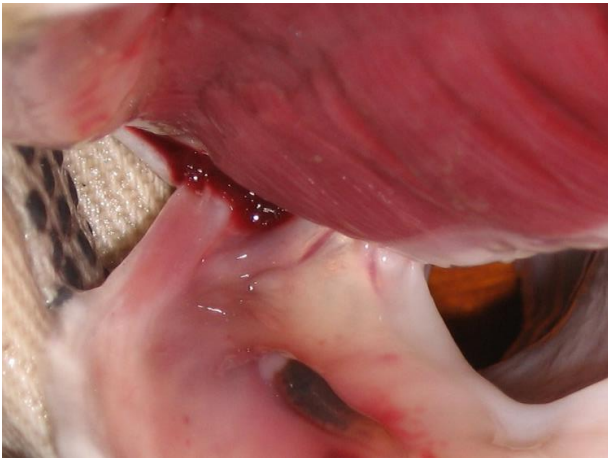


• **Have you seen anything like that?**

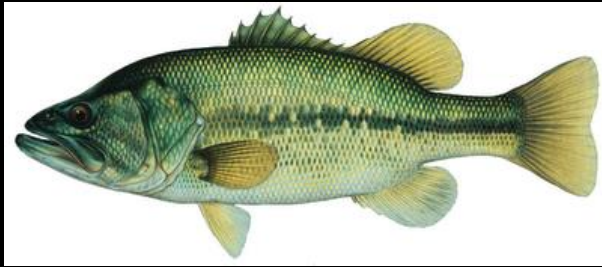


• **What could they be?**

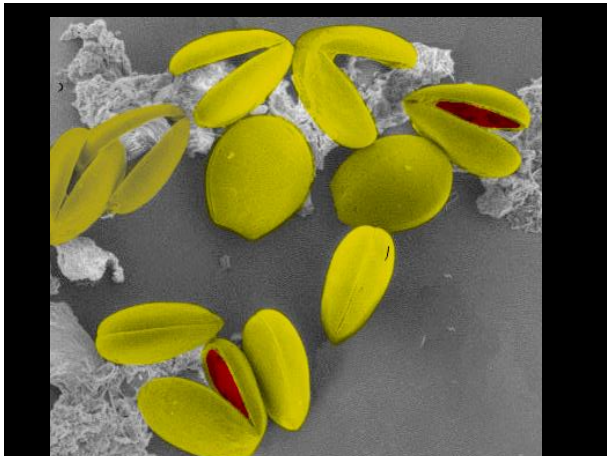
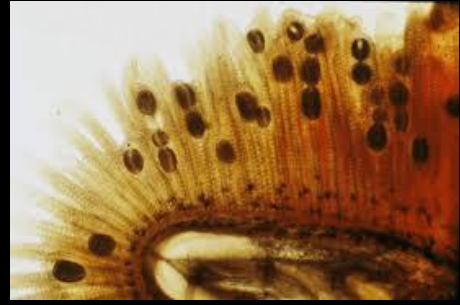




**Case 2
Largemouth bass**



LMB



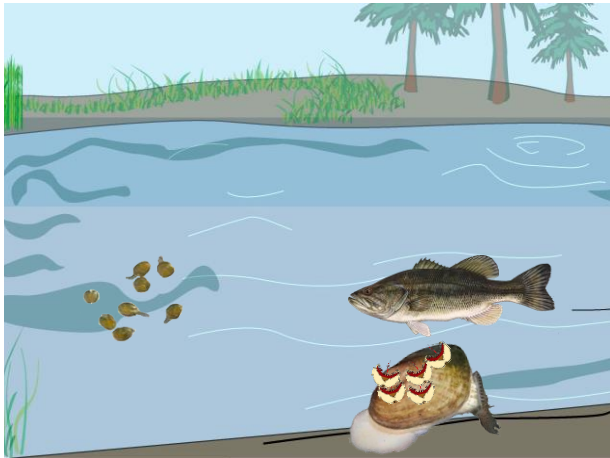
• **What do you think this could be?**

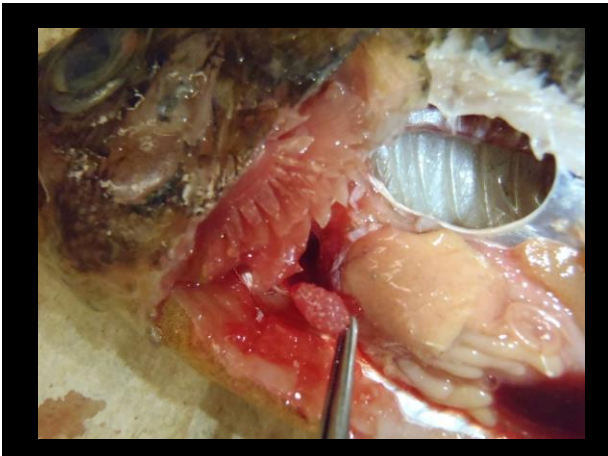
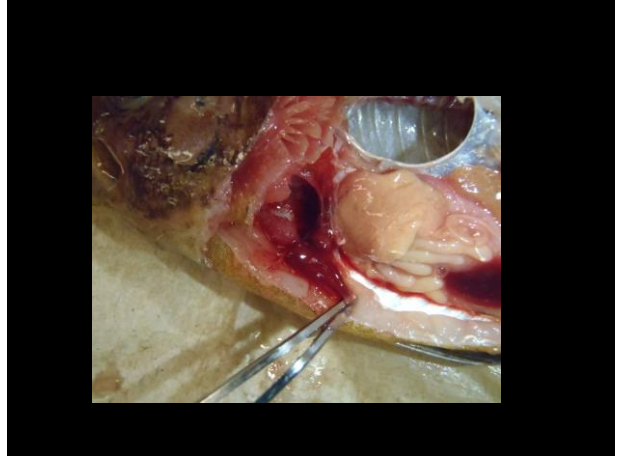
Glochidia

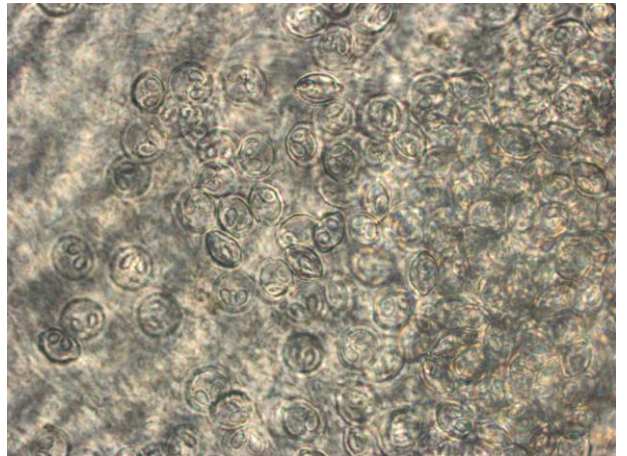
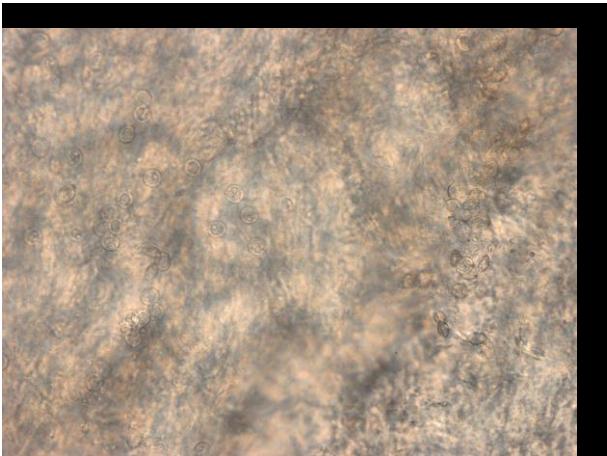
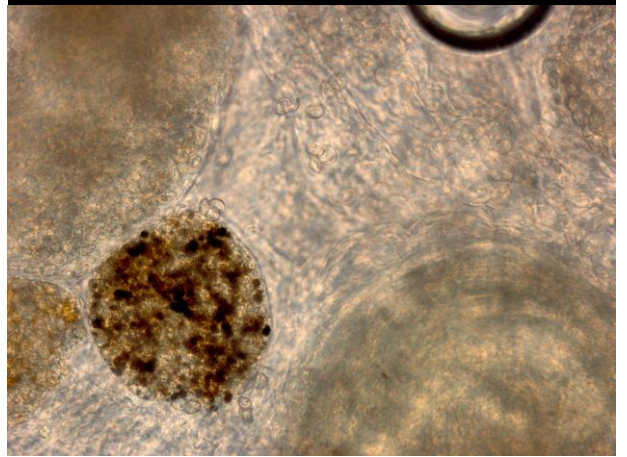
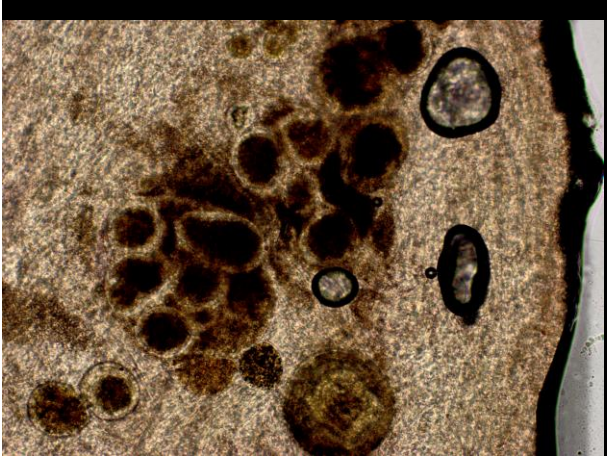


CASE 3

- **Rockbass**



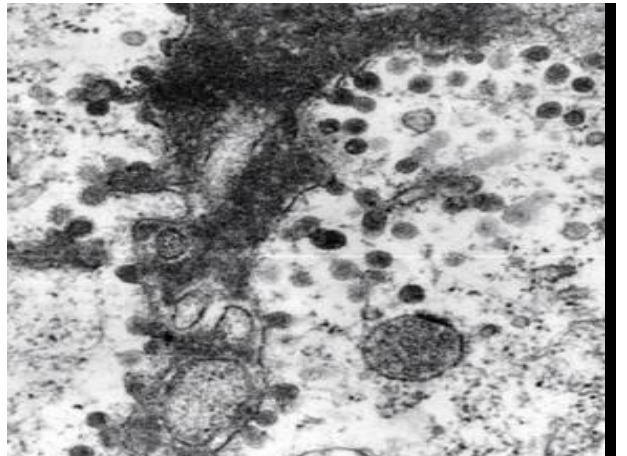
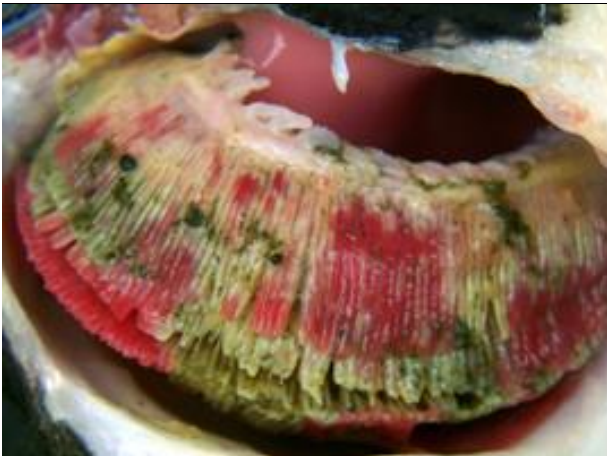




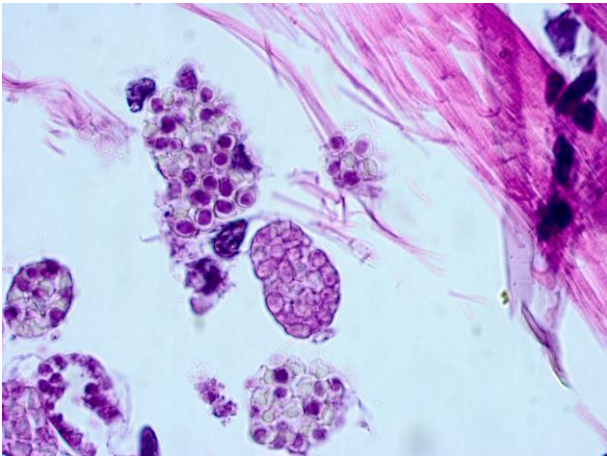
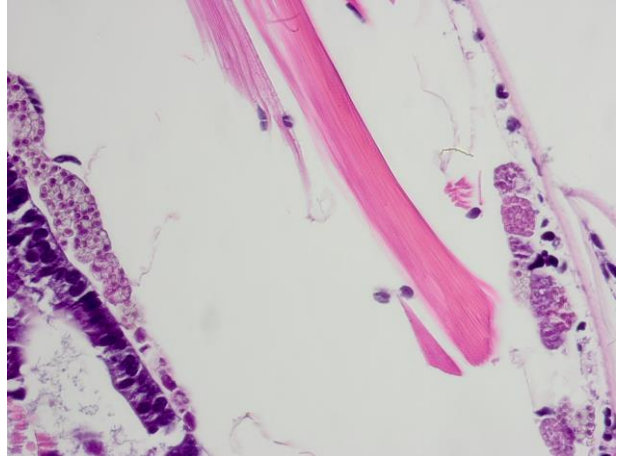
- Myxosporidian cyst

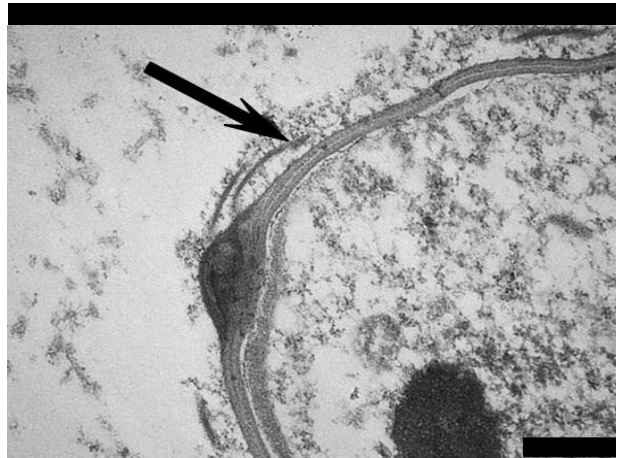
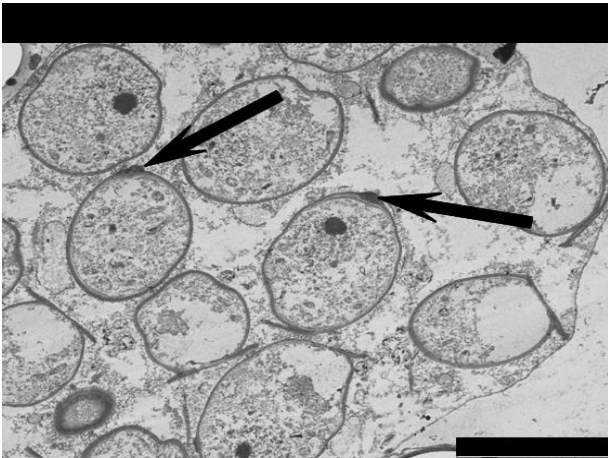
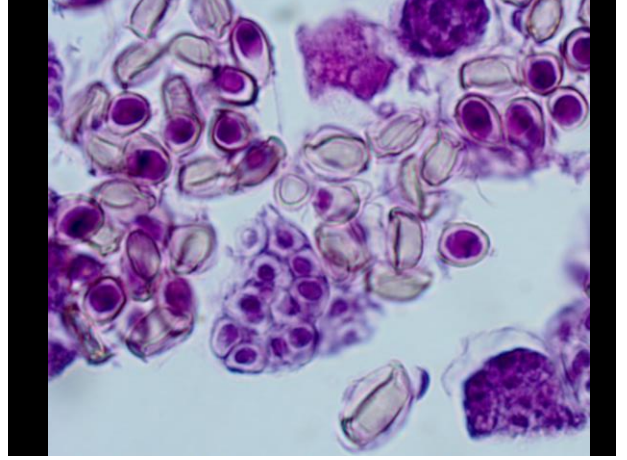
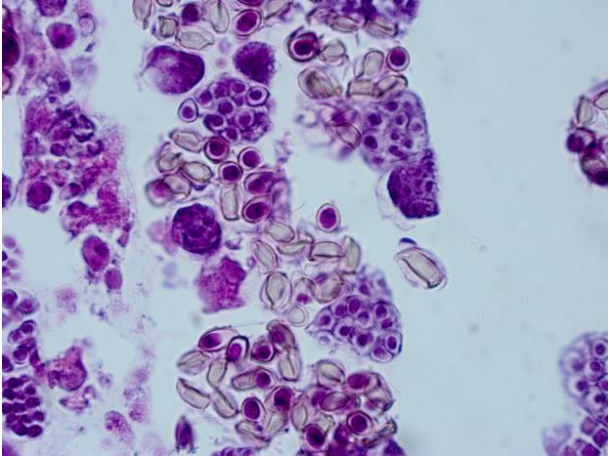
CASE 4

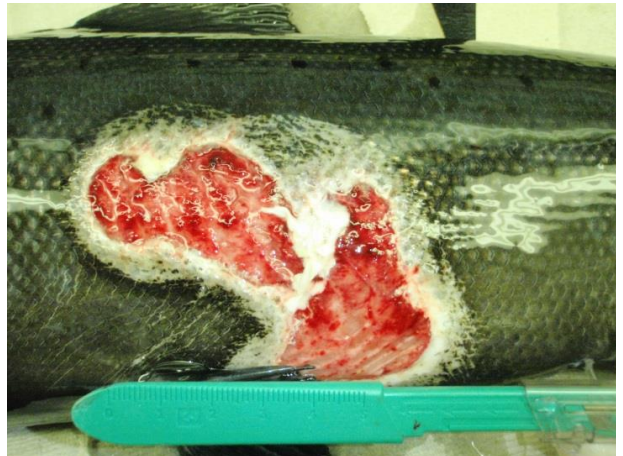
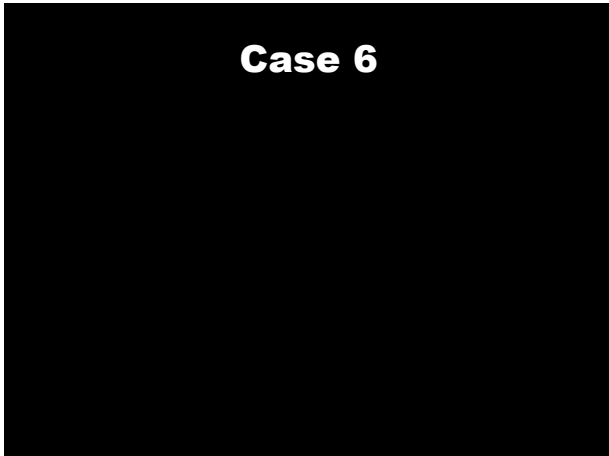
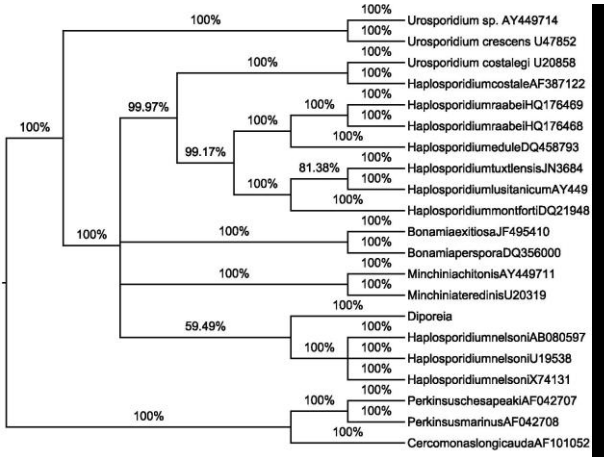
- Kent Lake
- Common carp kills

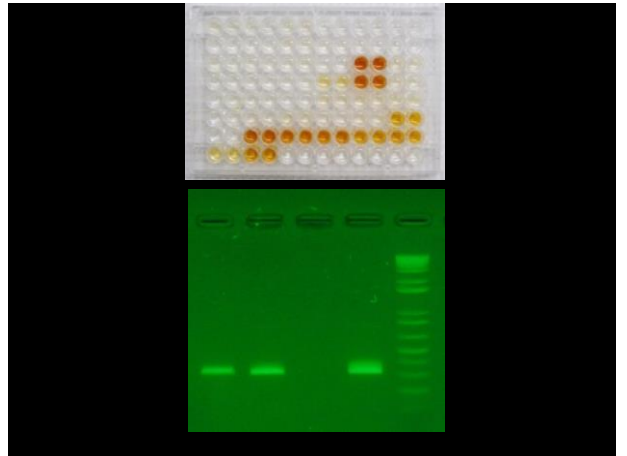
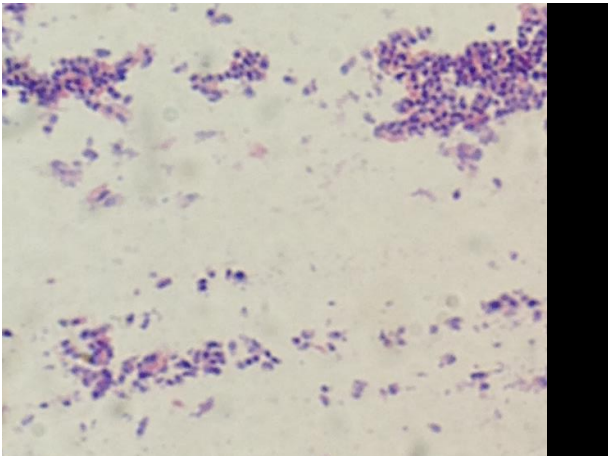
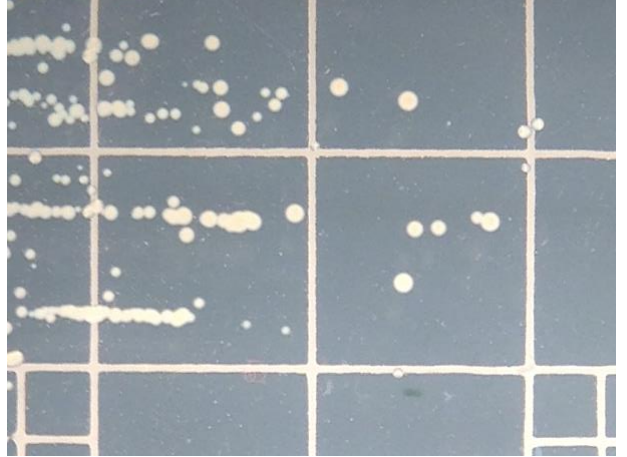


**Case 5
Diporeia**









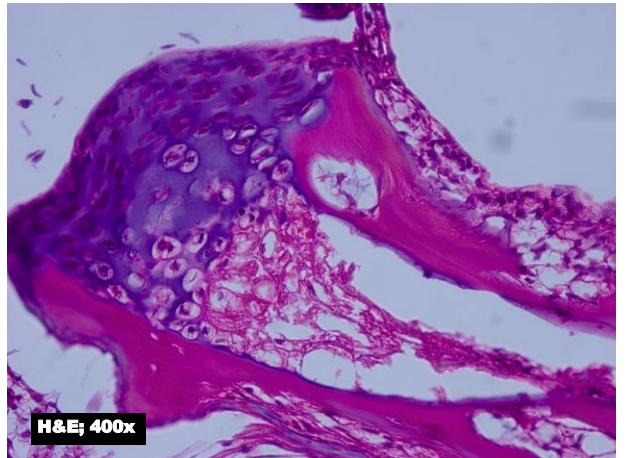
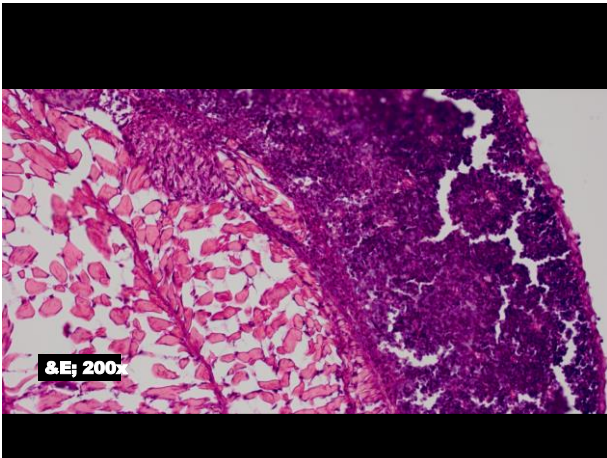
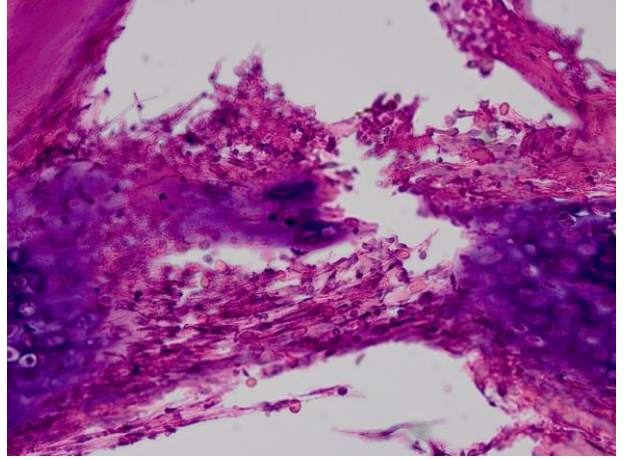
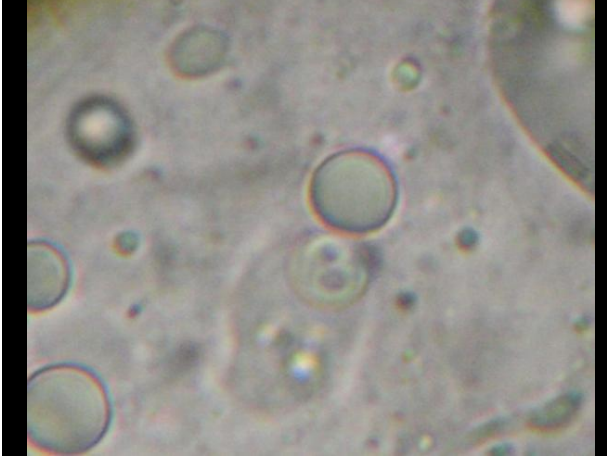
Diagnosis

- Based on clinical manifestations, colony morphology, sandwich ELISA, and PCR this case is Bacterial Kidney Disease caused by *Renibacterium salmoninarum*

CASE 7

- Atlantic salmon





Diagnosis

- **Typical clinical manifestations of whirling disease**
- **Histopathology is very suggestive of whirling disease.**
- **PCR negative**
- **Spores not present**
- **Diagnosis: Unknown**

Fish Disease Cases on Fish Farms

Myron Kebus, M.S., DVM
Wisconsin Department of Agriculture, Trade & Consumer Protection (DATCP)



Fish Health Medicine Certificate Program
<http://vetmedce.vetmed.wisc.edu/fhm>

Myron Kebus, MS, DVM
Christopher Hartleb, PhD

Wisconsin Department of Agriculture, Trade and Consumer Protection, Division of Animal Health
University of Wisconsin-Stevens Point—Northern Aquaculture Demonstration Facility

Diseases we will discuss

- ▶ Water quality problems
- ▶ Environmental disease
- ▶ Bacterial infections
- ▶ Ectoparasites
- ▶ KHV
- ▶ LMBV
- ▶ ISA
- ▶ SVC
- ▶ VHS

Case #1 Catfish Mortalities



Case #2
Trout Rearing Complications



Case #2
Solution

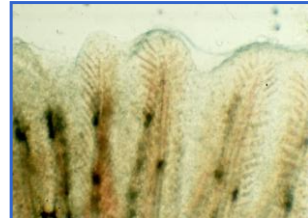


The baffles extended to two inches from the bottom of the tanks

Case #1
Analysis



Case #2
Trout Gill Disease

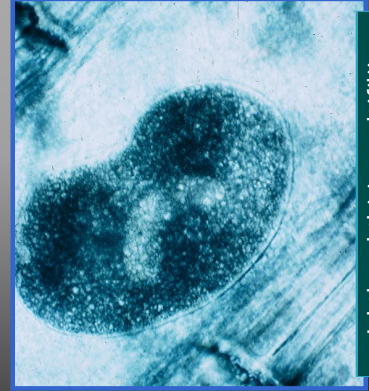


Gross and microscopic view of trout gills

Case #3
Analysis



Case #4
Analysis



Ichthyophthirius multifiliis

Case #3
Dying Perch Fingerlings



Case #4
Flashing Gold Fish



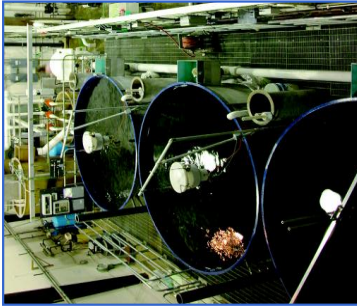
Case #5
Lesions on Fish



Case #6: Koi Disease
The Problem & Findings



Case #5
Reddened Trout Skin

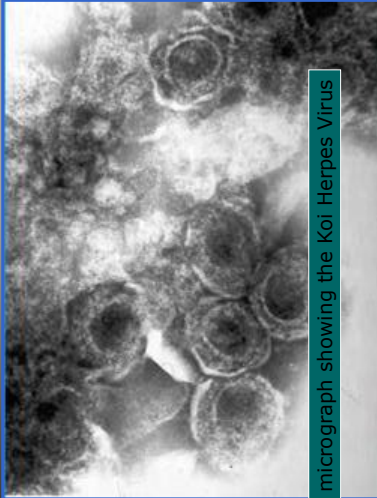


Case #5
The Problem



Clean-out

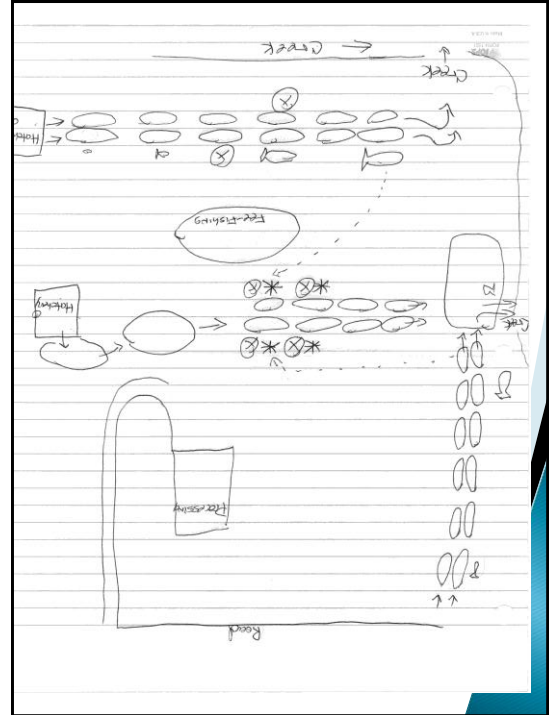
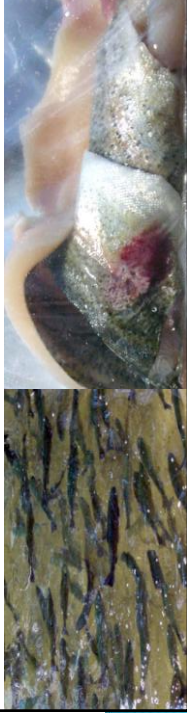
Case #6: Koi Disease The Outcome



Electron micrograph showing the Koi Herpes Virus

Case #7: History

- ▶ Red lesions on market-sized rainbow trout
 - Looks like furunculosis
 - Similar lesions last year
- ▶ Can't market due to unappealing appearance
 - Customers are mainly restaurants
- ▶ January 20th - Southern Wisconsin fish farm



The Farm


- ◆ Fish are raised in earthen raceways (12' x 50')
- ◆ Water comes from artesian (groundwater) source and flows back into creek
- ◆ Commercial floating feed
- ◆ No vaccination/drug protocol in use



Gills

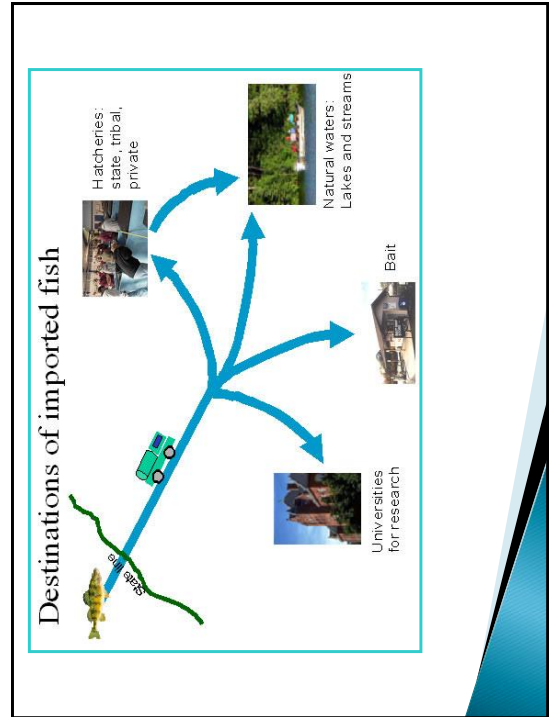


- Lesions on body wall of fish
- Furunculosis-like
- Behind gills/lateral fins
- Not contained to one raceway
- Similar location on each fish




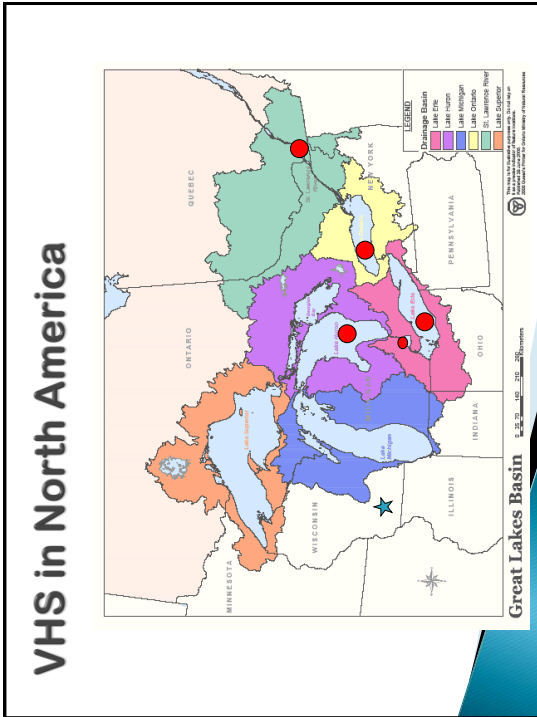
Farm visit

- Necropsy shows no internal lesions
- Happened on/off in past year, but no seasonal pattern
- Feed not contaminated
- Live morbid fish behaving normally
- Water turbid but no toxins; normal pH
- Culture of lesions: *Aeromonas hydrophila* (opportunistic)



VHS Lesions: Hemorrhage





- ▶ Mechanical trauma from old nets with holes
 - Some repaired with plastic ties
- ▶ Secondary opportunistic infection with *Aeromonas hydrophila*

FISH HEALTH AND RECIRCULATION SYSTEMS...Experience in Failures!

Myron J. Kebus, M.S., DVM
 Wisconsin Department of Agriculture,
 Trade and Consumer Protection

There have been few resources in the U.S. for research on diseases of yellow perch.

Discussion of **health** has been secondary to **feed training, rearing, nutrition and marketing**.



Producers often appreciate the importance of disease late in the process of learning to rear yellow perch.

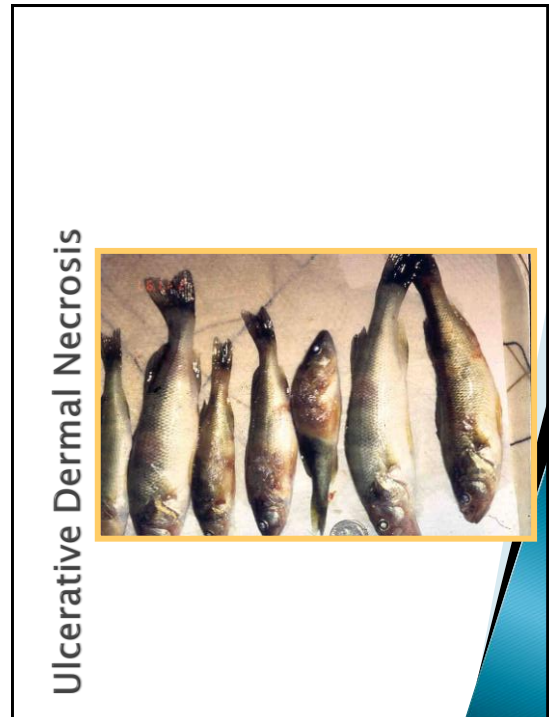
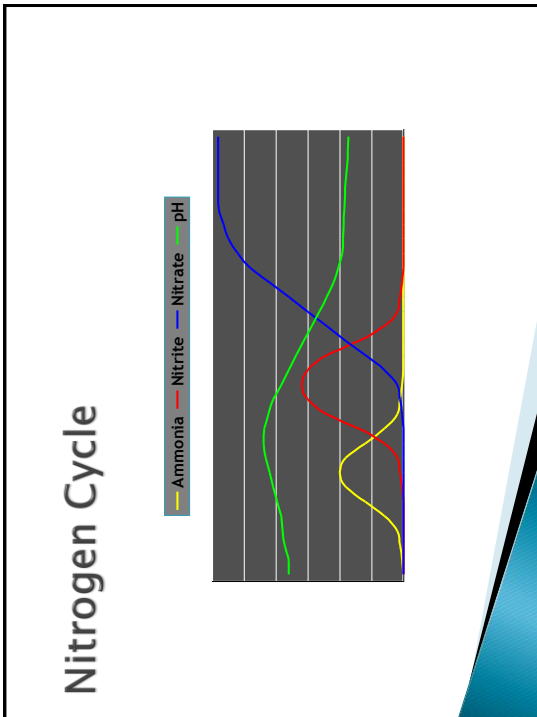
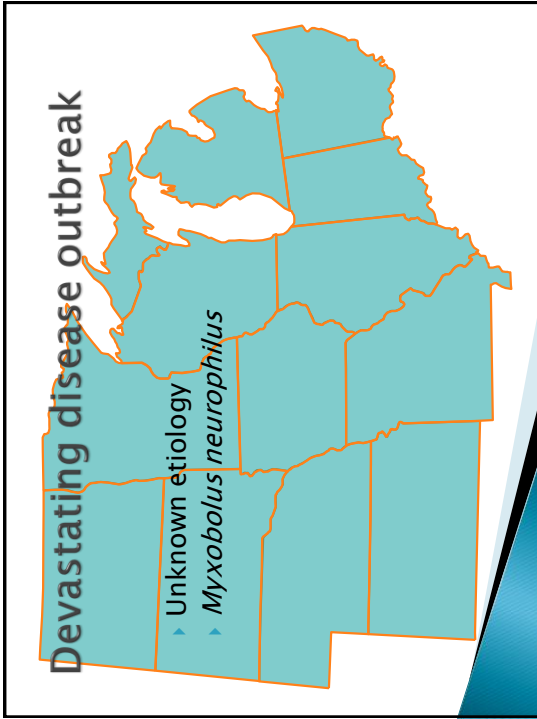


Diseases have limited production and contributed to financial failure of several major farms in the Upper Midwest.

Bacterial and parasitic soup

- ▶ *Trichodina*
- ▶ *Aeromonas*
- ▶ *Columnaris*
- ▶ *Costia*
- ▶ *Gyrodactylus*





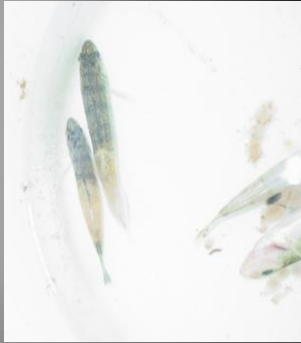
Recirculating Hydroponic Tanks



Visit www.growingpower.com to learn more about raising fish in hydroponic tanks

Questions

White Tail Syndrome



Streptococcus iniae



Detection of CTV (Cutthroat Trout Virus)-Where Are We Now?

Lacey Hopper
Virology Lab
USFWS-Bozeman Fish Health Center
Bozeman, MT

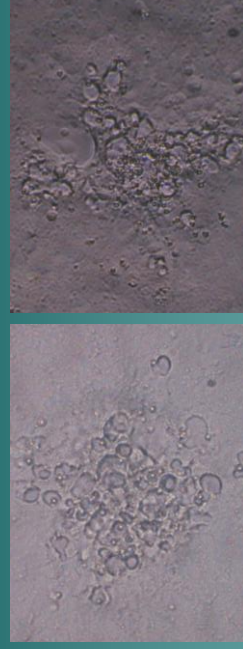
Cutthroat Trout Virus

- ◆ Small(31 nm), spherical SS RNA virus
- ◆ Related to *Hepatitis E virus*
- ◆ First isolated in mid 80's in CA
- ◆ Also detected in OR and ID
- ◆ Isolated from: (Adult) CUT, RBT, BNT, BKT, and Gila trout (NM)
- ◆ Experimentally infective, but no mortality

Interesting CTV Facts

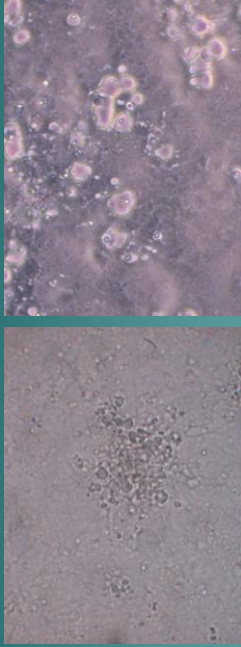
- ◆ CPE only in CHSE-214 cells
- ◆ Slow, focal type CPE
- ◆ Small syncytia formations
- ◆ No destruction of cell monolayer
- ◆ 7-28 d post-inoculation @ 15°C
- ◆ Easily mistaken for cytotoxicity
- ◆ Doesn't always show up in BP
- ◆ Dirty samples=no or very little replication
- ◆ Plate film helps!

CTV on CHSE-214 cells



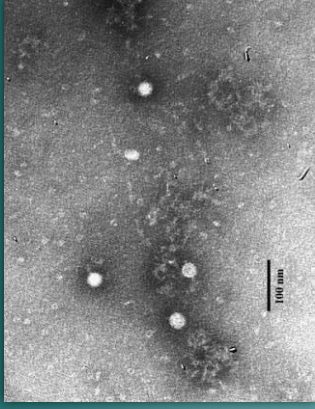
12 d post inoculation

More CTV on CHSE-214's



12 d post inoculation

Electron Micrograph of CTV



(Batts et al. 2011)

First Bozeman FHC CTV Isolate

- ◆ Spring, 2008
- ◆ **Glenwood Springs Hatchery, CO**
 - Recovered from RBT ovarian fluid
 - CPE observed at 14 d post-inoculation
 - Replicated only in CHSE-214 cells
 - Called "The Thing"
 - Later identified as CTV by USGS-WFRC (Seattle)

Other 2008 Isolates

- ◆ Spring-Summer, 2008
 - Colorado
 - Pitkin SFH (CUT OF)
 - Poudre Rearing Unit (Cut OF)
 - Wyoming
 - Jackson NFH (CUT OF)
 - Saratoga NFH (BRN K/S)
 - Montana
 - Murray Springs Hatchery (RBT OF)
 - Washoe Park SFH (CUT OF)
 - Ennis NFH (RBT OF & K/S)

2009 CTV Isolates

- ◆ **Colorado**
 - CDOW Fish Research Hatch. (RBT OF)
 - Pitkin SFH (CUT OF)
- ◆ **Wyoming**
 - Jackson NFH (CUT OF)
- ◆ **Montana**
 - Ennis NFH (RBT OF)
 - Murray Springs Hatchery (RBT OF)
 - Washoe Park SFH (CUT OF)

2010 Isolates

- ◆ **Colorado**
 - Crystal River SFH (CUT OF)
 - CDOW Fish Research Hatch. (RBT OF)
 - Poudre SFH (RBT OF)
 - Glenwood Springs SFH (CUT OF)
- ◆ **Wyoming**
 - Saratoga NFH (BNT OF)
 - Jackson NFH (CUT OF)
- ◆ **Montana**
 - Ennis NFH (RBT OF)
 - Yellowstone River SFH (CUT OF)

2011 Isolates

- ◆ **Colorado**
 - Glenwood Springs SFH (CUT & RBT OF)
 - Crystal River SFH (RBT OF)
- ◆ **Montana**
 - Spring Creek Trout Hatchery (BNT OF)
 - Ennis NFH (RBT OF)
 - Yellowstone River SFH (CUT OF)

2012 Isolates

- ◆ **Colorado**
 - Poudre SFH (RBT OF)
 - Durango SFH (CUT OF)
 - Fish Research (RBT OF)
- ◆ **Wyoming**
 - Jackson NFH (CUT OF)
- ◆ **Montana**
 - Washoe SFH (CUT OF)
 - Ennis NFH (RBT OF)
 - Yellowstone River SFH (CUT OF)

2013 isolates (so far...)

- ◆ **Colorado**
 - Glenwood Springs SFH (RBT OF)
 - Durango SFH (CUT OF)
 - Leadville NFH (CUT OF)
- ◆ **Wyoming**
 - Jackson NFH (CUT OF)
- ◆ **Montana**
 - Ennis NFH (RBT)
 - Yellowstone River SFH (CUT OF)
 - Washoe SFH (CUT OF)

CTV Isolations at Hatcheries

- ◆ Widespread panic at first
- ◆ Egg requests/transfers halted
- ◆ Emphasis on benign pathogen status began...



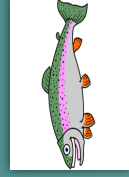
Infectivity Trial

- ◆ 3 different isolates used
- ◆ Juvenile RBT (~1g) intraperitoneally injected with high titer (10,000 virus particles/fish)
- ◆ Fish sampled at 5,8,12,16,20 days post-injection
- ◆ CTV isolated from only 1/12 fish
- ◆ Result: No mortality, no microscopic tissue changes

(LaPatra 2009)

Infectivity Results Suggest...

- ◆ CTV is NOT capable of causing pathology or mortality
 - > Very small, susceptible fish
 - > Very high virus concentrations
- ◆ Thus, CTV NOT shown to cause disease!



(Hedrick et al. 1991; LaPatra 2009)

Benefits of CTV Infection?

- ◆ Juvenile RBT infected with CTV showed increased resistance to Infectious Hematopoietic Necrosis Virus (IHNV)
- ◆ CTV infection increased survival by 70%, but only for 4 weeks
- ◆ Potent inducer of interferon-like activity

(Hedrick et al. 1994)

Financially “Benign?”

- ◆ Ennis NFH, Ennis MT
 - Largest RBT brood station in the US
 - Spawn ~23 million eggs annually
 - Ships eggs to 37 states
 - Significant impact on egg transfers

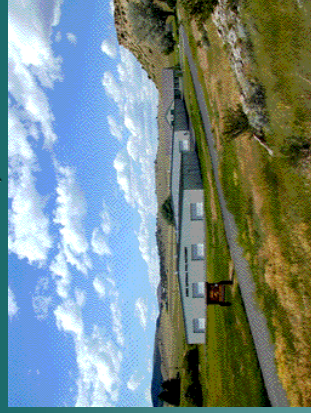
CTV Positive Status=Discarded 15% of total egg production=Approx. \$200,000 lost/year

Initial Effects of CTV

- ◆ Saratoga NFH Saratoga, WY
 - Impact:
 - Lost over **750,000** brood trout egg requests



Ennis NFH Ennis, MT



Study: Vertical Transmission and Persistence of CTV in Cultured RBT Using Standard Hatchery Protocols

CTV Vertical Transmission Study

- ◆ Identify infected OF by cell culture
- ◆ Rear progeny from infected adults separately for 3 years
- ◆ Include control groups
- ◆ Sample at intervals and sexual maturity for detection of virus
- ◆ Confirm any positives by PCR
- ◆ Sequence positives



Study Objectives

- ◆ Determine if CTV is vertically transmitted
- ◆ Monitor viral persistence
- ◆ Investigate CTV pathology in salmonids
- ◆ Provide clues of origin and viral movements



Results

- ◆ 325 pooled kidney/spleen samples negative by cell culture
- ◆ 360 individual ovarian fluid samples negative by cell culture
- ◆ 421 individual seminal fluid samples negative by cell culture
- ◆ 53 random cell culture fluid samples negative by PCR
- ◆ Histopathology = No microscopic tissue changes indicative of viral pathology
- ◆ No difference in mortality, no health problems
- ◆ K/S (survivors and morts) and OF PCR is pending...

What We Know Now

- ◆ Studies show no mortality or pathology attributed to CTV
- ◆ CTV is widespread and probably historically present in many trout broodstocks
- ◆ Standard hatchery protocols may greatly reduce the chance of vertical transmission

References

- ◆ Batts, W., S. Yun, R.P. Hedrick, and J. Winton. 2011. A novel member of the family *Herpeviridae* from cutthroat trout (*Oncorhynchus clarkii*). *Virus Res.* 158:116-123.
- ◆ Hedrick, R.P., S. Yun, and W.H. Wingfield. 1991. A small RNA virus isolated from salmonid fishes in California, USA. *Can. J. Fish. Aquat. Sci.* 48:99-104.
- ◆ Hedrick, R.P., S.E. LaPatra, S. Yun, K.A. Lauda, G.R. Jones, J.L. Congleton, and P. de Kinkelin. 1994. Induction of protection from infectious hematopoietic necrosis virus in rainbow trout *Oncorhynchus mykiss* by pre-exposure to the avirulent cutthroat trout virus (CTV). *Dis. Aquat. Org.* 20:111-118.
- ◆ LaPatra, S.E. 2009. Status of cutthroat trout virus (CTV) [White paper].

Any Questions?



Emerging pathogens of the Great Lakes region

Newly described microbial agents of fish

Nicholas Phelps, MS, PhD
 College of Veterinary Medicine
 Veterinary Diagnostic Laboratory

UNIVERSITY OF MINNESOTA
 Driven to Discover

Acknowledgements

- Dr. Anibal Armién
- Bill Batts
- Dr. Andy Goodwin
- Dr. Sagar Goyal
- Lacey Hopper
- Dr. Becky Lasee
- Sue Marcquenski
- Rebekah McCann
- Peggy Miller
- Dr. Sunil Mor
- Dr. Terry Ng
- Dr. Katey Pelican
- Paula Phelps
- Corey Puzach
- Ling Shen
- Dr. Tom Waltzek
- Dr. Jim Winton
- Many more...
- Minnesota's Environmental and Natural Resources Trust Fund
- Minnesota Rapid Agriculture Response Fund
- Minnesota Veterinary Diagnostic Lab and clients



Outline

What is new at the MVDL?

Heterosporis spp.

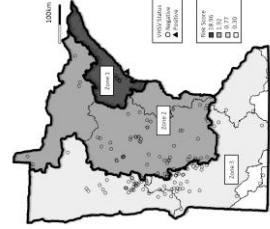
New viruses

Fathead minnow picornavirus

Other research activities

Viral hemorrhagic septicemia

- Regulatory and surveillance testing since 2007
- No VHSV positive waterbodies in Minnesota
- Except Lake Superior



Heterosporis spp.

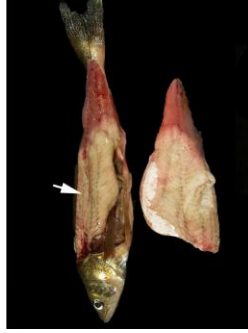
- Microsporidian parasite
 - Fungus-like, obligate intracellular organism
- Simple life cycle
 - No necessary intermediate hosts: Fish → Fish
- Parasite induced mortality
- Fish rendered unpalatable
- Up to 40% of population grossly infected
 - 2003: Leech Lake, Cass County, MN
- At least 15 susceptible species
- Zoonotic??



Heterosporis spp.

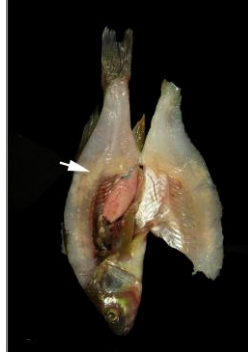
Walleye (Lake Wabana)

Submitted by MN DNR
*First report in this waterbody



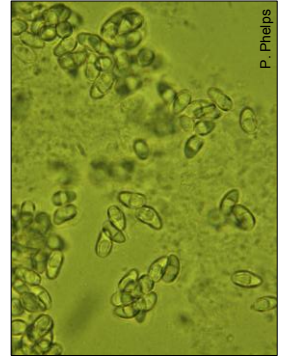
Yellow Perch (Leech Lake)

Submitted by resort owner
*Anglers reported "30% of YEP infected!"



Heterosporis spp.

- Wet mount of muscle
- Numerous spore-like organisms, consistent with microsporidian

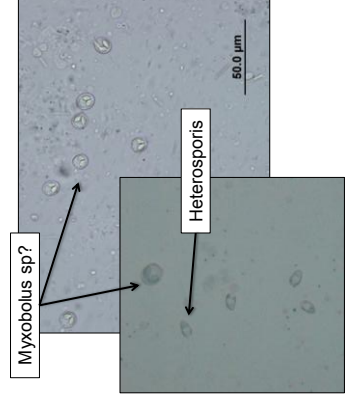
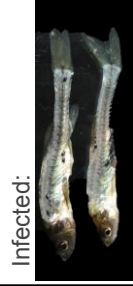


P. Phelps

Heterosporis spp.

Do not rely on clinical lesions

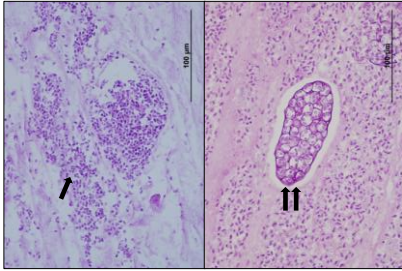
- FHM infection trial
- Control:



Heterosporis spp.

Histopathology

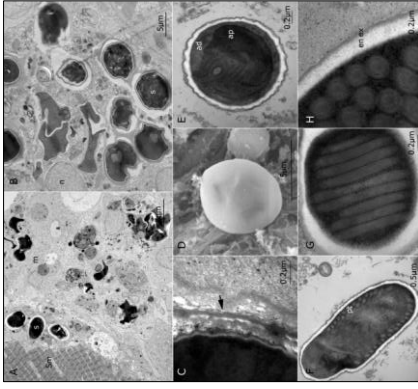
- Spores replicate within skeletal muscle cells, until cells rupture
- Fibrous connective tissue substitutes necrotic muscle fibers
- No spores observed in other organs



Heterosporis spp.

Electron microscopy

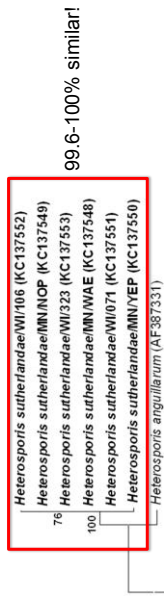
- Spore morphology consistent with *Heterosporis* spp.
- Degenerating and developing spores inside macrophages



Heterosporis spp.

Phylogenetic analysis

- Near complete SSU-ITS-LSU rRNA genes sequenced
- 3,646bp
- Included archived samples from Catfish Lake, WI
- Early *Heterosporis* sp. reports



0.01

Heterosporis spp.

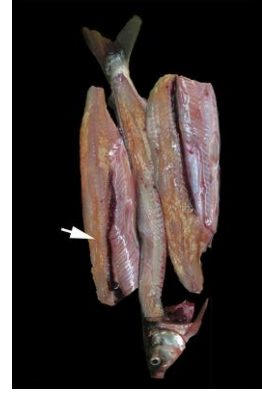
Cisco (Lake Superior)

Submitted by MN DNR

*First report in this waterbody and species

Compared to *H. sutherlandae*:

- Clinical lesions = Identical
- Wet mount = Identical
- Histo = Identical
- EM = Unknown
- Molecular, SSU-ITS-LSU = only 86.0% similar



Heterosporis spp.

Phylogenetic analysis

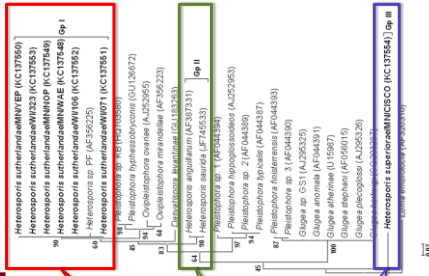
- Partial rRNA genes sequenced (750bp)
- Compared with variety of microsporidians

Similarities:

~97%

~88%

~89%



Heterosporis spp.

Diagnostic PCR

- Specific to *Heterosporis sutherlandae*
 - Detects 10 spores per reaction
 - Valuable for subclinical infections
 - Reproducible, accurate, etc.
- Specific to *Heterosporis superiorae*
 - In development

Research PCR

- Universal PCR for Heterosporis ID by sequencing
 - Also found new species from Brazil
 - Further genetic analysis pending

Heterosporis spp.

Conclusions

- Two distinct *Heterosporis* spp. in the Great Lakes region
 - *Heterosporis sutherlandae*
 - *Heterosporis superiorae*

MANY questions remain:

1. Population effects?
2. Environmental tolerance?
3. Hatchery control?
4. Zoonotic threat?
5. Improved diagnostics?
6. Recent introduction?



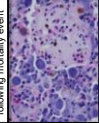
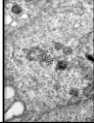

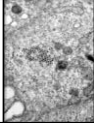
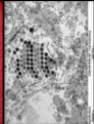
New viruses

The more you look, the more you find...

- VHSV surveillance
 - Hundreds – thousands of waterbodies tested for the first time
 - Non specific diagnostic methods
- Increased regulatory oversight
 - Find it, report it
- More awareness for fish health threats
 - Mortality events in wild fish
 - Aquaculture management
 - USFWS Wild fish health survey
- Funding
 - Fewer samples destined for freezer



New viruses

	Aquarium fish submitted following industry event	Walleye submitted for routine inspection	Bluefish submitted for routine inspection	Bluefish submitted with hemorrhagic lesions
History:				
Histology:		X	X	X
Cell culture:	X			X
EM:				X
Molecular:	BCIV	X	FV-3?	Papillomavirus

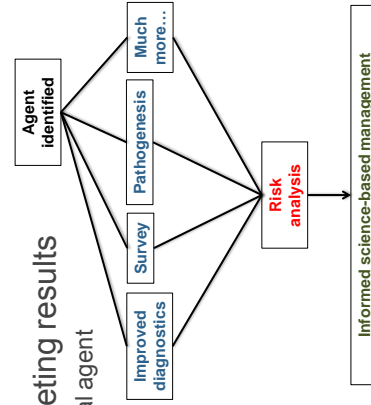
New viruses

- And there is more...
- Sturgeon Herpes Virus-2 (Dr. Waltzek, UF)
 - Calicivirus
 - Astrovirus
 - Bunyavirus (Dr. Hoffman, UW-LaCrosse)
 - Not surprising fish would have non-pathogenic viruses, everything else does!

New viruses

Use caution interpreting results

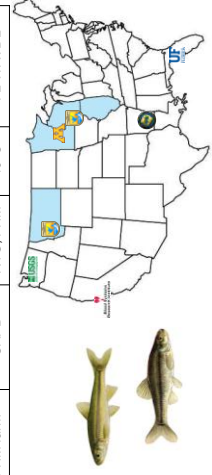
- Pathogen vs. microbial agent
- Viscera samples
- Cost vs Risk



FHM Picornavirus

Multiple unknown virus isolations over 10 years

Isolate ID	Time Period	Source	Primary Lab	Cell Line	Temp	Confirmatory Lab
1-8	2010-2012	MN/WI: Wholesale dealers	USFWS LaCrosse	EPC, CHSE	15, 25°C	MN VDL
9-19	2006-2011	MT: Wild baitfish	USFWS Bozeman	EPC, BF	22°C	USGS Seattle
20	2003	IL: FHM farm	UAPB	RTG, FHM	15°C	FL WAVDL



FHM Picornavirus

History: FHM collected during regulatory inspection

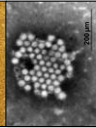
Histology: X

Clinical lesions?

Cell culture:



EM:

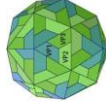


Molecular: Novel picornavirus

FHM Picornavirus

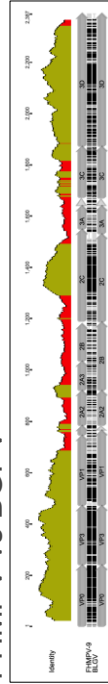
Picornavirus

- Small RNA genome (7-9kb), single polyprotein ORF
- 17 ICTV recognized genera
 - *Enterovirus* (rhinoviruses, polio virus), *Hepatovirus* (Hepatitis A virus), *Aphthovirus* (FMDV), *Avihepatovirus*, *Parechovirus*, *Kobuvirus*, *Teschovirus*, *Erbovirus*, *Cardiovirus*, *Aquamavirus*, *Cosavirus*, *Dictpivirus*, *Megrivirus*, *Salivirus*, *Sapelovirus*, *Senecavirus*, *Trenovirus*
- Fish: Sporadic reports of SPRV – Picornavirus?
 - Bluegill picornavirus
 - Mortality and hemorrhage in Wisconsin BL
 - Eel PV, Carp PV
 - Fathead minnow picornavirus

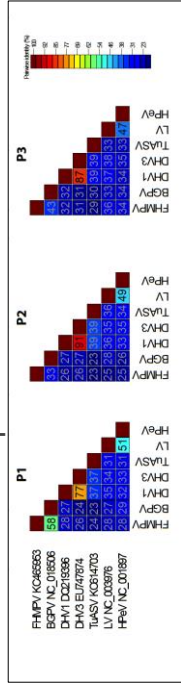


FHM Picornavirus

FHMPV vs BGPV



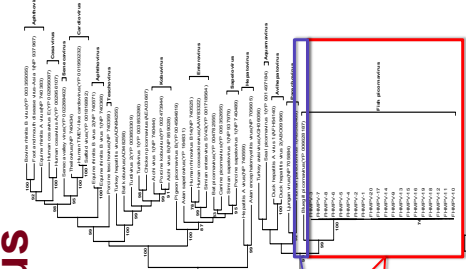
FHMPV vs other picornaviruses



FHM Picornavirus

20 isolates

- Sequenced 3D gene
- 1 isolate from each region
- Sequenced entire genome



43%

98.6-100%

FHM Picornavirus

Developed diagnostic assay

- RT-PCR and rRT-PCR available
 - Highly sensitive, specific, rapid
 - Surveillance and retrospective testing

Transmission to predatory fish

- Risk unknown

**No mortality or confirmed disease



FHM Picornavirus

Conclusions

- Novel FHMPV characterized
- No definitive association with morbidity/mortality
- Appears widespread

MANY questions remain:

1. Risk to predatory fish?
2. Risk to baitfish?
3. Source?
4. Environmental tolerance?
5. Relationship to other picornaviruses?

What else is out there...?



Continued research

Investigate new and archived agents

- Prioritize and characterize infectious agents
 - Identify emerging threats
 - Develop diagnostic and screening tools
 - Rapid response to new outbreaks

Establish formal risk analysis

- Many microbial agents, few pathogens...
 - Even fewer of concern
- Move towards "Risk-based management"
- Focus efforts



Continued research

Role of invasive species in pathogen introduction

- Invasive species = invasive pathogens?

Role of baitfish in pathogen dispersal

- Complex (and unknown) industry networks
- Model baitfish industry with hypothetical disease introduction
 - Identify hotspots, focus management, rapid response



Final thoughts from the lab

Communication

- Between labs, field, public, regulatory agency, funders, etc
- Early detection is key to limit impact of disease

Cooperation

- One lab can not do it all
 - At least in a timely and cost effective manner
- Identify regional experts and go-to labs



“Great Lakes lab network for emerging fish disease”

Questions??

**FISHERY RESEARCH PRIORITIES:
GREAT LAKES FISH HEALTH COMMITTEE
Great Lakes Fishery Commission**

Updated August 2013

This listing was compiled based on input from discussions within the Council of Lake Committees (for more information go to <http://www.glfcc.org/lakecom.php>) and the Great Lakes Fish Health Committee (<http://www.glfcc.org/boardcomm/fhealth/fhealth.php>). Order of listing does **not** imply relative ranking of priorities for the Fishery Research Program funding.

Research Priorities

- *What non-lethal field sampling methods and tissue/fluid samples are equivalent to conventional lethal field sampling methods to determine fish pathogen and/or disease status?*
- *What is the ecology of fish pathogens and diseases of concern in the Great Lakes Basin? Examples include (but are not limited to) viral hemorrhagic septicemia virus (VHSV) genotype IVb, Heterosporis sp., Epizootic Epitheliotropic Disease virus (EEDV), Flavobacterium sp., and emerging diseases.*
- *What is the effectiveness of the GLFHC disinfection protocols in eliminating key pathogens of interest from fish eggs? There is a need for a reliable disinfection methodology to prevent pathogen transmission via eggs and sperm.*

Additional Research Interests

1. Development and validation of new methods for detecting emerging fish pathogens or pathogens of concern in the Great Lakes Basin.
2. Disease Ecology and Epidemiology
 - (a) What is the susceptibility of Great Lakes fish species to emerging fish pathogens in the Great Lakes?
 - (b) Identification of reservoirs and vectors (including ballast water) for fish pathogens in the Great Lakes Basin
 - (c) What mechanisms affect the virulence and persistence of fish pathogens?
 - (d) What is the effect of population size on disease expression?
 - (e) What are the effects of multiple pathogens or combination of pathogens and nutritional deficiency and/or contaminant exposure on disease expression?
 - (f) What are the projected changes on fish pathogen prevalence and intensity as a result of climate change?
3. Nutritional Aspects of Fish Health in the Great Lakes.
 - (a) What is the role of lipids or other nutrients in determining and predicting health status?
 - (b) What is the role of thiaminase-producing organisms in Great Lakes ecosystems?
 - (c) What affect do invasive species have on nutrient stores in the Great Lakes and what are the associated effects on fish health?
 - (d) What is the effect of nutrition on reproductive success?

- (e) Does protein substitution in hatchery feeding formulations or extrusion manufacturing methods have a negative impact on survivorship, migratory behavior and reproductive success of hatchery-reared salmonids?
4. Fish Pathogen and Disease Management.
- (a) What are the effects of fish stocking and other management decisions on the manifestation of fish disease in the Great Lakes Basin?
 - (b) What effects does culling brood stock for pathogen control have on the genetics of production fish?
 - (c) When should fish not be moved past barriers (from a disease perspective)?
 - (d) Development of an emergency response plan for disease outbreaks in the Great Lakes Basin, including (but not limited to) training of field personnel and preplanning.
 - (e) What is the effectiveness of immunostimulants against key pathogens of interest in hatcheries?
 - (f) What is the effect of vaccination of hatchery fish on pathogen virulence?

Form RA-1. Risk Assessment for pathogen movements into a facility. Complete this form when importing fish or fertilized eggs into a hatchery from either the wild or from another hatchery.

1. Current prevalence of pathogen in the source population (5)		
High (67-100) – 5		
Medium (33-66) – 3		
Low (1-32) – 1		
None – 0		
2. Pathogen transmission through fish or their gametes (10)		
Vertical (and assumed horizontal) – 5		
Horizontal – 1		
Unknown – 5		
3. Current prevalence of the pathogen in the receiving facility (20)		
High – 1		
Medium – 3		
Low – 5		
None – 10		
4. Current prevalence of the pathogen in the effluent receiving waters (20)		
High – 1		
Medium – 3		
Low – 5		
None/ Unknown - 10		
5. Confidence in the pathogen test methods in the hatchery (15)		
Standard methods (Blue Book and/or OIE protocols) – 1		
Non-standard (non-representative) methods – 3		
No testing methods available (clinical signs only) – 5		
6. Describe the known potential for disease to other aquatic animals (10)		
One fish species affected – 1		
One fish family affected – 3		
More than one fish family affected – 5		
Multiple classes affected – 7		
7. Are effective treatments available to control infection and transmission with the pathogen? (10)		
Yes (e.g., egg disinfection, vaccinations, etc.) – 0		
No – 10		
8. Describe the potential for an epidemic in cultured and wild stocks (15)		
Known to cause elsewhere – 5		
Does not cause epidemics – 1		
Unknown – 5		
9. Knowledge of the fish species and its culture requirements (5)		
Adequate – 1		
Inadequate/Unknown – 5		
10. Source fish health history (last 10 years) (10)		
Parental history with no other Model Program pathogens – 1		
Parental history with other Model Program pathogens – 5		
No parental history – 5		

11. Population source location (10)		
Within the same Great Lake watershed – 1		
Between Great Lakes – 3		
An adjacent basin to the Great Lakes (e.g., Mississippi River, Hudson River, etc.) – 5		
Outside of the adjacent Great Lakes basins – 10		
Total Risk Score		

Form RA-2. Risk assessment for pathogen movements out of a facility. Complete this form when transferring fish to the wild during stocking events.

1. Current prevalence of the pathogen in the hatchery (10)		
High (67-100) – 5		
Medium (33-66) – 3		
Low (1-32) – 1		
None – 0		
Unknown – 5		
2. Current prevalence of the pathogen in the lot (10)		
High (67-100) – 5		
Medium (33-66) – 3		
Low (1-32) – 1		
None – 0		
Unknown – 5		
3. Pathogen transmission through fish or their gametes (10)		
Vertical (and assumed horizontal) – 5		
Horizontal – 1		
Unknown – 5		
4. Will effective treatment/disinfection measures be implemented for the pathogen? (10)		
Yes (e.g., egg disinfection, etc) – 0		
No – 5		
5. Current geographic distribution of the pathogen in the Great Lakes basin (10)		
Presence – 0		
Absence – 10		
6. Is the activity of introducing these fish likely to increase a pathogen's geographic range? (10)		
Yes – 10		
Maybe (presumed presence of the pathogen within the geographic range) – 5		
No – 0		
7. Is the activity of introducing these fish likely to increase a pathogen's prevalence in Question #5 of the receiving water? (10)		
Yes – 5		
Maybe – 3		
No – 0		
8. Prevalence of the pathogen in the receiving water (5)		
High – 1		
Medium – 3		
Low – 5		
None – 10		
Unknown – 10		
9. Is the receiving waterbody a broodstock source? (10)		
Yes – 5		
No – 0		

10. Indicate which vectors enable transmission of the pathogen in the receiving water (5)		
Choose all responses that apply and total the values.		
Commercial activities (ballast, weed harvesting, fishing) – 5		
Fish stocking and bait – 5		
Predators (birds and mammals) – 1		
Human activity (recreational fishing) – 3		
11. Describe the potential for disease to other aquatic animals (10)		
One fish species affected – 1		
One fish family affected – 2		
More than one fish family affected – 7		
Multiple classes affected – 10		
12. Describe the potential for an epidemic in wild stocks (20)		
Known to cause epidemics elsewhere – 5		
Does not cause epidemics – 0		
Unknown – 5		
13. Confidence in the pathogen test methods in the hatchery (5)		
Standard methods (Blue Book and/or OIE protocols) – 1		
Non-standard (non-representative) methods – 3		
No testing methods available (clinical signs only) – 7		
14. Fish health history of the lot (the last 2 years) (5)		
No history of other Model Program pathogens – 1		
History of other Model Program pathogens – 5		
No history – 5		
15. Fish health history of the current broodstock (the last 10 years) (5)		
No history of other Model Program pathogens – 1		
History of other Model Program pathogens – 5		
No history – 5		
16. Fish health history of the facility (the last 10 years) (5)		
No history of other Model Program pathogens – 1		
History of other Model Program pathogens – 5		
No history – 5		
17. Other pathogen presence (influence) in the receiving hatchery or waterbody (10)		
Comprehensive/Continual/Annual pathogen surveillance		
Other Model Program pathogen(s) detected – 7		
No other Model Program pathogen(s) detected – 1		
Limited/Sporadic pathogen surveillance		
Other Model Program pathogen(s) detected – 7		
No other Model Program pathogen(s) detected – 3		
No population/pathogen history – 7		
Total Risk Score		

Form RA-3. Risk Assessment Summary Information

Hazard Identification

Viruses:

Bacteria:

Fungi:

Parasites:

Other:

Comments:

Summary of the Request:

Summary of the Risk Assessment:

Statement on Overall Risk:

Signature of GLFHC Chairperson

Date

Cutthroat trout virus in Wisconsin

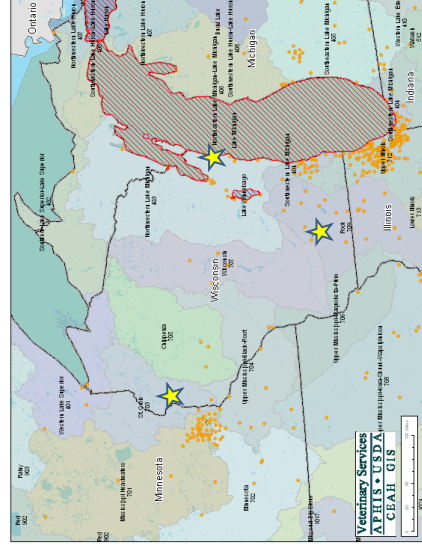
- CTV is a member of the Hepeviridae virus family. It is a small RNA virus that infects fish
- Diagnostic method: cell culture on CHSE-214 cell line
 - CPE is not dramatic and occurs late in the testing period- easily missed
- First identified/described in the late 1980's/early 1990's in California hatchery and wild trout populations. Has since spread to most Western states likely by movements of fish eggs and fish between hatcheries.
- Does not seem to cause disease or mortality in trout
- Susceptible trout species (1988-2010) cutthroat, rainbow, brown, brook, golden, apache, gila
- Little surveillance for CTV has been done on coolwater fish species or amphibians.

CTV in Wisconsin

- The Great Lakes Fish Health Committee developed an issue brief on CTV in 2008 after the virus was detected in two western federal hatcheries that supply trout eggs to other states. Major points:
 - Based on anecdotal evidence, the virus is likely an intra-egg virus and thus exports of infected eggs have assisted the spread of the virus. External egg disinfection protocols would not be effective on an intra-egg virus.
 - No significant disease has occurred in trout
 - However the GLFHC recognized that the effect of the virus on cool and warm water fish species is unknown
 - Their conclusion is the overall risk of CTV to the Great Lakes region is low, and that more research is needed.
 - GL states continued to import fish eggs from CTV positive federal hatcheries. WI ceased egg imports from CTV + states- but may have been too late

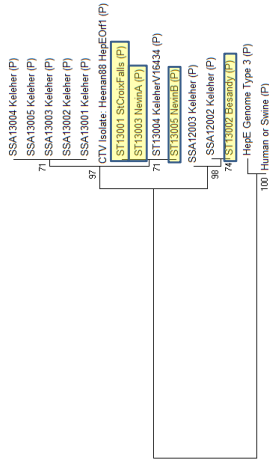
CTV in Wisconsin

- CTV first isolated in WI in Fall 2012 from :
 - St Croix Falls Hatchery SCF strain brown trout ovarian fluids (October)
 - Nevin Hatchery TC strain brown trout ovarian fluids (November)
 - Besadny Spawning Facility brown trout ovarian fluids and kidney/spleen tissue pools (November)
 - St Croix Falls Hatchery yearling TC strain brown trout (December)
- WIDATCP advised of the positive detections and chose not to take regulatory action based on the western experiences.
- FM board decision in January 2013 to allow fish from WI hatcheries that test positive for CTV to be stocked. Supported further CTV research
- CTV isolated from a PA hatchery in Fall 2012
 - Brown trout fingerlings



CTV in Wisconsin

- **Research underway:**
 - Develop a quantitative molecular method to detect CTV (qPCR)
 - Use the qPCR method to evaluate modifications of hatchery practices to reduce the exposure/transmission of the virus. Goal is to develop BMPs and share the findings with other states.
- **Research needed:**
 - Susceptibility of native cool and warm water fish species and amphibians to CTV
 - Long term population effects (post stocking)
 - Surveillance to track the spread in WI
 - Possible link between CTV infection and enlarged hearts in brown trout



Genetic relatedness of CTV isolates from WI
(T Waltzek U FL)

My Summer: Atlantic Salmon Virus and more

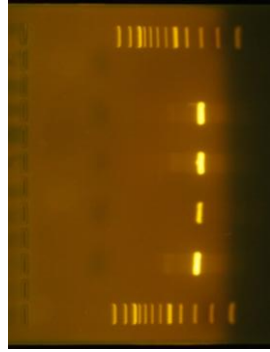
Justin Stilwell
Dr. Thomas Waltzek

Our Project

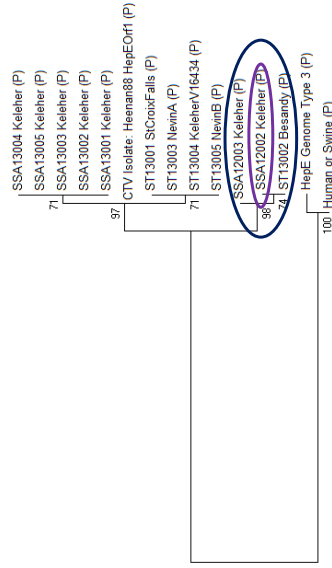
- Atlantic Salmon Virus – a novel Hepatitis E virus identified in farmed salmon
- Only the second Hepatitis E Virus identified in fish (CTV identified in northwest US)
- Importance: Zoonotic? Impact on salmon industry?
- Not associated with outward disease state
- Goal: Sequence the genome for the novel virus and develop a diagnostic assay

How We Did It

- RNA Extraction, RT-PCR, Electrophoresis, Gel Extraction, Sanger Sequencing
- Sequence Editing
 - CLC and BioEdit
- Primer Development
- Amplify and BioEdit
- Phylogeny
- Mega5.2



Appendix 10



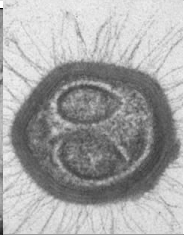
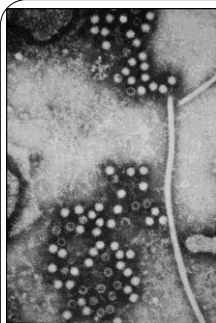
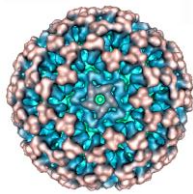
What We Got

- 5.9 kbs out of an estimated 7.3 kbs for SSA12002
- 3.4 kbs for SSA12003
- 73-78% Similarity to CTV

What else I did

Other viruses I worked with:

- CTV
- Mimivirus
- Paramyxovirus
- Aquareovirus



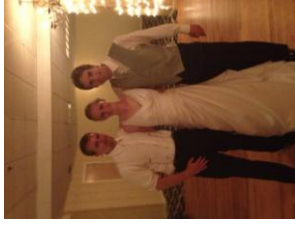
pathmicro.med.sc.edu
www.livescience.com
www.stanford.edu
www.reoviridae.org

What else I did

Gull Banding at Shoals Marine Laboratory



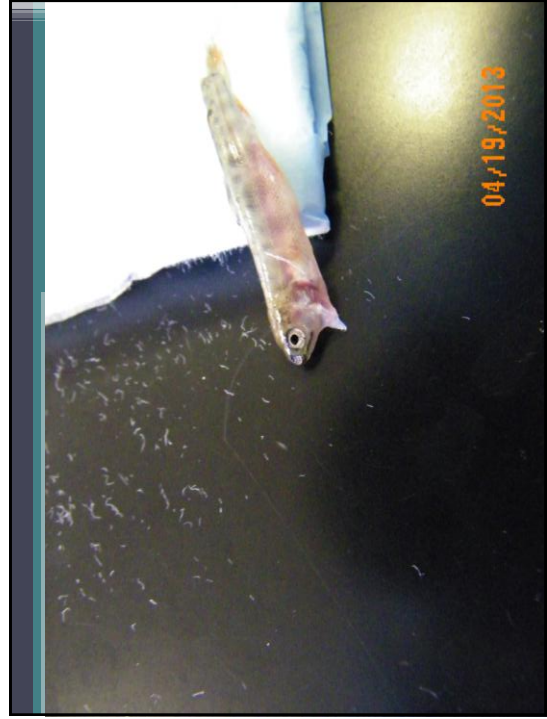
A Wedding in Vermont



Questions?

Mortality Event at Crystal Springs SFH

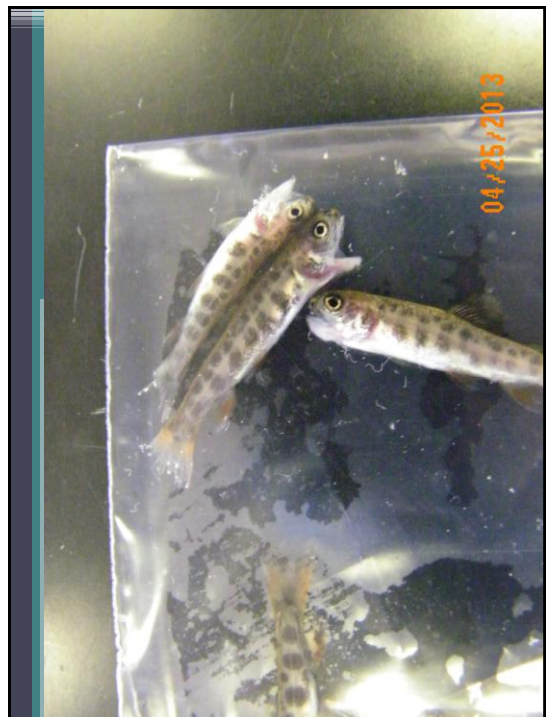
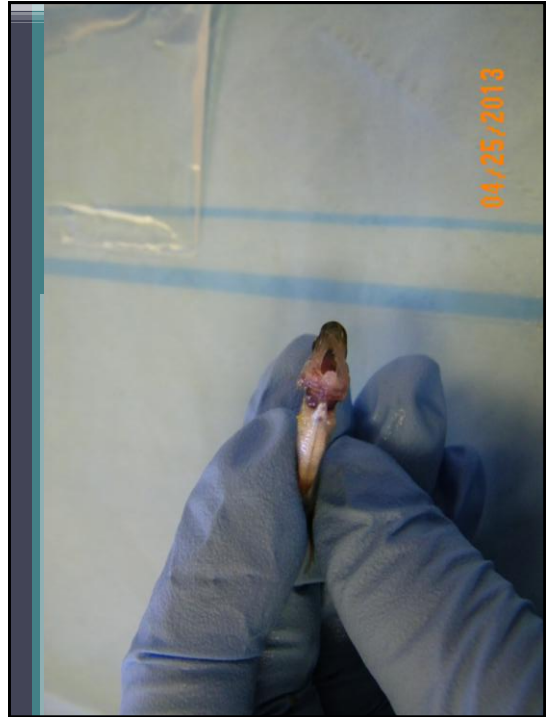
- Necropsy
 - Fish had distinct “Jaw dropping” phenomenon
 - Eroded tail
 - Hyperplastic gill
 - No parasites observed

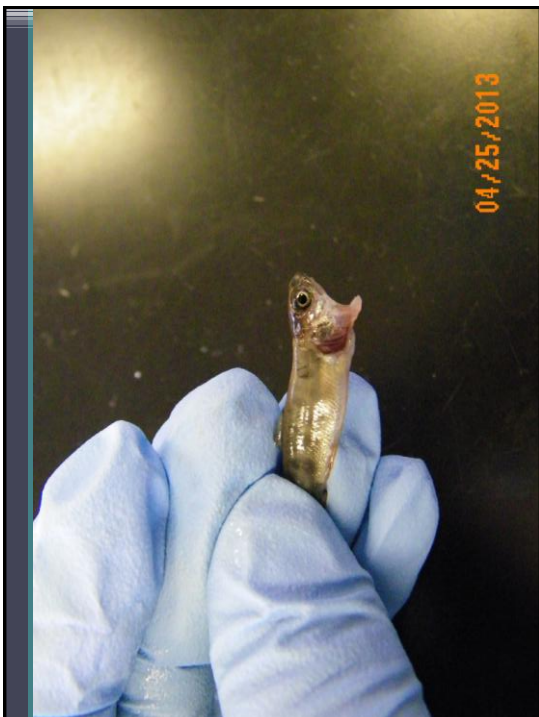
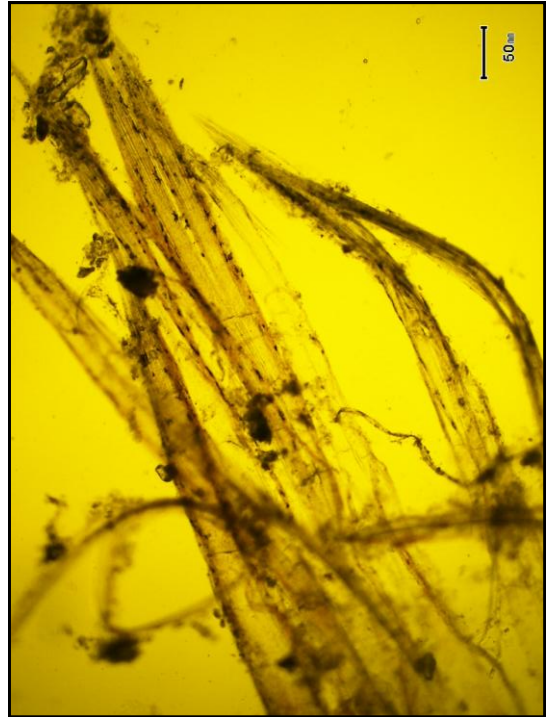


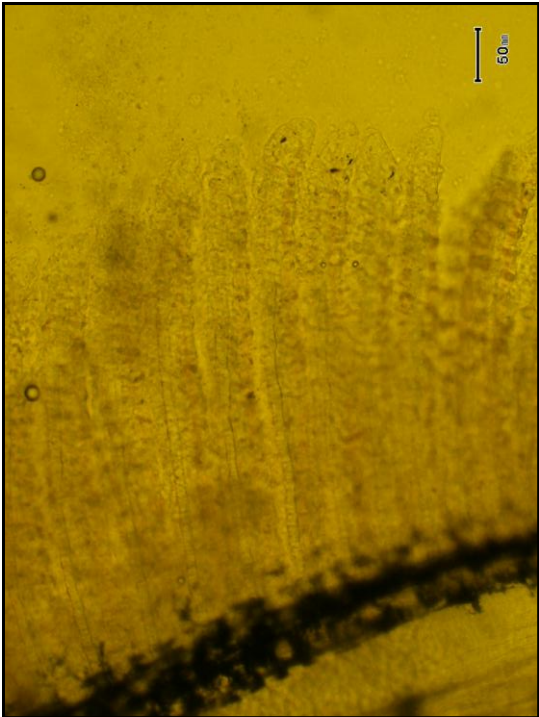
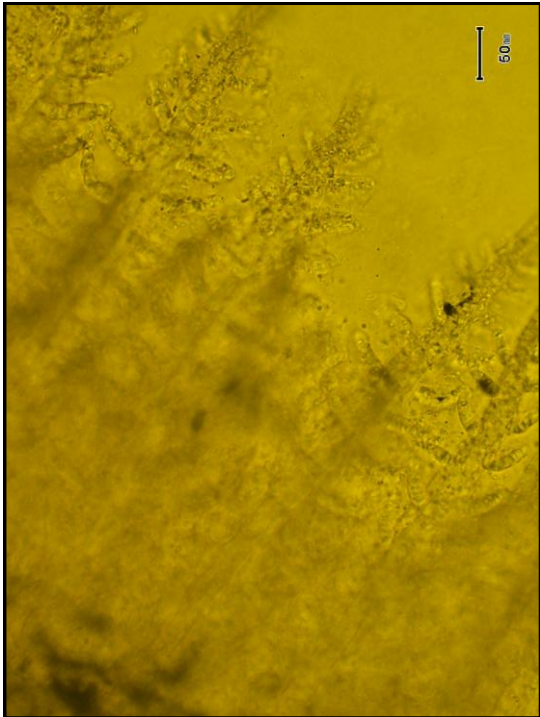
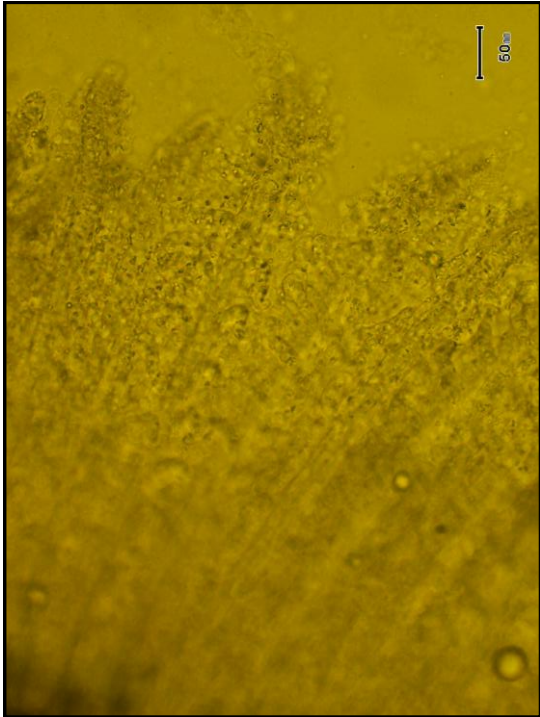
Mortality Event at Crystal Springs SFH

- Heavy rain occurred 4/9/13 three inch rain in 3hr
- Water from the spring turned into chocolate brown/foamy color
- Mortality started 4-5 days after the rain event
- Mortality started at 100/day, Climbed to 300-400/day



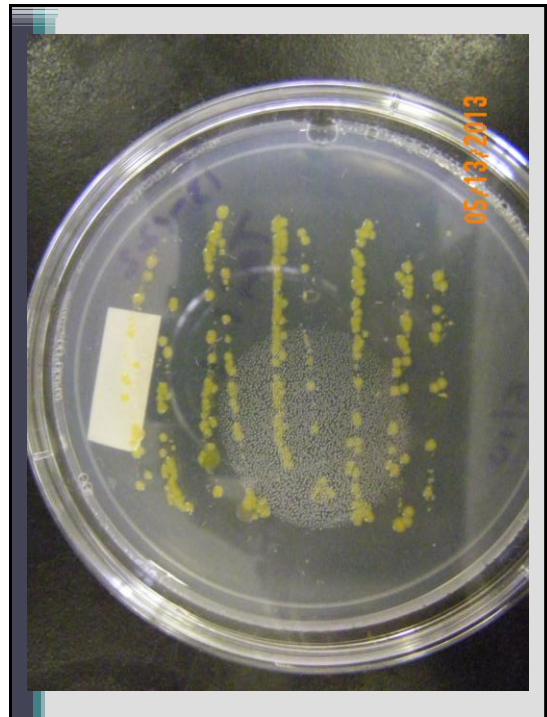
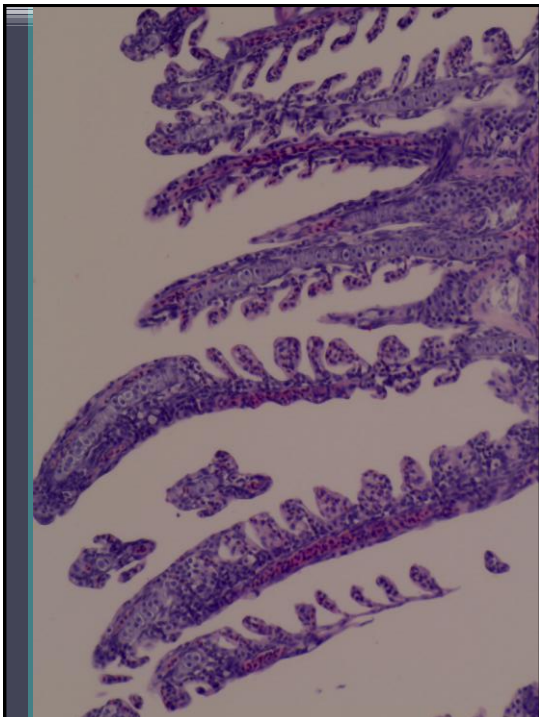
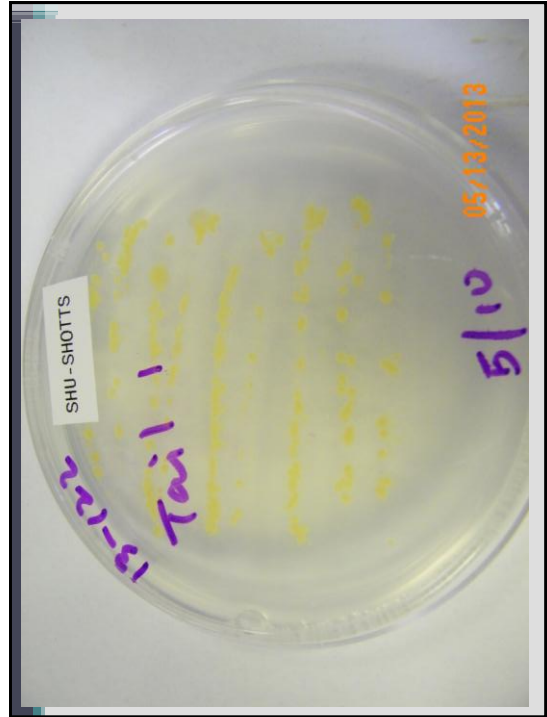






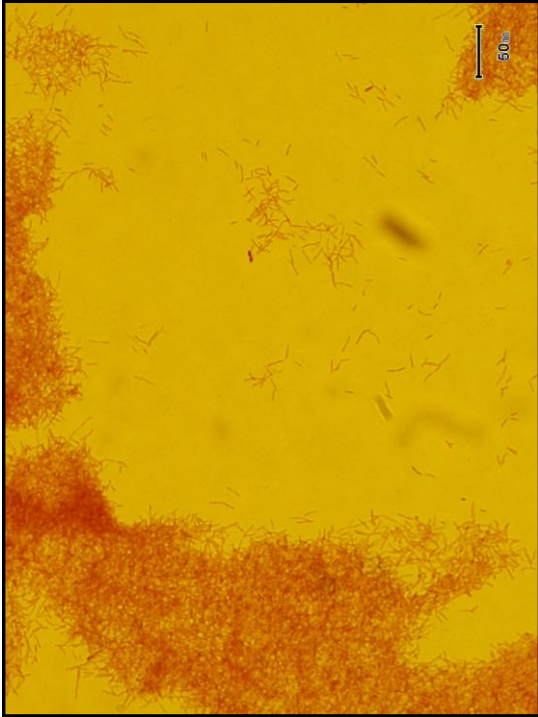
Lab Tests

- Virology
 - Sample were put on both EPC and CHSE cell line.
 - No CPE observed
- Bacteriology
 - growth obtained from both gills and tails on shu-shotts media



Mortality Event at Crystal Springs SFH

- Bacteria Identification
(Kennebec River Biosciences)
 - Both isolates from gill and tail were found to be biochemically nearly identical
 - Identified as: **Chryseobacterium chaponense**



Character of the bacteria

- Grow at 20c not at 15c
- Sensitive to several antibiotics including Oxytetracycline, Romet
- Fish did not respond to salt bath and Hydrogen oxide treatment. Mortality dropped to zero after fish been put on terramycin medicated feed for 10 days.