GREAT LAKES FISH HEALTH COMMITTEE

2013 Winter Meeting
South Bend, Indiana
February 5-6, 2013

Minutes
(with attachments)

Submitted By:

Christina Haska
Great Lakes Fishery Commission

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

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List of Attendees

John Coll	U.S. Fish and Wildlife Service- Pennsylvania
John Dettmers	Great Lakes Fishery Commission
Mohamed Faisal	Michigan State University
Christina Haska	Great Lakes Fishery Commission
Sunita Khatkar	Fisheries and Oceans Canada
Randy Lang	Indiana Department of Natural Resources
Kevin Loftus	Ontario Ministry of Natural Resources
Sue Marcquenski	Wisconsin Department of Natural Resources
Dave Meuninck	Indiana Department of Natural Resources
Andy Noyes	New York State Department of Environmental Conservation
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:

Martha Wahlgamood	Michigan Department of Natural Resources

Great Lakes Fish Health Committee Meeting

February 5-6, 2013
Salon B
DoubleTree Hotel
123 North St. Joseph Street
South Bend, IN 46601

Draft Agenda

(Revised January 31, 2013)

Tuesday, February 5

8:30 am - 8:45 am	Welcome & Introductions (Phillips)
8:45 am - 8:55 am	Approval of Meeting Minutes (Phillips)
8:55 am – 9:00 am	Communications (Phillips)
9:00 am – 9:15 am	Model Program Update (Phillips)
9:15 am - 9:40 am	Ranavirus Presentation (Waltzek)
9:40 am - 10:05 am	New Tools to Detect Emerging Fish Viruses (Waltzek)
10:05 am - 10:25 am	Break
10:25 am - 10:55 am	Unknown Viruses from Chinook Salmon in Ontario (Loftus)
10:55 am - 11:30 am	APHIS Update (Whaley)
11:30 am-1:00 pm	Lunch (on your own)
1:00 pm-1:45 pm	Conservation by the cup of water: The potential for indirect surveillance techniques to inform conservation management (Jerde)
1:45 pm-2:15 am	Michigan State University Research Update (Faisal)
2:15 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	Cutthroat Trout Virus (CTv) in WI DNR Brood Stocks (Marcquenski/Phillips)
3:00 pm-3:20 pm	Break
3:20 pm-4:20 pm	EEDv @ Marquette SFH (Whelan/Phillips)
4:20 pm-4:45 pm	GLFHC Webpage (Haska/Phillips)
4:45 pm-5:30 pm	Agency Updates (5-10 minutes each) (All)
5:30 pm	Adjourn for the day

Wednesday, February 6

8:30 am-9:00 am	Hydrogen Sulfide - From Aquatic Pollutant to Clinical Therapeutic, A Fish Story (Olson)
9:00 am-9:30 am	Resident Fishes Display Elevated Organic Pollutants in Salmon Spawning Streams of the Great Lakes Invited Paper (Lamberti/Chaloner)

9:30 am-10:15 am	Great Lakes Fish Health Center Discussion (Faisal/Marcquenski)
10:15 am-10:35 am	Break
10:35 am-11:35 am	Incorporating Non-Member Agencies—Presentations (Phillips)
	A. Indiana Board of Animal Health (Strasser)
	B. Ohio Department of Agriculture (Forshey)
	C. Wisconsin Dept. of Agriculture Trade & Consumer Protection (Kebus?)
11:35 am-1:00 pm	Lunch (on your own)
1:00 pm- 1:30 pm	Epitheliocystis Blue Jay Creek FCS (Lumsden/Loftus)
1:30 pm-2:30 pm	Agency Updates—Continued (All)
2:30 pm – 2:50 pm	Break
2:50 pm-3:50 pm	Review & Discussion of Research Pre-proposals (Phillips)
3:50 pm-4:10 pm	Selection of New GLFHC Vice-Chair (Dettmers)
4:10 pm-4:30 pm	Future meetings (Phillips/Dettmers)
	A. Number of Meetings/Year
	B. Dates/location for Winter 2014 Meeting
4:30 pm-5:00 pm	Incorporating Non-Member Agencies—Discussion (Phillips)
5:00 pm-5:30 pm	Meeting Wrap-up/Parking Lot (Phillips)
5:30 pm	Adjourn for the day

Tuesday, February 5, 2013

1. Welcome and introductions (Phillips)

2. Approval of meeting minutes (All)
The meeting minutes from July 2012 and the conference call in January 2013 were approved.

3. Communications (Phillips)
Ken was contacted by John Kerwin, Executive Secretary of the Pacific Northwest Fish Health Protection Committee (PNFHPC) regarding the SVC outbreak that occurred in Minnesota in 2011. Ken sent the excerpt from Minnesota's annual report that addressed the outbreak, a link to a communication in the Journal of Aquatic Animal Health, as well as Ranjit Bhagym's (co-author of the publication) and Ling's contact information.

4. Model Program update (Phillips)
The copy edits are done and the final version will be sent to Randy at the GLFC for formal typesetting. This will take about four weeks to finish, then the document will be published online. Hard copies will be printed after that. The online version can be updated annually, and the hard copy as needed. The winter agenda should include time to discuss any edits to the Model Program because that is when most members are present. It is likely any changes would be prompted by something happening throughout the year that would elicit an edit.

Consensus was reached that the document would be changed as needed. The writing subcommittee of Ken, Ling, Andy, and Gary will be in charge of edits. Inform Ling of any issues that need to be discussed, and she will start the appropriate chain of events.

5. Ranavirus presentation (Waltzek)
See Appendix 1 for the presentation.

- Tadpoles are the most susceptible. Amphibians are not challenged as adults.
- Pallid sturgeon fingerlings and tadpoles were the life stages used in the study.
- The only isolate from adults was from a white sturgeon epizootic in California.
- The studies were done by bath exposure, not by injection.
- The Frog Virus 3 that was found during VHS surveillance was not part of a disease outbreak. There may have been carriers, but it's unknown.
- Temperature extremes may stress fish and initiate mortality, but there are a lot of unknowns – e.g., if adults are carriers, transmission paths, etc.
- The water temperature at BPSFHS was 17 – 25°C in 2001 and 2009.

6. New tools to detect emerging fish viruses (Waltzek)
See Appendix 2 for the presentation.

- It is difficult to culture the pallid sturgeon iridovirus. It is tough to grow and you don't often see cytopathic effect. That's been a big challenge, so instead, they work from infected tissues.
- The cost of sequencing depends on the question and what you need. If the virus has been purified and has a short sequence, it is cheaper.
- The sequencing is done at the university sequencing core.
- The DNA extraction process to separate out the genome of the fish from that of the virus is to essentially purify for all viruses, which could result in a long list of which are present. When there is a co-infection, it can be challenging to know which is the culprit of the infection. The process is similar to that of purifying cell tissue: rupture fish cell; release viral cell; separate out debris; and treat with nucleases to destroy DNA or RNA outside the capsid. The virus remains.

7. Ontario Fish Health (Loftus)
See Appendix 3 for the presentation.

- The detection was 30% from the ovarian fluids and 30% from the kidney and spleens.
- The U.S. also has this issue and is watching the RTG work in Ontario for insight.
- Roz Stevenson would have information on the progress of sequencing the aqaureovirus.

8. APHIS Update (Whaley, via phone)
Stakeholder groups were asked to give recommendations regarding VHS. The GLFHC survey to its members is much appreciated, and she is looking forward to seeing all the responses.

Decisions have been made about next steps, which were sparked by industry groups who want the federal order removed immediately. APHIS is not rescinding this order. Other natural resources groups do not want the order removed until the groups have alternate methods in place.

Existing regulations are being reviewed. A risk assessment is looking at different approaches to reduce exposure of live freshwater fish species to VHSv. The risk of farm animals transferring the disease is also being investigated. Objectives will take about 6 months to complete, and they will be posted in public record to get feedback. The scope is in draft form and will consider the likelihood of VHS being introduced to farm fishes, the spread to susceptible species, and biological and economical consequences. It will NOT look at the effectiveness of state regulations. This information will help them move forward with decisions.

They will likely look into a voluntary VHS certification program. They are also looking for pilot research projects, pending funding, similar to that of ISA in the northwest. Resources are limited though.

- APHIS has not received any feedback yet from the industry sector about not rescinding the order. Janet will likely hear something when she meets with industry folks in Nashville.

- Recommendations from the original questions are still welcome. The project is in the scoping phase, but the risk assessment will likely start in the next few weeks.
- The Ford-Collins group is doing the risk assessment.
- The modeling approach is similar to that of ISAv. There were unconfirmed findings of ISAv in British Columbia in the Fraser River. The U.S. Congress asked the aquatic animal health task force to answer questions on U.S. capabilities to look for it. A surveillance and research plan were initiated from some seed money, and now an enhanced surveillance project is happening in Washington and Alaska. APHIS is working with various agencies for testing and screening. Positives will be confirmed by a USDA veterinary service. The pilot study will be working with industry and performing surveillance of captive Atlantic salmon. This all is just getting started.
- The survey is a two-year project depending on funding. APHIS had asked Congress for funding but did not receive it, so they are working off 2012 numbers.

If you have any comments or questions, please don't hesitate to contact her.

Thoughts on how to proceed with the GLFHC:
Go back and revisit the questions. Review what you have written and see if there are any changes. Ken will send out to the committee what was sent to Janet. The group can review the statements and see if any changes need to be made. The final version will be sent to the CLC chair and vice-chair prior to sending back to Janet for final submission. The deadline for everything is the end of February. The writing subcommittee could take charge, if needed.

9. eDNA presentation (Jerde)
See Appendix 4 for the presentation.

- The control water samples were a mixed slurry of non-target DNA and distilled water. These were placed in tubes with the caps open on the boat, and then were run through PCR to make sure they were not contaminated with carp DNA.
- qPCR was not used for this study, but others have used it in projects. The silver carp marker is more sensitive than the bighead carp marker.
- When measuring the sensitivity of the text, it is done in pure water with the template. eDNA is not mixed in. This prevents inhibition due to environmental contaminants.
- False negatives from PCR inhibition have been detected, and those are tough to figure out.
- There have been some DNA degradation studies, and the longest it has been found is 4 days. The environmental conditions make a difference (e.g., low water levels with high temperatures will decrease the amount of DNA).
- Purdue is testing if egg DNA can be found during spawning events. Milt is more likely to be found and amplified during an event.

10. Michigan State University Research Update (Faisal)

See Appendix 5 for the presentation.

- The team is not going to approach the FDA to get approval until another student's research is completed and the results are in.
- Coldwater disease is common at some weirs but not in steelhead; nonetheless, it shows up in steelhead at hatcheries. This could be from the Chinook salmon housed at the same hatchery. There are too many potential explanations for this.

11. CTv discussion (Marcquenski)

Sue had asked Ken to forward an email about CTv to the committee in January (Appendix 6). Ovarian fluids were tested from spawning broodstock at the St. Croix Falls hatchery (SCF). The fish appeared normal with no mortalities. The LaCrosse fish health facility found Chinook salmon cells were abnormal. It was confirmed in late November/early December that it was CTv. There is a strain of brown trout at Nevin hatchery (which is not close to SCF), which has been exchanging eggs with SCF for the last 30 years or so. Those fish were tested and CTv was found there also. Other sources of brown trout were tested and the virus was found there as well. In early December, every lot of fish was tested at SCF (fry, yearlings, adults, etc.). CTv was found in yearlings that had spent their whole lives within the hatchery, separated from the broodstock. Staff have said that every year, eyed eggs were disinfected at Nevin, transferred to SCF, disinfected again, and still had the virus as yearlings.

Currently, there is early life stage mortality in coho fry at Wild Rose hatchery. About ten days ago, technicians have started to see mortality in muskies. See the pictures in Appendix 7 for details.

Sue's recommendation was to not stock anything that tested positive, but her supervisors did not agree. When stocking fish with viruses, consideration should also be given to other species. See Tom's ppt in which viruses can be transferred between species. Sue is still concerned and will be testing for CTv above and below the dam in the St. Croix River in a variety of species. She is concerned because wild fish do not go into SCF or Nevin hatchery, so it's unclear what was first exposed. It could be a feed issue (bad pasteurization), but records of feed deliveries were not kept.

In the issue brief to her administrators, Sue expressed the need to have a hatchery system with a goal of a virus-free broodstock. Best management practices would be necessary. This could reduce the virus below a detection threshold, but more information is needed to know the susceptibility of coolwater species.

The water source at the hatchery is spring water. The CTv results were sent to Tom Waltzek to be compared with previous outbreaks, and this strain of CTv is not unique.

Nevin Hatchery does not stock fish in the Great Lakes, and SCF historically did but that has ended. Only Wild Rose Hatchery strains are stocked now in the basin.

Pennsylvania has had similar CTv issues (see email, Appendix 8). The hatcheries were experiencing fish mortalities but did not find IPN. They changed the feed, but fish were still dying. Finally, inspections discovered it was CTv. The brood they came from were IPN positive--- PCR tests are currently being run now and they are awaiting results. The broodstock is known to have genetic issues, and Coja is trying to get them to switch them out and bring in new, disease-free fish. The CTv fish are not scheduled to be stocked until 2014, so they will be kept for now. The fish likely won't be stocked in the Great Lakes basin. Next year, the fish will be cultured an additional week to see if the virus can be find earlier.

Mohamed commented on helper viruses. This occurs often with herpesvirus, in which only having one strain of the virus will not result in disease, but when two strains co-occur, they are activated and disease occurs.

Recommendation from the committee:
Is it possible that CTv has been here for a while but just hasn't been detected? It could be beneficial to process older samples to see if it was present. Prevalence may not be able to be calculated. The pathogenicity of the virus would be important to know. There are too many unknowns for the committee to be able to make a proper recommendation on how to handle this virus.

12. EEDv at Marquette State Fish Hatchery (Whelan/Phillips)
A document was sent from Gary through email (Appendix 9). In it, there is data on the cumulative mortality by raceway and lake trout strain.

Fish mortality is leveling off, and lesions are healing. The coloration of the fish is coming back. Titer levels are low and declining, indicating the fish are likely in recovery now. Splake were positive for EEDv, and subsequent testing has turned negative, so they may have cleared the virus. No positive brook trout are on the station. The broodstock may have an immune response from exposure in the wild.

EEDv is an endemic virus, and the fish appear to be in recovery. Hatchery personnel are expecting to see even lower titer levels when the fish get retested in a few weeks.

The LHTC does not want these fish stocked in Lake Huron. Gary's opinion is that these fish probably don't represent a high risk, but acknowledges that it's a herpesvirus and will be latent. These flare-ups may have been caused by stress from the conditions at the hatchery, which they likely would never experience in the wild.

All lake trout production units had positive fish within it (raceways). There was no mortality in the broodstock, but the pathogen was detected. This is the only lake trout hatchery in Michigan.

The current plan is to manage around this disease. No fish with clinical signs will be stocked. If there is a recurrence or if this happens on a regular basis, the stocking issue would be revisited.

Member Comments:

- Constituents may be upset that Michigan is stocking infected fish. If this virus is similar to BKD, that's a level 2 restricted pathogen in the Model Program, which recommends not stocking.
- EEDv has been found in Lake Ontario and Lake Superior, but it has not yet been looked for in Lake Huron.
- It's legitimate for the LHTC to be concerned, even if fish have been stocked before with latent virus. These fish are showing clinical signs, which increases the risk. They have the right to be worried about increasing the viral load in Lake Huron.
- On the other hand, this virus is likely ubiquitous in the Great Lakes, BUT the effects of stocking these fish could potentially be felt 20 years from now.
- The fish might be stocked in Lake Michigan, but that is currently unclear. This is not a simple management issue.
- The GLFHC could make a description of the risks, but not necessarily provide a recommendation.
- Neither La Crosse nor Lamar has done much testing in the Great Lakes for this virus. 59 fish from Lake Huron were sampled and no pathogens were detected. Two fish in Lake Ontario were positive. If this is endemic in the receiving water, they could be put in.
- It may be worthwhile to sample tissue of the survivors and also stress a subset of fish and see what happens. That could provide information that would feed into what to do. Nonlethal sample assays (e.g., WBC) would be great.

Michigan DNR will not stock fish with clinical signs, and will do subsequent testing of pathogen levels in the springtime. Right now, there's nothing to hint these fish could not be stocked in the Great Lakes. If the CLC recommends not to stock in the Great Lakes waters, these fish will likely be stocked inland instead.

When reporting to the CLC, the GLFHC should give the Risk Assessment with rationale for each value. Ken will look into the scales on the risk assessment and assure they are comparable to that of USFWS.

13. Hydrogen sulfide – from aquatic pollutant to clinical therapeutic (Olson)
See Appendix 10 for the presentation.

- There are deep thermal wells with H_2S at the Thompson SFH, which also has chronic disease issues with fish. The hatchery technicians think it might be sublethal toxicity.
- There are different susceptibilities in fish to H_2S. There appear to be physiological adaptations, such as carp being more resistant than trout. Different tissues from different species have been analyzed and there are large variations.
- Patents are in place for a new aspirin, and they are negotiating with drug companies to have the drug hit the market.

14. Resident fishes display elevated organic pollutants in salmon spawning streams (Chaloner)
See Appendix 11 for the presentation.

- Dam removal is an important issue right now in Michigan. Of concern is the population effects on native fish that are exposed once the dams are removed. Also, mink and bald eagle populations are increasing in the areas with spawning fish.
- Lake trout recruitment might be contributing to contaminant levels. It's hard to evaluate and there is not much in the literature. There is concern: dams probably shouldn't be removed without more research. There's no definitive word that it would/not have an effect. Should we be concerned about consuming these fish? There's the human health issue too.
- Gut analysis wouldn't necessarily tell what the source of the contaminants is (e.g., do sculpin eat the eggs?), which is why it would be beneficial to use stable isotopes to see the continuous flow.
- The control sites were up and downstream with barriers but without salmon.
- Managers make the decisions about negative consequences vs. benefits. Scientists determine lethal limits which aren't currently known. We need models to predict likely contaminant transfers. The current levels could be of concern, but we need more data. Each system should be looked at individually when making decisions to remove a dam or restore connectivity.

15. Great Lakes Fish Health Center discussion (Marcquenski and Faisal)
See Appendix 12 for the presentation.

Over the years, the GLFHC has faced challenges as a committee. Sue and Mohamed need a recommendation from the committee that having a Fish Health Center is a priority.

There was some concern from committee members that their agency would not be able to contribute money to the project. Nonetheless, it would be worth pursuing and investigating other funding opportunities as the project progresses.

It would be important to have fishery manager support as well. The Executive Board would be comprised of managers, fish health experts, and academics.

The first step will be creating a white paper to summarize these ideas, gain support from the GLFHC, and present to fishery managers to gain their insight. Gary, John, and Mohamed will work on the white paper together and send it out to the committee for comment.

16. Incorporating non-member agencies

Indiana Board of Animal Health (Strasser)

See Appendix 13 for the presentation.

- It would be nice to have a relationship with the GLFHC to help brainstorm when issues arise.
- If the U.S. develops an aquatic health plan, the Board's role may change depending on the regulations that become enforced. For example, if the USDA takes charge, she may have an aquaculture role.
- The Board contacts other states when drafting new regulations. It wants to be in line with what the surrounding states do, but each state has its own issues.
- Aquaculture in Indiana is not huge. There's only one large producer, but lots of farms raise freshwater shrimp.
- The DNR is in charge of the aquaculture registration process. There are currently 120 permit holders and 20-40 grass carp holders.

Ohio Department of Agriculture (Forshey)

See Appendix 14 for the presentation.

- There is a lot of fragmentation in the components of the industry because of varying communication styles between the DA and the Amish community.

17. Epitheliocystis Blue Jay Creek FCS (Lumsden)

See Appendix 15 for the presentation.

- At three weeks, there was a decrease in inclusions but an increase in mortalities. This is likely due to variation between species, and some have co-infections. In lake trout, microcolonies are present before early signs. They rupture, causing inflammation and lesions, and eventually cause mortality.
- When events occur, reduce the feed response. Tetracycline may help. Field trials are currently ongoing and they are awaiting results to give information on the course of the disease. The bacteria cannot be cultured.

18. EEDv Conversation Resumed (All)

Everyone is assigned to do the Risk Assessment independently and send it to the Christina, Ling, and John. They will summarize the reports and a conference call will follow to discuss the issue.

Agency comments

Wisconsin USFWS: Wisconsin cannot support stocking these fish. Not much is known about the virus and its distribution in the wild. Wisconsin hatchery managers are concerned these fish are shedding the virus into tributaries leading to Lake Superior. The fish which tested positive and had clinical signs of disease should be destroyed.

Minnesota DNR: This is considered an Emergency disease in Minnesota. The management would destroy the fish and not proceed with stocking. Lake Huron water levels are at records lows. This may induce stress and not be appropriate to stock diseased fish there.

Pennsylvania USFWS: Abstains from making a recommendation. More needs to be known--- prevalence, whether or not it would biomagnify, etc. It is out there already but no one knows where or to what extent.

Pennsylvania FBC: Stocking inland may be appropriate. If these fish went through another stress test and the disease reemerged, these fish should be destroyed. If not, stock inland, but not within the Great Lakes.

New York State DEC: If this happened in New York, the fish would be destroyed. Andy abstained from making a comment on behalf of his agency.

Indiana DNR (Lange): Draft out the facts and present what is known and what is unknown. Present this then to the LHTC and the LHC for a decision.

Wisconsin DNR: In 2009-2010, kidneys and spleens were tested and found positive for EEDv. The fish did not display any outward signs of disease. At the time, the GLFHC gave mixed advice. In the end, the fish were stocked. If there had been clinical signs, these fish would not have been stocked. Stocking known diseased fish on top of any background levels of the pathogen in the water is not supported.

Fisheries and Oceans Canada: Abstains from making a representative comment until consultation with colleagues. There is concern about stocking.

Michigan State University: Even fish which have not shown clinical signs are infected. The risk is the same for fish which are infected versus those which have the disease. Perhaps if we stock fish who have survived the disease, we will build a stronger stock (herd immunity).

Ontario MNR: Articulate the facts objectively and provide it to the CLC. They then can assess the risk and make the final recommendation. This does not appear to be a huge risk, but admittedly he is not the most qualified to make that decision. This would be an appropriate objective of a Fish Health Center!

Indiana DNR (Meuninck): Survivors are sitting in this hatchery and it may be beneficial to release them. It could be that every fish in the Great Lakes has some form of this infection---it's unknown.

Final recommendation: There likely won't be consensus about what to do. We can present these issues to the LHC and CLC and allow them to proceed as they see fit.

19. Research Pre-Proposals (All)

The GLFHC was asked to review 12 pre-proposals submitted to the GLFC's Fishery Research Program and provide recommendations about whether or not the projects merited full proposals.

20. Selection of New GLFHC Vice-chair (All)

Coja was nominated and accepted the nomination. Beginning after this meeting, the Chair will be Ling and the Vice-Chair is Coja.

21. Future meetings (All)

Prior to VHSv outbreaks in the Great Lakes, the committee only met once a year. There has been talk about going back to this because it is difficult for some members to get travel approval twice a year. The GLFC would prefer the committee met twice a year but recognizes this is the members' committee. Other committees do not have every member attend every meeting, and it's understandable when this happens.

One meeting a year **benefits**: not having to worry about travel.
One meeting a year **cons**: does not allow for great continuity; only routine business would be conducted without an opportunity to invite speakers and branch out to other researchers and interests.

Member comments:

- Two meetings/year would allow for information to be provided to the CLC at each of its meetings, if need be.
- We could have distance meetings, i.e., remote conference calls broken up into sections, Skype, Adobe Connect, etc.
- Many members never attend any meetings. It may be worthwhile to reestablish these members at distance meetings.
- Mohamed's proposals: 1) one in-person meeting and one electronic; 2) one in-person meeting and then again at a national conference in the summer

Decision:

For this summer meeting, the GLFHC will meet in-person. For those that cannot make it, there will be a call-in option. Ling will set up a tour of the local hatchery by Duluth.

Dates/Location for next winter:

Pennsylvania will host. February 4-5, 2014.
The committee should consider hosting some continuing education workshops for the winter meeting.

22. Incorporating Agriculture Departments (All)

How can the GLFHC ensure continuity with invitations to agriculture consultants?

- Develop a list of potential contacts that could be invited to meetings. Eg., Diane Elliott, Myron _, Janet Whaley, etc.

- Agencies can nominate who should be on this list. GLFHC members could contact those they know already and see what they suggest. Each committee member should figure out who is best to contact for their state.
- Only agencies signatory to the Joint Strategic Plan can be official members of the committee. This does not include Agriculture departments; nonetheless, their input and participation is wanted. Their presence is akin to technical advisors.
- Likely, representatives will only attend meetings when it is held in their area. This means that some representatives may only come to meetings once every 5-6 years. Invitations can be sent with agendas to involve their participation. Travel funding may help get them to attend (unsure where this would come from) OR they can attend via off-site capabilities.
- There is the potential to have the representative's presentation be a part of the continuing education part of the Winter 2014 meeting.

23. Final Comments (All)

The GLFHC website needs revisions. Ling, John, and Andy will form a subcommittee to review the website and decide the best course of action.

Asian tapeworm has been found in spottail shiners in Saginaw Bay. They were previously found in Lake St. Clair. Gary will send out additional email through email.

A press release from New York indicated the finding of furunculosis. The fish have since been destroyed. After 2 months, a similar situation happened to Rome SFH fish (~800k). The cause is unknown, but it may have been from challenging broodstock fry. Two weeks later, *A. sal* was detected. Mitigation approaches include using an ozone or UV system. Surveillance is ongoing.

24. Adjourn

RANAVIRUSES

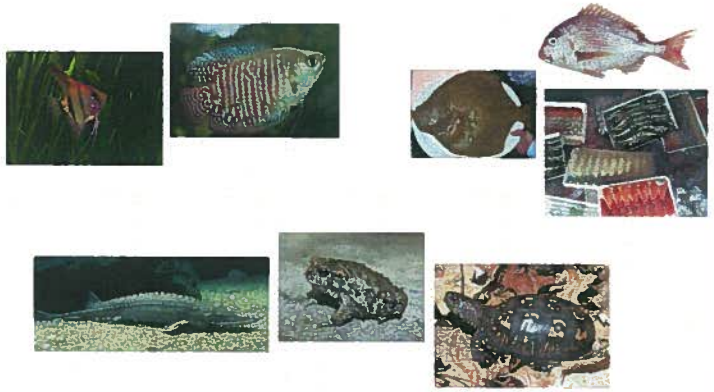
Emerging Threats to Aquatic Industries & Ecosystems

Thomas B. Waltzek MS, DVM, PhD
Assistant Professor
UF College of Veterinary Medicine
Department of Infectious Diseases and Pathology

UF College of Veterinary Medicine
UNIVERSITY of FLORIDA

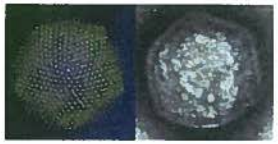
AQUATIC ANIMAL HEALTH

RANAVIRUSES
Emerging Threats to Aquatic Industries & Ecosystems



Family *Iridoviridae* Characteristics

- Diverse group of Nucleocytoplasmic Large dsDNA Viruses (NCLDV)
- Virion: enveloped icosahedral capsid (120-350 nm)
- Taxonomy
  - Genus *Iridovirus* (arthropod hosts)
  - Genus *Chloriridovirus* (dipteran host)
  - Genus *Lymphocystivirus* (fish hosts)
  - Genus *Megalocytivirus* (fish hosts)
  - Genus *Ranavirus* (fish, amphibian, & reptilian hosts)
  - Unassigned members
    - Missouri River (Pallid) Sturgeon IV (MRSIV/PSIV), White Sturgeon IV (WSIV)



Ranaviruses:

an emerging threat to ectothermic vertebrates

Friday, 8 July 2011
Minneapolis, MN, USA

THE UNIVERSITY of TENNESSEE

FIRST INTERNATIONAL SYMPOSIUM ON RANAVIRUSES

Joint Meeting of Ichthyologists and Herpetologists

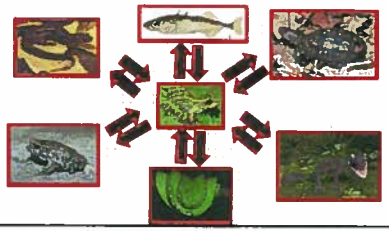
6 – 11 July 2011
Minneapolis, MN, USA

23 Speakers from 8 Countries!

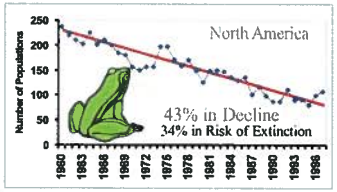
Ranavirus: past, present & future (Lesbarrères et al. 2011)

Ranavirus (RV) Characteristics & Taxonomy

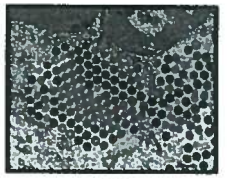
- Systemic diseases of fish, amphibians, & reptiles
  - Temperate to tropical 10 - 32 °C
  - Significant morbidity & mortality (10-100%)
  - **OIE reportable (amphibian outbreaks only)**
- 6 species recognized (USA – 3 endemic)
  - Santee-Cooper ranavirus (SCRV/LMBV)
  - Ambystoma tigrinum virus (ATV)
  - Frog Virus 3 (FV3) – low host specificity



Amphibian Decline & Emerging Infectious Diseases

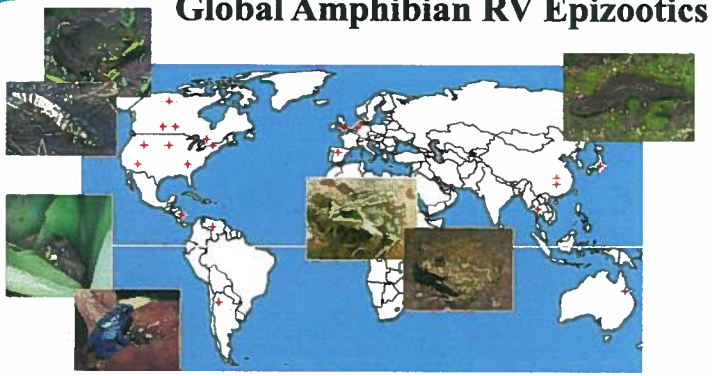


Chytrid Fungus



Ranavirus

Global Amphibian RV Epizootics



- Varied habitats across 5 continents
- 12 affected families of frogs, toads, salamanders, & newts

Amphibian RV Epizootics in North America

Families

Ranidae
Hylidae
Bufonidae
Ambystomatidae
Salamandridae

Lithobates sylvaticus

- 5 Canadian Provinces & > 30 States = 25 spp. amphibians
  - 2008-10: Repeated die-offs in green frogs and bullfrogs in Pallette, Escanaba, and Big Muskellunge Lakes
  - 2011: FWRI confirmed 1st RV outbreak in Florida (Landsberg et al. 2013)

Host Range of Frog Virus 3 like Agents

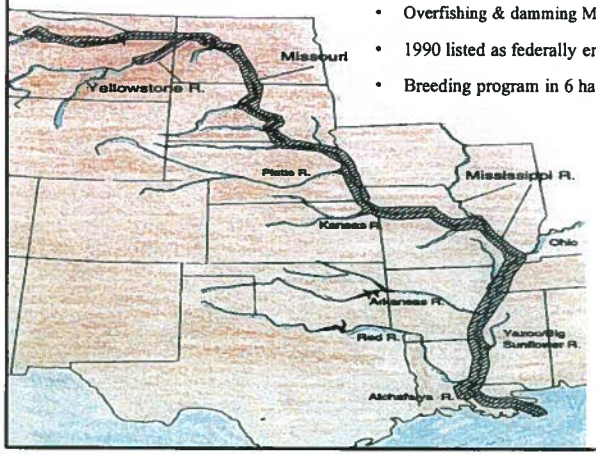
Frog Virus 3 like agents	Host Class	Host	Host common name	MCP % ID
Frog Virus 3 (FV3)	Amphibia	*Rana pipiens*	Leopard frog	/
Redwood Park Virus (RPV)	Amphibia	*Rana aurora*	Northern red-legged frog	100
Tadpole Edema Virus (TEV)	Amphibia	*Rana catesbeiana*	Bullfrog	99.6
Rana temporiana United Kingdom Virus (RUK)	Amphibia	*Rana temporaria*	European common frog	99.4
Rana gyrlio virus (RGV)	Amphibia	*Rana gryllo*	Pig frog	99.2
Rana utriculana virus	Amphibia	*Rana utricularia*	Southern leopard frog	100
Rana clamitans virus	Amphibia	*Rana clamitans*	Green frog	100
Bufo bufo United Kingdom Virus (BUK)	Amphibia	*Bufo bufo*	European toad	99.2
Bufo mannus Venezuelan Iridovirus 1	Amphibia	*Bufo marinus*	Cane toad	99.2
Desmognathus quadramaculatus Virus	Amphibia	*D. quadramaculatus*	Blackbelly salamander	99.1
Box turtle virus 3 (TV3)	Reptilia	*Terrapene carolina*	Eastern box turtle	100
Tortoise virus 5 (TV5)	Reptilia	*Testudo horsfieldi*	Russian tortoise	99.1
Gopher tortoise virus	Reptilia	*Gopherus polyphemus*	Gopher toritoise virus	100
Burmese star tortoise virus	Reptilia	*Geochelone platynota*	Burmese star tortoise	100
Leopard tortoise Virus	Reptilia	*Geochelone pardalis*	Leopard tortoise	99.4
Softshell turtle iridovirus (STIV)	Reptilia	*Trionyx sinensis*	Chinese softshell turtle	99.7
Stickleback Virus (SBV)	Osteichthys	*Gasterosteus aculeatus*	Threespine stickleback	100
Pallid sturgeon Ranavirus (PSRV)	Osteichthys	*Scaphirhynchus albus*	Pallid sturgeon	100



Isolation of Frog Virus 3 from Pallid Sturgeon (*Scaphirhynchus albus*) Suggests an Interclass Host Shift

Thomas B. Waltzek, Debra L. Miller, Bruce Drecktrah, Jeff T. Briggler, Beth MacConnell, Crystal Hudson, Lacey Hopper, Susan C. Yun, Kirsten V. Malm, Scott Weber, Roberto Brenes, Nate Hilzinger, Sean Roon, Matt J. Gray, Ronald P. Hedrick

Decline of Pallid Sturgeon within the Missouri River Basin (MRB)



- Overfishing & damming MRB led to PS decline
- 1990 listed as federally endangered
- Breeding program in 6 hatcheries

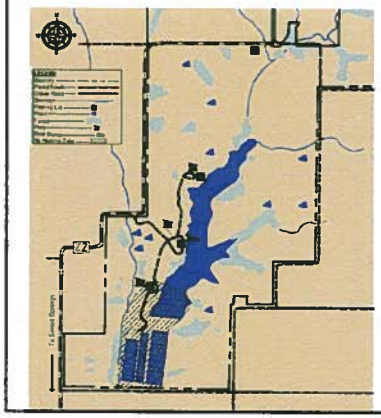
Pallid Sturgeon Restoration Effort

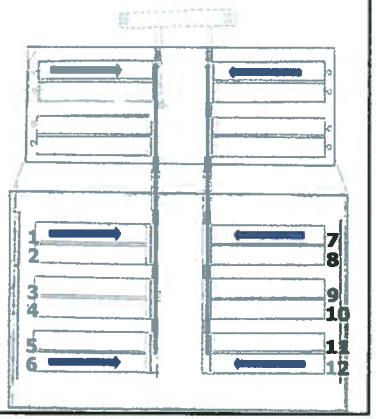
- Endangered adults are captured annually & transported to 1/6 hatcheries
- Adults spawned & then returned to the wild
- Progeny reared at 6 hatcheries for restocking 3 management zones within the MRB


Blind Pony State Fish Hatchery (BPSFH)

Blind Pony State Fish Hatchery, Sweet Springs, MO

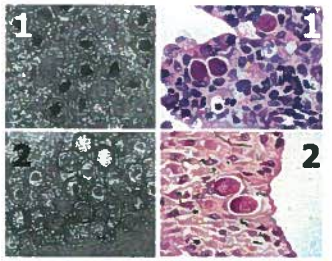
- Hatchery spawns & rears ~ 12,000 pallid sturgeon annually
- 12 raceways typically stocked at 2,000/raceway
- Raceways receive untreated H_2O by gravity from Blind Pony Lake Dam

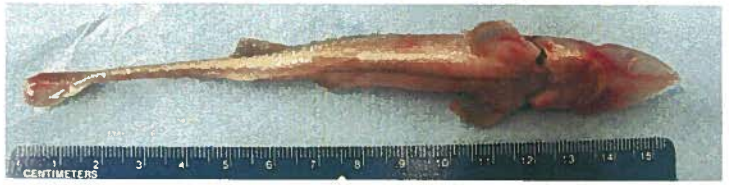




Missouri River Sturgeon Iridovirus

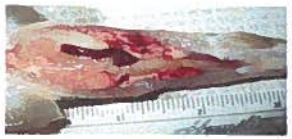
1. Pallid (PSIV) } MRSIV
2. Shovelnose

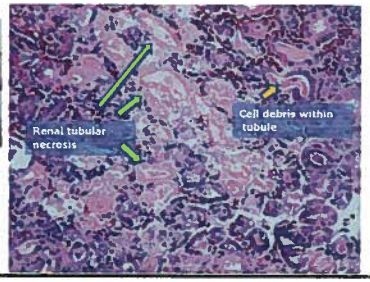




2009 Blind Pony State Fish Hatchery Epizootic

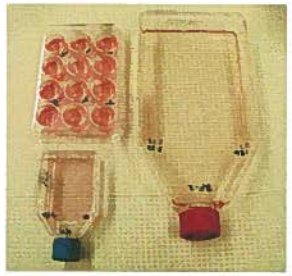
- July – Sept. YOY PS experienced heavy losses (80-100% over 3 months)
- Mortality (550/d) greatest at higher temps (15.5 – 25.5 °C)
- Dying fish displayed external and internal hemorrhagic lesions
- Histology revealed necrosis of hematopoietic (K, S) organs et al…
- Negative (-) by PCR for MRSIV

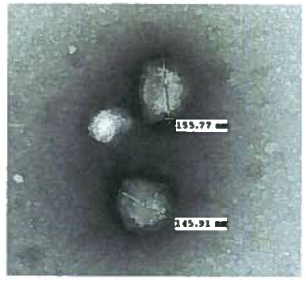




BPSFH PS Epizootic – Bacteriology, Cell Culture, EM

- Mixed pop. of bact. cultured but mort. continued despite repeated antib. Tx
- Replicating agent observed in sturgeon cell lines
- Negative staining EM revealed icosahedral particles (~ 150 nm versus 300 nm for MRSIV)

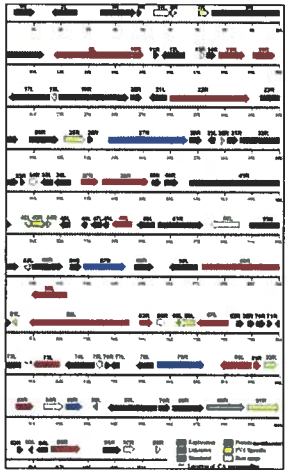




PS Isolate Genomic Characterization

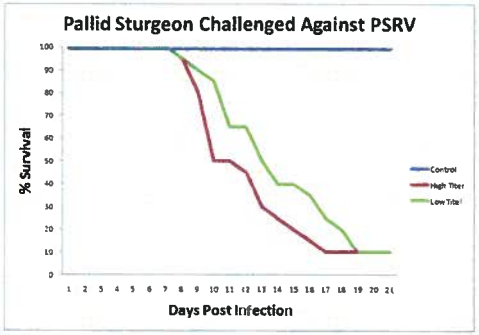
- PS isolate genome sequenced (Illumina)
  - > 99% ID to FV3 (105 kbp)

- PS represents a new fish host for Frog Virus 3 (FV3) = interclass host shift!
- PS Ranavirus (PRSV) the cause of the mortality???



PSRV Lethal to Pallid Sturgeon - Koch's Postulates Fulfilled

- 90% mortality in low & high titer exposures
- High virus titers recov. from 36/36 morts, 2/4 virus exposed survivors, 0/5 controls



Percent survival of individuals (n = 20/treatment) exposed to PSRV isolate. Bath exposure was 10^3 PFU/ml (low) or 10^4 PFU/ml (high); experiment duration = 21 d.

2009 BPSFH Epizootic – Summary

- 2009 BPSFH PS epizootic attributed to a FV-3 like agent
- Induced gross & microscopic lesions, virion architecture, & in vitro characteristics typical of systemic IVs
- Virus isolated from hatchery PS in Sept, Oct, & Dec 2009 during & after active outbreak. PS lots destroyed.
- Oct 28 2009: 8 adult bullfrogs tested for ranavirus by PCR (all negative).
- Sept 2010: Given the untreated intake water 31 adult bullfrogs, 26 adult plains leopard frogs, & 4 plains leopard frog tadpoles from adjacent watersheds were cultured for virus (all negative) .

Mysterious 2001 BPSFH PS epizootic

- Epizootic began in July with rising water temperatures
  - All raceways experience 100% mortality
  - Samples negative (-) for MRSIV
  - Mixed bag of bact. cultured but mort. continued despite repeated antib. Tx
  - External & internal hemorrhagic lesions
  - Necrosis of haematopoietic tissues and mesentery
  - Virus isolated on several cell lines
  - Negative staining SEM revealed icosahedral particles (~ 160 nm)
  - Comparison of the 2001/2009 full MCP seq. revealed they are identical!!!
- PSRV continues to threaten conservation effort
- What is the source of the virus???
  - Sympatric amphibians contaminating hatchery intake H_2O in 01 & 09 (2x HS)
  - Adult population infected & pass vertically to progeny during manual spawning
  - Another hatchery reservoir?

Host Range of Frog Virus 3 like Agents

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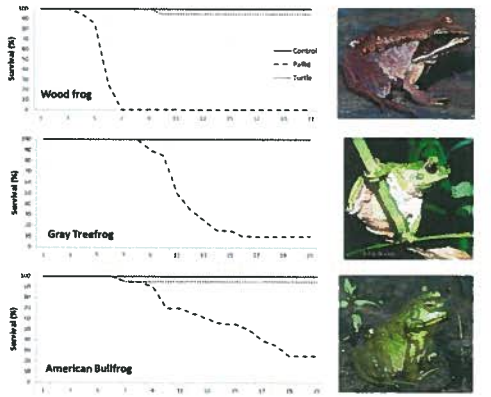
Pallid sturgeon Ranavirus (PSRV)	Osteichthys	*Scaphirhynchus albus*	Pallid sturgeon	100



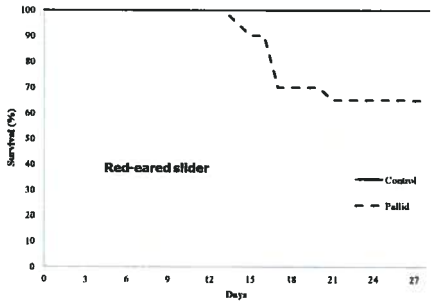
PSRV (FV3) Lethal to Amphibians



Decreasing Susceptibility to FV3

Percent survival of individuals (n = 20/treatment) exposed to one of two ranavirus isolates.
Bath exposure was 10^3 PFU/ml; experiment duration = 21 d.

PSRV (FV3) Lethal to Reptiles

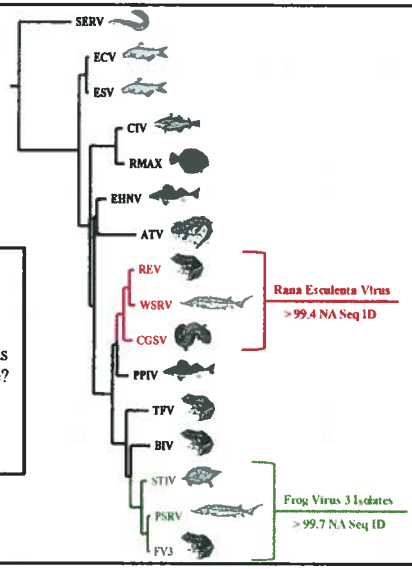


Percent survival of individuals (n = 20/treatment) exposed to PRSV (FV3).
Bath exposure was 10^3 PFU/ml; experiment duration = 28 d.

Ranavirus Tree of Life

- Lowest host specificity observed among dsDNA viruses?
- Implications for control/eradication efforts & thus regulatory implications
  - Only amphibian RVs reportable?
- Conservation concern
  - Endangered species
  - Ecosystem-level effects?

Bayesian Analysis: Full DNA Polymerase & Major Capsid Genes



Significance

- Ranaviruses are a global threat to both cultured & feral populations of poikilothermic vertebrates (fish, amphibians, & reptiles)
- RVs are especially concerning emerging pathogens given their high virulence & low host specificity (interclass host shifts/reservoirs)
- These epizootics are especially concerning given the federally endangered status of pallid sturgeon
- Temperature is an important cofactor in disease manifestation
- Survivors may become viral carriers

Future Directions

- **FV3-like Epizootics in Sturgeon**
  - **White Sturgeon RV (1998 CA epizootic)**
  - **2001 Pallid Sturgeon RV (2001 MO Hatchery Epizootic)**
  - **Russian/Lake Sturgeon RV (2004 GA Hatchery Epizootic)**
  - **Pallid Sturgeon RV (2009 MO Hatchery Epizootic)**

- **FV3-like Isolates in Other Species**
  - **Northern Pike RV (VHSV Surveillance)**
  - **Fathead Minnow RV (VHSV Surveillance)**
  - **Walleye RV (VHSV Surveillance)**

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* Clark Gray
* Maki Jenkins

Thanks for your attention! Questions?

Exploring the Phylogenomic Diversity of Emerging Fish Viruses

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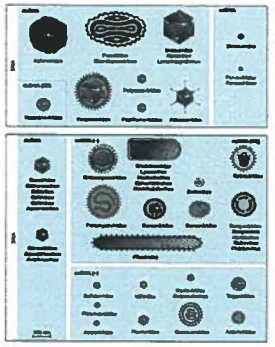
Department of Infectious Diseases and Pathology

UF College of Veterinary Medicine UNIVERSITY of FLORIDA

AQUATIC ANIMAL HEALTH

Objectives

- Sequencing revolution is advancing the discovery & understanding of emerging fish pathogens (viruses)
  - PCR/Shotgun Cloning/Sanger
  - Illumina/454
  - ***Pitfalls/Caveats/Lessons***
- Emerging Fish Viruses
  - DNA
    - *Alloherpesviridae, Iridoviridae, Adenoviridae, Mimiviridae, Poxviridae, Papillomaviridae*
  - RNA
    - *Picornaviridae, Orthomyxoviridae, Bunyaviridae*



Global Aquaculture Challenges

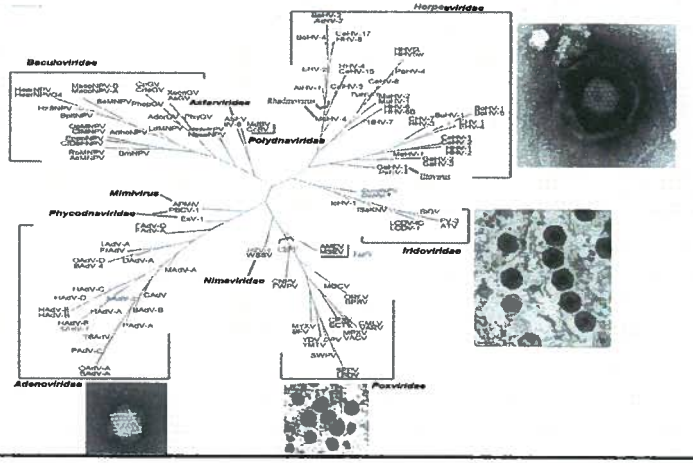
- Emerging viral diseases are the single greatest threat to global aquaculture
  - Losses due to disease >$3 billion/yr (World Bank 1997)
- **White spot syndrome** has devastated global shrimp production
  - Spread (22 countries) via trade in post-larvae (losses >$1 billion/yr)

- **Infectious salmon anemia virus** threatens Atlantic salmon aquaculture
  - Untold losses to angling & food fish industries

- **Koi herpesvirus disease (KHVD)** linked to international koi carp trade
  - Epizootics threaten global carp industry (ornamental, food, sport fisheries)

Tree of Life for dsDNA Viruses

(Gao and Qi 2007)



Order *Herpesvirales* Characteristics

- Diverse assemblage of large dsDNA viruses
- Virion: enveloped icosahedral capsid (90-110 nm)
- Recent taxonomic changes to the order *Herpesvirales*
  - Family *Herpesviridae* (mammalian, reptilian, & avian hosts)
    - 3 subfamilies (*Alpha-, Beta-, Gammaherpesvirinae*)
  - Family *Alloherpesviridae* (fish & amphibian hosts)
    - Genus *Ictalurivirus* (catfish and sturgeon hosts)
    - Genus *Cyprinivirus* (common carp/koi & goldfish hosts)
    - Genus *Salmonivirus* (salmon & trout hosts)
    - Genus *Batrachovirus* (leopard frog hosts)
  - Family *Malacoherpesviridae* (invertebrate hosts)
    - Genus *Ostreavirus*

Emergence of Koi herpesvirus (KHV)

- Mass mortality in common carp varieties (*Cyprinus carpio carpio/koi)*
  - Warmwater disease (20 - 28 C)
  - Incubation period 7-14 d
  - High mortality (70-100%)
  - Spread globally since 1996

- **Carp aquaculture** impacted (3.4 Million tonnes/yr)

- **Carp sport fishery impacted** (1 $Billion/yr)

- International ornamental **koi commerce impacted** (1 $Billion/yr)

- **Reportable** to OIE & USDA/APHIS

Novel Herpesvirus or Not???

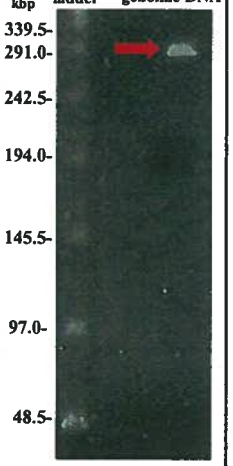
- Named Koi Herpesvirus (KHV)
  - Host, pathology, virion morphogenesis & architecture

- Other carp HVs
  - Sano et al. 1985 **(CyHV1)**
  - Jung & Miyazaki 1985 **(CyHV2)**

- KHV renamed Carp Nephritis & Gill Necrosis Virus (CNGV) (Ronen et al. 2003)
  - Large genome size (295 kbp)
  - Preliminary **PCR/Sanger** sequencing suggests KHV/CNGV may represent a novel dsDNA group…

Herpesvirus Genomics

- Family *Herpesviridae*
  - 53 mammal & bird herpesvirus genomes (150 – 250 kbp)
  - 5 fish & amphibian herpesvirus genomes (134 – 295 kbp)
- KHV sequence (4 genes sequenced by **PCR/Sanger**) reveals closest relatives are CyHV1 & 2 (Waltzek et al. 2005)
- KHV genome sequenced by **shotgun cloning/Sanger** (Aoki et al. 2007)
  - 295 kbp, 22 kbp terminal repeats, 156 ORFs
  - Immunomodulatory genes (vTNFR1 & 2, vIL-10, VIR)
  - ***14 conserved core genes****
    - Glycoproteins
    - Capsid structural proteins (triplex, MCP, capsid protease)
    - Replication proteins (primase, helicase, **polymerase**)
    - DNA packaging protein (**terminase**)
- Phylogenetics of the fish & amphibian herpesviruses (Waltzek et al. 2009)
  - Partial DNA polymerase & terminase sequences compared for 15 herpesviruses by **PCR/Sanger**



Aoki et al. 2007

Fish & Amphibian Herpesviruses

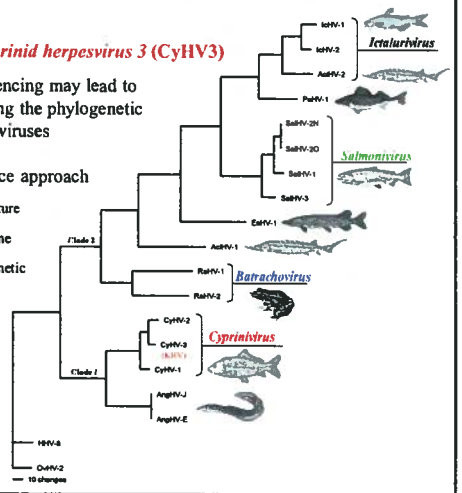
List in accordance with the 9th Report of the ICTV

Designation	Host	Reference
Cyprinid herpesvirus 1 (CyHV1, CHV)	*Cyprinus carpio*	Sano et al. 1985
Cyprinid herpesvirus 2 (CyHV2, GFHNV)	*Carassiusauratus*	Jung & Miyazaki 1995
Cyprinid herpesvirus 3 **(CyHV3, KHV, CNGV)**	*Cyprinus carpin*	Hedrick et al. 2000
Ictalurid herpesvirus 1 (IcHV1, CCV)	*Ictalurus punctatus*	Fijan et al.1970
Ictalurid herpesvirus 2 (IcHV2)	*Ameirus melas & I. punctatus*	Hedrick et al. 2003
Anguillid herpesvirus 1 (AngHV1)	*Anguilla japonicus & anguilla*	Sano et al. 1988
Acipenserid herpesvirus 1 (AciHV1)	*Acipenser transmontanus*	Hedrick et al. 1991
Acipenserid herpesvirus 2 (AciHV2)	*Acipenser transmontanus*	Watson et al. 1995
Salmonid herpesvirus 1 (SalHV1)	*Oncorhynchus nykiss*	Wolf & Taylor 1975
Salmonid herpesvirus 2 (SalHV2)(NeVTA)	*Oncorhynchus nerka*	Sano 1976
Salmonid herpesvirus 2 (SalHV2)(OMV)	*Oncorhynchus masou*	Kimura et al. 1981
Salmonid herpesvirus 3 (SalHV3, BEDV)	*Salvelinus namaycush*	McAllister & Herman 1989
Salmonid herpesvirus 4 (SalHV3, AS Papillomatosis)	*Salmo salar*	Shchelkunov et al. 1992
Esocid herpesvirus 1 (EsHV1)	*Esox lucius & masquinongy*	Yamamoto et al., 1983
Percid herpesvirus 1 (PeHV1)	*Stizostedion vitreum*	Kelley et al. 1983
Ranid herpesvirus 1 (RaHV1)	*Rana pipiens*	Granoff et al. 1989
Ranid herpesvirus 2 (RaHV2)	*Rana pipiens*	Rafferty 1965

Taxonomic Changes to the Fish & Amphibian Herpesviruses

- **ICTV designation for KHV is *Cyprinid herpesvirus 3* (CyHV3)**
  - **Caveat:** Partial genomic sequencing may lead to erroneous conclusions regarding the phylogenetic relationships of large genome viruses
  - **Solution:** Rely on total evidence approach
    - Virion morphogenesis & architecture
    - If possible sequence whole genome
    - Choose genes wisely for phylogenetic analyses (i.e. core genes)
    - Rely on multigene phylogenetic analyses (Gene vs. Species Tree)

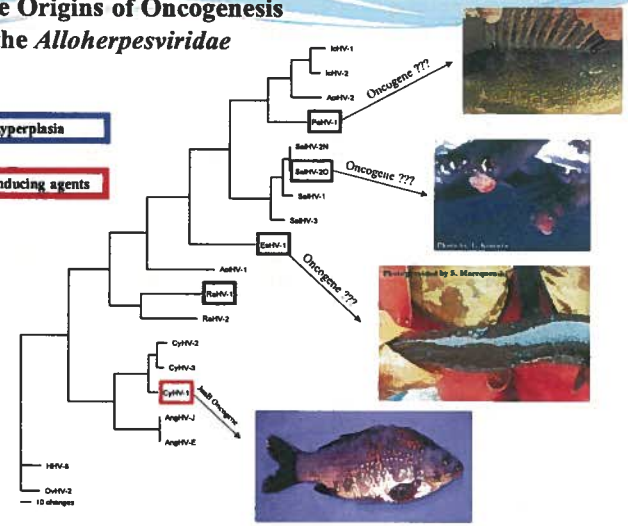
- **2 core gene (Pol/Term) sequences generated by degenerate PCR/Sanger**
- **Bayesian & ML analyses**



Multiple Origins of Oncogenesis within the *Alloherpesviridae*

Focal hyperplasia

Neoplasia inducing agents



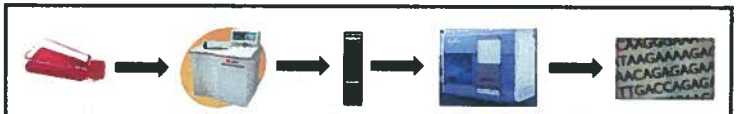
Herpesvirales Genomics

- **1st Generation Sequencing (Shotgun Cloning & Sanger Sequencing)**
  - 53 Mammalian & avian HVs
  - 5 Fish & Amphibian HVs
  - *Ostreid HV1* = 207 kbp (Davison et al. 2005)

  → KHV isolates (U, I, J) = **3**
  2-3x genome coverage
  2004 for **$30,000**
  (Aoki et al. 2007)

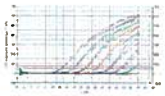
- **2nd Generation Sequencing (Illumina)**
  - *Haliotid HV 1* = 43 kbp (Chen et al. unpub.)
  - *Amphioxus associated HV1* (Savin et al. 2010)
  - *Cyprinid HV1* = 291 kbp
  - *Cyprinid HV2* = 294 kbp
  - *Salmonid HV1* = 180 kbp
  - *Salmonid HV2* = 182 kbp
  - *Salmonid HV3* = 185 kbp
  - *Acipenserid HV1* = 185 kbp
  - *Acipenserid HV2* = 142 kbp

  → 7 Herpes + 3 Irido = **10**
  20-50x genome coverage
  2011 for **$4,000**
  (Waltzek & Davison 2011)



Alloherpesvirus Genomics: Improved Diagnostics

- Sequencing KHV (CyHV3) & close relatives CyHV1-2 (Waltzek et al. 2005) allowed the development & validation of rapid/specific/sensitive
  - **Single Round PCR*** (Yuasa et al. 2005, Bercovier et al. 2005)
  - **Nested** (Bergmann et al. 2006, El-Matbouli et al. 2007)
  - **Multiplex** (Bigarre et al. 2009)
  - **Real-Time TaqMan PCR** (Gilad et al. 2004)
  - **LAMP** (Gunimaladevi et al. 2004, Yoshino et al. 2009)
  - **PCR/Southern** (Eide et al. 2011)
  - ***In situ* hybridization** (Bergmann et al. 2006)
  - **DNA Array** (Lievens et al. 2011)



Waltzek et al. 2007, 2012

- Sequencing the other fish/amphibian agents allowed consensus primer development for the discovery of emerging alloherpesviruses
  - DNA polymerase (Hanson et al. 2005)
  - Terminase (Waltzek et al. 2009)

Emerging Ornamental Alloherpesviruses

- Discus/angelfish HV
  - Mellergard and Bloch 1988, Petty and Fraser 2005, Waltzek & Yanong in prep.
- Guppy HV
  - Waltzek & Hedrick in prep.
- Glass Catfish HV
  - Waltzek & Kanchanakhan in prep.

2011 Discus HV Epizootic

2011 Glass Catfish HV Epizootic

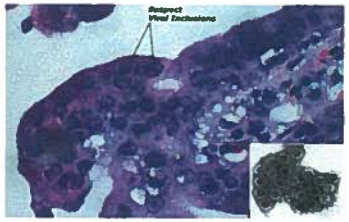
2002 Guppy HV Epizootic

Emerging Marine Food/Sport Fish Alloherpesviruses

- Smooth dogfish HV
- Japanese flounder HV
- Pilchard HV
- White sea bass HV (Waltzek & Okihiro submitted)
- Atlantic cod HV (Marcos-Lopez & Waltzek 2011)

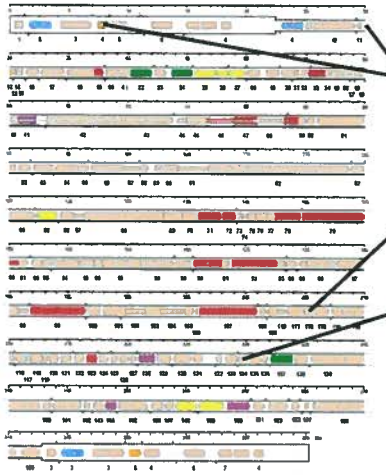
HV induced ulcerative dermatopathy in smooth dogfish herpesvirus

H&E, illustrating HV induced GI pathology from a 2009 white sea bass epizootic



H&E illustrating HV induced gill pathology from a 2009 Atlantic cod epizootic

Searching the KHV Genome for Genes Involved in Pathogenesis

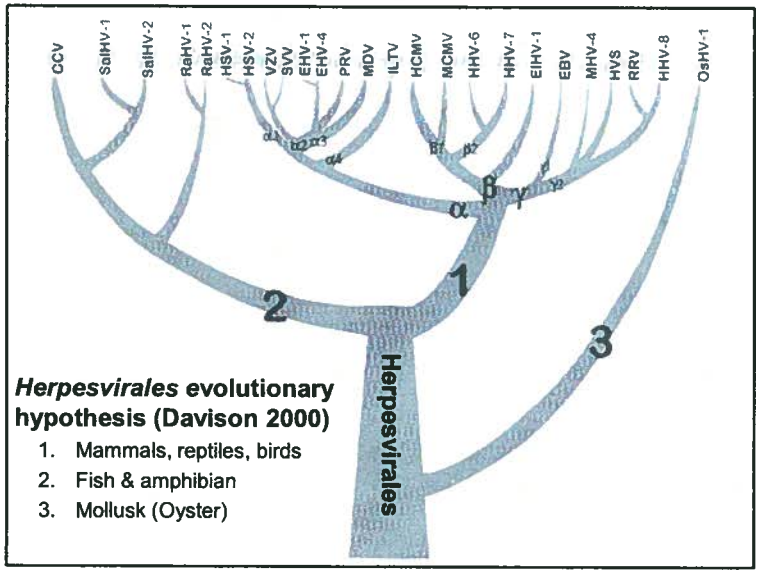


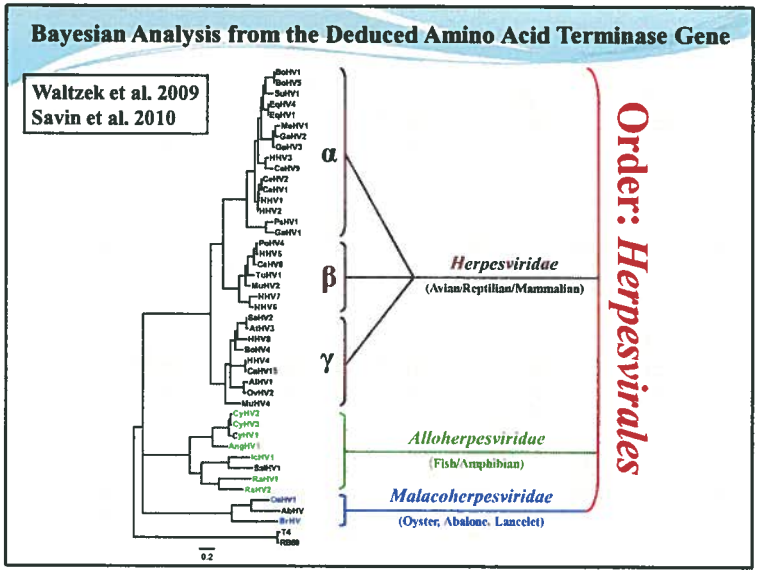
Tumor Necrosis Factor Receptor Genes 1 & 2
- Herpes (EBV, CMV), Pox (ORF)

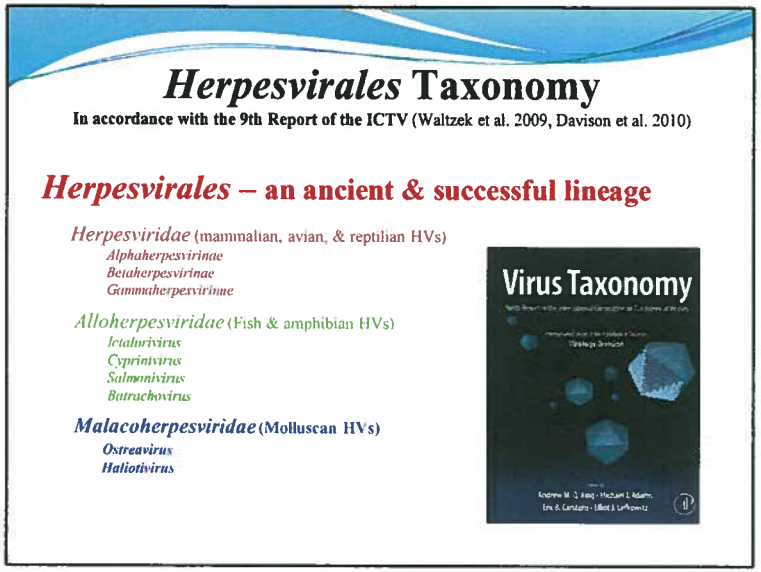
Virus Interferon Resistance Gene
- Poxviruses (VAC E3L)

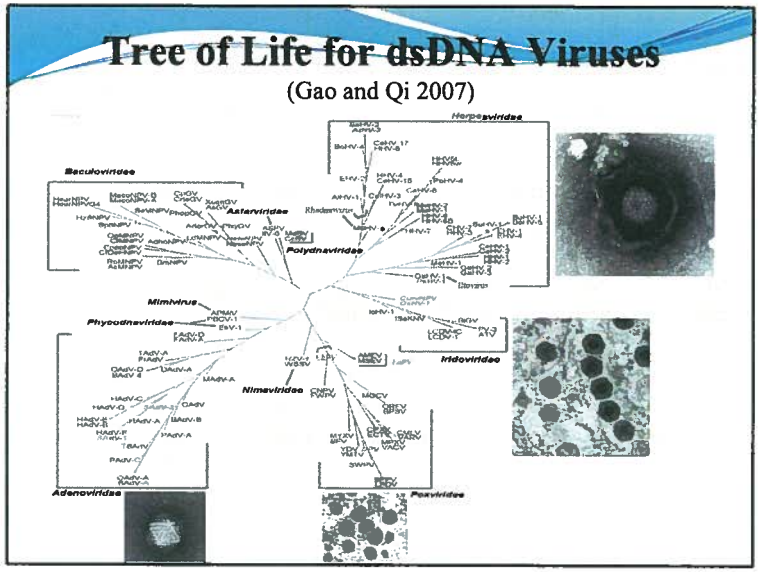
Interleukin 10 Gene
- Herpes (EBV, CMV, EHV2), Pox (ORF)

- **Viral piracy events result in immune evasion & pathogenesis?**


CCV
SalHV-1
SalHV-2
RaHV-1
RaHV-2
HSV-1
HSV-2
VZV
SVV
EHV-1
EHV-4
PRV
MDV
ILTV
HCMV
MCMV
HHV-6
HHV-7
EHV-1
EBV
MHV-4
HVS
RRV
HHV-8
OsHV-1
α
β
γ
1
2
3
Herpesvirales
Herpesvirales evolutionary hypothesis (Davison 2000)
1. Mammals, reptiles, birds
2. Fish & amphibian
3. Mollusk (Oyster)

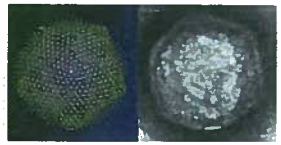

Bayesian Analysis from the Deduced Amino Acid Terminase Gene
Waltzek et al. 2009
Savin et al. 2010
α
β
γ
Herpesviridae
(Avian/Reptilian/Mammalian)
Alloherpesviridae
(Fish/Amphibian)
Malacoherpesviridae
(Oyster, Abalone, Lancelet)
Order: Herpesvirales
0.2


Herpesvirales Taxonomy
In accordance with the 9th Report of the ICTV (Waltzek et al. 2009, Davison et al. 2010)
Herpesvirales – an ancient & successful lineage
Herpesviridae (mammalian, avian, & reptilian HVs)
Alphaherpesvirinae
Betaherpesvirinae
Gammaherpesvirinae
Alloherpesviridae (Fish & amphibian HVs)
Ictalurivirus
Cyprinivirus
Salmonivirus
Batrachovirus
Malacoherpesviridae (Molluscan HVs)
Ostreavirus
Haliotivirus
Virus Taxonomy


Tree of Life for dsDNA Viruses
(Gao and Qi 2007)
Herpesviridae
Baculoviridae
Asfarviridae
Polydnaviridae
Mimivirus
Phycodnaviridae
Iridoviridae
Nimaviridae
Adenoviridae
Poxviridae

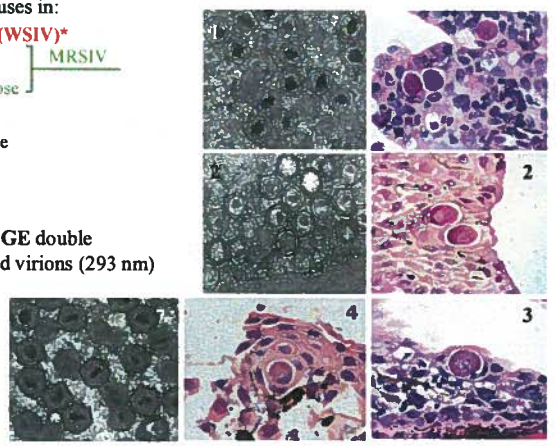
Family *Iridoviridae* Characteristics

- Diverse group of Nucleocytoplasmic Large dsDNA Viruses (NCLDV)
- Virion: enveloped icosahedral capsid (120-350 nm)
- Taxonomy
  - Genus *Iridovirus* (arthropod hosts)
  - Genus *Chloriridovirus* (dipteran host)
  - Genus *Lymphocystivirus* (fish hosts)
  - Genus *Megalocytivirus* (fish hosts)
  - Genus *Ranavirus* (fish, amphibian, & reptilian hosts)
  - Unassigned members
    - * Missouri River (Pallid) Sturgeon IV (MRSIV/PSIV), White Sturgeon IV (WSIV)



Sturgeon Iridoviruses

- Similar Viruses in:
  1. *White (WSIV)*
  2. Pallid — MRSIV
  3. Shovelnose — MRSIV
  4. Lake
  5. Shortnose
  6. Italian
  7. Russian
- Unique **HUGE** double encapsidated virions (293 nm)



White Sturgeon Iridovirus (WSIV) Genomics

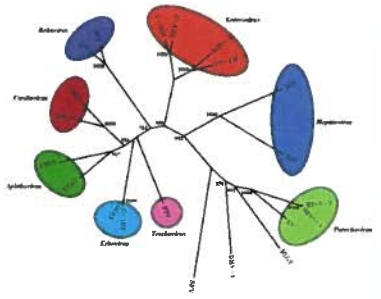
- Next Generation Sequencing (454) data
- > 100 genes recovered including structural (MCP) & replication (DNA Pol) proteins
- Most gene sequences show greatest homology with mimiviruses (WSMV)!
- Mimiviruses are the giant viruses that infect other microbes (Raoult et al. 2004)
  - Misidentified as intracellular bacteria by light microscopy (0.7 um)
  - Genomes of > 1 million bps!
  - Larger than a wide variety of prokaryotes!
- These sturgeon viruses are the 1st mimiviruses pathogenic to a vertebrate
- Giant sturgeon viruses (MRSIV/WSIV) to be reclassified
  - Mimiviruses unknown when sturgeon viruses discovered & named
  - **Lesson:** Much of what we think we know will soon be challenged as a result of the sequencing revolution

Discovery of Novel Fish RNA Virus

Sample & Host	Collaborator	454 Sequencing Result
Purified virus from LMB	Andy Goodwin	Complete genome - Novel highly divergent picornavirus (new genus)
Purified virus from GF	Andy Goodwin	Complete genome - Novel highly divergent picornavirus (new genus)
Purified virus from FHM	Andy Goodwin	Complete genome - Identical to above

Fish Picornaviruses

- Picornaviruses detected in barramundi, grouper, smelt, salmon, turbot
- Bluegill (Barbknecht 2009)
- Common eel (Philipps et al. 2010)
- **Cyprinid picornavirus radiation** (Phelps et al. in prep.)
  - Fathead minnow, goldfish, shiner



Barbknecht 2009

Discovery of Novel RNA Viruses

Sample & Host	Collaborator	454 Sequencing Result
Purified virus from Koi	Bill Batts/James Winton	Partial Genome - Novel highly divergent Orthomyxovirus
Purified virus from GF	Bill Batts/James Winton	Complete Genome - Novel highly divergent *Orthobunyavirus*

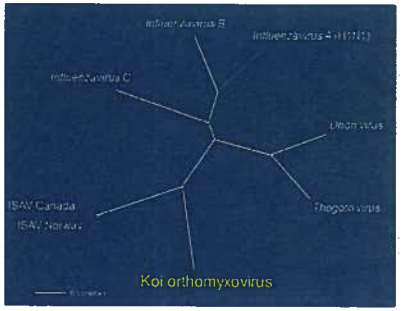
2nd Fish Orthomyxovirus

- Koi Orthomyxovirus closest relative ISAV

1st Fish Bunyaviruses

- Distant relative of viruses like Hantavirus
- Goldfish and White Sucker Bunyaviruses

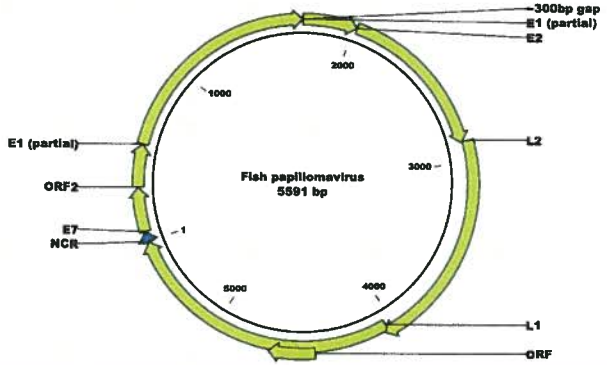
KOCH'S POSTULATES NOT FULFILLED! SEAFOOD SAFETY?



Significance - Phylogenomic Lessons

- Lack of knowledge of poikilothermic vertebrate viruses is the result of a lack of interest/resources & technology gap
- Sequencing revolution has revealed fish viruses are highly divergent from more familiar lineages indicating their ancient & successful status (e.g. alloherpesviruses may predate the invert/vert split some 500 MYA)
- Much of what we think we know will soon be challenged as a result of the sequencing revolution (e.g. creation of the order *Herpesvirales* & WSIV reclassification)
- Sequencing revolution has revealed the importance of total evidence phylogenomic approaches relying on multiple core genes to unravel viral relationships (e.g. some viral genes acquired via exchange with host)
- Future challenges will not be the discovery of novel fish viruses, but proving their significance in disease (Koch's Postulates)

Thanks For Your Attention! Questions?



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* Linda Archer

Aquatic Pathobiology Laboratory

Blood Systems Research Institute
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* Dr. Terry Fei Fan Ng

Blood Systems Research Institute

UM College of Veterinary Medicine
* Dr. Tom Molitor
* Dr. Sagar Goyal
* Dr. Nick Phelps
* Dr. Sunil Kumar

College of Veterinary Medicine UNIVERSITY OF MINNESOTA

USGS Western Fish Research Center
* Dr. Bill Batts
* Dr. James Winton

USGS science for a changing world

Ontario Fish Health Monitoring Update on Virus Isolations

... and other research interests

Steve Lord,
Melinda Raymond,
Roz Stevenson

Fish Health Laboratory,
Department of Molecular & Cellular Biology,
University of Guelph

Communicated by Kevin Loftus, Feb 2013

Detecting viruses by TISSUE CULTURE

CLINICAL SIGNS ?
viremia, virus loads in organs

gill, pyloric caeca, spleen, kidney

- macerated
- diluted
- centrifuged
- filtered

- samples applied to fish cell lines in tissue culture
- observed at 4, 10, 14, 21 days

re-pass

"WE HAVE LIFT-OFF ! " (CPE)

Mono-layers of FISH CELL LINES in culture are examined for evidence of CYTOPATHIC EFFECT

If CPE is observed, diluted samples are applied to new tissue culture cells, as a test for CYTOTOXICITY

Samples may also be BLIND-PASSAGED ....

..all this takes a while!

Routine testing for fish viruses has been carried out since 1977 ... and, mostly, it was pretty boring.

Infectious pancreatic necrosis virus (IPNV) was an issue until a hatchery on surface water was closed in the early 1980's.

In 1997 and again in 1999, tissue culture of a routine sample showed CPE, due to

→ AQUAREOVIRUS

"may you live in interesting times"

Since 2005, three significant viral pathogens were detected in Ontario:

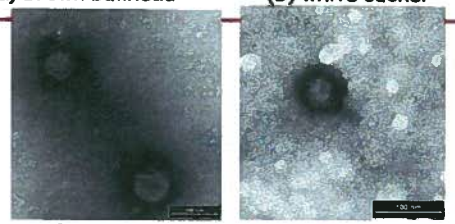
- **Viral Haemorrhagic Septicaemia virus**
  - Great Lakes VHSV IVb
  - 2005 Bay of Quinte, drum (and others)
- **Koi Herpes virus**
  - 2007, 2009 - Kawartha Lakes, carp
- **Spring Viremia of Carp**

... and some other viruses were detected as well, by presence of CPE -

v 5583 Samples from VHSV survey work in Hamilton Harbour by John Lumsden and Lowia Al-Hussinee, OVC

VHSV and two others in a multispecies die-off (May 2007)

(a) brown bullhead (b) white sucker



- CPE on RTG-2, EPC and CHSE-214
- icosahedral, ~ 80 nm ... envelope? .... RNA

FISH CELL LINES - Which to use ?
- How many ?

- RTG-2
- CHSE-214
- FHM Fat Head Minnow
- EPC (Epithelioma papulosum cyprinis)
- BF-2 (bluegill fry)

With the Ontario VHSV, CPE only on FHM; not on RTG-2 or CHSE cells ...

→ REALLY OF FAT-HEAD MINNOW ORIGIN !

BIODIVERSITY INSTITUTE OF ONTARIO

Isolates from routine screening of wild fish, during egg collections

AQUAREOVIRUS group

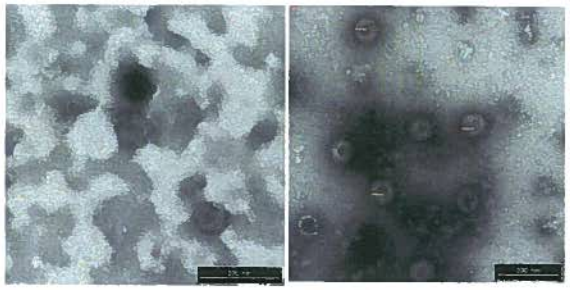
- v5915 coho salmon,Credit River, Lake Ontario 2009 (and chinook in 2010)
- v5909 Atlantic salmon (Lac St. Jean, PQ), 2009, *for potential import*

BACILLIFORM virus

- v5767 chinook salmon, Credit River, Lake Ontario, 2008
- re-isolated in 2011 and 2012
- *also from Credit River coho in 2012*

v5909 Atlantic salmon
Lac St. Jean, PQ Oct 2009

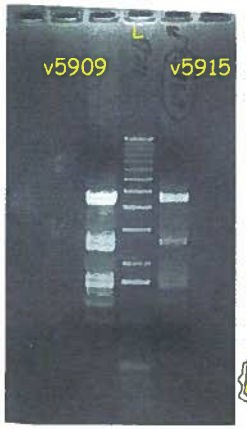
v5915 Coho salmon
Credit River, Nov 2009



- CPE on EPC, CHSE-214
- icosahedral ~100 nm

CHARACTERIZING THE VIRUSES

- electron microscopy of virus particles
  - *shape and size*
- RNA or DNA ?
  - *5-iodo-2'-deoxyuridine*
- Enveloped or unenveloped?
  - *chloroform effect on CPE*
- Polymerase Chain Reaction and reverse-transcription PCR with diagnostic primers
- Genome organization; Sequences



- RNA viruses
- genome on agarose gel consists of multiple bands in clusters
- patterns for V5909 and v5915 are different

Characteristics fit the Aquareovirus group
- significance for fish health ?

v5767 Bacilliform virus from Chinook salmon

- Credit River, spawning fish Sept 2008
- on RTG-2 only
- RNA virus
- Enveloped

RT-PCR for using primers for VHSV

OUTER PRIMERS → 950 bp VHSV product ✗

INNER PRIMERS → 558 bp VHSV product ✗

With the inner primers, v5767 *did* show a weakly-amplified 950 bp product.

NO SEQUENCE HOMOLOGY to VHSV

so presumed to be a random amplification

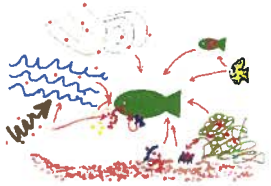
This sequence allowed confirmation that the same virus was re-isolated from Credit River chinook in 2011 and 2012, and coho in 2012.

From the rough sequence, new internal primers were designed, to amplify a 400 bp product from v5767.

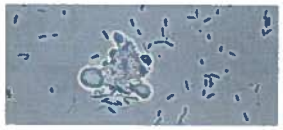
- no sequence homology to known viruses
- RT-PCR with primers negative for VHSV, IHNV ..

While the bacilliform shape made the fathead minnow nidovirus (Iwanowicz & Goodwin 2002) of interest, despite a different CPE.

However, the recently published sequence (Batts, Goodwin & Winton 2012) for this virus has no homology with the sequence from v5767 or other sequences available.



Amoeba as potential Environmental Reservoirs of Fish Pathogens



Trophozoites of amoeba rapidly form resting cysts, which are resistant to disinfection by chlorination and other biocides.

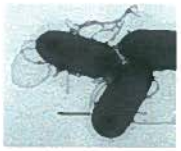
... and they can carry passengers!

THE TROJAN HORSE

A collaboration with Dr. Lucy Mutharia

Enteric Redmouth Disease

Yersinia ruckeri



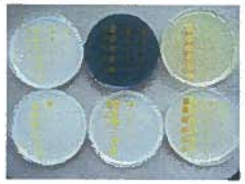
An opportunistic pathogen (stress-associated) with several serological variants

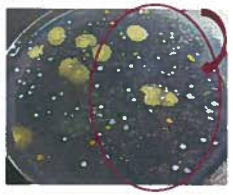
- are they all equally pathogenic ?
- can we tell them apart by genetic tests ?

Flavobacterium branchiophilum

Bacterial Gill Disease bacterium – can we do a better job of culturing those cells from gills ?

THEY ARE THE TINY COLONIES !!



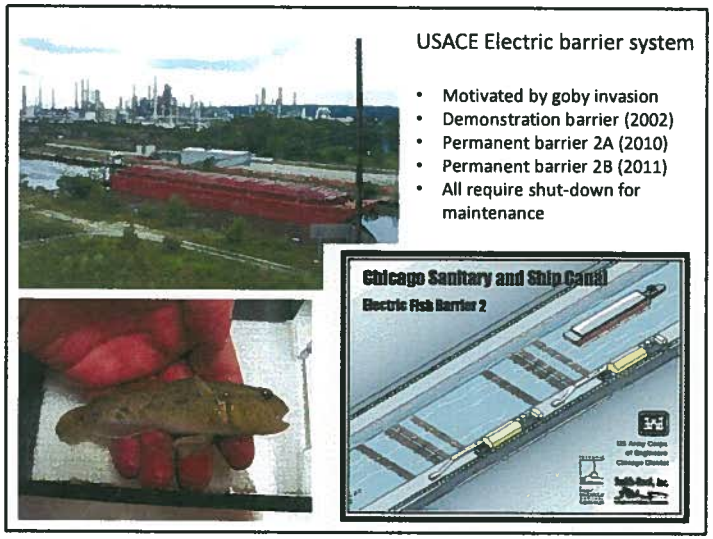


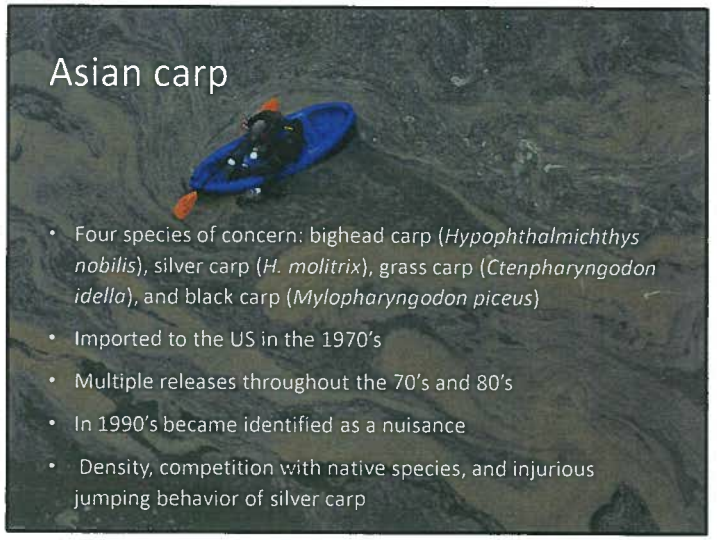
Conservation by the cup of water: The potential for indirect surveillance techniques to inform conservation management
Christopher Jerde, University of Notre Dame
Great Lakes Fish Health Committee Meeting, 5 February 2013

LAKE MICHIGAN
Mississippi River Basin
Great Lakes Basin

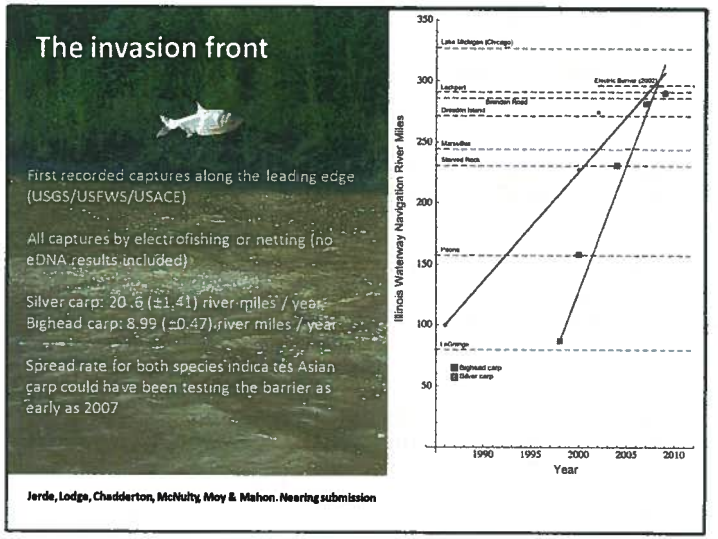
The Chicago Sanitary and Ship Canal

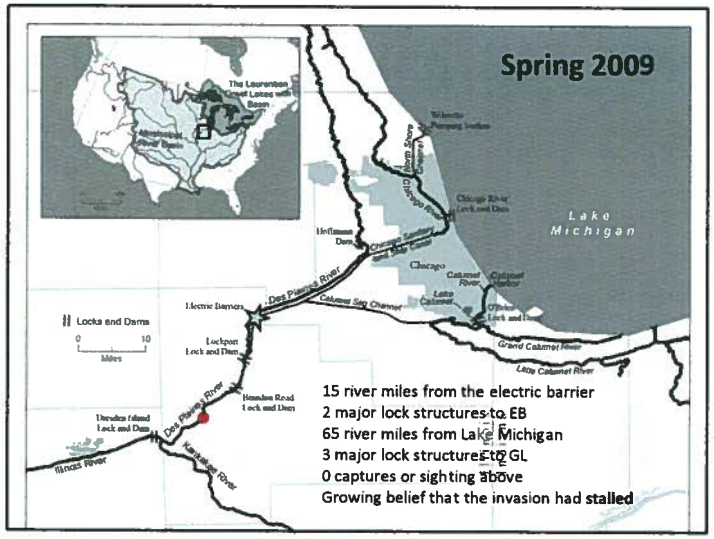
The Chicago Sanitary and Ship Canal
LAKE MICHIGAN
CHICAGO SANITARY AND SHIP

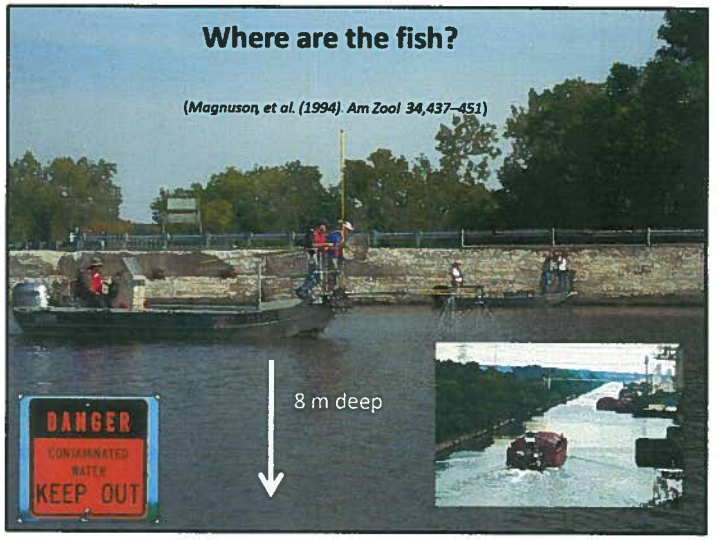

USACE Electric barrier system
Motivated by goby invasion
Demonstration barrier (2002)
Permanent barrier 2A (2010)
Permanent barrier 2B (2011)
All require shut-down for maintenance
Chicago Sanitary and Ship Canal
Electric Fish Barrier 2


Asian carp
Four species of concern: bighead carp (Hypophthalmichthys nobilis), silver carp (H. molitrix), grass carp (Ctenpharyngodon idella), and black carp (Mylopharyngodon piceus)
Imported to the US in the 1970's
Multiple releases throughout the 70's and 80's
In 1990's became identified as a nuisance
Density, competition with native species, and injurious jumping behavior of silver carp

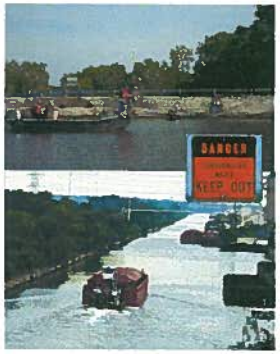

If you see this, it's too late!


The invasion front
First recorded captures along the leading edge (USGS/USFWS/USACE)
All captures by electrofishing or netting (no eDNA results included)
Silver carp: 20.6 (±1.41) river miles / year
Bighead carp: 8.99 (±0.47) river miles / year
Spread rate for both species indicates Asian carp could have been testing the barrier as early as 2007
Illinois Waterway Navigation River Miles
Year
Bighead carp
Silver carp
Jerde, Lodge, Chadderton, McNulty, Moy & Mahon. Nearing submission



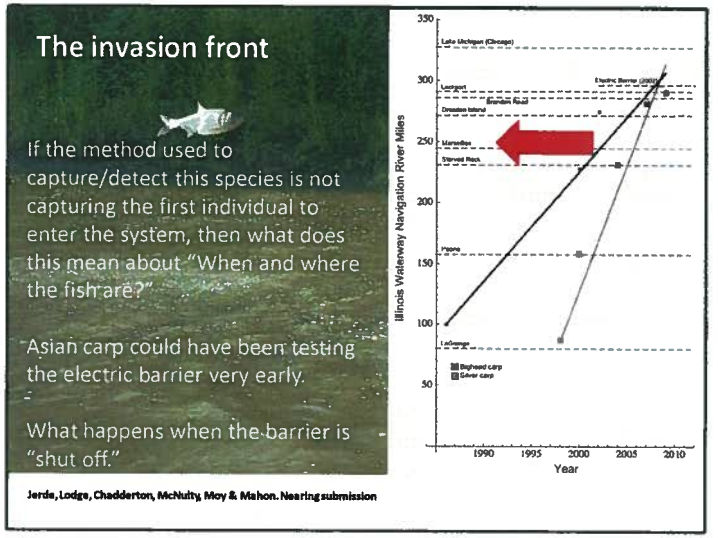


Electrofishing detection



"Electrofishing for large bighead carp and silver carp is notoriously difficult, especially where the water is deeper than the electric field. For example, in 2004 in the Lamine River, a slow-moving tributary of the lower Missouri River, trammel nets were used to trap telemetered fish in short segments (approximately 200 m) of the river and two electrofishing boats were used for 2 days in an attempt to capture the fish or to force them into the nets. The attempt was unsuccessful at capturing the telemetered fish, and the fish were traced apparently moving under the electrofishing boats on multiple occasions (D. Chapman, USGS, personal observation)"

From Moy, Polls, & Dettmers. 2011. The Chicago Sanitary and Ship Canal Aquatic Nuisance Species Dispersal Barrier. In Invasive Asian Carps in North America (Chapman & Hoff, eds.) pg 136.



The eDNA research group

Christopher Jerde, University of Notre Dame
David Lodge, University of Notre Dame
Andrew Mahon, Central Michigan University
Lindsay Chadderton, The Nature Conservancy

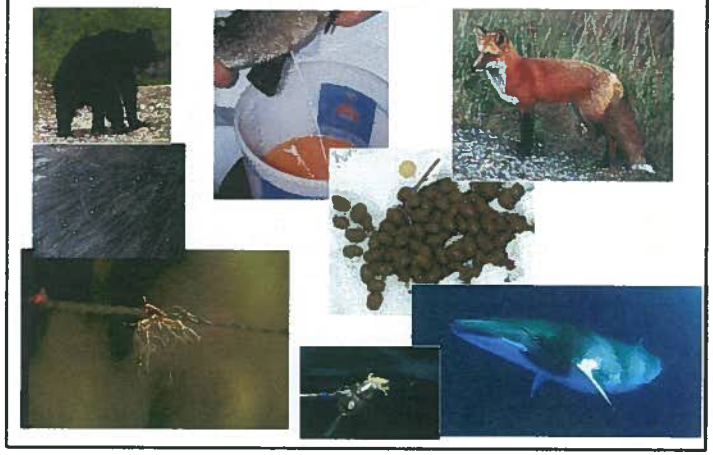



CMU CENTRAL MICHIGAN UNIVERSITY

The Nature Conservancy Protecting nature. Preserving life.

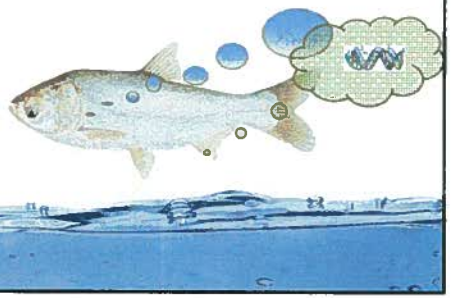
UNIVERSITY OF NOTRE DAME

The Idea! All organisms shed DNA

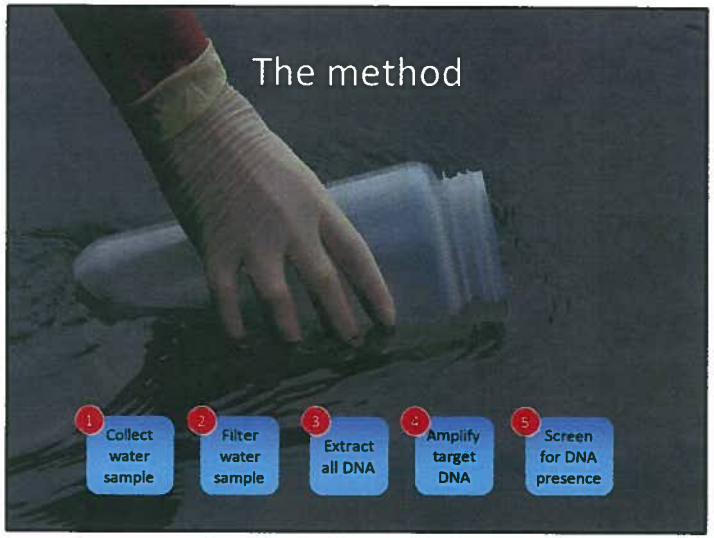


Using eDNA for detection of invasive carp

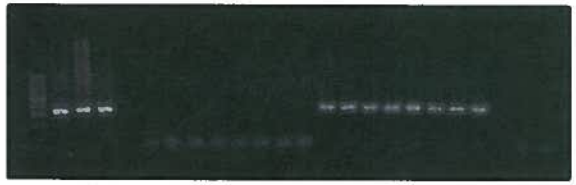
- Fish naturally shed sloughed cells in mucus, scales, feces, and urine
- Some cellular material will remain in suspension and can be collected
- Asian carp produce large quantities of feces and urine



The method



The outcome

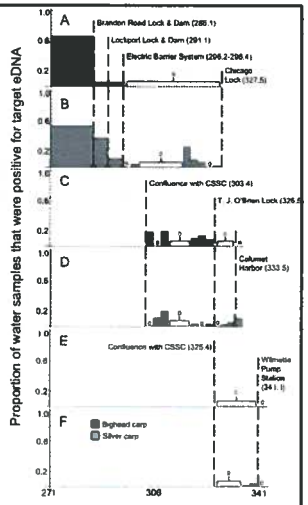


Inference: Presence of target DNA is an indication of presence of the target organism

Study overview

- Molecular markers for bighead (312bp) and silv er carp (191bp) tested against available sequences and tissues of known species in the area
- Approximately 1000, 2 liter water samples collected and screened for bighead and silver carp
- Adaptive sampling design with more effort placed at presumptive leading edge of the detection
- June 2009 to May 2010, 15 different sampling trips
- QAQC: cooler blanks of deionized water, equipment controls for all samples, sequencing of positive samples
- Marker specificity, collection, processing, and screening of eDNA procedures reviewed by independent audit by US EPA (Blume et al. 2010) and by Independent External Peer Review (USACE 2011)

Blume et al. 2010. Laboratory audit report: Lodge Laboratory, Department of Biological Sciences, University of Notre Dame. US Environmental Protection Agency Great Lakes National Program Office, Chicago.

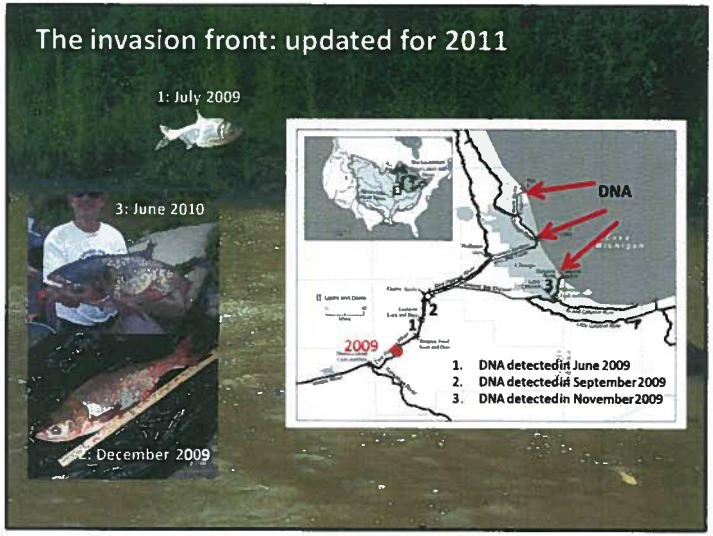


Pattern indicative of the leading edge of an invasion front

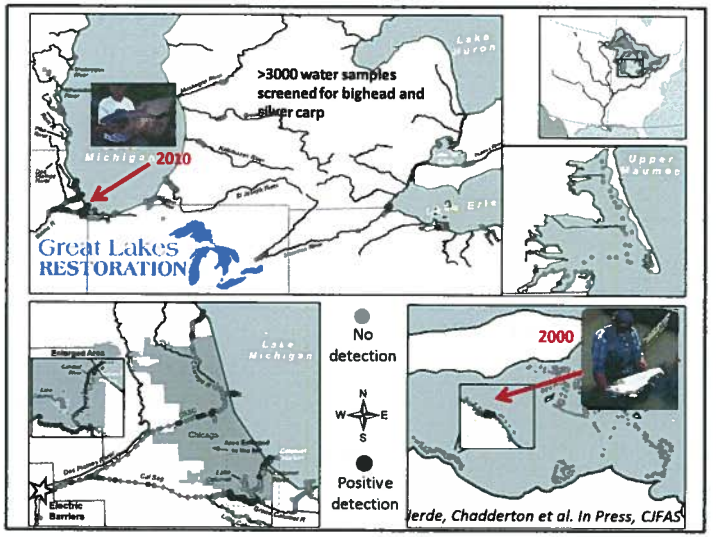
Jump dispersal (accumulation below locks)

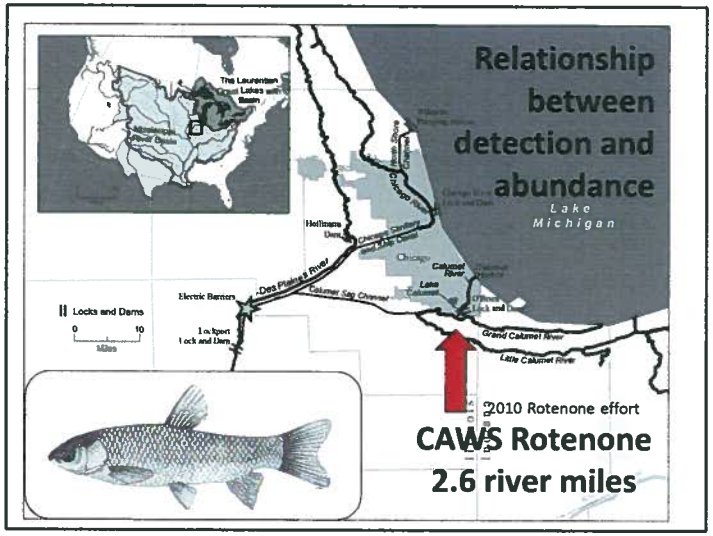
First indication that Asian carp were present above the electric barrier

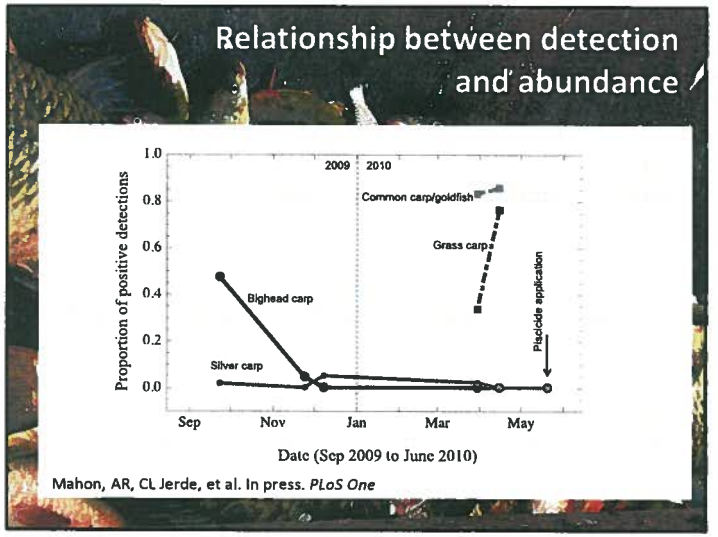
Jerde et al. 2011. 'Sight-unseen' detection of rare aquatic species using environmental DNA. Conservation Letters. 4(3): 150-157.




Alternative sources of DNA or explanations


>3000 water samples screened for bighead and silver carp
Michigan 2010
Great Lakes RESTORATION
Lake Huron
Lake Erie
Upper Maumee
Lake Michigan
Chicago
Electric Barriers
No detection
Positive detection
2000
Jerde, Chadderton et al. In Press, CJFAS


Relationship between detection and abundance
Lake Michigan
Chicago
Des Plaines River
Calumet River
Calumet Sag Channel
Lake Calumet
Grand Calumet River
Little Calumet River
Electric Barriers
Locks and Dams
Lockport Lock and Dam
2010 Rotenone effort
CAWS Rotenone
2.6 river miles


Relationship between detection and abundance
Proportion of positive detections
1.0
0.8
0.6
0.4
0.2
0.0
2009
2010
Common carp/goldfish
Grass carp
Bighead carp
Silver carp
Piscicide application
Sep
Nov
Jan
Mar
May
Date (Sep 2009 to June 2010)
Mahon, AR, CL Jerde, et al. In press. PLoS One

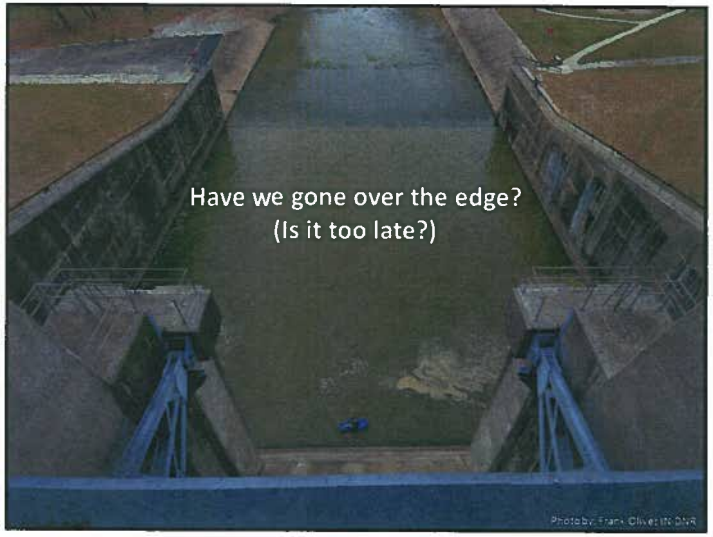
Relationship between detection and abundance

Tetra-tech
>12,000 CC/GF
43 grass carp
No bighead carp
No silver carp
No black carp

Approximately 50% of water samples tested positive for grass carp DNA.
Approximately 86% of water samples tested positive for CC/GF DNA

eDNA detections [amount of DNA] is positively correlated with fish abundance [biomass]

Mahon, AR, CL Jerde, et al. In press. *PLoS One*
Thomsen et al. 2012. *Molecular Ecology* 21:2565-2573
Takahara et al. 2012. *PLoS One* e35868



Current state of the problem:

1. Evidence from eDNA and captures show that some Asian carp are present in Great Lakes, but numbers and distributions are largely unknown (likely small).
2. The only direct source (swimming fish) is from the CSSC
3. Other sources of introduction are possible (fish stocking, bait, intermittent hydrological connections) but currently unmeasured
4. Changing the operation of the CSSC will be very expensive, but so may be not changing the operation of the CSSC. Need economic evaluations and public input

Methodological Developments

- Evaluation of marker sensitivity, calibration, and qPCR advancement
- Non-target surveillance with high throughput sequencing
- Estimating species richness and diversity
- Boarder and cargo inspection
- Real time detection (there's an app for that).

Thomsen PF, Kielgast J, Iversen LL et al. 2012. Monitoring endangered freshwater biodiversity by environmental DNA. Molecular Ecology. 21: 2565–2573.

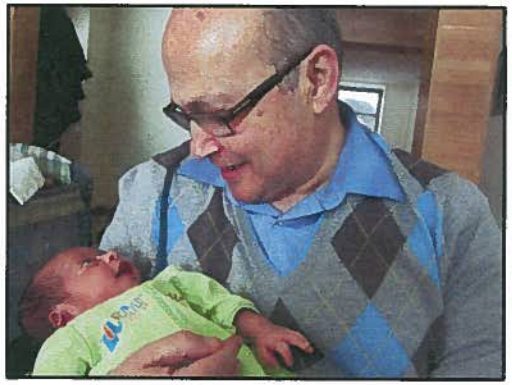
Lodge DM, Turner CR, Jerde CL et al. 2012. Conservation in a cup of water: estimating biodiversity and population abundance with environmental DNA. Molecular Ecology. 21:2555-2558

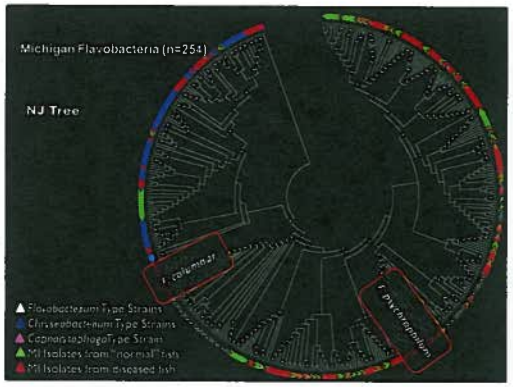
Fish Health and indirect surveillance

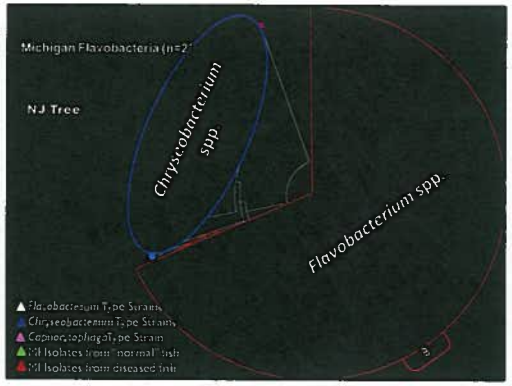
- Smith, Schmidt, Rosen, and Amaral-Zettler 2012. Microbial diversity and potential pathogens in ornamental fish aquarium water. PLoS One 7(9): e39971
- Found eleven bacterial species of human and fish health concern: Coxiella burnetii, Flavobacterium columnare, Legionella birminghamensis, L. pneumophila, Vibrio cholerae, V. mimicus, V. vulnificus, Aeromonas schubertii, A veronii Ahydrophila, and Plesiomonas shigelloides.
- What about fish haulers, bait dealers, water garden supply centers, hydrological connections,....

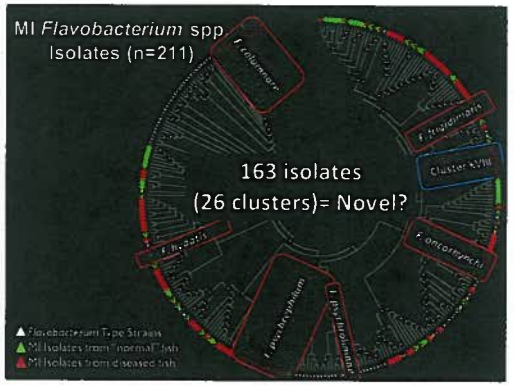
January 10, 2013 at 1:00 PM

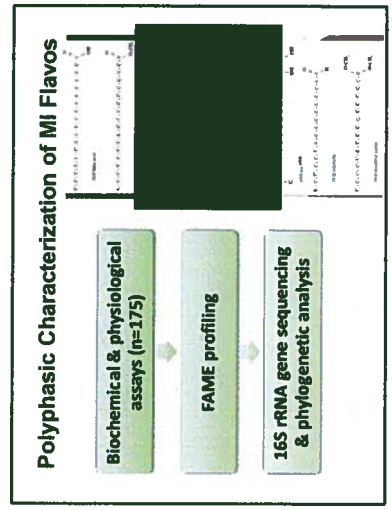
Zane arrived



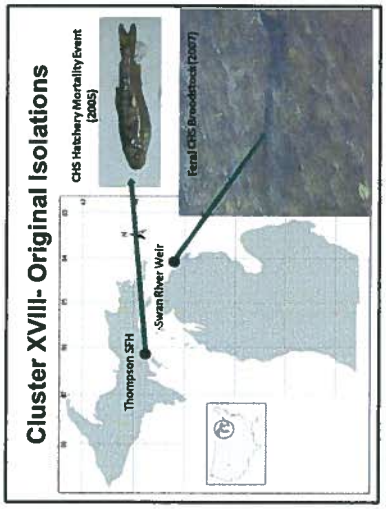


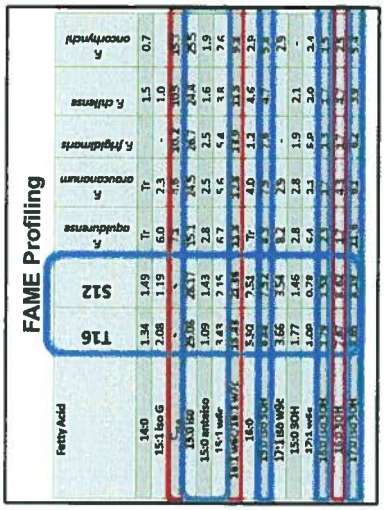


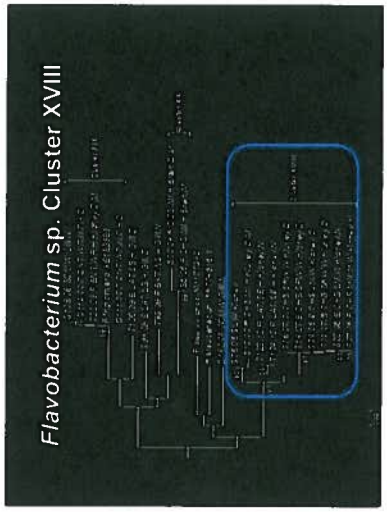


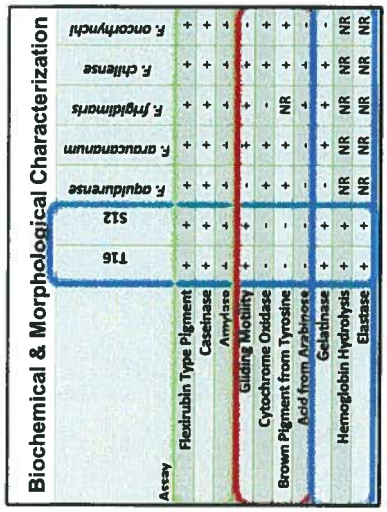

Polyphasic Characterization of MI Flavos
Biochemical & physiological assays (n=175)
FAME profiling
16S rRNA gene sequencing & phylogenetic analysis


Phylogenetic Analyses


Cluster XVIII- Original Isolations
CIS Hatchery Mortality Event (2005)
Thompson SFH
Swan River Weir
Feral CIS Broodstock (2007)


FAME Profiling
Fatty Acid
T16
S12


Flavobacterium sp. Cluster XVIII


Biochemical & Morphological Characterization
Assay
T16
S12
F. aquidurense
F. araucananum
F. frigidimaris
F. chilense
F. oncorhynchi
Flexirubin Type Pigment
Caselnase
Amylase
Gliding Mobility
Cytochrome Oxidase
Brown Pigment from Tyrosine
Acid from Arabinose
Gelatinase
Hemoglobin Hydrolysis
Elastase

Gross Pathology- F. spartani sp. nov.

Antibiotic Susceptibility
Isolate
T91 R (12.0) R (0) S (32.0) R (16.0) S (23.0)
T75 R (11.0) R (0) S (33.0) S (31.0) R (14.5)
T18 R (14.0) R (0) S (32.0) S (24.5) S (30.0)
T16 R (11.5) R (0) R (16.0) S (18.0) R (27.0)
S87 R (14.0) S (15.0) S (25.0) R (17.5) S (30.0)
S12 R (14.0) R (0) R (17.0) S (18.5) R (16.5)
S21 R (0) R (0) S (27.5) S (18.0) S (27.0)
T76 S (18.0) R (0) S (24.0) S (24.5) R (17.0)

Gross Pathology- F. spartani sp. nov.

Phylogenetic Analyses
PP=100
MP=88
T16T & S12 represent a novel Flavobacterium sp., for which the name F. spartani is proposed.

Gross Pathology- F. spartani sp. nov.

Gross Pathology- F. spartani sp. nov.

Chryseobacterium sp. T68

Assay	T68	T62	C. ginseno.	C. gregarium
pH 5.0	(+)	(+)	-	NR
4-5 °C	+	+	-	(+)
37 °C	-	-	+	-
2% NaCl	(+)	(+)	-	NR
Production of:				
Diase	-	-	+	-
Amylase	-	-	+	+
Arginine dihydrolase	-	-	+	+
Urease	-	-	+	-
Esterase	+	+	-	-
Cystine arylamidase	+	+	-	-
Degradation of:				
Trypsin	+	(+)	-	-
α-chymotrypsin	+	+	-	-
Assimilation of:				
D-Maltose	-	-	+	-
Acid from L-Arabinose	-	-	(+)	+
Acid from D-Sucrose	(+)	-	-	+

Chryseobacterium sp. T68

Fatty Acid	T68	C. ginsenosidimutans	C. gregarium
iso-C15: 0	30.9	50.3	35.1
anteiso-C15: 0	2.6	3.8	0.1
16:1 w6c/16:1 w7c	28.0	0.5*	-
16:1 w5c	1.5	-	-
C16: 0	7.0	-	Tr
iso-C16:3-OH	2.5	5.2	2.8
iso-C15 : 0 2-OH	-	-	10.6
iso-C17 : 1 w8c	1.4	9.3	16.9
16:0 3OH	5.1	-	1.2
iso-C17: 0 3-OH	16.1	21.9	10.0
C17:0 2-OH	1.8	-	Tr

Chryseobacterium sp. T68
T68 & T62 represent a novel Chryseobacterium sp., for which the name C. aahli is proposed.

C. aahli- Gross Pathology

C. aahli - Histopathology
H&E; 400x
H&E; 400x
H&E; 400x
H&E; 200x
H&E; 200x

Investigation of Virulence Factors
VS

F. spartani- Virulence Factors in vivo
ML=55 cm, MW=1.6 kg

In vitro Chambers

In vivo- Chamber Recovery

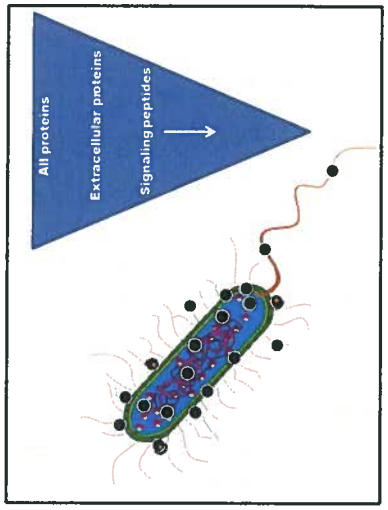
SSH Sequence Results

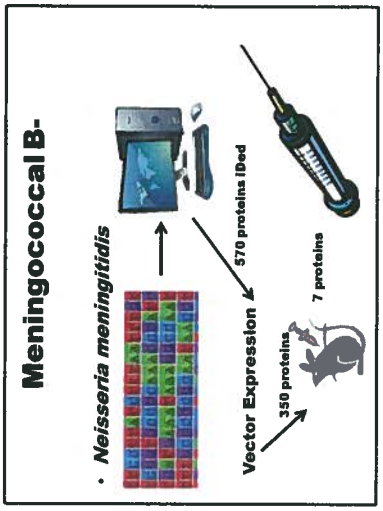
- Genes associated with bacterial cell-activation
- Transposase (*L. anguillarum, P. damselae*)
- Transporter protein (*P. damselae*)
- **Multiple proteins with unknown functions probably virulence factors**

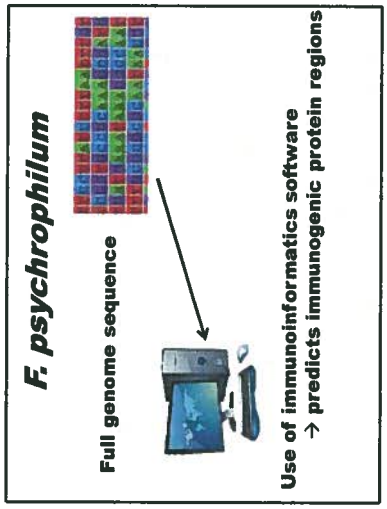
Bacterial Cold Water Disease (BCWD)

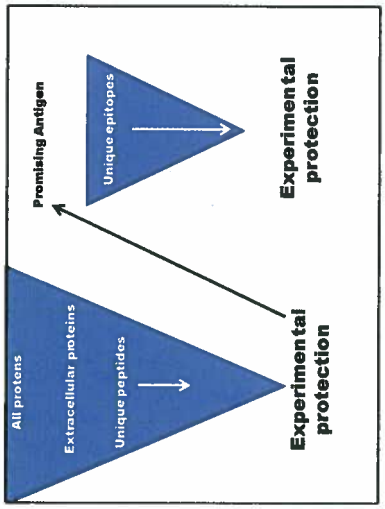
- **Can be resistant to antibiotic treatments**

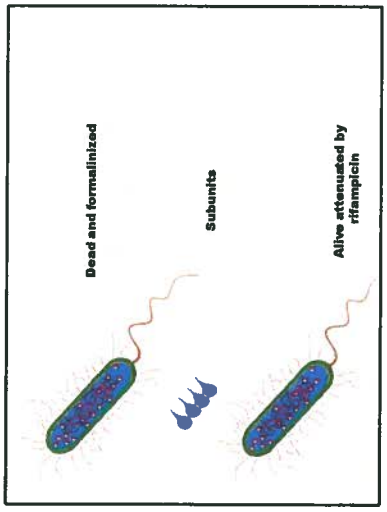
2011	**2**
2013	**4**

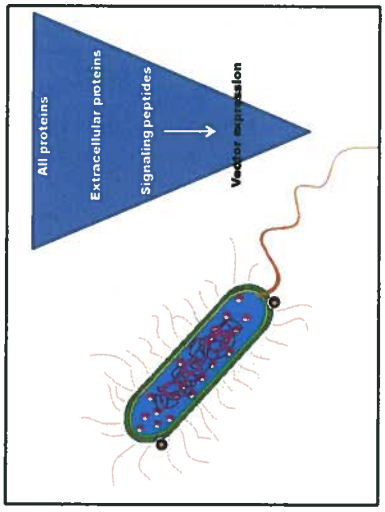

All proteins
Extracellular proteins
Signaling peptides


Meningococcal B-
· Neisseria meningitidis
570 proteins IDed
Vector Expression
350 proteins
7 proteins


F. psychrophilum
Full genome sequence
Use of immunoinformatics software
→ predicts immunogenic protein regions


All protens
Extracellular proteins
Unique peptides
Promising Antigen
Unique epitopes
Experimental protection
Experimental protection


Dead and formalinized
Subunits
Alive attenuated by rifampicin


All proteins
Extracellular proteins
Signaling peptides

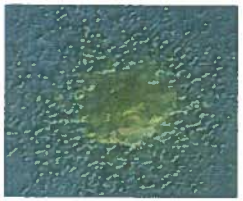
RV Fish examples

- **RV lead to effective vaccine candidates**

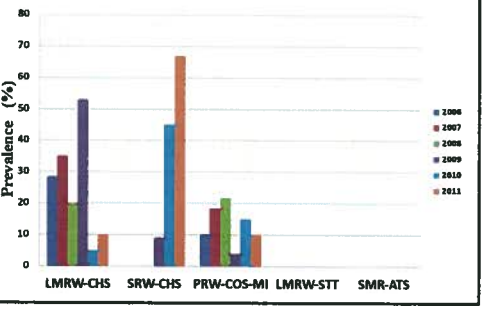
  →*Photobacterium damselae* (Italy, 2012)
  -370 proteins IDed
  -8 expressed via a vector

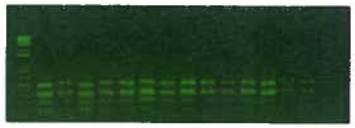
  →*Edwardsiella tarda* (China, 2012)
  -16 flagellar proteins IDed
  -10 expressed via a vector

***Flavobacterium columnare* in Michigan**



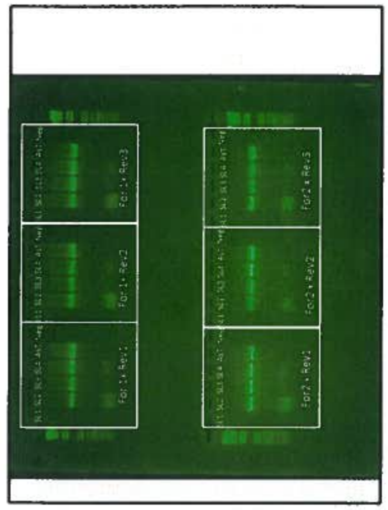
***F. columnare* in MI Salmonids**

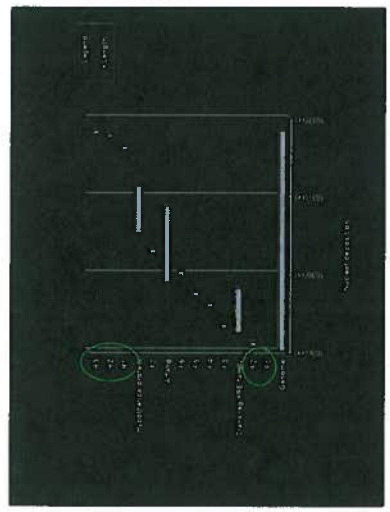




Test	Isolates As-SL2-4 (n=3)	Isolate As-SL1	A. salmonicida subsp. salmonicida	A. salmonicida subsp. achromogenes	A. salmonicida subsp. masoucida	A. salmonicida subsp. smithia	A. salmonicida subsp. pectinolytica
Oxidase production	+ (100%)	+	+	+	+	+	+
Brown pigment	+ (100%)	+	+	-	-	-	+
Indole production	- (0%)	-	-	+	+	-	+
Bile-esculin hydrolysis	+ (100%)	+	+	-	+	-	-
Gas from dextrose	+ (100%)	+	+	-	+	(+)	+
TSI Reaction	K/A	A/A	K/A	A/A	NR	NR	NR
Sucrose	- (0%)	+	-	+	+	d	+
Dark blue colonies on CBBA	+ (100%)	-	+	-	NR	NR	NR

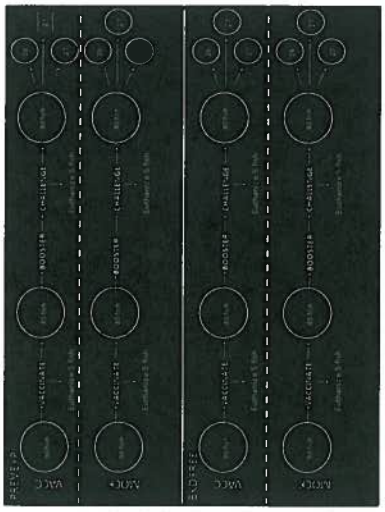
***A. salmonicida* in Sea Lamprey**

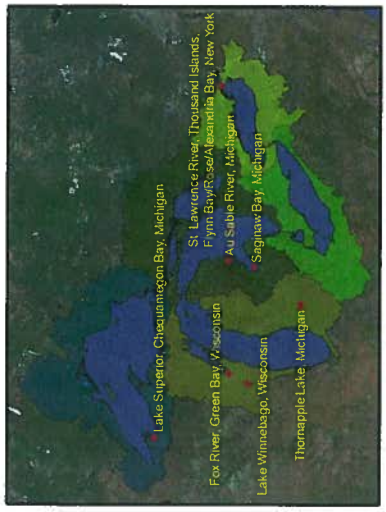




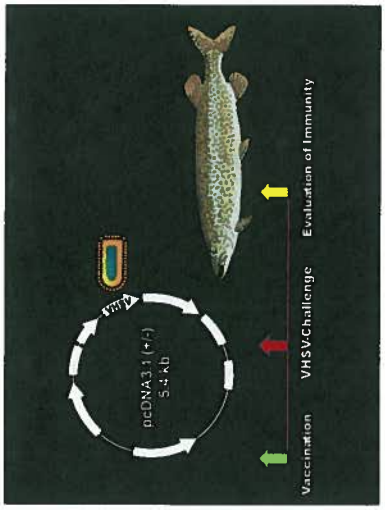
PCR on Sea Lamprey Isolates (Miyata Primers)

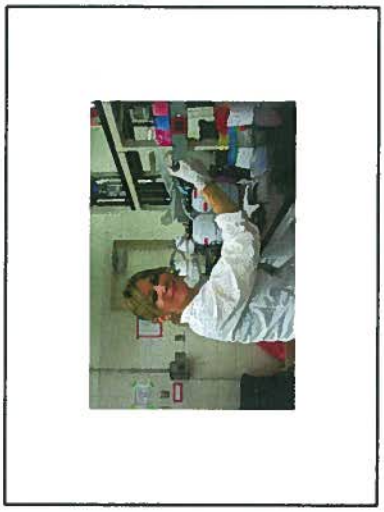
Appendix 5

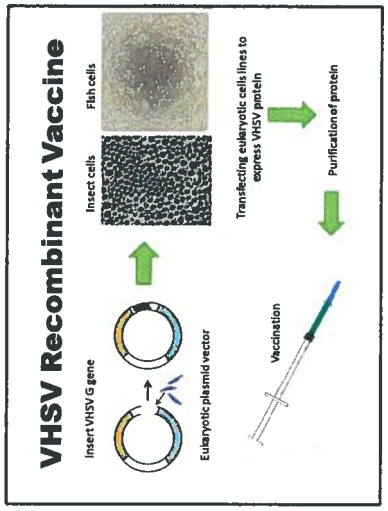


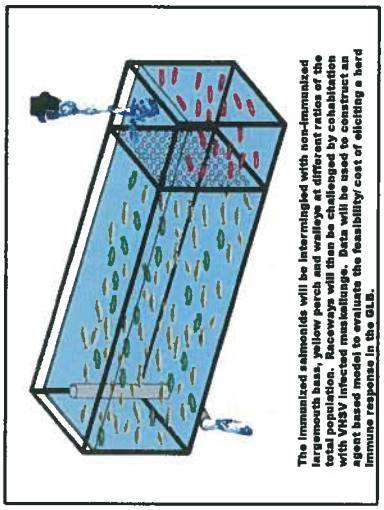


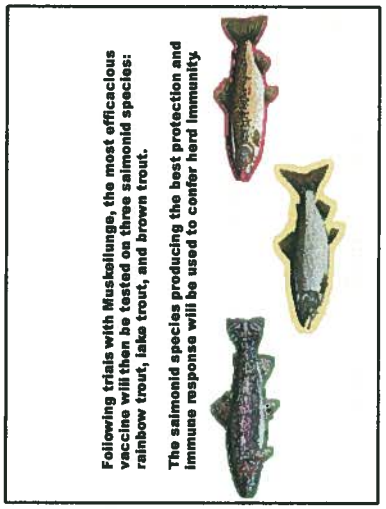
Sample	Prevalence	Kappa†	95% C.I.	Assumed Prevalence	Sample Size
Lethal	80% (142/178)	N/A	N/A	10%	N/A
Nonlethal	57% (101/178)	0.53	0.44-0.62	30%	980
Fecal	52% (93/178)	0.44	0.35-0.57	50%	445
Mucus	21% (38/178)	0.17	0.11-0.23	10%	383
Serum	31% (56/178)	0.26	0.19-0.31	30%	980
Blood	31% (55/178)	0.25	0.18-0.32	50%	485
				10%	403
				30%	2104
				50%	1178
				10%	1055
				30%	1818
				50%	814
				10%	756
				30%	1624
				50%	834
					759

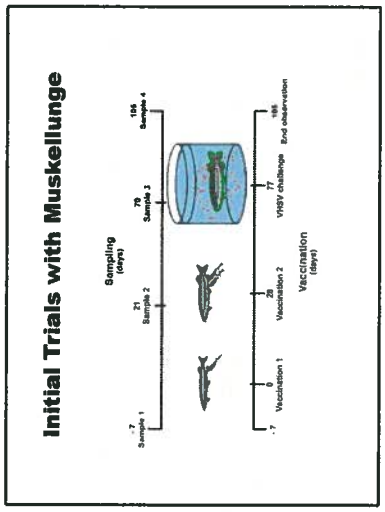





VHSV Recombinant Vaccine
Insert VHSV G gene
Eukaryotic plasmid vector
Insect cells
Fish cells
Transfecting eukaryotic cells lines to express VHSV protein
Purification of protein
Vaccination


The immunized salmonids will be intermingled with non-immunized largemouth bass, yellow perch and walleye at different ratios of the total population. Raceways will than be challenged by cohabitation with VHSV infected muskellunge. Data will be used to construct an agent based model to evaluate the feasibility/ cost of eliciting a herd immune response in the GLB.


Following trials with Muskellunge, the most efficacious vaccine will then be tested on three salmonid species: rainbow trout, lake trout, and brown trout.
The salmonid species producing the best protection and immune response will be used to confer herd immunity

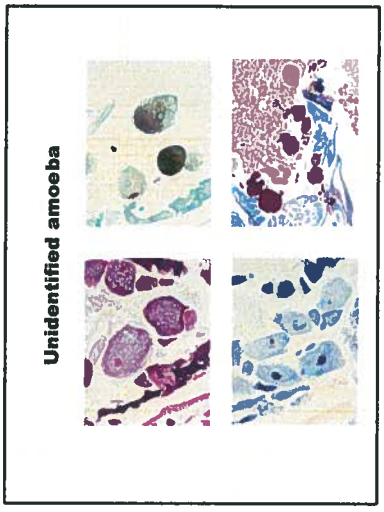

Initial Trials with Muskellunge
Sampling (days)
Vaccination (days)
Vaccination 1
Vaccination 2
VHSV challenge
End observation

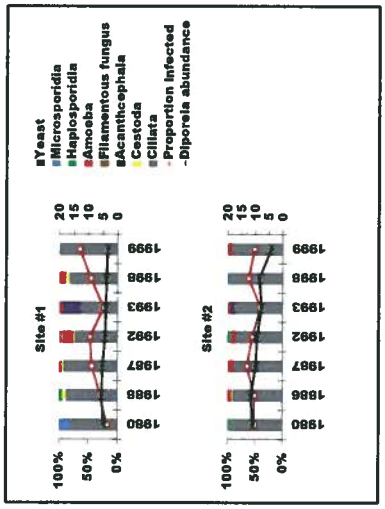
Diseases of Diporeia spp.

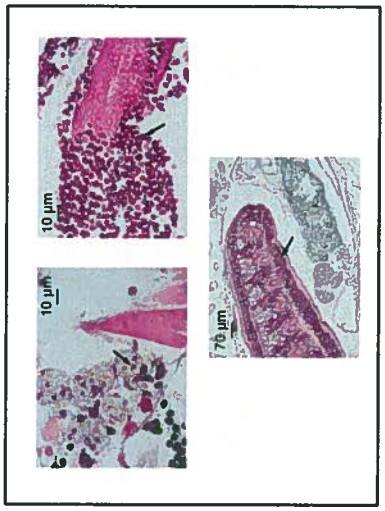
Parasites of Diporeia: 1980-2007

MI-47
MI-40
MI-27M
MI-18M
3
2
15
2
7
0
9
2
12
1
0
3
0
2
1
20

Lake Michigan Diporeia
MI-18M
MI-27M
MI-40
MI-47
Flavobacterium spp.
Methylotenera spp.
Pseudomonas spp.
Average relative abundance
T-RF (bp)


Unidentified amoeba


Yeast
Microsporidia
Haplosporidia
Amoeba
Filamentous fungus
Acanthocephala
Cestoda
Ciliata
Proportion infected
Diporeia abundance
Site #1
Site #2
1999
1998
1993
1992
1987
1988
1980
1986
20
15
10
5
0
100%
50%
0%


10 µm
10 µm
70 µm

From: Marcquenski, Susan V - DNR
Sent: Wednesday, November 21, 2012 12:39 PM
To: Jensen, Peter J - DNR; Branstad, David A - DNR; Bremness, Randy L - DNR; Kobernick, Vince H - DNR; Krueger, Deborah L - DNR; Walters, Becky J - DNR; Lindenberger, Gary A - DNR; Kaas, Alfred - DNR; Komassa, John J - DNR; Giehtbrock, David - DNR; Engel, Marty P - DNR; Andre, Matt R - DNR
Cc: DNR DL WD FH Fish Brd; Kebus, Myron J - DATCP; McGraw, Paul J - DATCP; Ehlenfeldt, Robert G - DATCP; Kenneth_Phillips@fws.gov; ling.shen@dnr.state.mn.us; Corey_Puzach@fws.gov; Becky_Lasee@fws.gov
Subject: Cutthroat trout virus isolated from adult BNT at the St Croix Falls Hatchery

Hello Everyone,

In routine testing of ovarian fluids (150 females sampled as five-fish pools for a total of 30 tubes) from the captive adult brown trout broodstock at the St Croix Falls hatchery this Fall, the La Crosse Fish Health Center isolated and confirmed Cutthroat Trout virus (CTV) in the samples (see Corey Puzach's e-mail below). The St Croix Falls hatchery has reared captive brook and brown trout broodstocks and their progeny for over 30 years and this is the first time a virus has been isolated from fish at the hatchery. Cutthroat trout virus was not isolated from the brook trout broodfish ovarian fluids, nor the fingerling brown trout and brook trout that were sampled in August prior to Fall stocking. There has not been any sign of gross pathology , morbidity or mortality in the adult brown trout at any time this Fall.

This is the first time that CTV has been isolated and confirmed in Wisconsin, and by this memo, we are reporting it as a foreign/ exotic pathogen to DATCP per ATCP 10.66 (1) (a).

One question that has come up is whether this is a new infection or whether it may have been present, but undetected in the recent past. In consultation with Dr Tom Waltzek at the University of Florida, it is possible this is a new infection. We have routinely tested the St Croix Falls broodfish and progeny, and the progeny that were transferred to other DNR hatcheries each year for the past 30+ years and CTV was not isolated. However, Dr Waltzek pointed out that certain cell lines may be more or less susceptible to various viruses, even if they are the "same" cell line, depending on the lineage of the cell line and how many passages of the cells have been made. This year we are using the La Crosse Fish Health Center instead of the WVDL to do most of our virus isolation work. It is possible that their CHSE-214 cell line is slightly different than that of the WVDL, and that might explain why we are detecting this for the first time. Also, the type of cytopathic effect that the virus causes in not dramatic- cells simply enlarge and become grainy as you can see in the attached images from Corey. So it is possible that this subtle form of CPE may have gone unremarked in the past. Dr Waltzek offered to sequence our isolate so that we can compare its genome with other CTV isolates, which may help us figure out the source of the virus. Corey will send our isolate to Dr Waltzek November 26 and the genetic testing will take a few days.

I have attached two scientific papers about CTV. This virus is in the Hepeviridae family, and this family of viruses also includes the human pathogen Hepatitis E virus. CTV has been isolated from ovarian fluids from many species of trout, including brown trout, in Western states and Atlantic salmon from New Brunswick. CTV infections have never been associated with disease or mortality. The virus is very stable in the environment, and anecdotal evidence suggests that infected fish may be life-long carriers, with viral shedding resuming during spawning periods when fish are immunosuppressed by spawning hormones.

I have also attached an issue brief on CTV that Ken Phillips and others of the Great Lake Fish Health Committee developed a few years ago to assess the risk to fish in the GL basin. It is an excellent document, with the key message that this virus is not likely a serious pathogen of trout, but its effect on coolwater species is unknown.

There has been no direct or indirect movement of eggs or fish from western states to the St Croix Falls Hatchery. The hatchery is located above the St Croix River and broodstock raceways are not enclosed. The hatchery water supply is obtained from a series of drain tiles that are buried under the city of St Croix Falls. No surface water is used at the hatchery. At present, there is no logical source of the virus. Batts et al. suggest that CTV is likely more widespread in the United States than is currently known.

We had planned to do our routine lethal testing of the SCF brook and brown trout broodstock in early December and will follow through with that December 4 and 5. We will collect tissues for virus isolation and bacteriology. Pending the CTV diagnosis, the hatchery is currently holding fingerling SCF strain brook trout, SCF strain brown trout and Timber Coulee strain brown trout that would usually have been stocked by now. We can include those groups of fish in our sampling if the FM board or DATCP so advises. The hatchery cannot hold these fish indefinitely, so we will need to discuss the disposition of the fish at the hatchery.

I would like to test fish from several locations in the St Croix River for CTV next spring to see if the river might be a reservoir of infection.

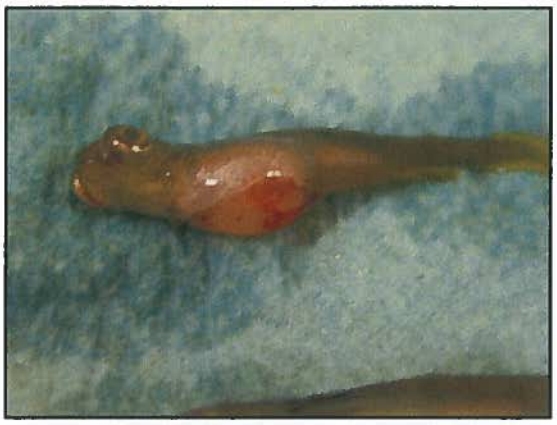
Peter Jensen and I have spoken about biosecurity at St Croix Falls and Osceola hatcheries and we feel existing day to day practices will contain the virus at the St Croix Falls hatchery. These practices include using separate equipment when working on different lots of fish, disinfecting equipment, sanitizing hands, and working with the adult brown trout broodstock last, if there is a need to work with more than one group of fish on a given day. Since St Croix Falls and Osceola are a work unit and staff travel daily between hatcheries, staff should have dedicated sets of raingear, waders, etc. for use at each hatchery. Pete and the hatchery staff will need to discuss how they accomplish their weekend rotational work duties, to minimize any spread of the virus between facilities.

In the long term, we will need to reflect on how best to operate the St Croix Falls hatchery and make the best use of its production. It is likely that the virus will persist in the broodfish.

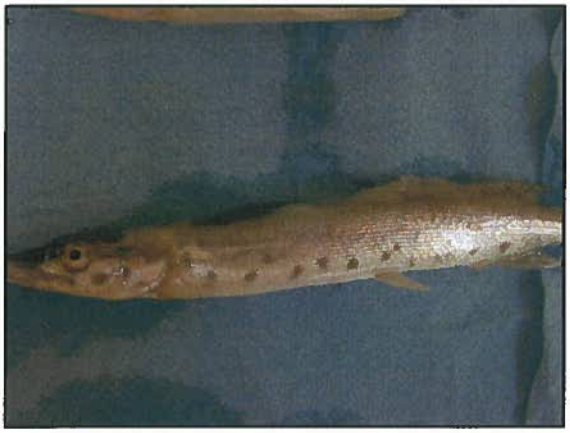
Ken, could you please forward this information to the GLFHC? The St Croix Falls hatchery is not in the Great Lakes basin, but I think the members will want to know about this isolation.

Please let me know if you have any questions,
Sue

Coho fry



Muskies



Appendix 8

From: Batts, William [bbatts@usgs.gov]
Sent: Thursday, January 24, 2013 7:05 PM
To: Barbash, Patricia
Cc: Coll, John; Jim Winton; Yamashita, Coja; William Batts; Gael Kurath
Subject: Bellefonte PA CTV sequencing results

Hi Trish (and all collaborators),

Your Bellefonte PA brown trout isolate (13-019) was indeed a cutthroat trout virus strain. It has a unique sequence signature when compared to nearly 100 other CTV isolates we have processed. It could not be compared to the Atlantic salmon isolate in New Brunswick, since my primer site is not in the GenBank accessioned sequence by Kibenge. They reported a dual infection with ISAV.

The region I compare is 262 nt long and lies between the two primers that you have. Bellefonte CTV is grouping separately from the large sub-lineage "A" that our paper in Virus Research describes with greater than 19 nt differences from the next closest virus isolate. This results in a 7 to 10% nt difference for the majority of those isolates in "A". We are putting together with Scott LaPatra another paper that will describe another sub-lineage "B" from Idaho, which is about 25% nt different from the "A" isolates.
See below for some of the relevant comparisons with the Bellefonte isolate, starting with some viruses that were the closest by a ClustalW distance tree, then includes other relevant isolates, such as ones from brown trout.

	nt different	% different
Glenwood Springs, CO RT 2008	19	7.3
Poudre, CO CTT 2008	19	7.3
Pitkin, CO CTT 2008	20	7.6
Saratoga, WY BNT 2008	25	9.5
Mt Shasta, CA BNT 1988	22	8.4
Ford, WA BNT 1990	24	9.2
Heenan Lake CA CTT 1988 (type strain)	22	8.4
LaPatra UA5 ID (sub-lineage "B")	66	25.2

I hope this makes some sense to you. It would be nice to know how close Bellefonte is to the Wisconsin and New Brunswick isolates. Maybe you can check with the Wisconsin lab to see if they used the same primers that you used. According to our paper, the New Brunswick isolate should have been 7% different than the Heenan Lake 1988 isolate. Not far off of yours compared to the Heenan isolate too (8.4%). The top four (Glenwood to Saratoga) were closer by the distance method program I used, not the ideal one for publication grade data, but usually informative.

Bill

Draft Epizootic Epitheliotropic Disease Virus (EEDv) at Marquette State Fish Hatchery Briefing Paper

February 3, 2013

Hatchery Background

Marquette State Fish Hatchery (MSFH) is located at N46.468056, W87.355833 in Marquette County, Michigan. This facility is the State of Michigan's char broodstock and production unit and rears brook trout (Assinica strain - AS) and lake trout (Lake Superior Inshore Lean – LS and Seneca strains - SE) along with splake. Captive broodstocks are maintained for Assinica strain brook trout and Lake Superior Inshore Lean lake trout. The Lake Superior Lean stocks were derived from wild Lake Superior Lean stocks taken from various locations from the Marquette area west to the Apostle Islands in Wisconsin in 2001, 2003 and 2004. All Seneca strain lake trout are from federal hatchery broodstocks. Of the approximately 250 to 300,000 yearling Seneca strain lake trout reared annually at MSFH, 100,000 of these fish are reared to meet 1836 Tribal Consent Decree requirements. Annual production is between 32,000 to 48,000 kg annually depending on fisheries management needs. FY2012 production is detailed in the following table and the projected FY2013 rearing is similar.

Table 1. FY2012 Marquette State Fish Hatchery Production

Species	Stage	Number	Kgs
BKT AS	A	1,133	650
BKT AS	FF	31,695	913
BKT AS	SF	12,215	100
BKT AS	YR	53,343	4,644
LAT SE	SF	27,045	148
LAT SE	YR	275,964	6,042
LAT LS	A	1,487	1,103
LAT LS	YR	146,571	4,149
SPL	YR	218,397	15,978

The production facility has 14- 7 m^3 indoor tanks (98 m^3 total) and 11- 100 to 114 m^3 outdoor raceways. There are an additional 8 - 100 m^3 outdoor broodstock raceways. The indoor tank room uses up to 6,400 lpm from protected groundwater wells which goes directly to the hatchery

discharge at this time. The outdoor raceways use 100% surface water (Cherry Creek). The broodstock building is at the head of the water flow and receives 11,300 lpm of UV treated Cherry Creek water as first pass water. After passing through broodstock, the water is bypassed to the effluent treatment system with no serial reuse in the production raceways. The rest of the outdoor rearing complex receives 22,700 lpm from Cherry Creek which is not UV treated at this time. The upper production raceways are divided into two groups with five raceways in the first building (RW1-5) and six raceways in the second building (RW6-11). The upper production raceways receive the flow as first pass water and the effluent is serially reused in the lower production raceways before it goes to the effluent treatment ponds.

EEDv Background

Epizootic epitheliotropic disease virus (EEDv) is a hexagonal, unenveloped herpesvirus (Alloherpesviridae) that is 100-105 um in size (Bradley et al. 1989). The virus affects lake trout skin and has not been shown to affect any other species except for lake trout (McAllister and Herman 1989). The virus often kills quickly following non-specific clinical signs such as epithelium lesions (often with fungal infections), lethargy, and unusual swimming patterns (Plumb and Henson 2011). The only internal clinical sign is swelling in the spleen. It is a stress mediated disease agent that causes epizootics at temperatures from 6-15° C with peak activity between 8-10° C (Plumb and Henson 2011). There is evidence for both horizontal (Bradley et al 1989 and McAllister and Herman 1989) and vertical transmission (McAllister 1993 and Kurobe et al 2009). Since this is a herpesvirus, it is likely to stay in a latent form in both cultured and wild reservoirs (Plumb and Henson 2011). Currently, there are no cell culture techniques available for this virus and the only detection methods are PCR assays, full genome sequencing, and histopathology.

The virus was first described in the late 1980s at MSFH along with Iron River and Pendill's Creek National Fish Hatcheries and Bayfield and Westfield State Fish Hatcheries in Wisconsin. The initial epizootic killed approximately 15 million fish in these hatcheries from 1985-1989 (Plumb and Henson 2011). Similar mortality events, although with smaller losses, were also seen at New York State Fish Hatcheries in the early 1980s (John Schachte, NYDEC, Personal Communication). Mortalities from 65-95% in production lake trout were documented from 1985-1987 at MSFH with a total mortality of 4.1 million fish at that facility and similar high mortalities were noted at all affected facilities. It is suspected that the epizootic started in 1983 as the result of extreme drought conditions along with unusual rain events that created nitrogen supersaturation issues at MSFH which caused the loss of 75% of the fingerling lake trout by August 8, 1983. These fish were noted to have some similar clinical signs to those observed later in the epizootic. Additional analysis of historical information for MSFH from the 1950s has found evidence for clinical signs of a pathogen that looks very similar to EEDv and caused some mortality at that time.

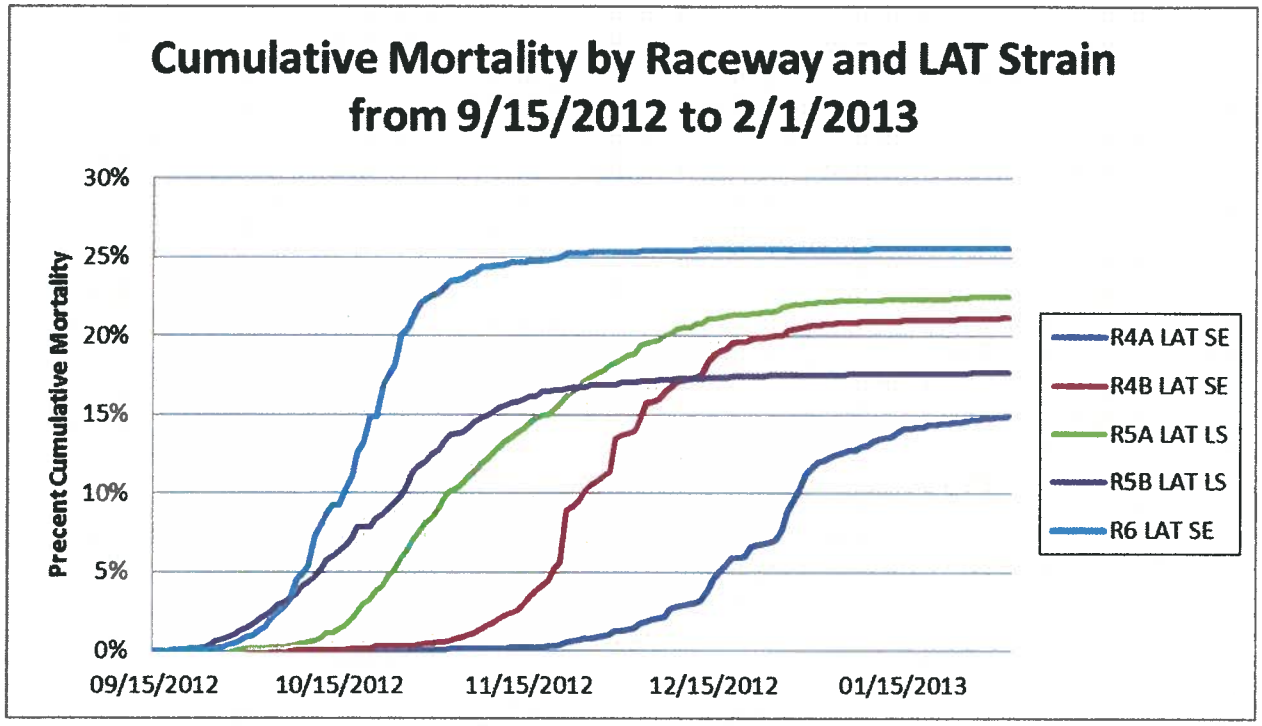
In 1988, the Michigan Department of Natural Resources (MDNR) decided to fully depopulate and disinfect MSFH and to disinfect by chlorination (20 mg/l chlorine) the Cherry Creek watershed upstream of the hatchery through and including the outdoor raceways on June 15, 1989. In May 1988, a total of 232,423 yearling lake trout weighing 5,014 kg (mean size of 46 fish/kg) from MSFH were buried with 1,000 lbs. of quick lime (John Driver, Interoffice Communication May 9, 1988). Similarly in April 1989, 431,500 yearling splake weighing 17,400 kg (mean size of 24.8 fish/kg) and 703,000 fingerling lake trout weighing 10,800 kg (mean size 65.1 fish/kg) were buried in the K&W Landfill in Ontonagon County, Michigan (John Driver, Interoffice Communication April 10, 1989). The outbreak in the 1980s at MSFH has been speculated to be related to high gas supersaturation issues, drought conditions, and likely excessively high rearing densities. The combination of these factors could have created excessive stress that may have triggered the 1980s EEDv epizootic at MSFH.

The most recent prior detection of EEDv in the Great Lakes Basin was from Wisconsin DNR's Bayfield State Fish Hatchery in 2003 and were from Lake Superior Apostle Island stocks. There were also possible detections of EEDv in Wyoming waters and hatcheries in 2009 (Steven Sharon, Wyoming Game and Fish Department, Personal Communication). The current opinion among fish health professionals in the US is that EEDv is likely to be an endemic virus to the Great Lakes Basin.

MSFH 2012 Disease Chronology to Date

Initial and unusual increases in lake trout mortality were noted in the period from September 15-20 and mortalities continued to increase through November and December with one raceway series showing increases through early January. Mortalities in all five production lake trout raceways have leveled off and are unchanged for some time. Observations of the fish have also indicated that lesions are healing and fish are recovering from the infection. No other increased mortalities were documented for any of the broodstock or production lots. Figure 1 displays percent mortality by raceway and strain.

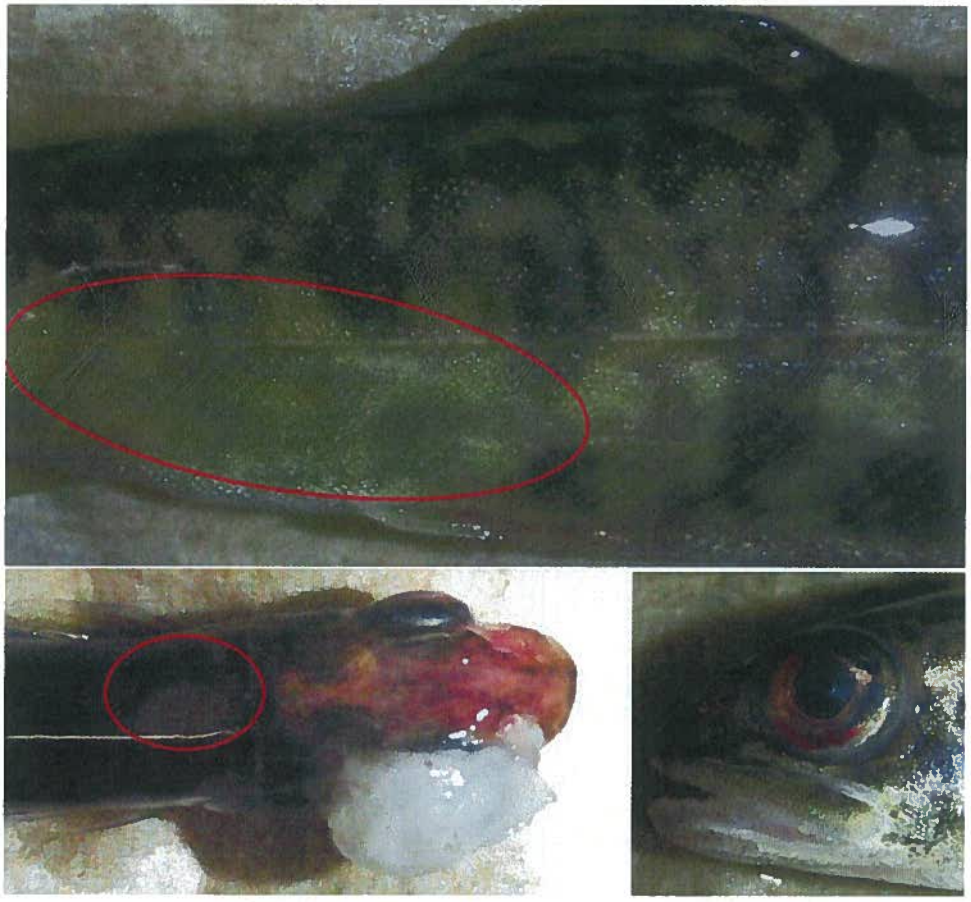
Figure 1. Cumulative percent mortality of lake trout at MSFH from September 15, 2012 to January 15, 2013.



Initially, it was thought that the two MSFH production lots (P-LAT-LS-D-11-MA, P-LAT-SE-D-11-HI-MA) that demonstrated prolonged mortalities had a Bacterial Gill Disease (BGD) or Coldwater Disease (CWD) as they exhibited typical CWD symptoms such as ulceration, secondary fungal infections, and the presence of large levels of *Flavobacterium psychrophilum*. In September, MSFH Staff observed that lake trout in Unit RW5b had what looked like BGD with fish near the surface, stressed, and "jumpy" with staff movement. Fish were sampled from this unit, gills checked and some bacterial presence was confirmed upon examination. A study request was submitted to treat these raceways for the BGD, under the Chloramine-T Investigational New Animal Drug (INAD) program. After a study number was assigned, the raceway was subsequently treated with Chloramine-T on 9/18/2012 and 9/24-26/2012. Fish were sampled from RW5A (LAT-LS), RW5B (LAT-LS), and RW6 (LAT-SE) and sent to Michigan State University-Aquatic Animal Health Laboratory (MSU-AAHL) for analysis on 9/26/2012. MSU-AAHL reported that CWD disease was consistent with the pathology and *Flavobacterium psychrophilum* bacteria were found and the fungal infection was likely a secondary issue. A treatment of Aquaflor was recommended by MSU-AAHL and rearing units RW5A (LAT-LS), RW5B (LAT-LS), and RW6 (LAT-SE) were treated with Aquaflor on 10/08-19/2012 and 10/29-11/4/2012 with little or no effect. Units R4A and R4B (LAT-SE) were treated with Aquaflor on 11/5- 11/15/2012 with no effect. MSFH staff increased biosecurity measures, picked all mortalities at least daily, and increased raceway cleaning.

September samples were analyzed and no viruses could be isolated by early November. MSU-AAHL staff noticed dermal changes such as pale patches, hemorrhages in the eye, and patches of fungal infections and suspected EEDv was the causative agent (Figure 2). Additional samples were sent from MSFH to MSU-AAHL on 11/8/2012, shared with Gavin Glenney (USFWS-Lamar), and were presumptively determined to be EEDv positive on 11/14/2012 by him.

Figure 2. Photos of MSFH lake trout with dermal changes (MSU-AADL Lab Photos).



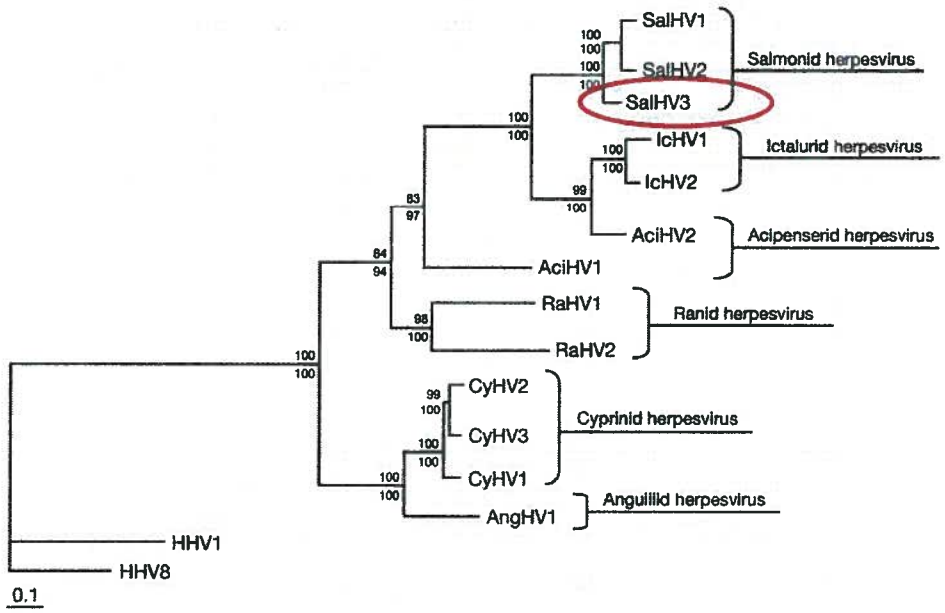
The following 11/14/2012 email message to Dr. Mohamed Faisal summarized Gavin Glenney's findings:

> *"I have finished running your samples with the real-time assay and the first round of a nested assay we use. Both tests indicate your samples are positive for EEDV. I separated out the gills and skins you had pooled and ran those individually. For the real-time assay, I ran 8-gills, 8-skins, and the 8-kid.spleen samples for Case 121108-1 (P-LAT-SE-D-11-HI-MA). I only ran 8-skins and 8-kid/spleens for the real-time assay for Case 121108-2 (P-LAT-LS-D-11-MA) due to space constraints on the 96-well plate. Every sample came up positive for EEDV. Some of your samples were hotter than my plasmid, which was nice to see I did not contaminate the samples. It was odd for me to have such hot EEDV samples, since we are used to barely detecting it in the wild fish we generally look at. I then picked 6 of the hottest samples you gave me and ran the first round of a nested assay we have. All these samples were positive for the first round band of 324bp (photo attached). This assay uses a different primer set than the real-time assay, so these samples are both positive by two different primer sets for different location on the same gene. Both of these assays have been*

tested and have shown negative results for both SALHV I and II, and CCV. However, neither of our assays are published and since we can not culture this virus, we usually sequence to make sure it is not anything else. Did you want us to do this, or would you like to do that in your lab to verify? Do you have easy access to sequencing equipment? We have to schedule a time to use a sequencer in the genetics lab here at the North East Fishery Center, and sometimes it takes a while till the machine if free. Now you know where to get plenty of positive tissues. "

To confirm this finding, MSU-AAHL sequenced EEDv from the same samples on 11/27/2012. The calculated phylogenetic dendogram (Figure 3) based on this sequencing showed almost 100% identity between MSFH isolates and previous samples from Wisconsin. Sequencing also showed substantial differences with other viruses in the same family which ruled out other potential viruses using data in part from Waltzek et al. (2009).

Figure 3. EEDv phylogenetic dendogram based on Waltzek et al. (2009).



MSU-AAHL has also analyzed fish from Lake Superior strain lake trout that originated from the donor broodstock lake trout that founded the current captive broodstock at MSFH. These fish were brought into the MSU-AAHL Quarantine Facility for fish health clearing and are progeny of 2003-2004 fish from Marquette Harbor MI area reefs. No EEDv have been detected in these fish which indicates that this broodstock did not carry EEDv.

We have examined the available length-weight information for these lake trout lots and overall, they continue to grow at or above expected values.

Some unusual abiotic conditions have been noted at MSFH including a severe long-term drought with likely higher than normal water temperatures and a number of intense precipitation events at about the time of the start of the mortalities which greatly increased sediment loads into the

production fish raceways. Additional analysis will be done on these and other abiotic factors to see if they could be a trigger for outbreaks.

Next Steps

The MDNR is treating this epizootic as we would any other restricted disease outbreak. Our strategy is to determine where it is in the hatchery and the prevalence in positive rearing units along with changes in these rates over time. This information will used to decide the disposition of these fish. Initial position is to use a similar control strategy as we do for Bacterial Kidney Disease (BKD) and not stock fish in excess of 10-15% prevalence in Great Lakes waters, although we may use these fish in inland waters. We will also take into consideration the titer levels of the virus as part of the stocking decision.

To obtain the needed information, MDNR and MSU-AAHL developed a tiered sampling strategy to fully delineate the positive parts of the hatchery and to begin the trace back process on the origin of the virus (Appendix 1). The first tier of this strategy was implemented by MSU-AAHL and MDNR staff and non-lethally sampled broodstock and lethally sampled production fish during the week of December 18, 2012. The following samples were collected that week:

Marquette State Fish Hatchery EEDv Sampling
12/2012

Unit	Sex	Species	Lot Year	Lot Size	Likely Positive	Purpose	Sample Size	Pool Size	Total Pools	Lethal/ Nonlethal
BT-3		BKT		1,058	Unknown	P/A	60	5	12	L
BT-4		BKT		1,394	Unknown	P/A	60	5	12	L
R-01A		BKT		13,384	Unknown	P/A	60	5	12	L
R-01B		BKT		10,648	Unknown	P/A	60	5	12	L
R-02A		BKT		12,016	Unknown	P/A	60	5	12	L
R-02B		BKT		12,256	Unknown	P/A	60	5	12	L
R-03A		BKT		17,530	Unknown	P/A	60	5	12	L
R-03B		BKT		16,882	Unknown	P/A	60	5	12	L
R-04A		LAT-SE		123,747	Yes	Prevalence	60	1	60	L
R-04B		LAT-SE		111,293	Yes	Prevalence	60	1	60	L
R-05A		LAT-LS		69,800	Yes	Prevalence	60	1	60	L
R-05B		LAT-LS		58,883	Yes	Prevalence	60	1	60	L
R-06		LAT-SE		41,396	Yes	Prevalence	60	1	60	L
R-08		SPL		60,062	Unknown	P/A	60	5	12	L
R-09		SPL		66,694	Unknown	P/A	60	5	12	L
R-10		SPL		63,191	Unknown	P/A	60	5	12	L
R-11		SPL		62,644	Unknown	P/A	60	5	12	L
R-16A	F	BKT	2010	301	Unknown	P/A	30	5	6	N
R-16B	M	BKT	2010	301	Unknown	P/A	30			
R-17A		BKT		489	Unknown	P/A	60	5	12	N
R-17B	F	BKT	2009	87	Unknown	P/A	30			
R-20A	MIX	LAT-LS	2009	419	Unknown	P/A	30	5	6	N
R-20B	MIX	LAT-LS	2004	201	Unknown	P/A	20			
R-21	F	LAT-LS	2003	150	Unknown	P/A	60	5	12	N

R-22	MIX	LAT-LS	2009	420	Unknown	P/A	20	5	4	N
R-22B	M	LAT-LS	2004	264	Unknown	P/A	20			
R-23	F	LAT-LS	2003	149	Unknown	P/A	60	5	12	N
R-24	M	LAT-LS	2003	125	Unknown	P/A	30	5	6	N
R-25	F	LAT-LS	2001	99	Unknown	P/A	30	5	6	N
R-26	M	LAT-LS	2001	124	Unknown	P/A	30	5	6	N
R-27	F	LAT-LS	2001	100	Unknown	P/A	30	5	6	N
Federal Broodstock		LAT-SE					120	5	24	

Sampling will continue at MSFH as outlined in the sampling plan in Appendix 1.

Test Results

During the week of December 17, a total of 1620 lake trout, splake and brook trout were collected and submitted for EEDv analysis to the MSU AAHL. A summary of the test results received from Dr. Mohamed Faisal through February 1, 2013 are detailed in Appendix 2

Brook Trout. A total of 24 5-fish pools of brook trout from 2 broodstock rearing units and 52 5-fish pools from 6 production rearing units were tested with qPCR. All tests were negative for brook trout. No additional sampling or testing will be done at this time for brook trout.

Splake. A total of 12 5-fish pools of splake from 4 rearing units were tested with qPCR and initially 2 of the rearing units (3 of 4 pools) were initially positive for EEDv. Retesting of positive pools and all remaining pools are found to be negative. No additional sampling or testing will be done at this time for splake.

Lake Trout. A total of 48 5-fish pools of broodstock lake trout from 10 rearing units were tested with qPCR. Fish from 2 rearing units (RU 22 and 22B – Same physical unit) of broodstock lake trout were positive for EEDv (3 of 4 pools). Additional sampling and testing will be conducted to determine prevalence and intensity.

All 5 rearing units of production lake trout were positive for EEDv. Prevalence for LAT-SE ranged were 58% and 78% for the two raceways with completed samples. The majority of the LAT-SE samples demonstrated low or very low titers with individual samples from one raceway to be completed. Prevalence for LAT-LS ranged were 10% and 97% for the two raceways sampled. Similar to LAT-SE, LAT-LS titers were low or very low for individual samples. There is also some evidence that titer levels are declining with recovery of the fish.

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Appendix 1. Marquette State Fish Hatchery EEDv Sampling Strategy - December 13, 2012

First Round of Testing

- Objectives
  - Determine locations of pathogen in hatchery and system including Cherry Creek
  - Determine prevalence in known and presumed infected rearing units
- Test – Samples are arranged in testing order
  - Sample and test non-symptomatic fish randomly in uninfected lots for presence/absence
    - Use sample size of 60 fish in each rearing unit with lots of 5 fish (12 samples)
    - For broodstocks, randomly and non-lethally sample raceways with combined samples where raceways are shared. Use sample size of 60 fish in each raceway with lots of 5 fish (12 samples). We will need Tom Loch's assistance with this sampling.
  - Sample and test non-symptomatic fish randomly in known infected LAT rearing units for prevalence
    - Individually sample fish with a total sample size of 60 fish per rearing unit. Fish should not be pooled.
  - Sample and test LAT-SE federal broodstocks that were source of LAT-SE in production for presence/absence
    - Determine contributing lots of federal fish to fish reared at MSFH.
    - If tissue samples are available from the LaCrosse National Fish Health Laboratory, test at least 60 fish from each lot using pooled samples of 5 fish (12 samples).
    - If tissue samples are not available, randomly and non-lethally sample raceways at the contributing federal hatcheries with combined samples where raceways are shared. Use sample size of 60 fish in each raceway with lots of 5 fish (12 samples).
- Laboratory Methodology
  - qPCR and confirm all positive samples with sequencing or other agreed upon method

Second Round of Testing

- Objectives
  - Determine prevalence in known infected rearing units
  - Determine pathogen presence/absence in Cherry Creek
  - Develop data to begin to determine pathogen reservoirs and re-emergence
- Test– Samples are arranged in testing order
  - Sample and test fish randomly in infected rearing units from Round 1 for prevalence
    - Individually sample non-symptomatic fish with a total sample size of 60 fish per rearing unit

    - Test archived samples of past production lots of LAT-LS and LAT-SE to determine history of presence/absence of EEDV using 60 fish samples pooled into 5 fish pools of each lot with available tissue.
    - If LAT broodstocks are found to be positive in Round 1
        - Test archived broodstock samples back four years at a time until negative for two consecutive years.
        - Use 60 fish samples pooled into 5 fish pools of each lot with available tissue.
    - Test archived Cherry Creek samples from last three years for presence/absence
        - Use sample size of 60 fish for each species with lots of 5 fish (12 samples max per species)
- Laboratory Methodology
    - qPCR and confirm all positive samples with sequencing or other agreed upon method

Third Round of Testing

- Objectives
    - Determine prevalence of EEDv in past production lots of LAT-LS and LAT-SE
    - Determine of pathogen prevalence in Cherry Creek
- Testing
    - Test archived LAT-LS and LAT-SE for lots for which tissue is available using 60 fish samples for each lot.
    - If Cherry Creek is found to be positive in Round 2
        - Test all available archived samples individually
        - Collect and test new samples above and below hatchery with samples sizes of 60 fish per species
- Laboratory Methodology
    - qPCR and confirm all positive samples with sequencing or other agreed upon method

Appendix 2. Initial EEDV test results for Marquette State Fish Hatchery by Rearing Unit as of February 1, 2013

Production Brook Trout (tissues included pools of gills, kidneys, and spleens):

BT-3 (12 pools of 5 fish) – 0/12 positive by qPCR

BT- 4 (12 pools of 5 fish) - 0/12 positive by qPCR

01A (12 pools of 5 fish)- 0/12 positive by qPCR

01B (12 pools of 5 fish) - 0/12 positive by qPCR

02A (12 pools of 5 fish) - 0/12 positive by qPCR

02B (12 pools of 5 fish) - 0/12 positive by qPCR

03A (12 pools of 5 fish) – 0/12 positive by qPCR

03B (12 pools of 5 fish) – 0/12 positive by qPCR

Production Splake (tissues included pools of gills, kidneys, and spleens):

08 (12 pools of 5 fish) – 0/12 positive by qPCR

09 (12 pools of 5 fish)- 0/12 positive by qPCR

10 (12 pools of 5 fish)- 0/12 positive by qPCR

11 (12 pools of 5 fish)- 0/12 positive by qPCR

Brook Trout Broodstock (tissues included mucus samples collected from the body and within the opercula of fish):

16A (B-BKT, 4 pools of 5 fish)- 0/4 positive by qPCR

16B (B-BKT, 4 pools of 5 fish)- 0/4 positive by qPCR

17A (B-BKT, 4 pools of 5 fish)- 0/4 positive by qPCR

Lake Trout Broodstock (LS strain) (tissues included mucus samples collected from the body and within the opercula of fish):

20A (B-LAT-LS, 4 pools of 5 fish)- 0/4 positive by qPCR

20B (B-LAT-LS, 4 pools of 5 fish)- 0/4 positive by qPCR

21 (B-LAT-LS, 4 pools of 5 fish)- 0/4 positive by qPCR

22 (B-LAT-LS, 4 pools of 5 fish)- 2/4 positive by qPCR

22B (B-LAT-LS, 4 pools of 5 fish)- 1/4 positive by qPCR

23 (B-LAT-LS, 4 pools of 5 fish)- 0/4 positive by qPCR

24 (B-LAT-LS, 6 pools of 5 fish)- 0/6 positive by qPCR

25 (B-LAT-LS, 6 pools of 5 fish)- 0/6 positive by qPCR

26 (B-LAT-LS, 6 pools of 5 fish)- 0/6 positive by qPCR

27 (B-LAT-LS, 6 pools of 5 fish)- 0/6 positive by qPCR

Production Lake Trout (LAT-SE) (tissues included gills):

04A- 35/60 individual LAT (K/S, gills) positive - Prevalence = 58.3%

- Titers - 4H-2M-3L-26VL

04B- 4/10 individual LAT (K/S, gills) positive – Individual results pending

06- 47/60 individual LAT (K/S, gills) positive – Prevalence = 78%

- Titers - 1L-46VL

Production Lake Trout (LAT-LS) (tissues included gills):

05A- 6/60 individual LAT (K/S, gills) positive – Prevalence – 10%

- Titers – 1 M, 5 VL

05B- 58/60 individual LAT (K/S, gills) positive – Prevalence – 97%

- Titers - 7L-51VL

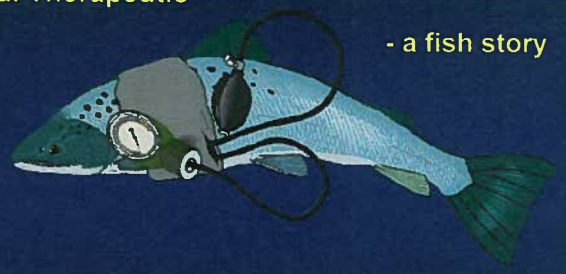
Table 1. MSFH EEDv results through February 1, 2013.

Unit	Species	Sample #s	Pool?	Tissue Type	qPCR	Viral Titers
BT-3	BKT	#1-60	5/p	K/S/G	0/12 pools	
BT-4	BKT	#1-60	5/p	K/S/G	0/12 pools	
R-01A	BKT	#1-60	5/p	K/S/G	0/12 pools	
R-01B	BKT	#1-60	5/p	K/S/G	0/12 pools	
R-02A	BKT	#1-60	5/p	K/S/G	0/12 pools	
R-02B	BKT	#1-60	5/p	K/S/G	0/12 pools	
R-03A	BKT	#1-60	5/p	K/S/G	0/12 pools	
R-03B	BKT	#1-60	5/p	K/S/G	0/12 pools	
R-04A	LAT-SE	#1-60	indiv	K/S; gill	35/60 indiv	4H-2M-3L-26VL
R-04B	LAT-SE	#1-60	indiv	K/S; gill	4/10 indiv	
R-05A	LAT-LS	#1-60	indiv	K/S; gill	6/60 indiv	1M-5VL
R-05B	LAT-LS	#1-60	indiv	K/S; gill	58/60 indiv	7L-51VL
R-06	LAT-SE	#1-60	indiv	K/S; gill	47/60 indiv	1L-46VL
R-08	SPL	#1-60	5/p	K/S/G	0/12 pools	
R-09	SPL	#1-60	5/p	K/S/G	0/12 pools	
R-10	SPL	#1-60	5/p	K/S/G	0/12 pools	
R-11	SPL	#1-60	5/p	K/S/G	0/12 pools	
R-16A	BKT	#1-20	indiv	mucous	0/4 pools	
R-16B	BKT	#1-20	indiv	mucous	0/4 pools	
R-17A	BKT	#1-20	indiv	mucous	0/4 pools	
R-20A	LAT-LS	#1-20	indiv	mucous	0/4 pools	
R-20B	LAT-LS	#1-20	indiv	mucous	0/4 pools	
R-21	LAT-LS	#1-20	indiv	mucous	0/4 pools	
R-22	LAT-LS	#1-20	indiv	mucous	2/4 pools	
R-22B	LAT-LS	#1-20	indiv	mucous	1/4 pools	
R-23	LAT-LS	#1-20	indiv	mucous	0/4 pools	
R-24	LAT-LS	#1-30	indiv	mucous	0/6 pools	
R-25	LAT-LS	#1-30	indiv	mucous	0/6 pools	
R-26	LAT-LS	#1-30	indiv	mucous	0/6 pools	
R-27	LAT-LS	#1-30	indiv	mucous	0/6 pools	

H=>1000 copies
M=medium (>100 copies)
L=low (50-100 copies)
VL=very low (<50copies)

Hydrogen Sulfide – From Aquatic Pollutant to Clinical Therapeutic

- a fish story



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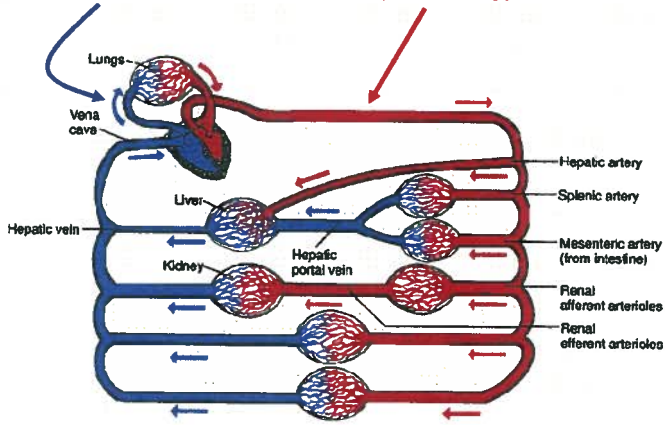
Pulmonary Hypertension vs Systemic Hypertension



Systemic Hypertension: 95% Primary (Cause?)

Models

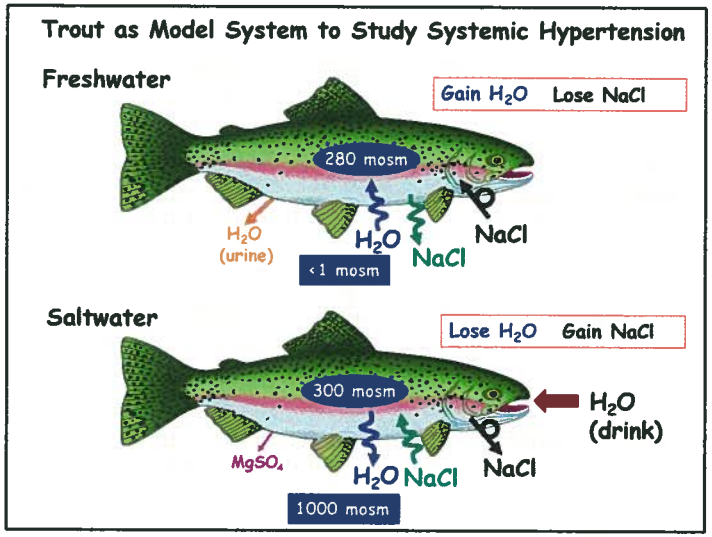
- Humans
- Lab animals (mammals)
  1. Pharmacologically treated
  2. Genetically manipulated
  3. Surgically modified

Pulmonary Hypertension: >80% Secondary (hypoxia)

Mechanism?

Models: mammalian pulmonary blood vessels

Trout as Model System to Study Systemic Hypertension

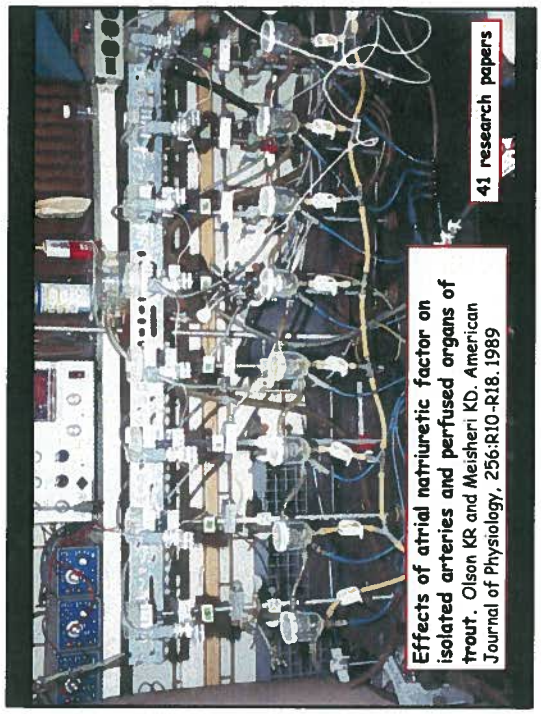


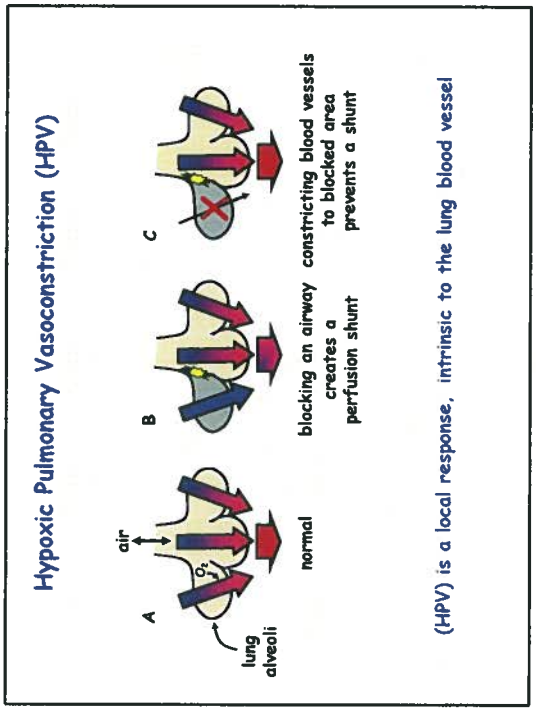
Experimental Paradigms
volume
salt
blood pressure
Saltwater
22 mmHg
Freshwater
24 mmHg
FW-NaCl Diet
26 mmHg

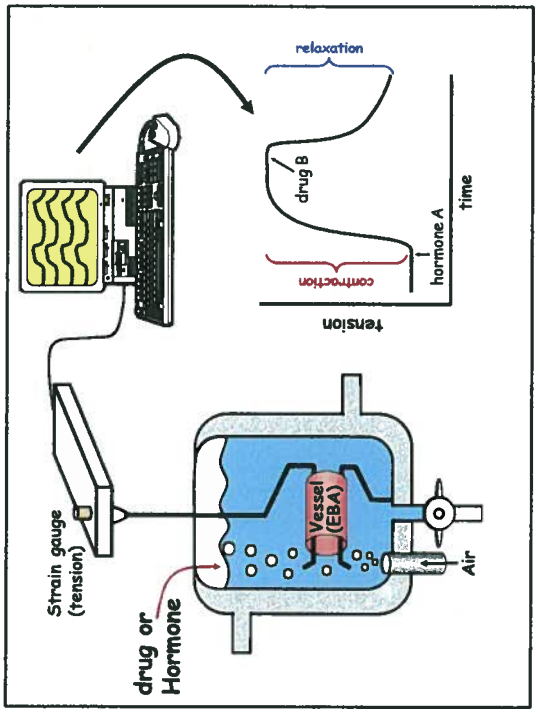
EBA
EBA

In vivo cardiovascular physiology
CO
P_{VA}
P_{VEN}
P_{DA}
urine
CO, cardiac output: P_{VA}, P_{VEN}, P_{DA}: pressures in ventral aorta, central vein (ductus Cuvier) & dorsal aorta

In vitro measurements can be compared to in vivo studies
Efferent branchial artery (EBA)
ductus Cuvier
P_{VEN}
P_{VA} & CO


Effects of atrial natriuretic factor on isolated arteries and perfused organs of trout. Olson KR and Meisheri KD. American Journal of Physiology, 256:R10-R18.1989
41 research papers


Hypoxic Pulmonary Vasoconstriction (HPV)
air
A
B
C
lung alveoli
normal
blocking an airway creates a perfusion shunt
constricting blood vessels to blocked area prevents a shunt
(HPV) is a local response, intrinsic to the lung blood vessel


relaxation
drug B
hormone A
time
contraction
tension
Strain gauge (tension)
Vessel (EBA)
Air
drug or Hormone

However, if Po_2 low in all alveoli...

Global Hypoxic Pulmonary Vasoconstriction

leads to Pulmonary Hypertension

1. High altitude
2. Ventilatory problems (obesity hypoventilation syndrome, emphysema, diffuse chronic obstructive disease (COPD))
3. Sleep apnea

Because the mechanism of O_2 sensing is unknown – treatment is difficult

Hydrogen sulfide as a biological signal

H_2S

The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. Hosoki et al., BBRC, 237:527-531, (1997)

Am J Physiol Regul Integr Comp Physiol 266: R678–R685, 2004;
10.1152/ajpregu.00419.2003.

Hydrogen sulfide as an endogenous regulator of vascular smooth muscle tone in trout

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Submitted 25 July 2003; accepted in final form 15 December 2003

Olson et al., Am.J.Physiol. 280: R198, 2001

O_2-Dependent H_2S Metabolism and O_2 Sensing

In Normoxia:

$$2RS^- \xrightarrow[\text{CAT/ 3-MST}]{\text{CBS CSE}} 2H_2S \xrightarrow{O_2} S_2O_3^{2-} \xrightarrow{O_2} SO_4^{2-}$$

In Hypoxia:

$$2RS^- \xrightarrow[\text{CAT/ 3-MST}]{\text{CBS CSE}} 2H_2S \xrightarrow{O_2} S_2O_3^{2-} \xrightarrow{O_2} SO_4^{2-}$$

(H_2S shown above $2H_2S$ and $S_2O_3^{2-}$)

Oldest known cells (3.4 bya) were sulfur-metabolizing
(Wacey et al. Nature Geoscience 4: 698, 2011)

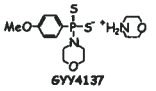
Aquatic hypoxia is also...
1. Seasonal: ice, drought, temperature
2. Periodic: phytoplankton blooms, vulcanic, wind
3. Diurnal: nocturnal respiration
4. Tidal
5. Even in utero in mammals
Kiel Fjord, September 1882
% O2 (100% = nitrogen saturation)
Day of Month
%O2 sat
H2S increases in...
1. Hypoxia
2. Pollution

H2S - Oxygen and Origin of Eukaryotes & Vertebrates
Sarcopterygii (lungfish)
Polypterniformes (reedfish)
Holostreans (bowfin, gar)
Teleosts (bony fish)
amphibians
reptiles
birds
mammals
first eukaryotes
1.5 bya in <2% O2
Euxinic oceans
H2S
O2
90% ocean
70% land
C O S D C P Tr J K T
Percent O2 in Atmosphere
Millions of Years
2000 1000 600 400 200 P
* Mass extinctions (WordSci. Am. 2006)

Wikimedia Commons

H_2S "donating" compounds

NaHS Na_2S



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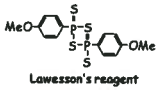
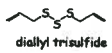
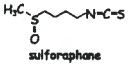


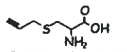
Lawesson's reagent



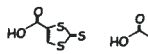
diallyl trisulfide



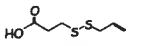
sulforaphane



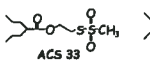
S-propargyl cysteine



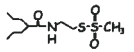
ACS5



ACS81



ACS 33



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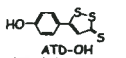
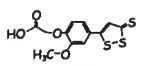
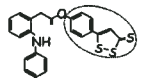


ATD-OH
(anethole trithione)



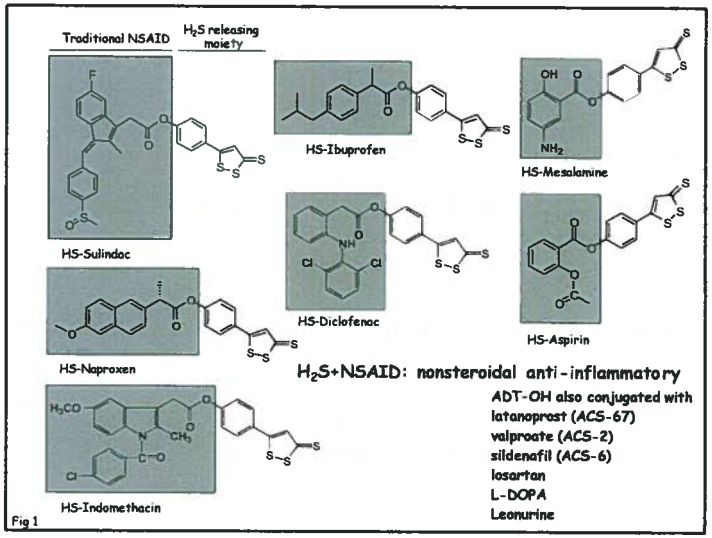
ACS50



S-diclofenac; ATB-337, ACS 15

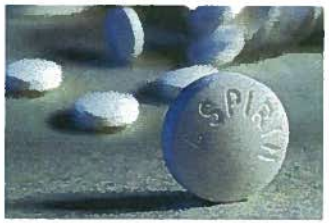
ATB: Antibe Therapeutics
ACS: CTG Pharma

ATB-429 (mescalamine) Inflammatory Bowel Disease (IBD): first stage clinical trials
ATB-284 (?) Irritable Bowel Syndrome (IBS): pre-clinical
ATB-346 (?) joint pain pre-clinical

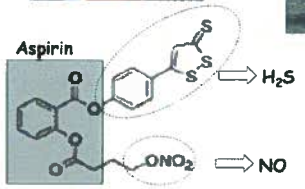


Khosrow Kashfi

City University of New York Medical School

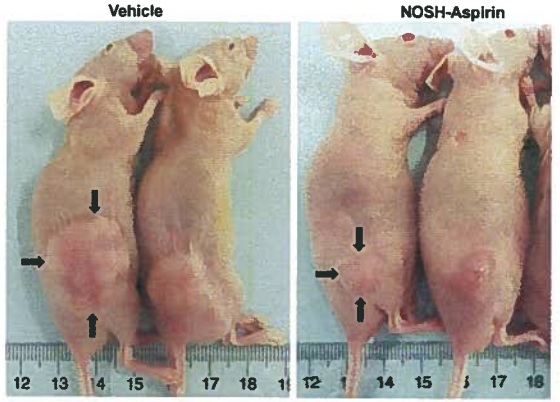


"Iranian scientist creates gas-filled aspirin to fight cancer"
New York Times



NOSH-1
NOSH-aspirin

NOSH-ASA inhibits tumor growth in athymic mice injected with HT-29 cells



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John Angus Erskine Fund
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Contaminant Transport to Great Lakes Tributaries by Pacific salmon

D.T. Chaloner[1], D.J. Janetski[2], A.H. Moerke[3], R.R. Rediske[2], J.P. O'Keefe[2], and G.A. Lamberti[1]

[1]Dept. of Biological Sciences, University of Notre Dame
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UNIVERSITY OF NOTRE DAME

GRAND VALLEY STATE UNIVERSITY

LAKE SUPERIOR STATE UNIVERSITY

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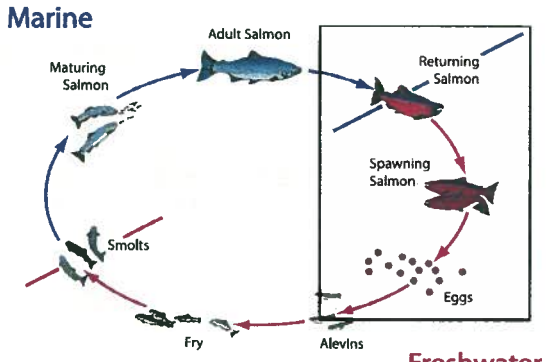
- **Funding** – Great Lakes Fishery Trust, Arthur J. Schmitt Foundation, University of Notre Dame Center for Aquatic Conservation

Great Lakes Fishery Trust

Pacific Salmon Lifecycle



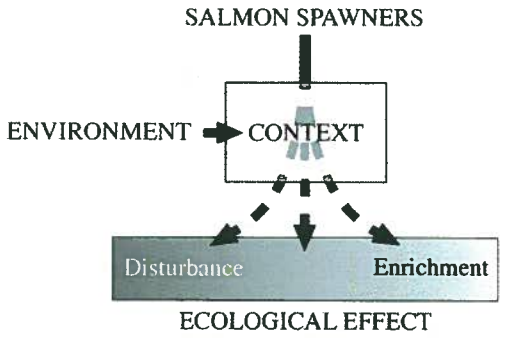
- Anadromous
- Semelparous

Ecological Effects of Pacific Salmon Spawners

Resource subsidies (enrichment)

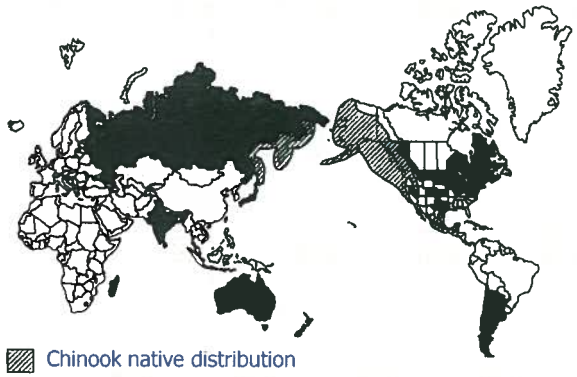
Ecosystem engineers (disturbance)

Role of Environmental Context



Modified from Janetski et al. (2009) *Oecologia*

Worldwide Introduction of Pacific Salmon



Chinook native distribution

Chinook introductions

Source: Crawford and Muir (2008) *Rev. Fish. Biol. Fisheries*

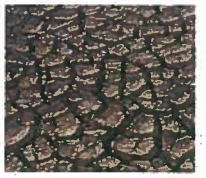
Great Lakes Environmental Issues

Invasive species

Resource use

Land use changes

Pollution



Climate change

The Great Lakes' Toxic Legacy

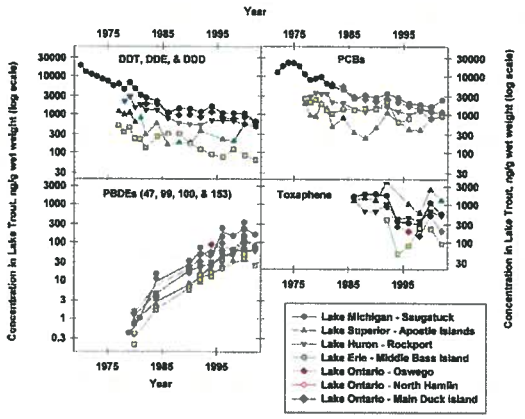
Great Lakes fish contain **organic** and **heavy metal** contaminants at reduced levels

http://www.saferchemicals.org/resources/great-lakes.html

Persistent Organic Pollutants

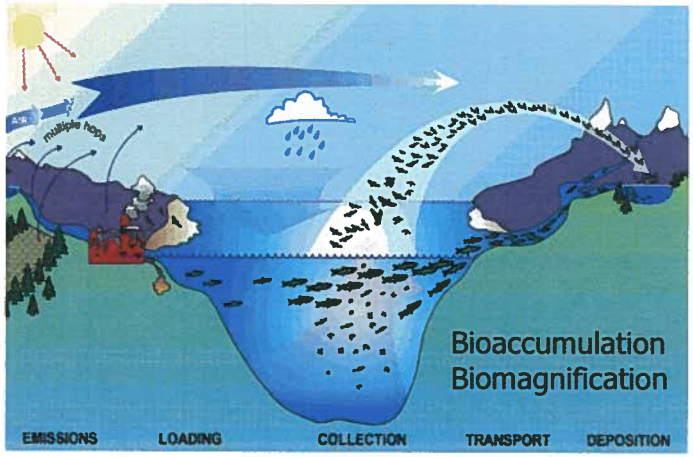
- Persistent organic pollutants (**POPs**) are synthetic organic compounds resistant to breakdown
- Polychlorinated biphenyl (**PCBs**)
  - Fluid in transformers and capacitors
  - Banned 1979
- Polybrominated diphenyl ether (**PBDEs**)
  - Flame retardents
  - Banned in some states
- Dichloro diphenyldichloroethylene (**DDEs**)
  - Degradation product of DDT
  - Regulated since 1972

Great Lakes Organic Contaminants



Source Carlson et al. (2010) *Env. Sci. Tech.*

Contaminant Biotransport



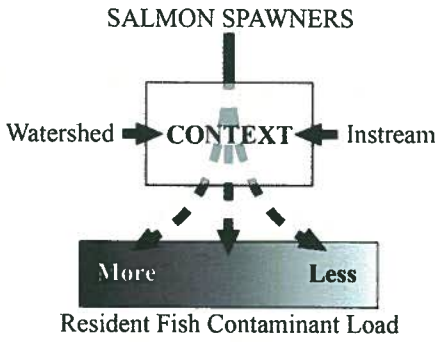
Source Blais et al. (2007) *Env. Sci. Tech.*

Contaminant Biotransport by Fish

- Fish are known to transport **organic** (e.g., PCBs) and **metal** (e.g., mercury) anthropogenic contaminants

- Less known about **salmon** biotransport in **Great Lakes**:
  - extent of contaminant transfer to resident fish
  e.g., are some fish more impacted than others?
  - predominant mechanisms of contaminant transfer
  e.g., are direct more important than indirect pathways?
  - role of environmental context in magnifying or mitigating contaminant transfer
  e.g., is contaminant transfer more pronounced under specific environmental circumstances?

Contaminant Transfer between Fish



Modified from Janetski et al. (2009) *Oecologia*

Overall Objectives and Hypotheses

- Determine the extent of POP transfer from salmon spawners to stream resident fish

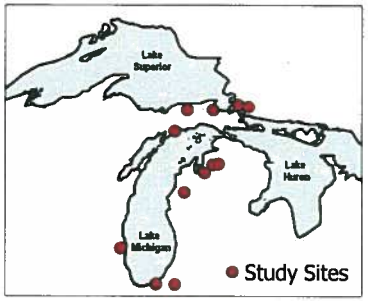
  POP load in stream resident fish determined by presence of salmon spawners

- Establish role of environmental context in POP transfer from spawners to stream resident fish

  Environmental context determines magnitude of salmon-derived POP load of stream resident fish

Study Design

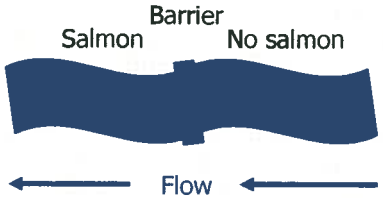
- Study sites:
  - tributaries of upper Great Lakes
  - presence of Chinook and/or coho salmon
  - Salmon migration barrier (e.g., dams)
  - Similar characteristics (e.g., discharge)



- Salmon spawners (Chinook, coho) and stream resident fish (brook trout, dace, sculpin) sampled for analysis

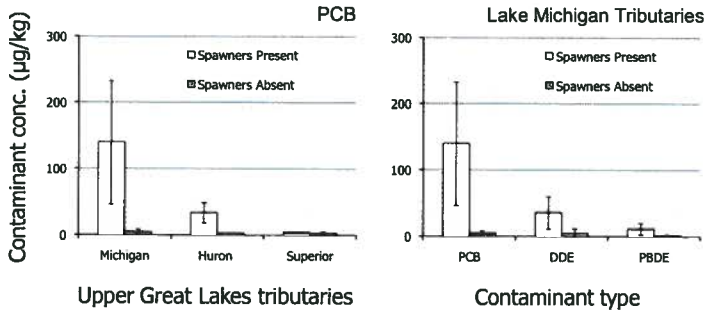
Sampling Design

- Sampling design for **stream resident fish**:
  - **before** and **during** salmon runs
  - **upstream** and **downstream** of migration barrier



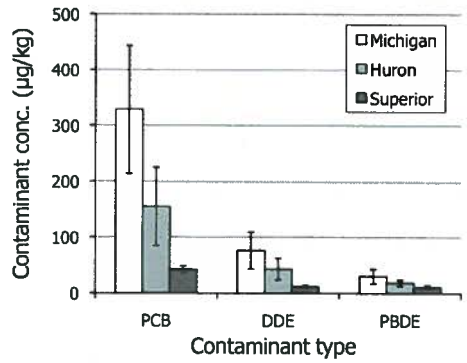
- Whole fish (minus eggs) ground and analyzed for **major** POPs including PCBs, PDBEs, and DDEs

Organic Pollutants in Resident Fish: Contrasts among Lakes & Contaminants



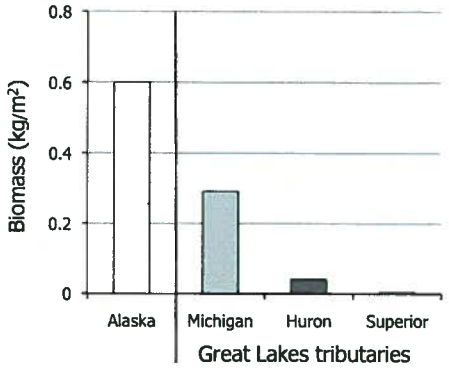
- POP load **higher** in presence of salmon spawners
- POP load in resident fish **differs** among tributaries
- Organic pollutants **differ** in the magnitude of increase

Organic Pollutants in Salmon Spawners



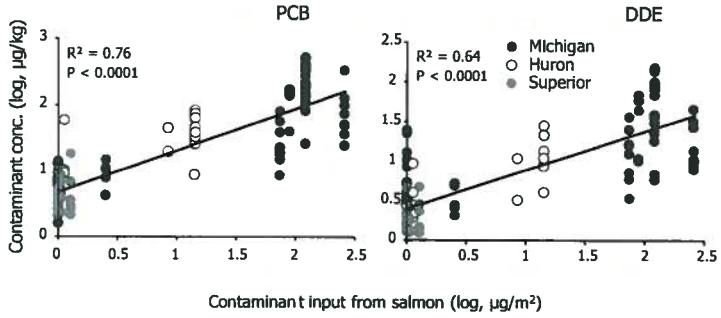
- Salmon POP load **differs** among the upper Great Lakes
- Organic pollutants **differ** in prevalence in salmon

Salmon Spawner Biomass



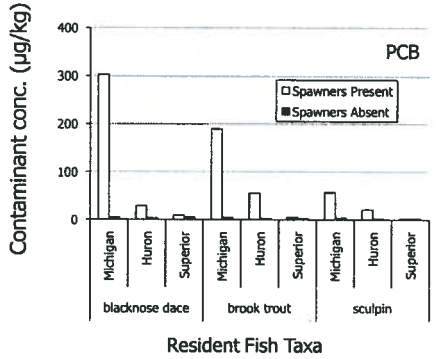
- Salmon spawner biomass **less** than in Alaska
- Biomass **variable** across upper Great Lakes tributaries

Relationship between Salmon Spawner and Resident Fish Contaminant Load



- Contaminant input **predicts** contaminant load of resident fish
- Similar relationship for **all** POPs measured in resident fish

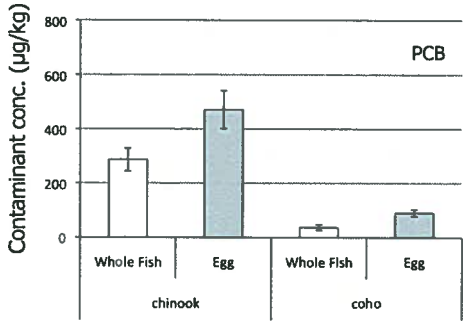
Organic Pollutants in Resident Fish: Contrasts Among Taxa



- Resident fish taxa **differ** in their POP load

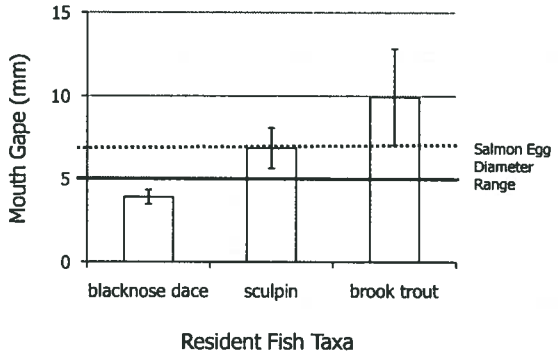
Pathways of POP Transfer

Organic Pollutants in Tissue and Eggs



- Salmon eggs appear **more** replete with PCBs

Mouth Gape of Resident Fish



- Brook trout and sculpin **likely** consume salmon eggs
- Blacknose dace **unlikely** to consume salmon eggs

Broader Context: Wildlife and Human Health

- Salmon and stream resident fish are consumed by both wildlife and humans
- **Consumption advisories** established by state health agencies (e.g., MI Dept of Community Health)
- **No Observable Adverse Effect Concentration** (NOAEC) used to establish safe contaminants concentrations, primarily for wildlife of concern

Human Fish Consumption Advisories

Basin	Salmon	Stream-resident fish	
		With salmon	No salmon
	(µg/kg tissue wet weight)		
Lake Michigan	**328**	**139**	5
Lake Huron	**154**	34	3
Lake Superior	36	4	3

One meal per week advisory for **women and children** is triggered by >50 µg/kg (Michigan Dept of Community Health)

Lake Michigan fish likely to trigger advisories

No Observable Adverse Effect Concentration (NOAEC)

Species	With salmon	No salmon
Brook trout	**172**	4
Dace	**303**	7
Sculpin	55	4

Dietary NOAEC for **bald eagles** is <143 µg/kg (Giesy et al. 1995) and for **mink** is <72 µg/kg (Giesy et al. 1994)

Stream resident fish exceed NOAEC wildlife advisories

Conclusions

- Pacific salmon are important **vector** of POPs to Great Lakes tributaries where they spawn
- **Multiple types** of POPs are likely involved, both already **established** and **emerging**
- **Transfer of POPs** from salmon to resident fish probably involves many **direct** and **indirect** pathways
- **Environmental context** may be involved by virtue of salmon biology or local conditions, and deserves further attention

Implications

- **Stocking** of Pacific salmon in Great Lakes
- Other **existing** (e.g., mercury) and **emerging** pollutants (e.g., pharmaceuticals)
- **Restoration** of ecosystem function and connectivity (e.g., dam removal)
- Native species **conservation** (e.g., brook trout)



Future Work

- **Expand** dataset to include heavy metals, especially **mercury**, and **more** sites
- Establish **pathway** of contaminant flow
- Explore role of **landscape features**
  - Land-use (e.g., urban versus forest)
  - Geomorphic features (e.g., large wood)
- Develop **predictive model** for managers
  - **mitigate** contaminant transfer to resident fish
  - **manage** the impacts of dam removal

GL Fish Health Center

- Emerging pathogens
- History and sequence of events not well documented
- No ability to respond to an emerging problem
- Interrupted wildfish surveys
- No evidence-based preventive measures
- Inconsistency in lab testing

- No resources
- Expertise and resources are not universal in all GL states
- Retirement
- No political will
- SEVERE LACK OF KNOWLEDGE

Collaboration

- Networking
- Consortium
- Center with an Executive Board

From idea to reality

1. Declare as a priority need
2. Find a hub at a place where there is a high likelihood of success
3. Seek seed money: from the host, grant funding agencies, and member agencies
4. Hire a secretary and seek more money
5. Hire a fixed term associate director
6. GLFHC determines satellite laboratories based on needed expertise

Center and satellites

Sources of funding

- Center grants (NOAA, EPA, NIH, NSF, DOE, USDA)
- Training grants (T38)
- NGOs
- IDCs

Expenditures

- Core activities in all centers:
  - Core funding

What would a center do that we currently can not?

- Design long term strategies
- Bring unique expertise together
- Proper training for fish health professionals thereby creating a cadre of specialists
- Brainstorming
- Archive of materials and decisions

What is needed now?

- Two lines that this is a priority!

Indiana State Board of Animal Health

"Office of the State Veterinarian"

Jennifer Strasser, DVM
District 1 Field Veterinarian
Aquaculture Director
jstrasser@boah.in.gov
(574) 274-3244

State Board of Animal Health's Charge

"the prevention, detection, control and eradication of infectious, contagious and communicable diseases affecting the health of animals… and processing and distribution of products derived from animals to control health hazards that may threaten public health"

IC 15-2.1-3.11

Guiding Principles

- **Access**
  - To information, resources, answers
- **Expertise**
  - Professionals in touch with constituent issues
- **Outreach**
  - Education, awareness, training
- **Representation**
  - Constituent input on specific issues
- **Responsiveness**
  - Ability to address situations quickly, with priority

State Board of Animal Health

Primary Missions:

- **Animal Health**
  - Pseudorabies, tuberculosis, scrapie, rabies
- **Food Safety**
  - Meat & Poultry, Dairy Inspection
- **Emergency Preparedness**
  - SAVE, Animal Issues in Disaster, bioterrorism
- **Animal Care**
  - Dog breeders, Livestock standards

Other Animal Agencies

USDA APHIS VS

- imports/exports
- national disease programs
- veterinary accreditation

State Department of Agriculture

- Certified Livestock Producer Program

State Department of Natural Resources

- Indiana's wildlife

State Board of Animal Health

Other lesser-known functions

- Brand registrations
- Renderer licensing
- Dead animal disposal oversight
- Livestock market licensing

Board of Animal Health

The Board

↓

The Agency

BOAH: The Board

11 members

- Appointed by governor
  - Industry nominations
- 4-year terms
  - Up to two consecutive terms

Meets quarterly

- January, April, July, October

BOAH: The Board

General Veterinary Medicine | Purdue Veterinary School | Livestock Markets | General Veterinary Medicine

Poultry | Sheep | Small Animal Veterinarian | Equine | Dairy

Swine | Beef

*No more than 4 members of a single political party

*Political balance must be maintained

BOAH: The Agency

- State Veterinarian is agency head
- 101 staff members
  - Primarily field staff
    - Veterinarians
    - Animal health technicians
    - Meat & poultry, dairy inspectors
  - Administrative/program support

BOAH Field Veterinarians

BOAH Field Veterinarians

- Coordinated multi-agency mapping
  - Indiana Department of Homeland Security
  - Indiana State Department of Health
- BOAH districts coincide with IDHS Districts
  - Assists with disasters and preparedness

Duties of the Field Veterinarians

- Disease monitoring and sampling
- Oversee a program within BOAH
  - Ex. Scrapie, Johne's, Brucellosis
- Serve on local public health boards
- Investigate neglect situations

BOAH: Advisory Committees

Opportunity for industry input

- Directly to Board representative
- Forum for debate early in process
- Avenue for feedback and information exchange

BOAH: Advisory Committees

Swine
Equine
Cervid
Sheep
Poultry (ISPA)
Farm Animal Care
Bovine/Johne's
Dairy Processors
Companion Animal
Livestock Markets
Renderers

Role in Fish Health

Historically no regulations
VHS put aquaculture on radar
coordinating Federal Order reqmts
Training private veterinarians to work with aquaculture producers
Resource for IAAI
Work in concert with DNR

VHS Surveillance

Funding - APHIS cooperative agreement

APHIS – BOAH – DNR – Purdue ADDL

Wild fish only

Separate sampling by producers

VHS Surveillance

DNR personnel collected the fish

VHS Surveillance

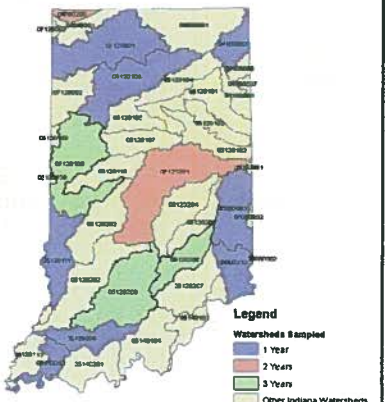
BOAH personnel-
transported fish to ADDL & collected samples

VHS Surveillance

WATERSHEDS –
HUC 8 VHS Testing

2009 through 2012

6 HUC's/ year
170 fish/ site



VHS Surveillance

Spring Sampling
45 – 65 F
Total of 4103 samples
22 different species

ALL NEGATIVE

VHS Surveillance

APHIS funding discontinued

DNR - some waters

BOAH – aquaculture operations

Next Steps

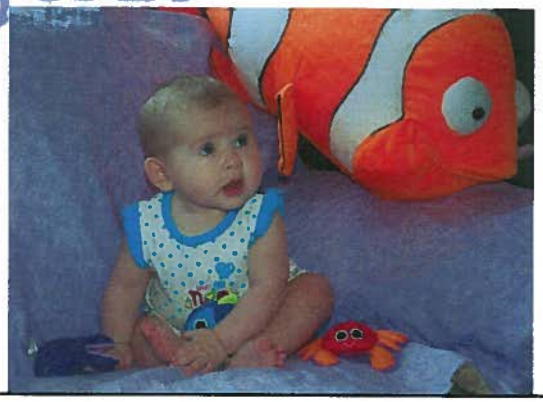
Encourage producers to test

Continue educating vets

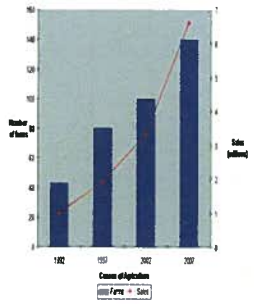
Work with other states to increase uniformity of aquatic regs

Aquaculture advisory committee??

Questions?

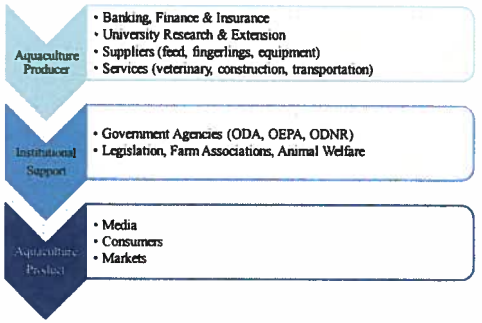


Aquaculture is Growing in Ohio



- 173 farms with aquaculture permits in 2010
- 80% in business less than 10 years
- 87% plan to maintain or expand production
- 66 farms reported
  - 75 full time employees
  - 81 part time employees

Complex Aquaculture Industry



Ohio Aquaculture Plan

Key Task Force Recommendations

- *Aquaculture Health, Food Safety and Marketing*
  - Create a State Aquaculture Coordinator position within the Ohio Department of Agriculture.
  - Provide support for the Ohio Department of Agriculture Aquatic Health Laboratory and Aquatic Health Advisory Committee
- *Aquaculture Business*
  - Develop a revolving loan program to stimulate new entries into the aquaculture industry.
  - Conduct a Feasibility Study for aquaculture in Ohio
  - Develop enterprise budgets for aquaculture facilities in Ohio by collecting "real-world" production data from aquaculture facilities.
- *Aquaculture Education*
  - Continue to promote aquaculture education
    - Hocking College Aquaculture Program
    - Finalize the Ohio State University "Triangle Plan"
    - Provide on-farm site consultation and training to assist growers in the industry.
- *Aquaculture Research*
  - Continue to support funding for applied and basic research addressing identified needs of the industry.

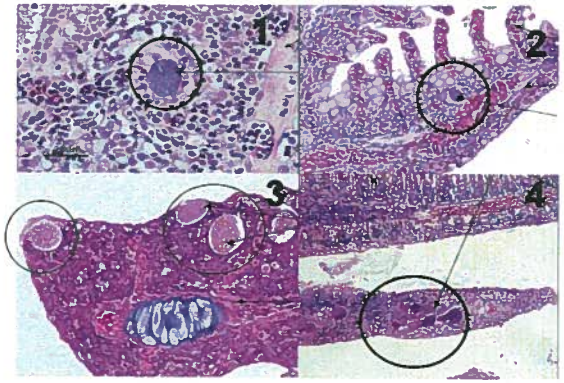
New Ohio Initiatives

- Ohio produced fish feeds – Premier Feeds
- Ohio produced fish meal replacement - Enviroflight
- Increase awareness in financial industry
- Growth in interest in aquaponics
- Leader in Midwest in aquaculture genetics research
- Aquaculture Boot Camp to train new fish farmers

Epitheliocystis in Lake and Rainbow Trout

Contador E, Frasca S, Lillie B, Lumsden JS

Epitheliocystis



Epitheliocystis

- **Epitheliocystis agents**
  - Chlamydia-like organisms (CLO)
    Draghi *et al*, 2004, 2007, 2010
  - Burkholderiales ,
    Toenshoff *et al*, 2012
  - Rickettsia-like organism (RLO)
    Paperna *et al*, 1976, Zachary *et al*, 1977

Inclusions (cysts)

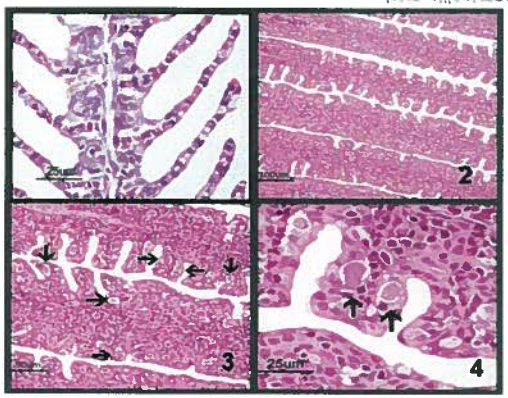
- **Others**
  - Herpes virus
  - Paramyxovirus
  - *Myxovirus-like organisms*

Associated

Problem # 1

- OMNR raises Lake trout for stocking in Ontario
- Lake trout epitheliocystis outbreaks in Dec/Jan/Feb; 6 week course
- Presence of intracytoplasmic granular inclusions in branchial epithelial cells occur during the early stages of disease

Light microscopy



Lesion summary in LT

Outbreak

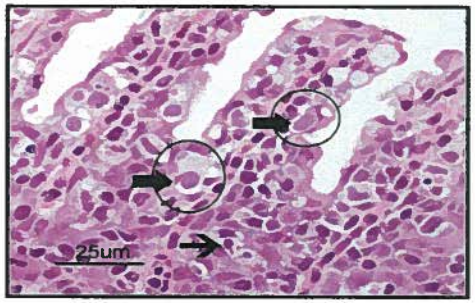
First week
- Intracellular inclusions
- Mild to moderate epithelial cell hypertrophy
- Epithelial proliferation
- Mild to moderate necrosis
- Limited mortality

Third week
- Decreased inclusions
- Increased inflammatory cell influx
- Moderate to severe necrosis
- Increased mortality

Fifth to sixth week
- No inclusions
- Decrease in inflammatory response
- Moderate necrosis
- No mortality

Problem # 2

- Rainbow trout first epitheliocystis outbreak in Ontario was detected in the summer of 2009. In summer of 2010, it was seen more commonly at several different fish farms



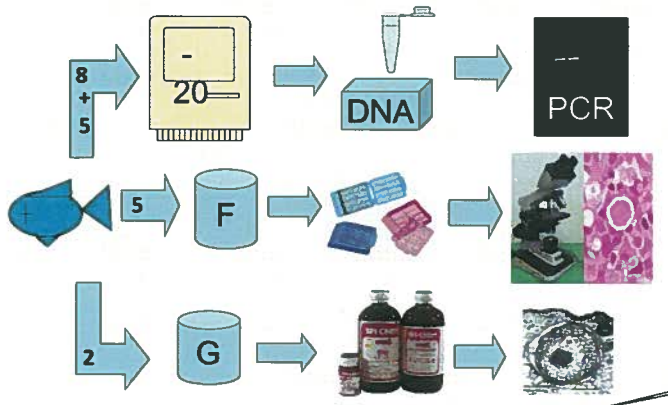
Hypotheses

1. The etiological agent of epitheliocystis outbreaks in Lake trout during each winter are CLOs
2. The etiological agent of epitheliocystis outbreaks in rainbow trout are CLOs, distinct from those in Lake trout
3. These organisms are found in fish without clinical disease

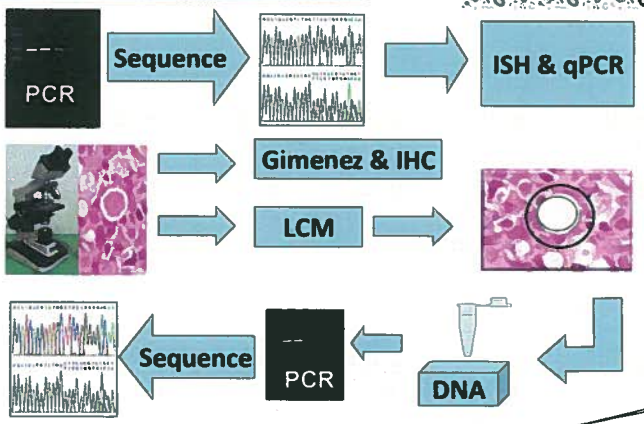
Objectives

1. Identify cases of gill disease in Lake trout and rainbow trout with **intracellular bacterial colonies** by light microscopy (epitheliocystis +)
2. Identify the organisms involved in this condition for both fish species and to localize the pathogens to inclusions using electron microscopy, laser capture microdissection, and *in situ* hybridization
3. Develop a qPCR to verify the presence of epitheliocystis agents in clinical affected and healthy fish populations at different times of the year

Materials & Methods



Methods

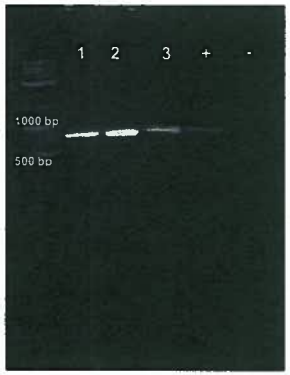


Results

	Histo H&E	Histo Gimenez	IHC Chla LPS ab.	PCR Chl primers Everet *et al*, 1999	16S RNA primers Relman et *al*, 1993
LT	**(+) ve n=15/45**	(-) ve n= 15	(-) ve n=15	(-) ve n=87	**(+) ve n= 24**
RT	**(+) ve n=15/20**	(-) ve n=5	(-) ve n=5	**(+) ve n=10**	**(+) ve N=9** (6=Brachiomona cyst and 3 for flavobacterium)

Results: PCR

- 16S universal bacterial primers (800bp)
  - 1: LT B214-11#2
  - 2: LT B214-11#3
  - 3: LT B214-11#4
  - + control: *E. coli*
  - - control: template
- Sequence results: Burkholderiales bacterium 483-L1 JN968376 (Toenshoff *et al*, 2012)

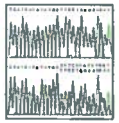


Results: LCM

LT B028-11 (winter 2010)
LT B214-11 (winter 2011)

First week submission "high number of inclusions"

PCR



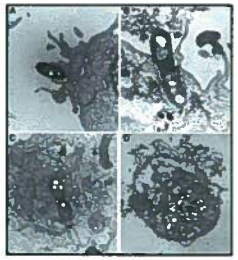
Sequencing

Burkholderiales JF800717.1 90% MI

Results: PCR

Burkholderiales bacterium 483-L1

- Maximum identity: 90%
- Query coverage: 85%
- Taxonomy:
  - Kingdom: Bacteria
  - Phylum: Proteobacteria
  - Class: Betaproteobacteria
  - Order: Burkholderiales
- Gram negative bacteria
- The order includes several pathogens for animals, humans and plants

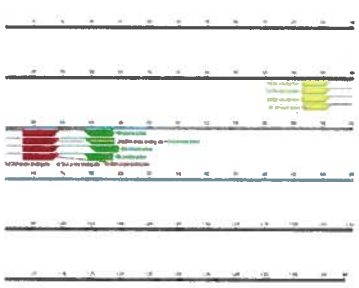


Conclusions

- Based on PCR and LCM using 16S universal bacterial primers and Chlamydiaceae primers, epitheliocystis is not consistently associated with a CLO in Lake trout.
- Results to date suggest that a Burkholderia-like organism is associated with cases of epitheliocystis
- More studies such as *in situ* hybridization are needed to support these results

Ongoing/future work

- Electron microscopy
- ISH probe developed
- qPCR probe to screen bacteria from fish samples collected at different times of the year



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