

GREAT LAKES FISH HEALTH COMMITTEE

2020 Winter Meeting
Ottawa, ON
February 4-5, 2020

Minutes
(with attachments)

Submitted By:

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Great Lakes Fishery Commission

The data, results, and discussion herein are considered provisional;
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GREAT LAKES FISHERY COMMISSION
2200 Commonwealth Blvd, Suite 100
Ann Arbor, Michigan 48105
Great Lakes Fish Health Committee

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List of Attendees

Andy Noyes	New York State Department of Environmental Conservation
Danielle Godard	Wisconsin Department of Natural Resources
Nicole Nietlisbach	Wisconsin Department of Natural Resources
Ken Phillips	U.S. Fish and Wildlife Service
Dave Meunick	Indiana Department of Natural Resources
Mitch Marcus	Indiana Department of Natural Resources
Gary Whelan	Michigan Department of Natural Resources
Tom Loch	Michigan State University
Kevin Loftus	Ontario Ministry of Natural Resources and Forestry
Coja Yamashita	Pennsylvania Fish and Boat Commission
Ling Shen	Minnesota Department of Natural Resources
Sunita Khatkar	Department of Fisheries and Oceans Canada

Invited Attendees:

Charise Dietrich	Department of Fisheries and Oceans Canada
Sherry Walker	Department of Fisheries and Oceans Canada
Joanne Constantine	Canadian Food and Inspection Agency
Kim Klotins	Canadian Food and Inspection Agency
Ellen Rae Melvin Walsh	Canadian Food and Inspection Agency

Great Lakes Fish Health Committee Meeting

Ottawa, ON

February 4-5, 2020

Monday, February 3rd, 2020

Check-in Delta Ottawa City Centre
101 Lyon Street North, Ottawa, ON
1-888-236-2427

Tuesday, February 4th, 2020

8:00-8:15 Welcome and Introductions (Godard & Khatkar)
- lunch & dinner options
- tour information

8:15-8:30 GLFC Update (Dettmers)

8:30-8:45 Joint meeting of the Fish Health Section-AFS and the Northeast Fish Health Committee in June (Dettmers)

8:45-9:30 CLC recommendations on KHV and Baitfish position statements (Whelan)

9:30-10:00 Baitfish testing next steps discussion – next steps (All)

10:00-10:15 **Break**

10:15-11:00 CFIA – NAAHP Program (TBD)

11:00-12:00 Update on MSU research (Loch)

12:00-1:30 **Lunch**

1:30-2:15 EEDv update – history and recent data analyses (Whelan)

2:15-3:00 DFO – eDNA (TBD)

3:00-3:15 **Break**

3:15-4:30 Update on recent EEDv work (Loch)

6:00 **Dinner**

Wednesday, February 5th, 2020

8:00-8:15 Reconvene and introductions (Godard)

8:15-8:45 Advice on fishery research proposals (fish health related) for the Research Board (Dettmers)

8:45-9:00 *Salmincola* update (Yamashita)

9:00-10:00 Agency Updates (All)

10:00-10:15 **Break**

10:15-10:45 Agency Updates (All)

10:45-11:45 Creative Dx/Rx innovations/Interesting Disease Cases (All)

11:45-12:00 Meeting Recap (Godard)
- Summer 2020 meeting location/dates – Thunder Bay, ON?
- Winter 2021 meeting location/dates – Windsor, ON?

12:00 **Adjourn**

1:30 **Tour** – OMNRF White Lake Fish Culture Station (Sharbot Lake, ON)

Summary of Decisions and Action Items

Action Item: Sunita will send out Joanne, Kim, and Ellen's contact information to the committee. *Completed June 11, 2020.*

Action Item: Gary Whelan will lead a team that includes Godard, Noyes, and Loch to develop a survey to collect information about each agency's baitfish industry and current testing methodologies to inform the committee about where it should start to address information gaps related to basin wide baitfish testing.

Action Item: The committee will stay in contact with Tom Loch about when is the appropriate time for GLFHC input into the Blue Book revision process. Representatives from individual agencies should also be working internally to proactively develop possible topics for inclusion in any revisions to the Blue Book.

Decision: The committee is supportive of moving the pre-proposal forward to a full proposal, given some questions will be answered.

1. **GLFC Update** (John Dettmers, GLFC)

The GLFHC remains very interested in sharing thiamine data from lake trout eggs. This information is analyzed by the USGS and the GLFHC wants to maintain strong ties to researchers in this field. USGS will be giving a presentation to managers at the upper lakes meeting in March 2020.

Discussion

What about inviting Jacques to future meetings? He is already a technical advisor, but he should be attending our meetings and being involved on a regular basis. His former graduate student may be more available as well.

2. **Joint meeting of the Fish Health Section-AFS and the Northeast Fish Health Committee** (John Dettmers, GLFC)

The joint meeting of the Fish Health Section-AFS and the Northeast Fish Health Committee will be this June in Burlington, VT. Several Great Lakes Fish Health Committee members are likely to attend. Tom Loch is the incoming president of the AFS Fish Health Section. Loch is currently on a committee that is in the process of substantially revising the AFS blue book. The GLFHC's input about possible blue book changes will be sought at future meetings. The committee brainstormed several topics that should be considered for inclusion in any revision. Loch and others are currently reviewing that. There are funds available to undertake some of these revisions. The primary focus of the discussions at the Fish Health Section meeting were concerns that the Blue Book is not up to date and very salmonid centric. Some members want shell fish and/or warm water fish information in there. Those that sit on the Blue Book oversight committee over represent some groups over others. Another concern was that there is not a lot of state-based input so far.

Discussion

Would any input from the committee be helpful for this revision process? Loch agrees the committee should have some input in the revision process. The timeline for the revision process is not solid and exactly what will be revised is not concrete. It will likely get moving in the next few months.

Action Item: The committee will stay in contact with Tom Loch about when is the appropriate time for GLFHC input into the Blue Book revision process. Representatives from individual agencies should also be working internally to proactively develop possible topics for inclusion in any revisions to the Blue Book.

3. **CLC recommendations about KHV and Baitfish position statements** (Gary Whelan, MDNR)

At the October 2019 CLC meeting, Gary Whelan presented a revised version of the KHv position statement. There was only one minor revision that was accepted and supported by the CLC. The final position statement can be found at:

<http://www.glfhc.org/pubs/fhealth/2019%20FHC%20Final%20KHv%20Position%20Statement.pdf>

Gary Whelan also presented the GLFHC baitfish position statement at the CLC meeting. It had supportive feedback from the CLC with consensus for the GLFHC to move forward with it, given some modifications that could allow it to become part of the GLFHC work plan on this topic.

4. Baitfish testing next steps discussion – next steps

Discussion

Where does the funding for this proposed baitfish work come from? It will continue to come through the associated agencies as always.

Does the GLFHC want to create a blanket statement for all the agencies or for each agency to create their own regulations and actions regarding controlling baitfish pathogens? The GLFHC may want to create a blanket statement and provide guidance about which pathogens the committee is concerned with and which need to be tested for all agencies to follow. Lobster bait tends to be prominent in the northeast. Discussions about testing lobster bait in the northeast are at the same level as the fish health committee is with baitfish.

Which agencies involved here are testing their baitfish? Michigan, Wisconsin, Pennsylvania Fish and Boat Commission (PFBC) and Minnesota Department of Natural Resources (DNR) test for VHS only.

If Ontario were to get funding to do this, is it possible to send samples to labs in the states? Typically, Wisconsin will notify customs ahead of time that samples are coming and they will be put through but sometimes it still gets stopped and held up, so that is something to consider. Ontario is doing a provisional baitfish review; it is not certain that Ontario will allow the export of baitfish for testing. Michigan will not allow import of baitfish without being tested.

Have fish chiefs been informed of the baitfish statement being approved by the CLC? As far as the Secretariat knows, agencies are not aware this is coming unless any CLC members have told their respective agencies since the meeting. From an agency perspective in Minnesota, the agency needs a solid foundation on what pathogens to test and why, and be able to describe what impacts these pathogens may have. The GLFHC should determine what it wants to do with the information rather than just collecting it. All agencies on the committee have some level of baitfish testing but it is highly variable. Each member agency should describe its baitfish testing methodology if any, as well as each jurisdiction's baitfish industry characteristics and policies to provide enough context to ask the right questions to move forward on this work plan from the CLC.

Action Item: Gary Whelan will lead a team that includes Godard, Noyes, and Loch to develop a survey to collect information about each agency's baitfish industry and current testing methodologies to inform the committee about where it should start to address information gaps related to basin wide baitfish testing.

Committee suggested questions for the survey:

- What pathogens associated with baitfish transport are your agency most concerned about?
- What pathogens is your agency currently testing for?
- What methodologies are being used for testing in the field and the lab?
- What is your agency's testing and collection capacity?
- What are viable numbers/sample sizes?
- What are the sources of bait within your jurisdiction?
- How is baitfish pathogen testing used in your jurisdiction to inform baitfish regulations?

- How would you like to see baitfish pathogen testing information used to inform decision making within your jurisdiction?
- What is the estimated number of baitfish going through or entering into each state and or province?
- What is your level of confidence in the chain of custody for samples taken?
- What, if any policies regulating baitfish transport are in effect within your jurisdiction?
- What is the size of each jurisdiction's bait industry i.e. its economic value?

Discussion continued

Is the Wild Fish Health Survey a source for asking for more funding? Potentially this might fall under the jurisdiction of Phillips with USFWS.

How long do we see this process panning out for? This would be ongoing as we continue to investigate and get more information down the road.

Are we going to call this a surveillance or investigation? Right now, we are still in investigation mode.

Do we want to do any sequencing of any kind when we find something to better understand where these infected fish came from? Yes, the committee agrees. It would be nice to get all labs to do the same level of testing and synthesize all the information they have been collecting so far.

5. NAAHP Program (CFIA Guest Speakers)

National Aquatic Animal Health Program in Canada

Under the Health of Animals Act, the Canadian Food Inspection Agency (CFIA) implements the National Aquatic Animal Health Program (NAAHP) and is the competent authority for aquatic animal health and welfare in Canada (OIE). The NAAHP addresses aquatic animal diseases of finfish, molluscs, and crustaceans, consistent with international standards. The primary goal of the NAAHP is to keep Canada's livestock free from disease as well as the eradication of known diseases or if not feasible, to confine or contain diseases of concern within a geographic area.

Domestic Disease Control Programs: CFIA must be formally notified for outbreaks of both domestic and exotic disease. This will initiate the disease response protocols that are incorporated in several hazard-specific plans. There are other diseases not listed by the OIE-I that are important to Canada.

CFIA operates a domestic movement control program by issuing permissions to test or disinfect and ship in closed premises as well as compartment recognition programs. Based on declaration of zones, compartments are recognized and declared as free areas. Commercial processing of finfish will be a new part of the NAAHP in addition to the commercial processing of molluscs. The CFIA has amended the humane transportation regulations under the Health of Animals Act regulations. These amendments will be enforced beginning this month and include movements made under stocking and enhancement. The amended regulations state that no one can move unfit or compromised individuals unless under veterinarian care. In addition to this change, the National Farmed Animal Care Council is writing a code of practice for farmed finfish and will have a chapter on transfer rigs which will eventually include enhancement facilities. Enhancement facilities are considered those that are creating commercial fishing opportunities, restoration for ecosystems, and stocking for recreational fishing.

The Aquatic Import Program: Regulates the import of all aquatic animals to Canada which include all finfish, molluscs, and crustaceans. Its regulations were revised in 2012 to define the list of animals considered by diseases they are susceptible to.

There is also the risk of release from importation. Regulations based on end uses are now also included (see slide for list of end uses). Regulated end uses for import may also include testing for aquarium fishes such as gold fish because there are many end uses in Canada that are not expected. Since 2015, the health status of animals by destination to and from is also considered. Permits are administered to importers with requirements for them and requirements for the exporting country. OIE has set new criteria for susceptibility of species. It is an ongoing and evolving process so it may be worth looking up and becoming familiar with. CFIA controls import of aquatic animal pathogens, they also control veterinarian biotechnologies such as vaccines, cell lines, serums, diagnostic tests etc.

If there is a country that cannot meet a requirement to import to Canada, there is the option of doing import quarantine although such a course requires substantial resources to complete. If a country of origin can certify disease freedom after quarantine, then that country and Canada can still be considered free of that disease.

The Aquatic Export Program: The CFIA provides export certification for shipments when required by the importing country, based on requirements from the OIE and the importing country.

Diagnostics: Test methods used are qPCR/PCR, virus isolation, and histology. A laboratory management information system is used (LIMS). Biocontainment involves CFIA standards for handling Aquatic Animal Pathogens. Level 2 biocontainment is in-vitro, and level 3 is in-vivo.

Network Laboratories: Policy on approval of external labs for the NAAHP. NAAHLS test method or CFIA-approved method that is on scope for accreditation. To maintain specific performance standards, and requires certification to at least AQC2 in vitro biocontainment standard.

Surveillance and Epidemiology:

Koi Herpes Virus and Spring Viremia Carp Virus have not had declared free areas yet.

Animal Health Risk Assessment Unit (AHRA):

Working on setting standards for wild fish populations to bring into the risk assessment process on a regular basis. The review process and documents are made available (See Slide).

Discussion

You mentioned two pathogens in the “free zones”. Of the pathogens that are known, there are 11 “reportable” diseases in Canada so those are being zoned for in those examples (see slide). When a free zone is declared it will be for controlling those pathogens. It can be just for one of them per zone. At most CFIA controls three pathogens in any one zone. For clarification, both reportable and immediately identifiable pathogens can be zoned. For mollusk pathogens, all salt-water pathogens are considered. CFIA asked the committee about its designation for *M. cerebralis* as it might have been detected in the Great Lakes Basin but is listed as a restricted pathogen. Yes, it has been in the Great Lakes for several decades. It has been in Michigan since 1967 after being introduced from private hatcheries. There are no clinical signs associated. It is not typically a disease agent, but it is still there. Currently the CFIA is calling Ontario a free area, has *M. cerebralis* been detected there? It is not known right now. We will need to ask Kerry to follow up with CFIA.

Action Item: Sunita will send out Joanne, Kim, and Ellen’s contact information to the committee.

Completed see discussion item 15.

6. Update on MSU research (Tom Loch- MSU)

Current members of Loch's Lab: Michelle Van Deuren, Dr. Megan Shavallier, Sean Burke, Courtney Harrison, Amber Johnston, Chris Knupp, Steven Sisolak, Eilleen Hendersen, Rachel London et al. Loch's lab collaborates strongly with Michigan DNR to understand aquatic infectious diseases in the management of hatcheries, aquaculture, and wild fisheries in Michigan waters. Scientists have been actively studying *F. psychrophilum* since the 1940s. However, there are still many knowledge gaps regarding its virulence, vaccinations, and antibiotic susceptibility. A USDA grant has funded research to understand the diversity of the Flavobacterium species *F. psychrophilum*. An overview on the genetic diversity map of *F. psychrophilum* was presented at the summer 2019 meeting.

F. psych antibiotic "susceptibility":

Loch's lab chose to focus on three antibiotics that are approved for use in the United States: Sulfa Trimethoprim, Oxytetracycline, and Florfenicol. The study size was 390 isolates. Another version of the *F. psych* diversity map is a Bayesian Reconstruction of North American *F. psychrophilum* phylogeny using seven house-keeping genes. The lab constructed phylogenomics and antibiotics susceptibility tests of 20 different strains to understand the mechanisms of virulence and antibiotic resistance. The entire genome was compared to the other 19 genomes. The phylogenetic tree that was presented shows green which means that isolate was not resistant, yellow was intermediate resistance, and red was 100 % resistance to the antibiotics. Sensitive isolates formed a clade, and mostly resistant isolates were in a clade. The large clonal complex 10 encompasses the clades that are most sensitive to antibiotics which came from captive fish. Whereas wild fish were associated with the clades of isolates that are not resistant.

In vivo treatment efficacy experiments:

Based on these results, what does sensitivity to antibiotics actually mean? Epidemiological cutout values for bacteria is a logical next step. Thus far, intraspecific *F. psych* diversity is substantial, but top "troublemakers" in the USA and the Great lakes have been identified. There is evidence of transmission via egg trade. The "troublemakers" are more likely to "resist" approved antibiotics. The mechanisms for this is still not understood. There are ongoing studies in vaccine development, improved egg disinfection, and elucidating *F. psychrophilum* disease ecology in hatcheries/aquaculture.

Michigan State Fish Hatcheries:

A summary of the various strains of *F. psychrophilum* that have been found in Michigan State Fish hatcheries.

Oden State Fish Hatchery (OSFH):

There are strong historical data for some of the Michigan facilities. Isolates of *F. psych* are archived from OSFH between 2010 and 2017, a particular strain that has not been reported anywhere else on a global scale. It has maintained itself in this hatchery only for almost 10 years. It is being recovered from broodstock and production fish.

Harrietta State Fish Hatchery:

Another strain was detected in 2017 at the Oden SFH has not been found anywhere else except at the Harrietta State Fish Hatchery, which received eggs from Oden SFH in 2019. Egg-associated transmission can be the origin of this strain. Egg disinfection methods should be improved to limit the transmission of *F. psych*. A strain that has been around since 1947 in the Green River, WA, the same river from which Michigan originally received salmon eggs. This is another indicator transmission via eggs may be possible. In summary the "unique" *F. psychrophilum* genotypes are ST286 which is likely egg-associated transmission from OSFH that caused BCWD outbreak in 2019, and ST349 which has an unknown source.

Platte River Weir and Platte River State Fish Hatchery:

The diversity of *F. psychrophilum* genotypes refers to a majority of them strongly associated with COS (here & elsewhere). Some (ST13) match Japanese, European, and Washington strains. Some may be “indigenous” to the Great Lakes (ST258, CC-ST252/256). Although other sources (e.g., water) cannot be ruled out, evidence suggests repeated egg-associated transmission. At least one isolate of ST13 serologically distinct from up and coming BCWD vaccine.

Little Manistee River Weir:

There are many different strains of *F. psych.* The Little Manistee River includes chinook that return in the fall, and steel head returning in the spring. These strains are present in feral fish that are returning while also being present in the broodstock fish.

Wolf Lake State Fish Hatchery:

Sequence type 10 was not seen in the broodstock but was found at WLSFH. In some cases, there is the reverse. Production fish at Wolf Lake had two new strains found in 2013 where is at the weir location those strains were found in the broodstock that are returning in 2017 and 2018 to Little Manistee River. Diversity of *F. psychrophilum* genotypes almost all belong to “RBT” clonal complexes and half belong to CC-ST10. The questions that remain are; is there evidence for infections that persist post-stocking, relation to life history, or evidence for egg-associated transmission; possibly other sources too?

Other Great Lakes Hatcheries

Tom’s lab is also trying to understand whether some isolates are unique to facilities throughout the Great Lakes from Wisconsin, Indiana, New York, Minnesota, and Pennsylvania. A few isolates are found to be unique in Wisconsin. One strain is circulating within a weir facility but is not causing any issues in the hatcheries. Further investigation will need more Wisconsin isolates to be sent to Loch’s lab. The ST258 isolate is causing issues in Michigan with coho salmon and has been detected at the Wild Rose facility during the last two years. This isolate is also present in Indiana coho salmon. The ST10 strain of the large founder group #10 is prevalent throughout the Great Lakes and was found from Rainbow trout in 2019 at the Bath State Fish Hatchery (SFH). Minnesota provided ST258 in the broodstock at Lanesboro SFH. Pennsylvania has several isolates that were analyzed. A colonial complex in #281 has strains only found in Pennsylvania from Lake Erie steelhead. It is being pulled from production fish and broodstock assuming egg associated transmission.

Flavobacteria Surviving Iodophor Egg Disinfection:

There were 69 flavobacterium isolates that survived at iodophore disinfection at 400ppm for 10 minutes. This is four times the dose currently being used and would not permit eggs to survive. Eyed eggs and unfertilized eggs were tested. standard iodophore disinfection protocol of 100ppm for 10 minutes is clearly not eliminating all flavobacteria, with 105 isolates surviving the standard disinfection protocol. Within this backdrop, Tom’s lab is seeking to improve disinfection methods for flavobacteria in eggs. Ongoing disinfectant experiments were conducted using the isolates recovered from the eggs that were disinfected at the 400ppm and 100ppm dosages. The lab exposed flavobacterial isolates to four common aquaculture disinfectants: formalin, chloramine-T, and iodine, for various amounts of time at various concentrations. Chloramine-T works very well at shorter exposures and lower concentrations than formalin. Chloramine-T produces 100% reduction for both chrysobacteria and flavobacteria when they are exposed for 30 minutes. For iodine, neither bacterium was inactivated by the iodine treatment. It is possible that there may be a biofilm protecting them or that some of the bacteria are inside the egg and are transmitted *intra ova*. It has been documented that bacteria are more successful at forming a biofilm when associated with other bacteria as compared to being isolated. Flavobacteria may be interacting with other microbes and this may be why they can survive some of the disinfecting methods currently being used. The lab is continuing to try to understand why some strains so successfully survive disinfectants.

Infectious Disease in Lake Sturgeon:

Lake sturgeon populations remain strongly suppressed compared to historical population sizes. Very little is known about infectious diseases of Great Lakes lake sturgeon. Is it possible that known diseases have contributed to some of their decline? Or are diseases hindering the rehabilitation of lake sturgeon? USFWS has funded Loch's MS graduate student Amber Johnston at Michigan State University that aims to understand which Great Lakes fish pathogens lake sturgeon may be susceptible to. Adult Great Lakes sturgeon sampling sites were the Black River, St. Clair River, and Peshtigo River. Blood and tissue biopsies as well as non-lethal external swabs were taken from all sturgeon collected for virological, bacteriological, histopathological, molecular, and hematological analyses. Several infected individuals were showing external signs. Blotted white spots and areas on fins and other extremities were suspicious of Acipenser herpes viruses that are seen in other sturgeon species. This was seen in 5% of the Black River fish, only 1% in St. Clair River fish, and 0% from the Peshtigo River fish (which had a much smaller sample size). The lesions are PCR positive. The virus is not known but there is a hit on a virus and will be sequenced this week. The health effects of an acipenser virus on lake sturgeon are not known, however it is potentially transmitted through reproductive fluids. Gross signs of disease were observed in both adult and juvenile Great Lakes lake sturgeon. However, there are no cytopathic effects observed so far. Numerous microbes were recovered but their identity and significance are still not known.

Discussion

How quickly is the evolution of the F. psychrophilum isolates occurring? We do not have a timeframe yet.

Is each strain highlighted in the Platte River Weir diagram from a specific species of fish? We estimate that 95% of all isolates in this colonial complex have come from coho salmon except the complex numbered 256 that the lab is calling the "Great Lakes endemic strain" as they all came from native Great Lakes fish.

Are you looking at different colonies to see if there is co-infection? Yes, we are taking many colonies and it has been documented that you can have multiple sequence types in one fish. In Japan, eggs are being disinfected with iodophor before fertilization. It was effective at eliminating F. psychrophilum. Wisconsin has tested similar lesions on sturgeon and identified Acipenser Herpes Virus 1. However, that testing was done with an invalidated test and is in the process of being validated.

7. EEDv update – history and recent data analyses (Gary Whelan- MDNR)

Gary Whelan presented a history of the detections and outbreaks of EEDv at the Marquette State Fish Hatchery (MSFH). After an initial outbreak of EEDv in the 1980s followed by depopulation and disinfection at MSFH, the virus was not detected again until 2009. The virus was detected again in 2009 with subsequent mortalities. Since then, repeated detection and mortalities following detection have occurred to the present. Large rainstorm events and water quality issues i.e. turbidity appear correlated with these detections and mortalities. After some genetic work, it was discovered that the strains from Michigan are the same as the strains from Bayfield, Wisconsin. It may be that this is an endemic virus to the Great Lakes. More than 90,000 lake trout production fish were lost from 2012 to 2013. In 2017 and 2018, EEDv was detected, but there were no unusual mortalities seen. Both Seneca and Lake Superior strain lake trout tested positive for EEDv. It appears that stress will cause an increase in the replication of the virus enough to detect it although not enough to cause mortalities. In 2018 more than 35,000 lake trout production fish were lost from EEDv.

Seneca strain lake trout had a significantly higher cumulative mortality for 2018 than Lake Superior strain. Surveying EEDv in the wild has been done in Bayfield, WI between 1988 and 2007. It was then sampled in Michigan waters of Lake Michigan and Lake Huron in 2010 and 2013, respectively. In 2018 Lake Huron, Lake Michigan, and Lake Superior were also surveyed in June, May, and then October respectively; positive results came back at very low levels for all three lakes. Although EEDv was almost certainly there, the timing of sampling will determine detectability. In 2019, Lake Superior was sampled in August, May, October, and June with only one or no positive results. It's likely that October is still too early for detection of EEDV in lake trout. MI DNR will continue to treat EEDv similarly to how BKD is treated and use similar procedures. Vaccines will be hopefully developed along with methods to cull infected adults. The DNR will determine positive locations in Michigan waters to allow stocking of fish without clinical disease. If these measures are unsuccessful, a new broodstock will need to be developed. In conclusion, it is best to look for EEDv closer to spawning time for lake trout or you will get low to no detections of it.

Discussion

Despite best biosecurity measures, movement of EEDv in the broodstock to production fish is possibly happening after spawning or those other higher stress events. Water temperature is very important in detecting EEDv. If the water is at 9 degrees C in the lab, fish can be infected, but external symptoms will not appear. If EEDv survives disinfection while not being within the egg, it may be protected by outer biofilms where the virus may be embedded. This is what MSU is seeing in testing for flavobacterium at MSU.

8. Expanding our toolbox: eDNA as a complement to conventional sampling (Charise Dietrich – DFO)

One way to analyze eDNA is by targeted eDNA detection or qPCR. It is mostly used for one to four species of interest with specific target primers that can be run simultaneously. Another method is eDNA metabarcoding, which is used to detect many species or a large group of species. There are many strengths to using eDNA; however, limitations exist. For instance, sex or sex ratios, reproductive status, age or size class, population size, condition, or whether the DNA is from a live or recently dead animal cannot be determined. DFO held a national eDNA workshop in 2018 to engage clients and scientists that are interested in using eDNA to identify information gaps, opportunities, technical challenges, and DFO research needs. The concerns or comments that were communicated from the workshop include that eDNA would be great for guiding regulations, there is a need to understand how to integrate eDNA into decision frameworks, and there should be a gold standard for its use. Based on another workshop from 2019, qPCR assays are ready for implementation by management, whereas metabarcoding has a lot of potential but is not there yet. A national eDNA Technical Working Group is working towards addressing many of the issues and developments from these workshops (see slide). eDNA standardization involves two parallel Canadian efforts involving Canadian Standards Association (CSA) and CSAS Science Response. DFO is moving forward to use eDNA to test for invasive species in ballast water. Opportunities and challenges exist with eDNA. eDNA is ideally used alongside conventional methods to detect species and is not considered the only methodology to be used or depend on. DFO will be developing accredited labs to assist with eDNA work.

Discussion

What process will DFO consider to create these accredited labs? It would consider eDNA work as a diagnostic practice.

Has DFO used eDNA for eradication purposes? Eradication of the invasive plant, water was done by OMNRF using eDNA. Michigan has been considering it for detecting parasites. IPN is a very hardy virus such that it will be easily recovered and preserved in fish. Therefore, if you are not finding it using eDNA something is not being done correctly.

Do you have an extraction kit that is best at eliminating PCR inhibitors? Some labs use DN-easy.

Do you ever concentrate the water? Usually a 1-2 liter sample of water is collected and passed through the filter paper. Others will centrifuge the water sample to get a more concentrated sample, however centrifugation does not work as well.

Does DFO conduct analysis of RNA? I do not know whether any is being done in Canada. It is being used now in the US because RNA is time sensitive, so this could touch on the issue of knowing the timing of a species occurrence. Some will use RNA along with DNA to get a timeframe associated with it because RNA does degrade more quickly and the signal is much weaker than DNA.

Is anyone working with VHS detections using eDNA? A lot that has been done for VHS in the past was before more advancements of eDNA technology and there were mostly false-positives for VHS.

9. Update on recent EEDv work with MSU and MI DNR (Tom Loch- MSU)

EEDv has strongly reemerged in 2017 to 2019. A qPCR was created for the detection and quantification of EEDv. Another study provided a field deployed detection method for EEDv. However, neither have been validated for use in reproductive fluids. EEDv in vivo infection and virus replication model is now used. EEDv genome is now sequenced. Species susceptibility to EEDV was tested as part of a graduate student study at MSU. Twelve Great Lakes species/strains of fish were tested including nine species of salmonidae, as well as cottidae, centrachidae, and esocidae. The injection challenge used was a low and high dose. There was no EEDv associated mortality or clinical disease in brook trout, brown trout, rainbow trout, Atlantic salmon, coho salmon, lake herring, largemouth bass, or musky. There was also no EEDv detection via qPCR.

EEDv susceptibility in lake trout strains:

None of the Seneca strain fish died. Only three of 10 Seneca strain fish survivors had EEDv prevalence. These Seneca's were producing a larger average EEDv load (copies/mg skin) over 100 days than over 66 days for the Lake Superior strain fish that had EEDv prevalence. There is varying susceptibility to EEDv for the Lake Superior and Seneca strain lake trout.

EEDv susceptibility in splake:

At the high dose, there was some disease signs that were consistent with EEDv early on. There was 30% mortality at three to ten days post infection. Only one out of 10 fish were EEDv-positive via qPCR. Some fish may be infected and clinically showing signs of EEDv but not necessarily dying because of infection.

EEDv susceptibility in mottled sculpin:

At the high dose, there was 60% mortality with no signs of EEDv. Zero out of 10 fish were positive via qPCR. At the low dose, there was 50% mortality without signs of EEDv. Only one of ten fish was EEDv-positive via qPCR. It is likely that mottled sculpin do not support EEDv replication.

Histology and Pathology of EEDv:

Lake trout (LAT) were challenged with EEDv. Six fish were euthanized of the replicates of the infected group and three fish from the control group. Tissues were collected from each fish and all were run using qPCR as well as histology using in situ hybridization. The objective of

the study was to determine what tissues are best to sample and when during the course of the disease.

Gross Pathology:

Signs of morbidity in the fish that were challenged via emersion with EEDv did not appear until about 10 to 15 days post challenge. Mortalities were not seen until about 21 to 25 days post challenge. Ten tissue types were tested. EEDv was detected in the eye tissue of one fish very early on. The first tissues to show up positive after that included the eye, the skin, and the fin. After that, every fish sampled tested positive. The eye, skin, and fin were consistently successful sampling targets, with large viral loads from day 18 and on compared to the internal tissues. Sampling was not changed based on presence of the lesions but there was testing in the same area each time.

Ability of lake trout to shed EEDv:

EEDv transmission via shedding

Fish shedding loads far exceeded their initial exposure concentration at one week after infection. At three weeks post-infection, every fish was shedding at far higher loads than they were exposed to, surpassing the lethal dose. Six fish survived to 58 weeks. Two of the fish were still shedding very low amounts after handling. Later on four out of the six fish were shedding at 10^5 virus copies. Some survivors may stop shedding but later on, they may continue to shed again when stressed. EEDv can be transmitted through equipment that has not been properly disinfected to naïve lake trout. Vikron is a successful treatment that can be used for disinfection. Based on 2019 wild lake trout EEDv surveillance, there is potential to start a new broodstock of lake trout that are EEDv free.

Developing Vaccines for EEDv:

Autogenous vaccines could be deployed immediately without approvals. The limitation is that the virus has to be grown in the lab with only small-medium dose vaccinations. A protein subunit vaccine would take a lot longer to be approved but would be able to be deployed on a much larger scale. The other objective is to validate EEDv molecular and serological tests for lake trout reproductive fluid screening. To validate using qPCR and qLAMP assays for EEDv. A third objective is to test for the transmission of EEDv via tagged and clipped fish. The working hypothesis is that it will be easily transmitted through tagging and clipping fish. The final objective is to develop easily applicable control measures to reduce lake trout losses during EEDv outbreaks. In conclusion, the outcomes of this work will be improved lake trout health in the hatcheries and in the wild.

10. Advice on fishery research proposals (fish health related) for the Research Board

(John Dettmers, GLFC)

A pre-proposal was submitted to the Commission's Research Board by Dr. J. Rinchar from SUNY Brockport titled, "Do lake trout eggs and alevins acquire thiamine during development in wild populations?"

Discussion

Andy Noyes supported moving it to full proposal. Gary Whalen suggests that it may be over priced but should be able to see where that money is spent in the full proposal. Danielle Godard asks for more detail in the full proposal to answer some questions such as how it will help mitigate poor health and recruitment. Tom Loch agrees, asks what changes in management will be made from the results of this work? Ken Philips agrees as well, and mentions that it doesn't say how the information will be used. *Attendee asked how much does the GLFC focus on the "applied" aspect?* The Board of Technical Experts is interested

in the applied aspect but is also interested in sound basic science that can underpin future management decisions.

Decision: The committee is supportive of moving the pre-proposal forward to a full proposal, given some questions will be answered.

11. *Salmincola* update (Coja Yamashita- PFBC)

Gill lice in Pennsylvania update:

Two parasitic copepods in Pennsylvania are species specific, targeting salmonids. *Salmincola edwardsii* is specific to rainbow trout and *Salmincola californiensis* targets brook trout. Gill lice were first documented in PA in the 1980s. *S. californiensis* was probably introduced first, likely as part of movement of fish from the west coast in the early 1980s. Gill lice were first described in 1761. A co-operative nursery had a large infestation in 2011. There is a strong relationship with private aquaculture stocking sites and wild fish in those areas that are infested. In 2016, PFBC discontinued stocking any fish that had gill lice or stocking fish in any positive waterways for gill lice. Since 2016, the PFBC began documenting sites of infestation throughout PA waters. Of 1,883 streams that have wild brook trout in them, about 4% have *S. californiensis*, consistent with commercial stocking of brook trout in those streams. Pennsylvania does not have as many wild populations of rainbow trout, but six of those streams that have been stocked with rainbow trout, are also about 4% that are positive for the *S. californiensis* parasite. In 2018, PFBC began requiring that any fish to be stocked for a fishing derby or any other special event, which normally are bought from private aquaculture facilities, must be certified as gill lice free. However, there was no process in place to certify fish as gill lice free and the PFBC also had to find someone to do it. Eventually a process was created using visual inspections for adult gill lice. A course was created for the veterinarians. Now, 15 veterinarians are recognized certifiers, as well as other state officials. Although the protocol and certificate states that fish are gill lice free, there is no process by which to notify the PFBC if any lots test positive. Commercial farms have the means of selling their fish as gill lice free now. PFBC has regular meetings with the Department of Agriculture on a regular basis to discuss the control of gill lice. In Pennsylvania, anyone is allowed to purchase fish from private hatcheries and stock them in any stream as long as it is not a protected trout stream or labeled a restricted stream in which to stock. There is no regulation about certifying fish before they are stocked in streams

Discussion

The committee believes it would be useful to look at gill lice samples from Michigan as well as possibly from Wisconsin and try to evolutionarily evaluate them to identify time of arrival and document the dynamics of its invasion since introduction. *Is the PFBC attempting to conduct any challenges with these two parasites?* No because it is not conclusive, yet in the literature that these parasites are driving brook trout or rainbow trout declines. In Michigan, the *S. edwardsii* parasite was found in a hatchery and UV light worked very well to eliminate them from brook trout. Hatchery fish are not seeing the same declines or drops in fitness as seen in wild populations. In the wild, there are more temperature changes and less control of environmental factors, resulting in wild brook trout populations sometimes declining rapidly. The PFBC is primarily trying to control the commercial hatcheries from stocking brook trout that could be infected with gill lice. However, there are some challenge studies involving fitness and swimming behavior. Trying these challenges with the two gill lice species in Pennsylvania wild fish could strengthen PFBC's case with controlling commercial hatcheries and private aquaculture facilities from stocking infected fish. It may help describe how the gill

lice is affecting the fitness of wild brook trout as the publication from Wisconsin describes effects of several other parasites and is not well defined as these two parasite species.

12. OMNRF Updates (Kevin Loftus – OMNRF)

Kevin Loftus provided an update about the work aimed at enhancing fitness of production fish in hatcheries, including why this work is relevant in terms of returns on investment and discussing the emerging concerns about post-stocking survival. Kevin also provided an update on bloater production and post stocking survival. A graduate student has been investigating methods for enhancing fitness at Normandale. Fish that were exposed to predators versus those that were raised under normal conditions were exposed to predator scent, and the previously exposed fish immediately dropped to the bottom of the tank. This work was expanded using the same design on fall fingerling size fish using a combination of four treatments. Fish were then grown to spring smolt stage and will be stocked in March, so results will be collected in the near future. A collaboration with Algoma U, Concordian U, Lake Superior State U, and MNRF submitted a pre-proposal about enhancing fitness to the Commission's fishery research program that was rejected, so it will be further developed. The bloater program began in 2011 with seven year classes to date. From the 2011-year class, less than 300 survived. However, there have been improved approaches in subsequent years. The survival of in-house broodstock bloaters to hatching was lower versus the survival to hatch for the wild bloater eggs. Once hatched, fish produced from in-house brood survived just as well as eggs from wild broodstock. Diet might be part of the problem with survival of in-house broodstock. In 2018, the program worked with a feed supplier and fish nutritionist to try to get the best diet plan for sufficient survival of in-house brood fish after hatching. Once stocked, some bloaters are traveling large distances and are detectable for a long time. Mortality rates are higher than anticipated. Some acoustically tagged fish descend to 50m or more within two minutes of stocking. Information from the telemetry tags suggest that many fish die shortly thereafter. MNRF would like to use the USGS decompression chamber to test the effect of rapid pressure changes on survival of hatchery-reared bloater.

Additional updates:

University of Guelph will take on the Ministry's fish monitoring contract when it expires in 2022. Aurora trout are a variation of brook trout that are found in northeastern Ontario. This species was virtually extinct at some point with only six females and three males in the wild. A recovery team was created to restore the species. There were serious signs of inbreeding with reduced survival in the hatchery. A rescue genetic technique will be used.

13. Agency Updates

Indiana DNR

This past July there was a loss of almost 100 steelhead, and two weeks later, there was a loss of another 142 after they were collected from Trail Creek. A theory is that it could be a combination of thiamine deficiency and bacterial infections of *aeromonis hydrophilla* or from OTC administration along with unstable water temperatures. This year, Indiana had the fewest ripe steelhead females available for the first egg takes starting in early January. The water temperature is constant at 54 F. The eggs look normal and healthy. No change was observed in the condition of the males. The only difference for this year than in more

successful years is the number of cloudy days this winter, with many more overcast days than normal. There could be a photoperiod effect in addition to water temperature. The fish are not fed in the hatchery as it is typically unsuccessful. Indiana has had the second warmest winter on record. Over the years at the Pond River State Fish Hatchery, the fall musky fingerlings were not surviving as well so there was a push for stocking larger musky. The musky were overwintered in an outdoor pond to get them very large. Over the last three seasons, the hatchery has been on a well supply. Since then, the over wintering musky did not survive. Steelhead and coho were put in and survived the winter but musky did not. Then, hatchery staff added fathead minnow as a forage fish, which all also died. The musky were tested and had five different species of *aeromonis*. They were severely bacterially compromised along with fungal infections. Virology testing was submitted and results will be in soon. One point is that there was no aeration system or degassing system in the pond. The pond is clay lined and filled. Fisheries and wildlife health sections are now combined. A position for a veterinarian will be filled soon. This will include fish health, contaminants, etc.

Discussion

Given the water temperatures, were the steelhead also being cultured for flavobacteria?

No, they were not. However, Indiana does tissue sampling of ovarian fluid for bacteria. Incorporating testing for flavobacteria may be beneficial if this continues.

Were there any external signs of disease on steelhead?

After they come in, they develop eroded fins and lesions but not on coming from the wild.

Are the number of returning fish still normal?

There are fewer numbers; however, the fish are much larger.

In Michigan, steelhead will start spawning in February in much colder waters than in Indiana streams. Fish will not ripen in 60 F water.

DFO

There has been a lot of effort in controlling and understanding the shrimp pathogen *Vibrio vulnificus* that is affecting fish in North America. DFO is using qPCR to detect the pathogen. Three fish tested positive. As a result, it was decided to close the border to transfer of shrimp from the US into Canada. CFIA wants a second test to confirm using histology in addition to qPCR detection. Canada is still trying to figure out what shrimp tissues to test, what life stage of the shrimp to test, etc. There is a lot of research going on between CFIA and DFO to further understand the pathogen.

Michigan DNR Largemouth bass virus continues to move north. The disease behaves differently in fish as it moves north. There is lower genetic diversity of the disease the further north it gets. Bass fishermen are complaining of lower catches. There has been a strange gas super saturation event occurring immediately below the Ford Dam on the Huron River that flows into Belleville Lake. There was a lot of evidence of barotrauma with gas emboli, blown out swim bladders etc. There have been changes to the spill gates and the lower slide gates. When they switch operation between the two, it tends to cause the issue. However, the literature does not match up in terms of gas super saturation events associated with these dam operations.

A large gizzard shad die-off occurred on the River Rouge in spring 2019. VHS was detected yet there were no classic external signs of the pathogen on the fish. However, there was a change of temperature of 10 degrees during that time that was likely the cause despite the

media communicating to the public that it was from the refinery nearby. Distressed steelhead have been recorded from angler photo and video and the situation is being closely monitored. There are no mortalities in the river yet, but egg takes will be closely monitored this spring. Arctic Grayling are a glacial remnant population. In Michigan, their native range was from the Muskegon River north to the Jordan River and all the interior streams, but never anywhere else. They were essentially extirpated in 1910 from over exploitation. Michigan DNR will attempt to reintroduce fluvial graylings with an Alaskan broodstock source. Alaska has a long-term fish health history. There was an egg collection from last year that are now in quarantine and going through fish health inspections. There has been some research looking into seeing how these fish will survive with the existing fish communities they will be stocked with. Brown trout may be the greatest predator threat to them. Eventually all the broodstock will be moved to Marquette SFH to take advantage of its constant water temperatures that grayling thrive in. The upper Manistee River will probably be the first targeted area of reintroduction. Grayling make very long-distance migrations so connected systems are key.

Minnesota DNR

This year's state hatchery inspections detected no certifiable diseases. There was one gill disease issue from the Peterson Hatchery. This is the first year that the MN DNR brought heritage brook trout eggs into the hatcheries. In past years, they always had bacterial kidney disease (BKD). After three years of testing, no positive tests were found. One of the hatcheries that the eggs were going to had also downgraded its BKD classification, so eggs were brought in. Once fish were spawned, using paired spawning of 71 fish, 35 of them were BKD positive. In addition, some eggs were lost because some females did not produce enough ovarian fluid. However, there is progress in introducing the disease-free healthy eggs of the heritage strain brook trout. During the last two years, MN DNR started looking into changing the testing requirements for VHSv. The state had over 10 years of testing for the virus to allow the declaration of three zones: freedom, risky, and positive zones as long as surveillance testing is continued in all zones. The change requires testing three waters in each basin in the freedom zone, six water bodies in each basin in risky zone, and no change in the risky zone. This process was started last year and will continue for the rest of 2020. The only issue is there are private groups that are stocking within the zones so the statute has to be changed; that is being worked on. A shrimp importation statute was established in 2018. Before the statute was enacted, once the certification was free of disease, shrimp were allowed into the state. For the shrimp industry, they only test every three weeks. Now, MN DNR asks for 36 months of OIE pathogen free testing results for the shrimp to come in. If this process is not followed but the lot is tested and is disease free, that shipment must be still quarantined before arrival into the state. PFAS has become a big concern. Because of this, the Department of Health proposed a change in the panfish consumption advisory from unlimited to two meals per week. However, the proposal was brought down because of concerns about the effect on fishing. Brook trout from Crystal Springs Hatchery developed strange lesions that appear primarily in males, where the lesion is consistently near the eye. *Vibrio vulnificus* was isolated from the eye lesions of four of the fish. *Aeromonas hydrophila* was isolated from the eye of two fish, and *Pseudomonas* was isolated from the kidney of one fish. Kidney lesions were reported from Lake of the Woods cisco. An oozy fluid emanated from the lesions when they were opened up. These were collected from a fisherman and only photos were sent. The fish from the fishermen had come in the early spring. In a fall survey, this was seen in 50% of the cisco surveyed. In Ling's lab, bacteriology did not reveal bacterial pathogens. Tom Loch's lab at MSU received samples and did detect a low *A. sal* antigen in one of the two fish samples. The USGS lab's initial diagnosis was a lymphosarcoma or a lymphoid tumor. Histopathology results are pending.

Discussion

How do you know if there is no human mediated transfer of pathogens in these free areas?
There is a permit process to help control any introductions.

For the brook trout eye lesions, are the fish held in concrete raceways? Could the lesions be abrasions from contact? Yes, they are held in concrete raceways.

How was vibrio vulnificus identified? It was found using APIs.

These clinical signs and prevalence of the kidney tissues in these wild fish at Lake of the Woods is something to pay attention to.

Yes, we are asking different regions within the state to check for these signs but there is nothing like this reported anywhere else. It may be useful to check with others that share the Lake of the Woods water.

USFWS Midwest Region

All broodstock at the Iron River Hatchery were de-populated due to *Vagococcus salmoninarum* (*V. sal*). Two-year-old fish were spawned this year. There have been no mortalities consistent with *V. sal* as seen in the past but there will be an inspection done in the next two weeks. Wild fish from Louis Lake, WY were brought in to Iron River. The fall inspection was negative for *V. sal*. There are a few articles about *V. sal*. being prepared for publication. One will describe the initial case at Iron River Hatchery; other will be on the tropism and qPCR development that Isaac Standish did. Bloaters were not collected from Lake Michigan last year due to the government shutdown; this year collections have been successful. The last collection will happen this week. It has been going well overall. DOI agencies including USFWS, USGS, NPS, etc. will all have the same regional boundaries now. It will not affect the USFWS in terms of the Great Lakes. The LaCrosse lab is still in interior region number three. This will likely be in effect by 2021. The Assistant Regional Director of Fisheries retired July 3, 2019. Aaron Woldt is the acting ARD.

PFBC

Aeromonas is still detected in hatcheries. This year it was detected in tiger musky. IPN is also seen in many hatcheries. The Cory Hatchery remains free of IPN since getting rid of brook trout. eDNA is being used to detect IPN but staff are not sure whether that effort will yield meaningful results. Waiting to hear back on a few results from a whirling disease report. Belafonte Hatchery is positive for whirling disease. It has been detected there off and on over the years. There were a few spores in the samples this time. If anyone needs positive samples to use, they can be sent. BKD is still in the same four hatcheries that have had it for some time. Trophy golden trout were positive for *Y. Ruckeri*. They were treated with OTC and have not found it since then. This summer, there were cutthroat trout virus issues. Since this summer, we have not detected the virus. There is an issue with a cooperative nursery with IPN detection. Although the fish were scheduled to be de-populated, some that tested negative but which were from the infected facility were still stocked out due to miscommunication. In a survey of the hatchery's water source, agency personnel found rainbow trout so they shocked out the fish. The water was tested positive for IPN using eDNA but qPCR techniques tested negative. Fish were still positive for IPN, so they depopulated the hatchery. Raceways remain dry. During walleye brood collections this year, many common carp were present in the trap nets. These carp were lethargic and barely moved. In

2018, a large fish kill from koi herpes virus was detected. Fish were sampled and sent to MN and came back positive for carp edema virus. It is in other surrounding states. It is only being found in common carp and not any other fish. PFBC started a freshwater mussel hatchery. White Sulphur Springs National Fish Hatchery needed to get rid of their mussels. Mussel production will start this spring. Brood from wild mussels were collected. The PFBC is looking for protocols in bringing in wild brood into a facility, as well as for literature and guidance about what the agency should be looking for in terms of health and pathogens. PFBC holds about five species right now. PFBC also is looking for largemouth bass that are certified disease free to use as hosts. The Department of Agriculture is putting restrictions on bringing tilapia into the state due to lake tilapia virus. Fish are being vaccinated for cold water disease to test its effectiveness on different species. Rainbow trout will be the next species, to see if there is a response difference between them and steelhead. This year, several lots were vaccinated but still treated for cold water disease. PFBC is not seeing the same lesions or mortality rates that were seen in the non-vaccinated fish.

Wisconsin DNR

Thirty fish health inspections were conducted this year with no significant findings for any of them. The WDNR veterinary team is conducting grow-out surveillance with musky and walleye to monitor for pathogens from feeding them bait fish. Golden shiner virus (GSv) was detected in musky, but was not found in the walleye. The walleye also had no clinical signs. Broodstock surveillance was done for testing of cultured and wild broodstock used for spawning. Pathogens of note included *F. psychrophilum*, *columnare*, *A. salmincola*, and EEDv. The surface water of three hatcheries that is fed from four lakes was tested for VHS. For stocking, lakes & hatchery fish need to be VHS negative (150 fish/lake tested). The result was no significant finding. Eight lots of forage fish were tested resulting in GSv in three lots (three facilities), and an unknown replicating agent in one lot (one facility). There were four wild fish transfers. There were unusual mortalities and morbidities seen in black crappie, walleye, and smallmouth bass. Sarcoma is suspected in the black crappie and walleye. A biologist submitted a small mouth bass out of concern that it could have VHSv. The clinical signs were inconsistent with VHSV and samples from this fish were negative for VHSv. Ongoing cases for wild fish include the AciHV1. This year, sampling will focus on the Wisconsin River to see if it is ubiquitous around the state. There may be a link with sex, as more females are affected. Black Crappie sarcoma is an ongoing concern since the 1980s. This year, WI DNR will be doing PCR, TEM, and ISH testing.

NYSDEC

The Rome Hatchery is a mile downstream from Delta Lake which was recently discovered to have zebra mussels. New York is now trying to find suitable stocking locations that already have dreissenid mussels present for fish reared at the hatchery. In the next five years, money will need to be spent to remove zebra mussels from the hatchery. The intake line from the lake will be shut off. The hatchery is currently fed with 1500 gallons per minute of spring water and 4000 gpm from Delta Lake. The recirculation system will need to be updated to accommodate that difference. The problem is the spring is only at 54 degrees Fahrenheit at its coldest so the brook trout Romiskany strain will need to be moved off site to hatcheries with colder waters.

14. Meeting Recap

Summer 2020 meeting:

Thunder Bay, ON. August 5-6, 2020

Winter 2021 meeting:

Windsor, ON. February 2-3, 2021.

Summer 2021 meeting:

Charlevoix, MI

15. Guest contact information

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GREAT LAKES FISH HEALTH COMMITTEE
TECHNICAL ADVISORS

February 2020

Bacteriology

Diane Elliot (U.S. Geological Survey)
Hui-Min Hsu (Wisconsin Veterinary Diagnostic Laboratory)
Thomas Loch (Michigan State University)

Virology

James Winton (U.S. Geological Survey)
Tom Waltzek (University of Florida)

Molecular

Nick Phelps (University of Minnesota)
Sharon Clouthier (Fisheries and Oceans Canada)

Nutrition

Wendy Sealey (U.S. Fish and Wildlife Service)
Ann Gannam (U.S. Fish and Wildlife Service)

Quantitative Fish Health Data Analysis

Dominic Travis (University of Minnesota)
Travis Brenden (Michigan State University)

Epidemiology

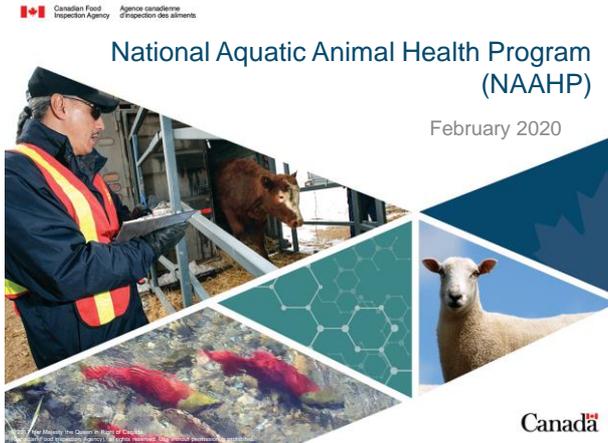
Lori Gustafson (U.S. Department of Agriculture)

Parasitology

David J. Marcogliese (Environment Canada)

Thiamine Deficiency

Jacques Rinchar (SUNY Brockport)
Don Tillitt (USGS)



Overview of the NAAHP

- Canadian Food Inspection Agency (CFIA), under the *Health of Animals Act*, implements the NAAHP and is the Competent Authority for aquatic animal health, and animal welfare [OIE also addresses antimicrobial resistance issues]
 - prevents introduction and spread of diseases
 - diagnostic laboratory and research support provided by Fisheries and Oceans Canada (DFO)
- Addresses aquatic animal diseases of finfish, molluscs and crustaceans
 - applies to wild and farmed aquatic animals
 - applies to emerging diseases (risk intelligence; identification of impacts; evaluation of CFIA's responsibility/role)
- Consistent with international standards set by the World Organisation for Animal Health (OIE)

NAAHP Components

- Foreign aquatic animal disease controls
- Domestic aquatic animal disease controls
- Import controls
- Export certification
- Science support
 - National Aquatic Animal Health Laboratory System/Network Laboratory System
 - Research
 - Surveillance and Epidemiology
 - Risk Assessment

Foreign Aquatic Animal Disease (FAD) Control Programs

- CFIA currently controls 24 foreign aquatic animal diseases.
 - Import controls to prevent introduction into Canada
 - Hazard specific plans and disease factsheets for management of FADs
 - Eradication (when feasible) or containment of disease spread
 - Disease response in collaboration with other government partners, industry, stakeholders, and rights holder groups

Domestic Disease Control Programs

- CFIA must be notified (both domestic and exotic diseases)
- Disease response – hazard specific plans
 - Current diseases are not human health or food safety issues
 - CFIA works primarily with the provinces, municipalities, and industry (aquaculture); but also with other federal departments and rights holder groups
 - CFIA confirms the suspect disease and reports to Canadians and the OIE
 - Non-enzootic areas: goal is eradication
 - Enzootic areas: goal is geographic containment; prevention of significant impacts on wild fish

Domestic Disease Control Programs

- Domestic Movement Control Program
 - Issuance of permissions: test and/or disinfect and ship; closed premises
 - Compartment recognition
- Based on declaration of zones, susceptible species
 - Recognized compartments are declared as free areas



Aquatic Import Program

- Regulates the import of ALL aquatic animals into Canada
 - Non-susceptible aquatic animals – declaration requirements
 - Susceptible aquatic animals – import requirements
 - <http://www.inspection.gc.ca/animals/aquatic-animals/diseases/susceptible-species/eng/1327162574928/1327162766981>



Susceptible species of aquatic animals

- Canadian importers require an Aquatic Animal Health Import Permit from the CFIA
- Required documentation from exporting country:
 - Zoosanitary export certificate negotiated with the CFIA
- Conditions on export certificate are based on
 - Species and the diseases that it is susceptible to
 - Health status of the origin
 - Health status of the destination
 - Commodity type (i.e. live vs. dead)
 - End use of the aquatic animals (i.e. culture vs. further processing)

Regulated End Uses For Import

- Aquarium - commercial
- Aquarium - private
- Live bait
- Dead bait
- Canadian good returning to Canada
- Ceremonial or religious use
- Culture
- Diagnostic testing
- Display in a zoo or public aquarium
- Outdoor holding unit – commercial
- Outdoor holding unit – private
- Feeding aquatic animals to aquatic animals
- Food service
- Further processing for human consumption
- In-transit
- Impoundment
- Manufacturing feed for aquatic animals
- Non-viable, for decorative purposes
- Research and education
- Retail use
- Show or exhibition
- Stocking, enhancement or refresh
- Traveller's and personal use not for resale or distribution

Aquatic Export Program

- Based on OIE and requirements from the importing country
- CFIA provides export certification for shipments when required by the importing country
- Support for export certification:
 - national level import controls
 - domestic aquatic animal health program
 - Surveillance for regulated diseases
 - mandatory notification of OIE and diseases of concern to Canada

National Aquatic Animal Health Laboratory System (NAAHLS)

- DFO provides:
 - Diagnostic Services
 - Research
 - Scientific Advice focusing on sampling and testing methodologies
- National Coordination – Ottawa
- Regional Laboratories
 - Fresh Water Institute- Aquatic Animal Health Laboratory, Winnipeg, MB (FWI-AAHL)
 - Pacific Biological Station - Aquatic Animal Health Laboratory, Nanaimo, BC (PBS-AAHL)
 - Gulf Fisheries Centre - Aquatic Animal Health Laboratory, Moncton, NB (GFC-AAHL)
 - Gulf Biocontainment Unit - Aquatic Animal Health Laboratory, Charlottetown, PEI (GBU-AAHL)

Diagnostics

- Test methods:
 - qPCR/PCR, Virus Isolation, Histology
- Quality Management - ISO 17025
 - FWI-AAHL, GFC-AAHL, PBS-AAHL
- Laboratory Information Management System (LIMS)
- Biocontainment
 - CFIA Containment Standards for Handling Aquatic Animal Pathogens
 - Level 2 *in-vitro* (FWI-AAHL, GFC-AAHL, PBS-AAHL)
 - Level 3 *in-vivo* (GBU-AAHL)

NAAHP Research

- Centre for Aquatic Animal Health Research and Diagnostics (CAAHRD)
- Current research priorities:
 - Test method validation as per OIE validation pathway
 - Improvements to laboratory processes
 - Level 3 *in-vivo* laboratory used for disease challenge trials

Network Laboratories

- Policy on approval of external laboratories for the NAAHP (<http://inspection.gc.ca/animals/aquatic-animals/exports/external-laboratories/eng/1473978994666/1473979063533>)
 - Accreditation to ISO 17025 standard
 - NAAHLS test method or CFIA-approved method that is on scope for accreditation
 - Maintain specific performance standards
 - Certification to at least AQC2 *in vitro* biocontainment standard

Surveillance and Epidemiology

- Designs and coordinates disease surveillance in cultured and wild aquatic animal species to (use of disease risk maps to target surveillance):
 - enable early detection of disease;
 - detect disease in specific geographic areas;
 - gather the required data used to evaluate the likelihood of disease freedom; and
 - support trade requirements
- Gathers data in collaboration with multiple stakeholder and rights holder groups
- Analyzes the data in the context of disease freedom for aquatic animal diseases that meets the *Health of Animals Regulations* and is consistent with international standards set by the OIE
- Samples collected under the surveillance program are tested by DFO-NAAHLS or a CFIA-approved network laboratory
- Results of surveillance activities are shared through semi-annual reports
 - can be requested through AquaSurv@inspection.gc.ca and CAHSS
- Canadian Animal Health Surveillance System (CAHSS) is an initiative with broad based collaborative support of industry and governments. It has been designed to fill the need for strengthened animal health surveillance in Canada. <https://www.cahss.ca/>
 - Individual network groups are self-organizing and self-governing; linked through shared purpose and principles
 - Aquatic network page <https://www.cahss.ca/surveillance/aquatic/>

Animal Health Risk Assessment Unit (AHRA)

Application of appropriate science advice tools:

- Literature review
- Hazard identification
- Likelihood assessment
- Full risk assessment
- Country evaluation

Risk assessment is a structured, systematic process to determine the likelihood of the occurrence of an event and the likely magnitude of the consequences following exposure to a hazard.

- qualitative or quantitative, and variability, uncertainty and assumptions are documented.

Country evaluation is a modular evidence-based one-health suite of tools assessing a foreign country's aquatic animal health status and programs.

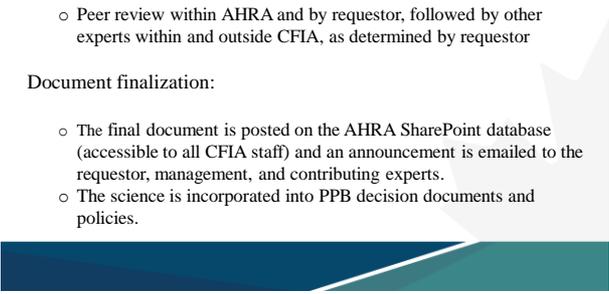
AHRA Document Review and Availability

Documents are requested by: Policy and Programs Branch, CFIA

The review process consists of:

- Peer review within AHRA and by requestor, followed by other experts within and outside CFIA, as determined by requestor

Document finalization:

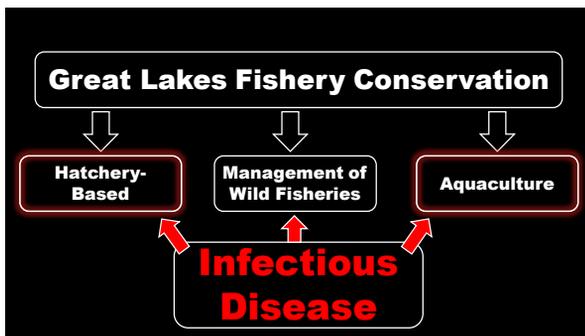
- The final document is posted on the AHRA SharePoint database (accessible to all CFIA staff) and an announcement is emailed to the requestor, management, and contributing experts.
 - The science is incorporated into PPB decision documents and policies.
- 

MSU-AAHL Research Updates

Dr. Thomas P. Loch

Michigan State University – Aquatic Animal Health Laboratory
Depts of Fisheries & Wildlife & Pathobiology & Diagnostic Investigation
Michigan State University

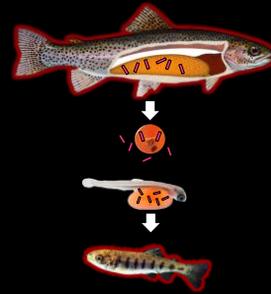
Email: lochthom@msu.edu



Bacterial Coldwater Disease

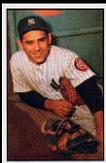


Vertical Transmission of *F. psychrophilum*



CARE AND DISEASES OF TROUT

By H. S. DAVIS
Revised edition, 1946



Why Have Adequate BCWD Prevention & Control Measures Yet to be Realized?

Virulence?



Vaccination?

Antibiotic Susceptibility?

Flavobacterial Diversity and its Effect on Disease in Aquaculture

T.R. Loch^{1,2}, D. Call³, K. Cain⁴, G. Wiens⁵, C. Knupp¹,
T.J. Bruce⁴, J. Ma⁴, M. Faisal^{1,2}

¹Department of Fisheries & Wildlife, Michigan State University, East Lansing, MI; ²Department of Pathobiology & Diagnostic Investigation, Michigan State University, East Lansing, MI; ³Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA; ⁴Department of Fish and Wildlife Sciences, University of Idaho, Moscow, ID; ⁵United States Department of Agriculture - Agricultural Research Service, Kearneysville, WV 25652



F. psychrophilum from Across the USA

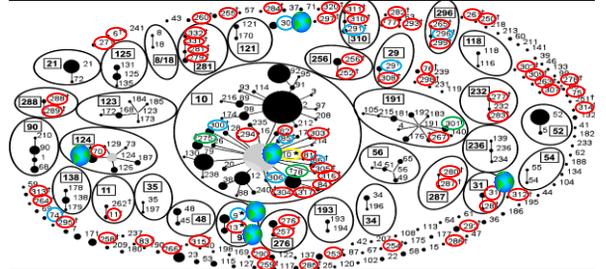


- >470 *Fp* isolates (& growing!)
- 23 States, 1 Province
- 4 decades (1981-2019)

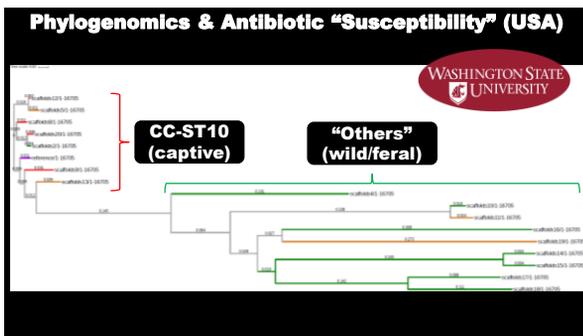
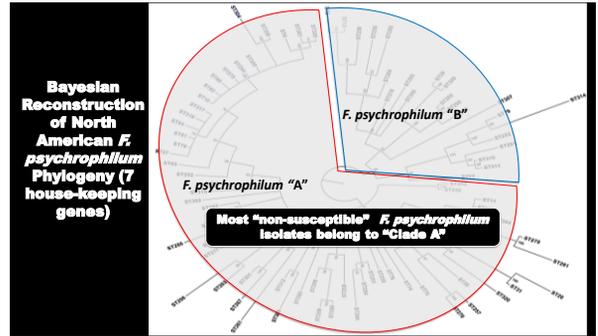
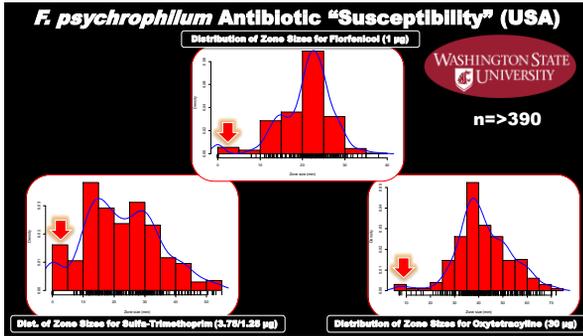


Christopher Knupp, PhD Student

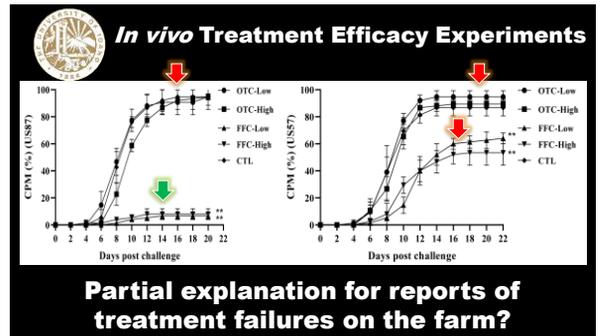
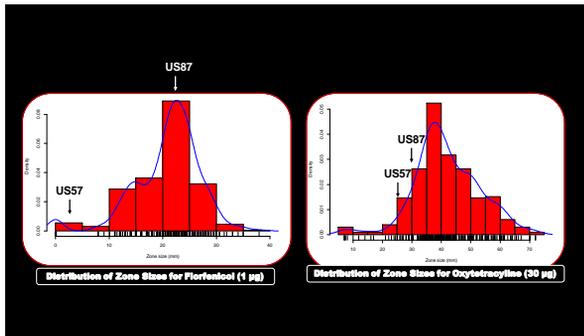
F. psychrophilum Genetic Diversity (Multilocus sequence typing)



Woods, C., Wiens, G.D., Faisal, M., Call, S.B., Cain, K.A., Wootton, F., Van Wert, E., Yonushonis, C., Ferguson, J.M., Hootman, D., Hill, D.H., Baker, S.B., Olson, L., Loch, T.R., 2019. Long-term analysis of *F. psychrophilum* multilocus sequence typing genotypes recovered from North American salmonids indicates that both newly identified and re-emerged clonal complexes are associated with disease. *Appl. Environ. Microbiol.*, 85.



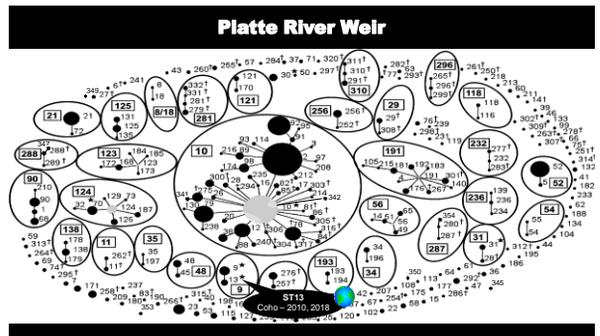
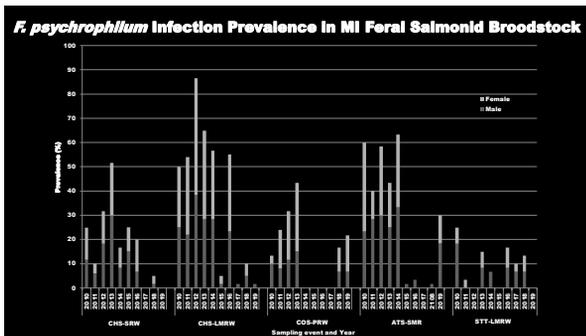
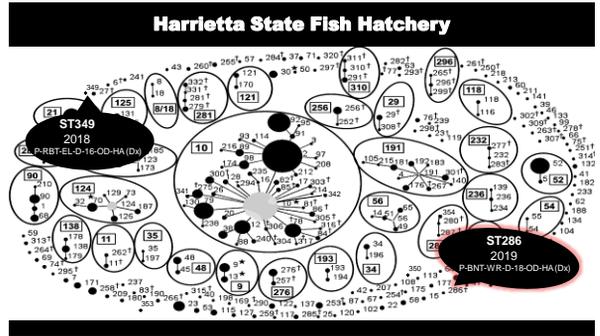
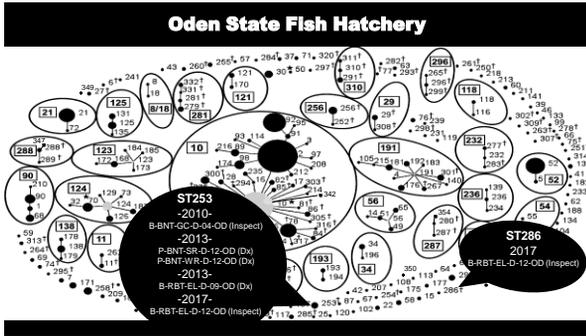
Is Observed "Reduced Susceptibility" Clinically Relevant?



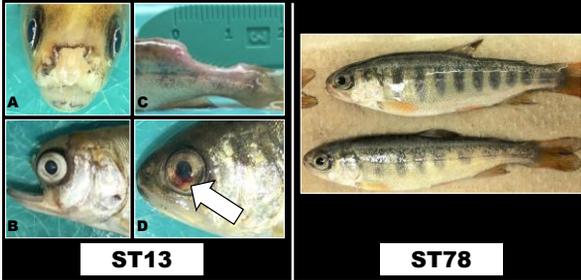
Conclusions (Thus Far)

- Intraspecific *F. psychrophilum* diversity is substantial, but top “troublemakers” in the USA (& GL) identified
- Evidence for transmission via egg trade
- “Troublemakers” more likely to “resist” approved antibiotics (mechanism(s) = ???)
- Ongoing studies: vaccine development, improved egg disinfection, and elucidating *F. psychrophilum* disease ecology in hatcheries/aquaculture

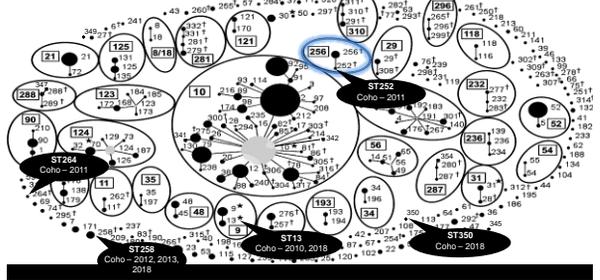
***F. psychrophilum* Epidemiology: Great Lakes States**



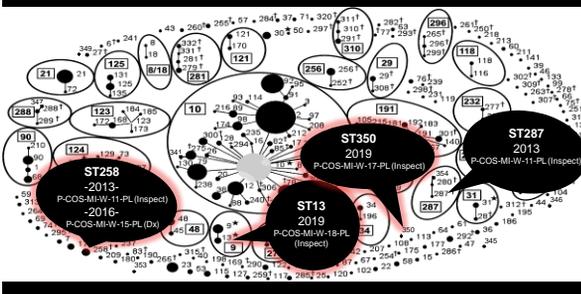
Reminder from Summer Section Meeting



Platte River Weir

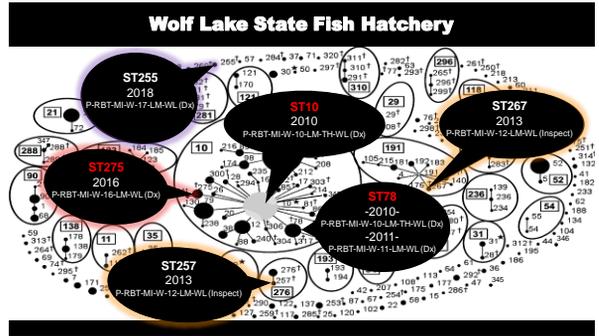
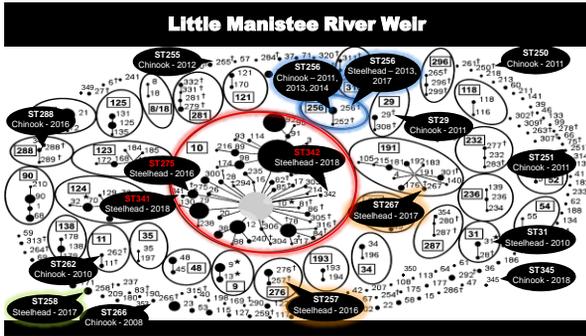


Platte River State Fish Hatchery



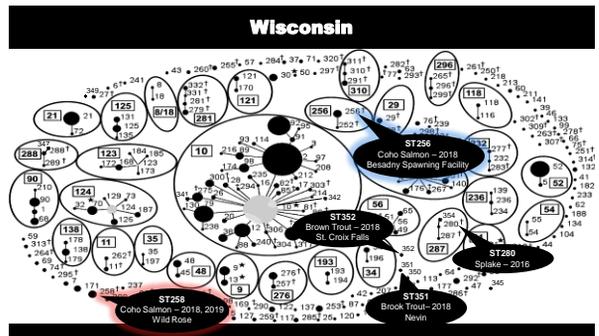
PRW/PRSFH

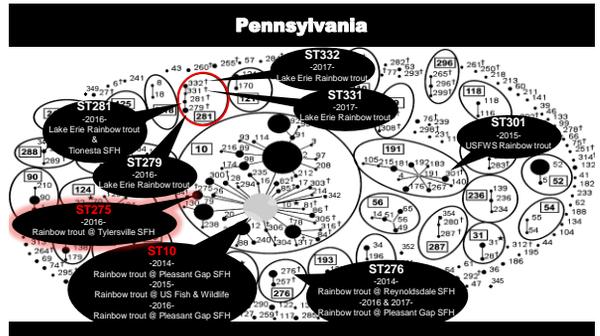
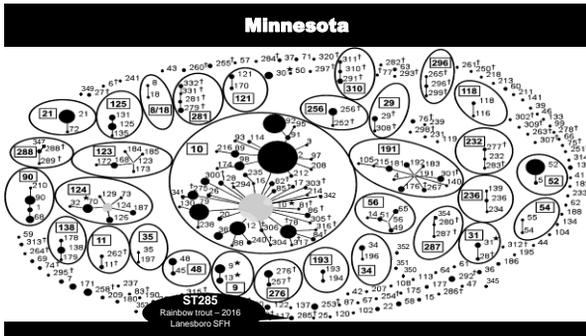
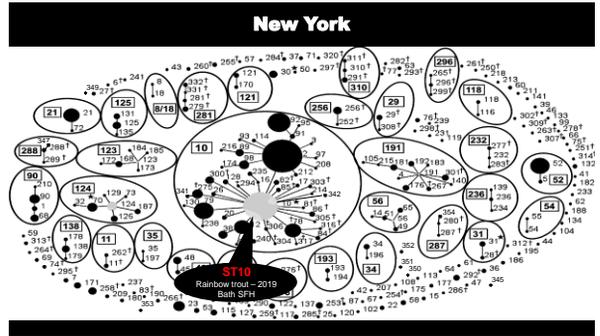
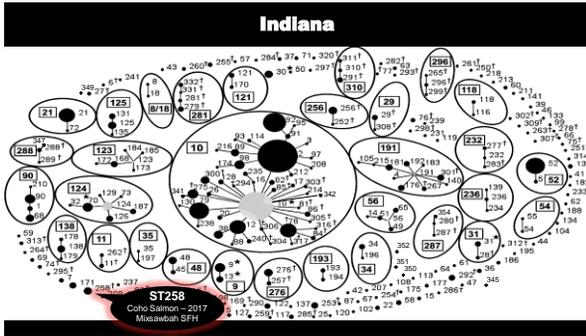
- **Diversity of *F. psychrophilum* genotypes**
 - Majority strongly associated with COS (here & elsewhere)
 - Some (ST13) match Japanese, European, and Washington strains
 - Some “indigenous” (ST258, CC-ST252/256) to Great Lakes?
- **Although other sources (e.g., water) can’t be ruled out, evidence suggests repeated egg-associated transmission?**
- **At least 1 isolate of ST13 serologically distinct from up and coming BCWD vaccine**



WLSFH

- **Diversity of *F. psychrophilum* genotypes**
 - Almost all belong to “RBT” clonal complexes
 - Half belong to CC-ST10
- **Evidence for infections that persist post-stocking? Relation to life history?**
- **Evidence for egg-associated transmission; possibly other sources too?**





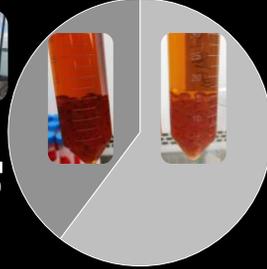
Still Unanswered Questions, But Some Epidemiological Clarity in MI Gained...

- Evidence for egg-associated *Fpsy* transmission for several hatcheries = need for effective egg disinfection
- Some *Fpsy* strains effective “perpetuators”
- Some *Fpsy* strains only recovered during “co-infections”
- We now need serological clarity (i.e., towards vaccine)

Flavobacteria Surviving Iodophor Egg Disinfection



69
Flavobacterium
isolates survived
400ppm (10 min)

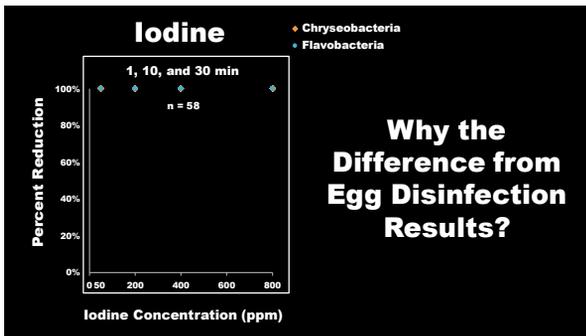
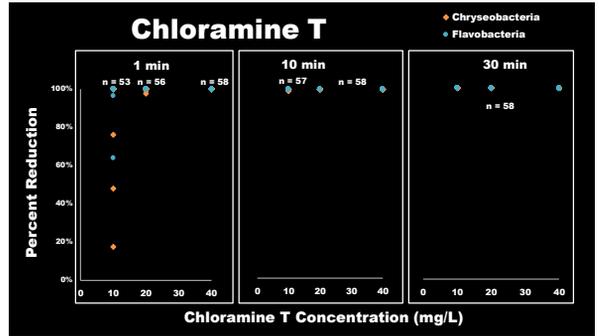
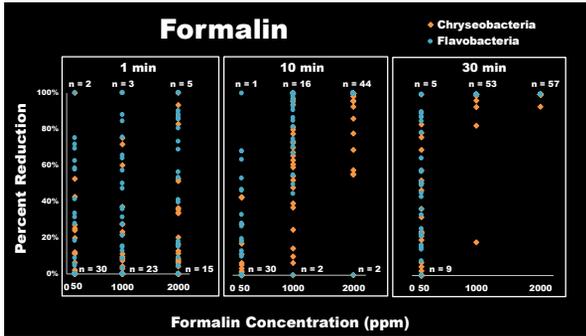


105
Flavobacterium
isolates survived
100ppm (10 min)

Ongoing Disinfectant Experiments

- Expose flavobacterial isolates to:
- 4 common aquaculture disinfectants
 - Formalin, Chloramine-T, Iodine, **Hydrogen Peroxide**
 - 3-4 concentrations per disinfectant
 - 3 exposure time points (1,10,30min)
- Colony counts → % Reduction compared to control





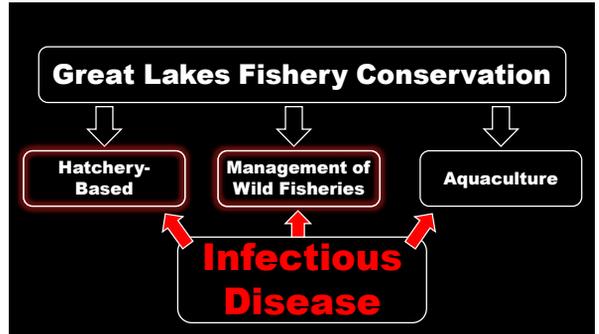
Biofilm?

Intra Ova
Transmission?

Dr. Terry Marsh

More Details to Come!

Questions/Discussion?



Great Lakes Lake Sturgeon



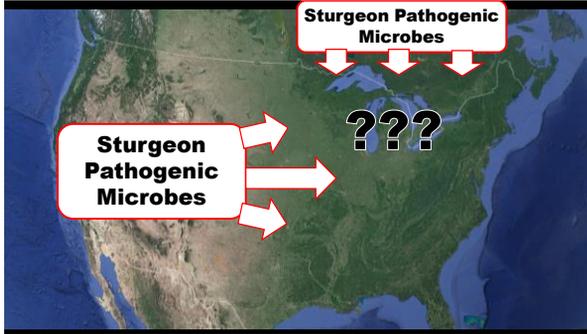
Infectious Diseases of
GL Lake Sturgeon

⇒ ???

Streamside Rearing of GL Lake Sturgeon



Mortality Events of
Unknown Cause(s)



Assessing the risk of emergent and endemic fish pathogens to Great Lakes lake sturgeon

Amber Johnston, Masters Student

Amber E. Johnston, Megan A. Shavaller, Kim T. Scribner, Esteban Soto, Edward A. Baker, Douglas Larson, Thomas P. Loch

Adults GL Lake Sturgeon Sampling Sites

Site	# Sampled (2019)
Black River	137
St. Clair River	76
Peshtigo River	19

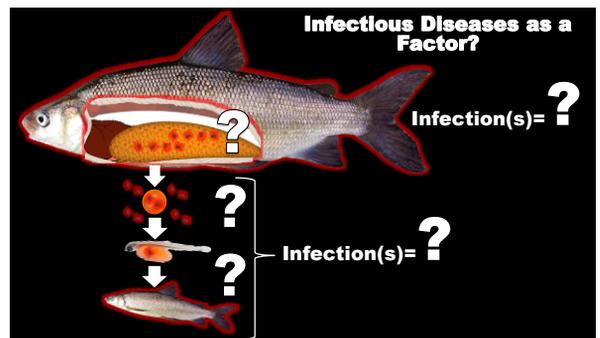
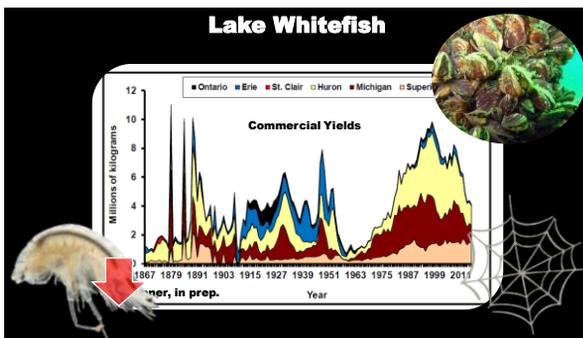
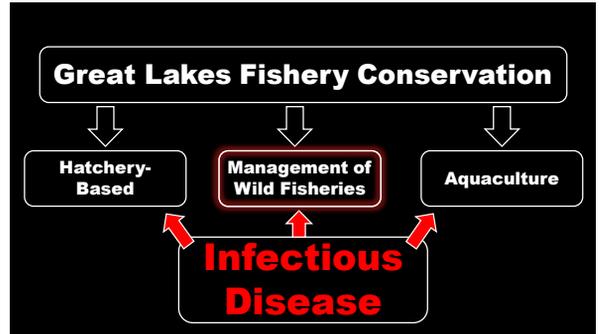
Blood & Tissue Biopsy Collection (Adults)

Virological, bacteriological, histopathological, molecular, hematological analyses



Conclusions Thus Far & What's Next?

- Gross signs of disease were observed in both adult & juvenile GL LST
- A multitude of microbes were recovered, the identity & significance of which are being determined



Investigating Infectious Diseases As A "Bottleneck" To Lake Whitefish Recruitment

Courtney E. Harrison, Travis O. Brenden, Mark P. Ebener, Chris K. Knupp, Michelle R. Van Deuren, Amber E. Johnston, Megan A. Shavaliar, & Thomas P. Loch



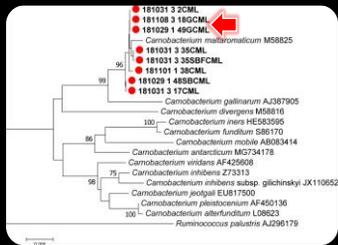
Courtney Harrison, Masters Student



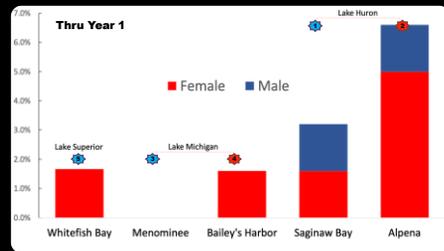
Spawning Phase LWF Collection



Pathological & Microbiological Analyses



Carnobacterium Infection Prevalence



Infectious Diseases in Juvenile LWF?



2019 Age-0 LWF Collections

- Bailey's Harbor (LM), n=150
- Thunder Bay, Alpena (LH), n=150
- Whitefish Bay (LS), n=24
- Caspville (LH), n=1
- Menominee (LM), n=0

What are their effects on juvenile LWF health?

Conclusions Thus Far & What's Next?

- Well-known fish pathogens recovered from gametes/reproductive of GL LWF for the first time
- A multitude of microbes were recovered, the identity & significance of which are being determined

Acknowledgements

Funding Agencies:

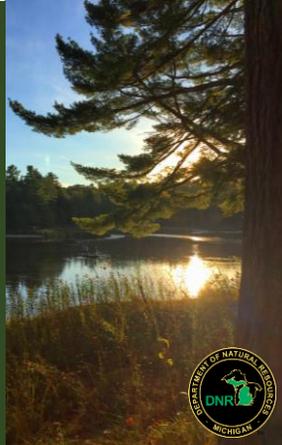
- MDNR Fisheries Division
- USDA - NIFA (2016-67015-24891, 2016-70007-25756)
- USFWS - GLFWRA (F19AP00229)
- GLFT (2018.1806)



- Gary Whelan, Martha VanAmberg, Ed Eisch, Jan VanAmberg, Aaron Switzer, Jim Aho, Randy Espinoza, Dan Sampson, Paul Stowe, Jon Jackoviale, Matt Hughes, Roger Grell, Seth Herbst, many others from MDNR Fisheries Div.
- Kim Scribner, Esteban Soto, Ed Baker, Doug Larson, Todd Wills, Jon Bauman, Michael Donofrio, Henry Quinlan, Glenn Miller, Andrew Briggs, Brad Utrup, Roy Beasley, Jeremy Maranowski
- Pierre Micolon, Giandla Doobald, Katre Osbourn, Danielle Van Vleet, Coja Yamaashita, Jayde Ferguson, Dave Neunhock, Hui-Min Hsu, Bridget Baker, Ling Shen, Geoff Grocock, Carl Smith, Danielle Godard
- Travis Brendan, Mark Ebener, Todd Williams, Sam McMurtry, Tony LeBlanc, Ralph Witcox, Todd Stuth
- Little Traverse Bay Bands of Odawa Indians
- Michelle Van Deuren, Dr. Megan Shavallier, & other past and present MSU - AAHL Colleagues

The EEDv Saga – Find me if you can

Gary Whelan
GLFHC Meeting
February 2020



History of EEDv Detections/Outbreaks at MSFH

- 1980s – Initial mortality events
 - Large scale mortalities
 - Depopulated and disinfected MSFH
- 2009-2010 - Detection
- 2012-2013 - Mortality
- 2017-2018 - Detection
- 2018-2019 – Mortality
- 2019-2020 - Detection



EEDv 2009 Detections

- Hendrick develops genetic test
 - CA SFH positive
 - WI Testing – Les Voigt - Bayfield SFH Strains positive 30/30 fish
- MI Testing
 - Ovarian Fluid – Kurobe (U-Cal Davis) – 9/30
 - 2001 Broodstock – 15/41 positive
 - 2004 Broodstock – 11/45 positive
 - Ovarian Fluid – Kurobe (U-Cal Davis) - 10/21
 - 2001 Broodstock – 3/10 positive
 - 2004 Broodstock – 4/10 positive
 - Similar to Bayfield SFH Strains
 - Primer questions on specificity



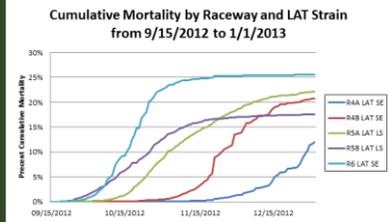
EEDv 2009 Detections

- WY Testing – Story SFH Positive
 - Fish were from Jenny Lake with no introductions since 1967
 - Hatchery received some Lewis Lake fish in 1980s from Saratoga NF
 - 98% match with Bayfield SFH fish



Appendix 4.

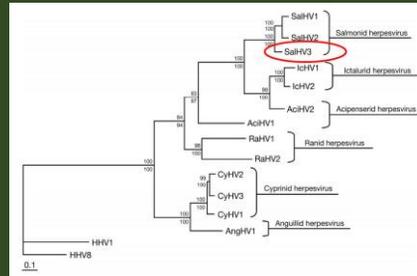
EEDv 2012-13



- 90,656 LAT Production Fish Lost



EEDv 2012-13 Strain Map

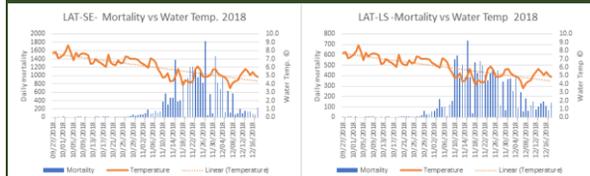


EEDv 2017-2018 Detection

- No unusual mortalities seen
- Samples run to examine for stocking
 - SE
 - February 52/60 positive – Ct mean = 27.1
 - March 44/60 positive – Ct mean = 29.3
 - April 18/60 positive – Ct mean = 32.2
 - May 33/60 positive – Ct mean = 32.5
 - LS
 - February 59/60 positive – Ct mean = 27.1
 - March 28/60 positive – Ct mean = 29.5
 - April 16/60 positive – Ct mean = 32.3
 - May 47/60 positive – Ct mean = 30.9



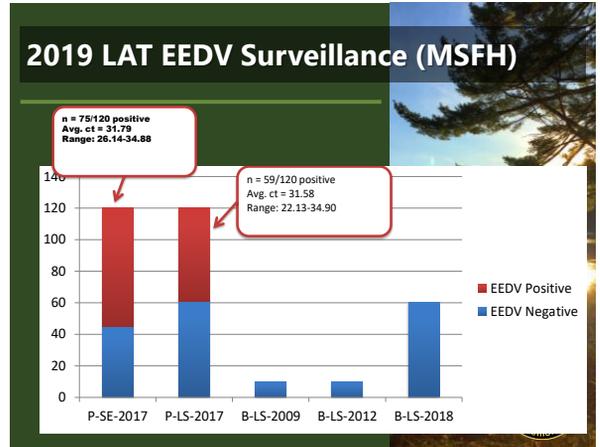
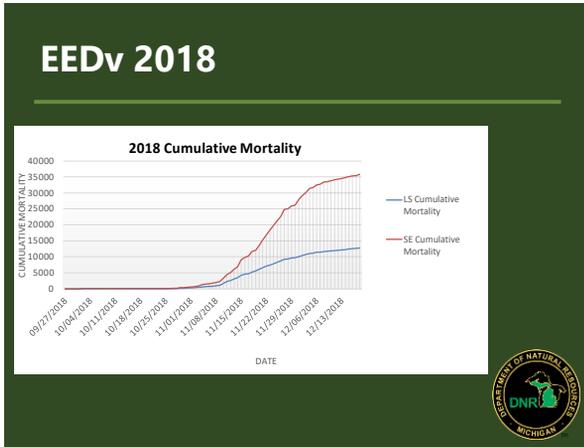
EEDv 2018



- 35,779 LAT Production Fish Lost



Appendix 4.



- ### Wild EEDv Surveillance – Kurobe
- 1988 - Bayfield, WI Skin + + 2/2
 - 2001 - Bayfield, WI Skin n/a + 0/5
 - 2003
 - Bayfield, WI Skin + + 1/1
 - Apostle Islands L Superior Ovarian fluid + - 3/10
 - 2006 - Bayfield, WI Skin - + 9/10
 - Apostle Islands L. Superior Ovarian fluid - - 2/6
 - 2007 Bayfield, WI Skin n/a - 2/11
- Michigan Department of Natural Resources logo

- ### Wild EEDv Surveillance
- 2010 - USFWS
 - Lake Michigan - **Positive**
 - Wisconsin waters, 20 LAT (4/20 positive via PCR, no sequencing)
 - Illinois waters, 20 LAT (6/20 positive via PCR, no sequencing)
 - Sturgeon Bay, WI, 20 LAT (7/20 positive via PCR, no sequencing; 3 of the positives were analyzed via Gavin Glenney's realtime PCR, 1/3 positive)
 - Mid-lake reef, 20 LAT (4/20 positive via PCR, no sequencing)
 - 2013 – MI DNR Lake Huron
 - 0/120 with PCR
- Michigan Department of Natural Resources logo

Appendix 4.

2018 Wild EEDv Surveillance

- Lake Huron – **Very Low Positive**
 - Northern Lake Huron – 0/10
 - Southern Lake Huron – 1/90 (low titers) – June 6, 2018
- Lake Michigan – **Low Positive**
 - Northern Lake Michigan (Arcadia) – 2/60 positive – May 29, 2018
- Lake Superior – **Positive**
 - Marquette Harbor - 58/60 positive – October 25, 2018
 - Ct values ranged from 22.8 – 34.3
 - average Ct=31.4; 2/60 w/ Ct >35



2018 Wild LAT Surveillance



2019 Wild LAT EEDv Surveillance

- Lake Superior
 - MN – August 12, 2019 - 1/60 positive
 - Isle Royale – May 30, 2019 - Lean – 0/60
 - MQT Harbor – October 9, 2019 - 0/60
 - Big Rock – June 24, 2019
 - Fat – 0/58
 - Lean – 0/60



2019 LS Wild LAT Surveillance



Disease Control Direction

- Treat it like BKD and use similar procedures
- Vaccine development
- Develop methods to cull infected adults
- Determine positive locations in Michigan waters to allow stocking of fish without clinical disease
- If unsuccessful, develop new broodstock



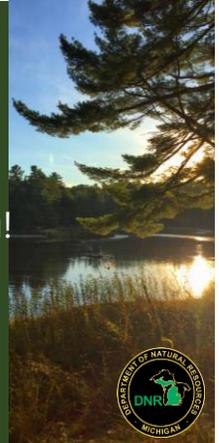
WALK SOFTLY



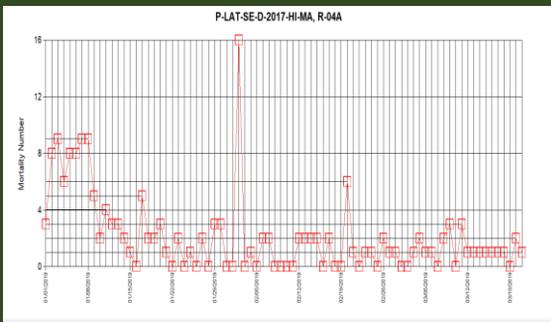
AND CARRY A BIG FISH

Gary E. Whelan
Michigan DNR
whelang@michigan.gov
517-284-5840

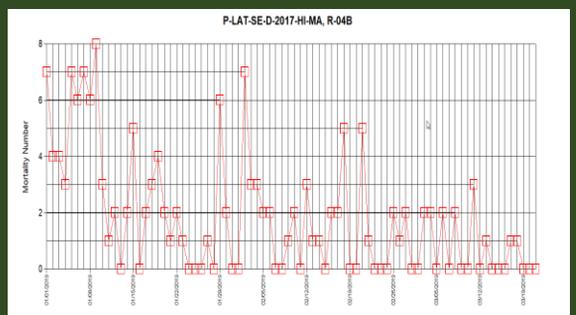
Thank You!



EEDv 2019



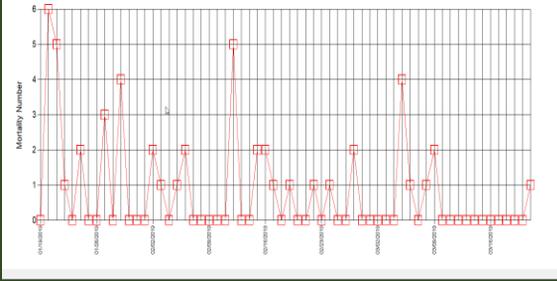
EEDv 2019



Appendix 4.

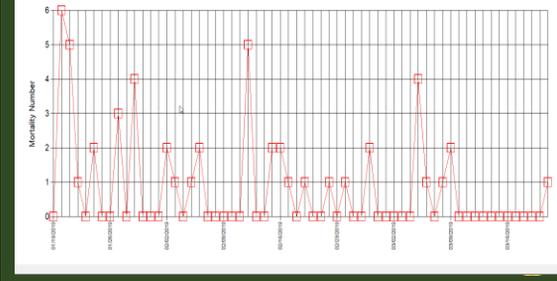
EEDv 2019

P-LAT-LS-D-2017-MA, R-05A



EEDv 2019

P-LAT-LS-D-2017-MA, R-05A



Appendix 5.

Fisheries and Oceans Canada / Pêches et Océans Canada

Environmental DNA: a powerful tool for monitoring

Charise Dietrich and Sherry Walker
GLFHC, February 4-5, 2020

Canada

Fisheries and Oceans Canada / Pêches et Océans Canada

Presentation overview

- Introduction to environmental DNA (eDNA)
- Status of eDNA at DFO:
 - National DFO Environmental DNA Workshops 2018 & 2019
 - National DFO eDNA Technical Working Group
 - Development of standards for eDNA (CSAS and CSA)
- eDNA research and management applications at DFO
- Challenges and opportunities of using eDNA
- Vision for eDNA at DFO

2

Fisheries and Oceans Canada / Pêches et Océans Canada

What is eDNA?

- Environmental DNA (eDNA): molecules of DNA that are shed into the environment by organisms via their feces, skin cells, gametes, etc.
- eDNA can be analyzed from water/sediment to assess species presence (indirect detection).

eDNA technologies are increasingly being used at DFO and globally to monitor aquatic organisms and inform management decisions

3

Fisheries and Oceans Canada / Pêches et Océans Canada

Two ways to analyze eDNA

Targeted eDNA detection

eDNA Metabarcoding

Targeted detection

Quantification

Community monitoring

Biodiversity

4

Appendix 5.

Strengths and Limitations of eDNA

eDNA technologies can:

- Allow for low-cost, broad-scale monitoring
- Monitor invasives, pathogens, viruses: early detection, expansion fronts, new introductions
- Detect rare or cryptic species and life stages (e.g., larvae, viruses)
- Detect shifts in community composition
- Be used where conventional sampling is logistically difficult or hazardous
- Inform/target conventional sampling
- Cause minimal habitat and species disturbance
- Engage citizen scientists for sample collection

eDNA detection cannot assess:

- Sex or sex ratios;
- Reproductive status;
- Age/size class;
- Population size;
- Condition; and
- Whether the DNA is from a live or recently dead animal.

5

National DFO eDNA Workshop 2018

- Objectives:
 - Engaged clients and scientists across DFO, identified information gaps, opportunities, technical challenges, and DFO research needs;
 - Reviewed a State of Knowledge paper on eDNA (Baillie et al. 2019)
- Key findings:
 - AIS and SAR programs are already using eDNA to inform management
 - Minimum standards are needed (data collection, analysis, and interpretation)
- Aquatic Animal Health Client needs:
 - *For regulation: QA/QC*, integrate eDNA and other evidence, 'gold' standard
 - *For surveillance in wild populations*: eDNA has minimal impact (species at risk, etc.), and can circumvent difficult sampling logistics
 - *For disease outbreak management*: assess pathogen spread, effectiveness of cleaning and disinfection, and appropriate following time

6

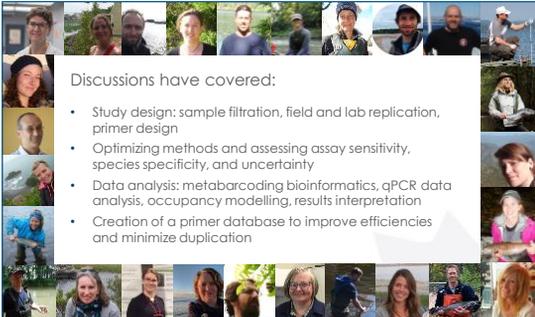
National DFO eDNA Workshop 2019

- The Workshop included research talks, management case studies, an eDNA-specialist panel discussion, and an eDNA training course
- Key findings:
 - qPCR assays are ready for implementation by management (presence)
 - eDNA metabarcoding can detect broad-scale community shifts and simultaneously detect many species
 - Robust study design is critical for meaningful research findings and effective management decisions (species ecology is key)
 - Management wants to implement eDNA, but lab infrastructure/capacity and education are needed



7

National eDNA Technical Working Group



Discussions have covered:

- Study design: sample filtration, field and lab replication, primer design
- Optimizing methods and assessing assay sensitivity, species specificity, and uncertainty
- Data analysis: metabarcoding bioinformatics, qPCR data analysis, occupancy modelling, results interpretation
- Creation of a primer database to improve efficiencies and minimize duplication

8

Appendix 5.

eDNA Standardization

Two parallel Canadian efforts towards eDNA standardization:



Canadian Standards Association (CSA):

- define consistent terminology for eDNA reporting across applications and sectors
- set baseline requirements for the generation and reporting of eDNA results
- increase the reliability of eDNA survey data and interpretations to increase regulator and public confidence

Publication in 15-18 months



CSAS Science Response:

- define the scientific terms and concepts associated with eDNA technologies and techniques
- provide interim minimum reporting guidelines and reporting template ...
- Provide an accompanying guidance document ... explanation of associated limitations, caveats and best practices ...

Science advice by April, 2020

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eDNA research at DFO

- eDNA research underway in all Regions (often collaborative)
- Current project objectives include:
 - Clarifying SAR distributions (Brook Floater, Salamander Mussel, etc.)
 - Detecting AIS (Green crab, Striped Bass, dreissenids, etc.)
 - Monitoring biodiversity (coastal, Arctic, deep sea, Marine Protected Area)
 - Detecting AMR near aquaculture facilities
 - Modelling eDNA dynamics/ecology (e.g., dispersion, persistence)
 - Correlating eDNA to species abundance
 - Assessing the ecological risks of vessel traffic and port development



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eDNA management applications

- eDNA analysis can inform monitoring and management:
 - Test ballast water for AIS (IMO Convention)
 - Monitor species distributions and biodiversity
 - Monitor effectiveness of eradication and control measures
 - Broad-scale community analysis (metabarcoding) to set baselines
 - Assays for regulated species, emerging species, and species at risk
 - Tunicates, Smallmouth bass, Green crab, Brook floater, Irish Moss
 - Preserve and archive samples for forensic analysis



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Opportunities and challenges

- eDNA sampling can be used to:
 - Trigger further sampling (early detection)
 - Target conventional sampling to optimize spending
 - Fill gaps when conventional sampling is unsafe or impractical
 - Complement conventional sampling (paired sampling for increased sensitivity, detection of multiple species, etc.)
- Resource Manager feedback:
 - Management action is expensive and can have ecological impacts, so uncertainty must be quantified (e.g., likelihood of false positives)
 - Advice needed: how should management respond to eDNA positives?
 - Management wants to implement eDNA, but standards, lab infrastructure/functional capacity, and education are needed

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Moving Forward

Needs	Solutions
Minimum reporting standards for clients to evaluate study quality and data reliability	<ul style="list-style-type: none"> CSAS Science Response CSA Standard
Research to validate assays for new applications (e.g., new species, habitat, region), to test new technologies, and to fill knowledge gaps (ecology of eDNA)	<ul style="list-style-type: none"> Identify designated funding for eDNA Renew Genomics Strategy
eDNA analysis and advice to support management, policy, and regulatory decisions; but DFO lacks infrastructure and functional capacity to meet demand	<ul style="list-style-type: none"> Establish eDNA capacity and designated accredited labs Seek certification

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Thank you

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Sherry Walker
 National Manager, Biotechnology and Genomics
 Sherry.Walker@dfo-mpo.gc.ca

*Recorded course on eDNA available upon request

14

Epizootic Epitheliotropic Disease Virus (EEDV) Research Updates

Thomas P. Loch

Michigan State University – Aquatic Animal Health Laboratory
Depts of Fisheries & Wildlife and Pathobiology & Diagnostic Investigation
Michigan State University

Email: lochthom@msu.edu

Epizootic Epitheliotropic Disease Virus (EEDV)

Bradley et al. 1989

15 Million Lake Trout Dead (3 GL States, 7 Hatcheries)

Photo Credit: Sue Marcquanski, Bayfield, WI, 1987

“Quiet”, Until... 2012, 2017-2019

MSFH, MI

% CUMULATIVE MORTALITY

Date

- **Substantial LAT losses**
- **Stocking modifications**
- **Detections in captive broodstock**
- **Reproductive fluid transmission?**

Great Lakes Fishery Trust

DEPARTMENT OF NATURAL RESOURCES
DNR
MICHIGAN

Appendix 6.

Recent EEDV Research Advances

Journal of Aquatic Animal Health

A Quantitative Polymerase Chain Reaction Assay for the Detection and Quantification of Epizootic Epitheliotropic Disease Virus (EEDV, Salmonid Herpesvirus 3)

David W. Grunney, Patricia A. Birkhead & John G. Cole

Journal of Virological Methods

Development of a loop-mediated isothermal amplification assay for the detection and quantification of epizootic epitheliotropic disease virus (salmonid herpesvirus-3)

Wenli Dong¹, Yuanli Shen¹, Xian Zhang¹, Xian Zhang¹

- EEDV *in vivo* infection (& virus replication) model
- EEDV genome

Species Susceptibility to EEDV

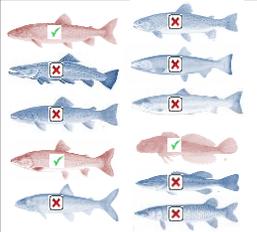


- **11 Great Lakes species/strains**
 - Salmonidae (LAT-LS, LAT-SE, BKT, SPL, BNT, RBT, ATS, COS, LHR)
 - Cottidae (MOS)
 - Centrarchidae (LMB)
 - Esocidae (MUS)
- **Injection challenge**
 - Low dose (4.75×10^3 copies/fish)
 - High dose (4.74×10^5 copies/fish)



EEDV Species Susceptibility

Species tested	Experiment length (days)
Eastern brook stickleback	65
Fathead minnow	100
Rock bass	93
White sucker	62
Brook trout	55
Roundnose dace	56
Atlantic salmon	45
Largemouth bass	23
Cape cod scup	22
Menhaden	13
Sablefish	56
Atlantic herring	56



- **No EEDV-associated mortality or clinical disease in BKT, BNT, RBT, ATS, COS, LHR, LMB or MUS.**
 - No EEDV detection via qPCR

EEDV Susceptibility in LAT Strains

	Strain LS	Strain SE
EEDV prevalence	10/10 (HD)	3/10 (HD)
Mortality	80% (HD), 0% (LD)	0% (HD), 0% (LD)
Average virus load (virus copies/mg skin) in dead	5.45×10^8 (n=8)	NA
EEDV prevalence-Survivors	2/2	3/10
Average EEDV load in survivors (virus copies/mg skin)	3.62×10^4 (66 days)	4.03×10^7 (100 days)

Varying EEDV Susceptibility by Strain?

Appendix 6.

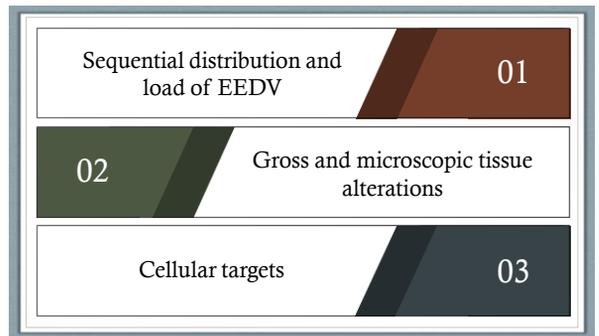
EEDV Susceptibility- Splake



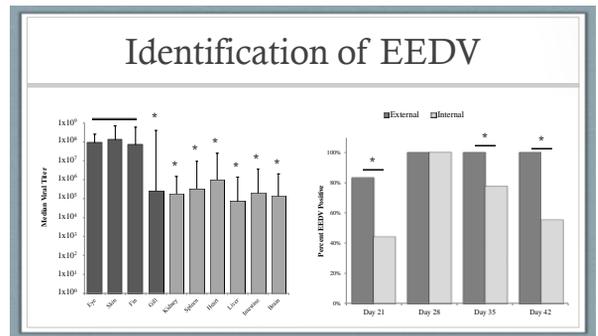
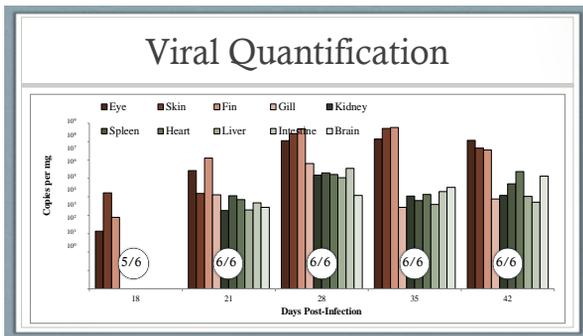
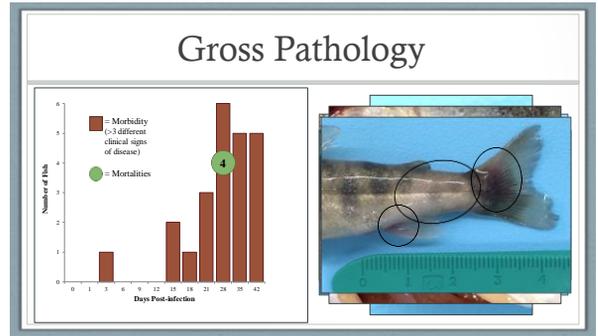
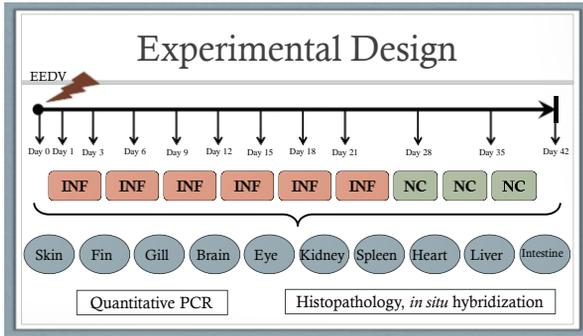
- **High dose:**
 - Some disease signs consistent w/ EED early on (e.g., lethargy, gill pallor, skin discoloration)
 - 30% mortality (3-10 days PI)
 - 1/10 fish EEDV-positive via qPCR (day 10; 3.86×10^3 Virus copies/mg skin)
- Potential as short term EEDV reservoir?

EEDV Susceptibility- Mottled Sculpin

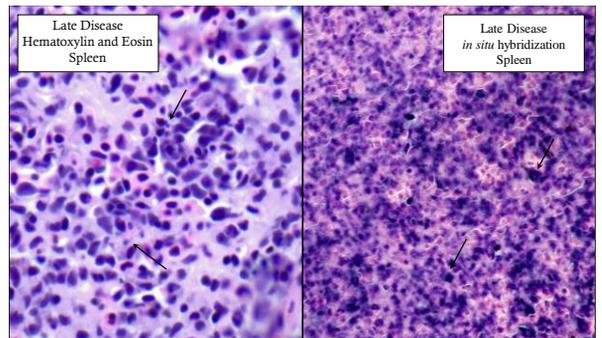
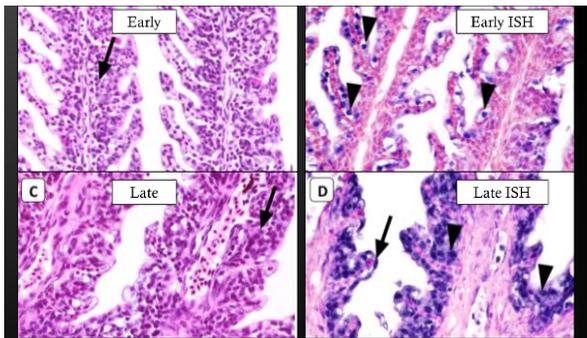
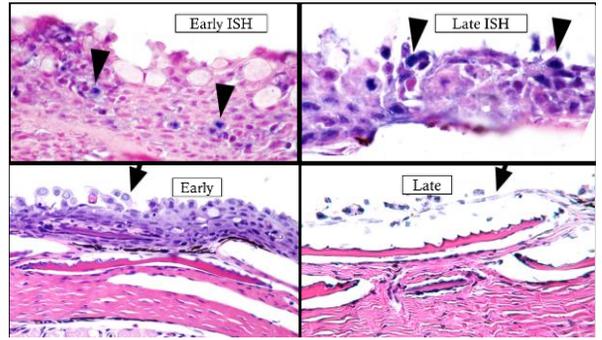
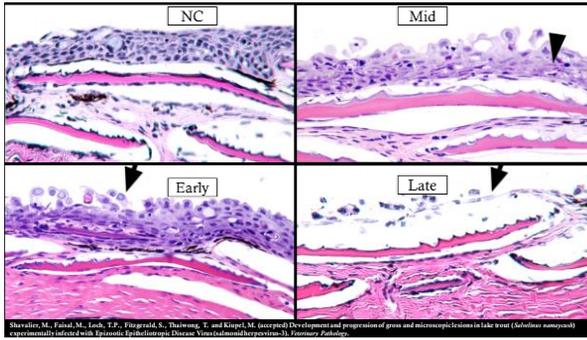
- **High dose:**
 - 60% mortality (no signs of EED)
 - 0/10 fish EEDV-positive via qPCR
- **Low dose:**
 - 50% mortality (no signs of EED)
 - 1/10 fish EEDV-positive via qPCR (day 8 PI; 1.10×10^2 virus copies/mg skin)
- **Likely do not support EEDV replication**



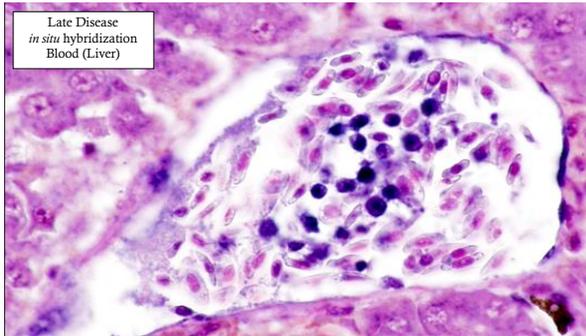
Appendix 6.



Appendix 6.



Appendix 6.



Pathology and Tissue Tropism – Conclusions



Primary viral targets

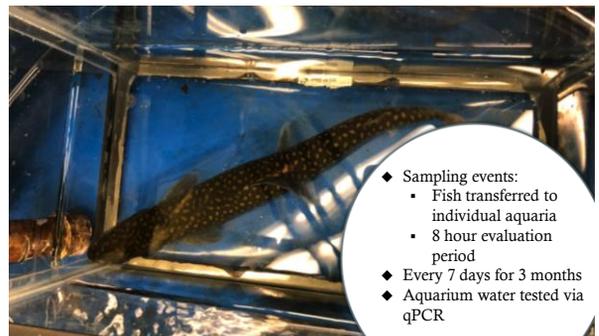


Viremia and systemic disease

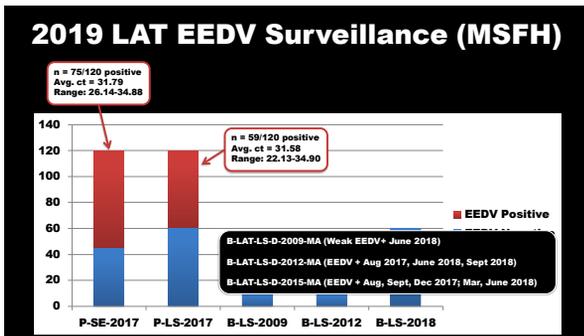
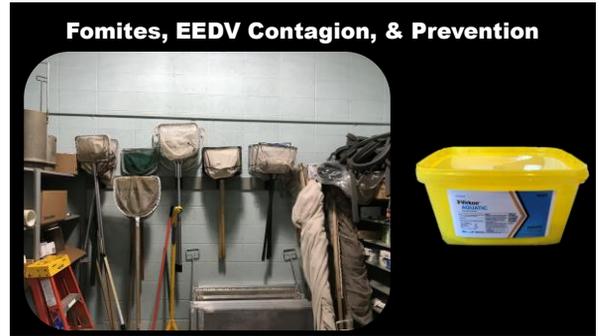
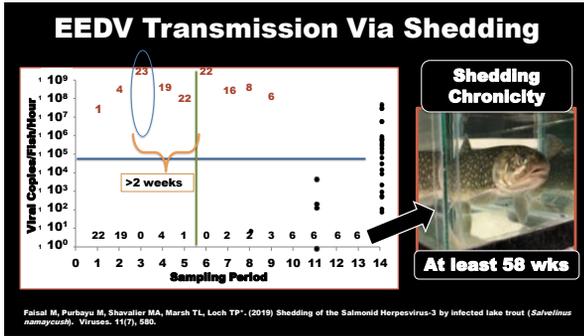


Practical applications

EEDV Shedding



Appendix 6.



What is Prevalence of EEDV in the GL & Can EEDV-Free LAT Broodstock be Developed from Wild LAT?

Appendix 6.



What We Don't Have (but Need)

- Validated EEDV assays for screening reproductive fluids
- EEDV vaccine!
- Therapeutants for reducing EED-associated losses
- An understanding of how tagging/clipping contributes to EEDV transmission

Improving Wild And Hatchery-Reared Lake Trout Health Via Development Of Novel Epizootic Epitheliotropic Disease Prevention Methods

Dr. Thomas Loch, Dr. Thomas Waltzek, Dr. Megan Shavliier, Dr. Kutti Subramaniam, Dr. Matti Kiupel, Mr. Gary Whelan

Dear Dr. Loch,

Congratulations! The Board of Technical Experts has recommended your proposal titled "Improving wild and hatchery-reared lake trout health via development of novel Epizootic Epitheliotropic Disease prevention methods" for funding. Funding is contingent on Commission approval, availability of funds, and satisfactory response to board comments. Please contact your co-investigators (if any) concerning this notification.

Appendix 6.

Obj. 1- Develop & Test EEDV Vaccine Preparations

Autogenous Vaccine

Virus Inactivation

Advantages:

- Whole virus; possibility for immediate deployment

Limitation:

- Small-medium scale vaccination only

Obj. 1- Develop & Test EEDV Vaccine Preparations

Protein Subunit Vaccine

G protein expression

Purify via Hi-tag

Vaccinate using purified glycoprotein

Amino acid sequence of the membrane glycoprotein

Dr. T. Waltzek, Dr. K. Subramaniam, UF

Vaccination Experiments

Vacc. + EEDV	Unvacc. + EEDV	Vacc. Only

$RPS = [1 - (\% \text{ mortality of vaccinated fish} / \% \text{ mortality of non-vaccinated fish})] \times 100$

Deliver: Vaccine(s)...

Vaccination route, need for booster vaccination, & vaccine "dose" also to be evaluated

Obj. 2- To Validate EEDV Molecular & Serological Tests for Lake Trout Reproductive Fluid Screening

Appendix 6.

Validation of qPCR & qLAMP Assays for EEDV Detection in Ovarian & Seminal Fluids

- Spawning fluids from known EEDV + LAT lots, & "spiked" fluids, to be used to determine:
 - Assay sensitivity, specificity, robustness, repeatability, & reproducibility
- ELISA to assess previous EEDV exposure (serum; try repro fluids)

Deliver: EEDV-free brood; non-lethal detection; assess exposure



Obj. 3- To Reduce EEDV Transmission Potential During Routine Hatchery Procedures (e.g., Fin-clipping/Tagging) Via Development of Disinfection Procedures

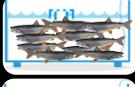
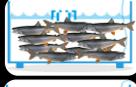
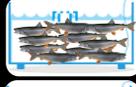
1) Tag & Clip +  +  = ?

2) Tag & Clip, **Disinfect** (Virkon®, sodium hypochlorite) = ?

Deliver: Reduced risk of EEDV transmission



Obj. 4- To Develop Easily Applicable Control Measures to Reduce Lake Trout Losses During EED Outbreaks

EEDV, NaCl Tx	EEDV, H ₂ O ₂ Tx	EEDV, GM-T Tx
		
		
		

RPS = [(1-% mortality of Tx fish / % mortality of un-Tx fish)] x 100

Deliver: Knowledge on deployable Tx options



Forecasted Deliverables

- Improved lake trout health (hatchery & wild)**
 - EEDV vaccine development (short & long-term)
 - Non-lethal EEDV detection
 - Tools for developing EEDV-free broodstock
 - Future assessments of previous EEDV exposure
 - Reduced risk of EEDV transmission
 - Amelioration of EED-associated losses

Appendix 6.

Acknowledgements

- **Great Lakes Fishery Trust**
• Grant # No. 2014.1455
- **MDNR Fisheries Division**
- **Great Lakes Fishery Commission**
- **MSU-AAHL Crew**
– Mogan Shavaller




Thanks to Many!!!



Gary Whelan, Martha VanAmberg, Ed Eisch, Jan VanAmberg, Jim Aho, Ed Baker (& many others from MDNR Fisheries Div.), Ling Shen, Kerry Hobden, Ken Phillips, Chuck Bronte, John Dettmers, Jun Li, Tom Waltzek, Kutti Subramaniam, Matti Kiupel, more

1. Check program applying to: [Check only **one** program]
 [Sea Lamprey Research Program](#) [Fishery Research Program](#)

2. Do you meet **all 5** new investigator criteria? _____ Yes No
<http://www.glfrc.org/research/Newinvestigatorcriteria.pdf>

TITLE: DO LAKE TROUT EGGS AND ALEVINS ACQUIRE THIAMINE DURING DEVELOPMENT IN WILD POPULATIONS?

PROJECT LEADER(S): Jacques Rinchar, SUNY Brockport, 350 New Campus Drive, Brockport, NY14420; 585-395-5750; jrincar@brockport.edu. **Matthew Futia**, mfutia@uvm.edu, and **Ellen Marsden**, ellen.marsden@uvm.edu. U. Vermont; **Allison Evans**, allison.evans@oregonstate.edu, Oregon State U. **Sergio Sañudo-Wilhemly**, sanudo@usc.edu, U. S. California.

COSTS: 1st yr: USD \$95,491 Total: \$183,481 PROJECT DATES: Sept. 1, 2021 – Aug. 31, 2023

RATIONALE: Thiamine deficiency complex (TDC) is a reproductive disorder affecting health and recruitment of Lake Trout in the Great Lakes region. Although the ultimate cause of TDC is unknown, it has been linked to high consumption of Alewife. Lake Trout consuming Alewife may allocate insufficient thiamine to their eggs, resulting in high offspring mortality. Thiamine deficiency signs are widespread in hatcheries and are mitigated by thiamine treatment. However, no studies have quantified thiamine levels or rates of TDC in wild alevins. Alevins have been observed at many sites where TDC is known to occur in the Great Lakes and Lake Champlain, but captured alevins may represent only those without TDC that were able to swim into a trap (i.e., there is survivorship bias). Alevins in hatcheries will not take inert food until 5-6 weeks old (after TDC has manifested), but wild alevins eat zooplankton within two weeks of hatching; wild alevins with a thiamine-rich diet may mitigate TDC. We postulate that both eggs and alevins may acquire thiamine from natural sources (e.g., algal breakdown products and alevin diets). Our recent research showed that thiamine levels in Lake Trout eggs reared on treated lake water unexpectedly increased between fertilization and hatching. We also found that when a given family's eggs were reared on two different water sources, the incidence of TDC differed. The purpose of this project is to establish whether TDC can be mitigated by absorption of free thiamine by eggs and consumption of thiamine-rich prey by alevins. We will test two hypotheses: (1) Lake Trout eggs can acquire dissolved thiamine from ambient water during overwinter incubation and (2) access to natural food increases thiamine in wild alevins and reduces their incidence of TDC. Previous studies have measured thiamine in unfertilized eggs and evaluated the effect of low thiamine by quantifying TDC signs in late-stage alevins under hatchery conditions; we will evaluate thiamine in lake water, unfertilized eggs, at hatching, and at yolk sac adsorption. If thiamine can be acquired throughout egg development or at the alevin stage, then rates of TDC in the wild may be reduced.

OBJECTIVES: Determine if (1) Lake Trout egg thiamine levels change during incubation, dependent on water source; (2) thiamine content and occurrence of TDC in wild alevins is different between individuals that have access to natural food, relative to those reared in hatchery water.

METHODS: Lake Trout eggs through alevin stage will be reared in natural environments as well as under controlled hatchery settings to determine whether thiamine can be acquired throughout egg development or at the alevin stage under natural conditions. Thiamine concentrations will be monitored from pre-fertilization throughout post-swim-up stage for offspring sampled from both rearing conditions (wild and hatchery) to assess any potential differences. Thiamine will be measured in lake water, eggs prior to fertilization, eggs after water-hardening, and eggs shortly before hatching to determine if thiamine from the surrounding environment can transfer into the eggs during their development. In addition, thiamine will be measured in offspring shortly after hatching and five weeks after hatching to determine if wild alevins are capable of increasing their thiamine concentrations relative to hatchery alevins by acquiring exogenous thiamine. An additional sample of eggs will receive thiamine treatment to ensure adequate concentrations for healthy development, and to compare post-hatch survival and feeding success. We will focus on two Lake Trout populations presenting different success of natural recruitment: lakes Champlain (successful recruitment) and Ontario (limited recruitment). In the field, three sets of egg bags will be seeded with eggs from each female, two with untreated eggs and one with thiamine-replete eggs. One set of bags will be retrieved as soon as the lake is accessible after ice-out; eggs will be separated from newly hatched alevins for measurement of pre-hatching and post-hatching thiamine. The second and third (thiamine replete) sets of bags will be retrieved approximately 5 weeks later, and survival and stomach contents of hatched offspring will be assessed.

RELEVANCE TO PROGRAM: This project addresses the Re-establishment of Native Deepwater Fishes theme area; specifically, we address hypothesis 3 of the white paper from Zimmerman and Krueger (2009): Lake Trout and Alewives are capable of coexisting under specific conditions, and the related question: Do alevin predation and early mortality syndrome affect the recruitment of lean Lake Trout in Lakes Huron, Michigan, and Ontario?

DELIVERABLES/PRODUCTS: Deliverables will include an annual progress report following year 1, a final report at the conclusion of the study, presentations at local, national, and international conferences, as well as several publications in peer-reviewed journals. Data will also be available to fisheries managers from the Great Lakes region.

Ministry of Natural Resources and Forestry

Exploring In-Hatchery Strategies for Enhancing Post-Stocking Survival in Atlantic Salmon at Normandale Fish Culture Station

Fish and Wildlife Services Branch
Provincial Services Division

Prepared by Kevin Loftus, Manager, Fish Culture Section

July 2019

Ontario 

Context

Why investigate In-hatchery Strategies for Enhancing Post-stocking Survival?

- 2006 MNRF and partners launched the LOASRP
- Ambitious program – 3 strains, 3 life stages, 3 rivers
- In 2014, 8 years into the program, MNRF & partners had stocked roughly 5 M Atlantics, but concern was growing about the low number of adult returns.
- Science workshop – to see if we could make some adjustments to improve outcomes.
- FCS immediately started to ramp up the sizes of all 3 life stages that we were stocking
- Now, 13 years into the program, we have stocked ~9 million Atlantics
- Returns are still lower than hoped for, though we are seeing encouraging signs
- While fisheries managers remain hopeful, they NEED to see evidence of significantly improved success.
- If not enough success the program will be at risk...with two potential impacts
- (1) staffing levels, and (2) it could also mean that a program that could have succeeded might not succeed.

2 Presentation Name

Ontario 

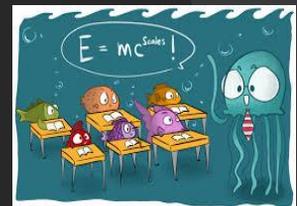
Why Explore In-hatchery Strategies to Enhance Post-stocking Survival?

- Why is it that captive-reared fish generally have lower fitness (and survival) in natural environments after stocking than their wild counterparts?
- The answer stems, in part, from
 - the fact that hatchery environments are homogeneous and impoverished compared to natural environments (Johnsson et al. 2014)
 - and the fact that fish reared in such environments often exhibit behavioural deficits (Brown and Day, 2002)



Early Learning in Fish

- Behavioural development in fish is strongly influenced by learning experiences early in life, including encounters with predators, prey, competitors and complex habitats.
- If hatchery fish are deprived of opportunities to learn these life skills prior to stocking, their ability to survive will likely be impaired (Shumway, 1999; Kellison et al., 2000).
- Fish destined for stocking in the wild should be trained for a life in the wild **while in the hatchery** and should, to the extent feasible, have the behavioural repertoire of wild fish at the time of stocking (Brännäs & Johnsson, 2008).



Ontario 

Findings from Other Research

Enriching the captive environment of fish- why and how
6.11.2018, Oslo



Pekka Hyvärinen
Natural Resources Institute Finland (Luke)
Kantaniemi Fisheries Research Station
Pattaniemi, Finland
www.kts.fi, www.luke.fi




Findings from Other Research

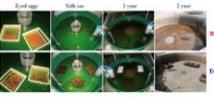
Objectives

Developing cost effective methods to produce young fish that are more viable and better adapted to natural environmental conditions

- Improve fish welfare
- Better results in fish stocking and conservation.

Basic Design

Enriched rearing for production scale



- Same densities (maximum used 3125 fish/m², 14 kg/m³) and food in standard and enriched tanks
- In enriched tanks also:
 - Gravel and shelters + changes in current direction, speed and water level



6

Results

Results - Benefits of enriched rearing vs. standard

- Higher feeding rate of natural food
- faster growth and changes in nature after release
- Increasing survival to release
- less fish for stocking needed → cost efficient
- Less vulnerable to angling → more difficult to capture
- more fish alive for reproduction
- Enhance disease resistance of fish in rearing tank
- less medicine needed
- cost efficient

Labour costs of enriched rearing are 5 % + standard but benefits are higher



Enriched rearing increased survival during smolt migration

In Temperate:

Group	Survived	Standard results
Enriched	2 x enriched benefits	2 x wild smolts
Standard	Survived	Standard results




These are the results that we need




Our Plan

Species/Life Stage

- LaHave strain Atlantic salmon destined to be stocked as fall fingerlings

Hypothesis

- Fish exposed to enriched conditions during rearing will survive better following stocking to the time of smolt migration

Basic Design

- 6 control tanks – follow standard rearing practices, no enrichment
- 6 treatment tanks – standard rearing practices plus
 - continuous exposure to in-tank structure
 - periodic exposure to live prey
 - periodic exposure to live predators
- Day/night stocking

Assessment

- fish will be pit tagged
- pit tag reader arrays

7

Our Team



Justine McAndrews,
MSc candidate,
U Windsor

Dr. Aaron Fisk,
Professor,
U Windsor

Dr. Trevor Pitcher,
A/Professor,
U Windsor



Dr. Culum Brown,
Professor,
Macquarie U,
NSW, Australia



Dr. Chris Wilson,
Research
Scientist, MNRF



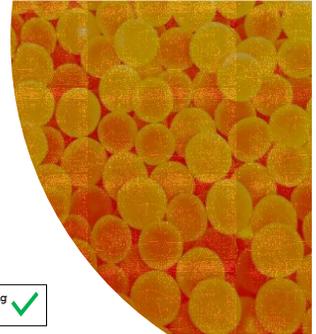
WISCONSIN DNR

Agency Update (Summary of 2019)



HATCHERY FISH HEALTH

- **FHC Inspections**
 - 35 fish health inspections (23 state, 2 co-op, 4 wild fish transfers, 1 private)
 - Results: NSF
- **Grow Out Surveillance**
 - To monitor MUE/WAE for pathogens from forage fish feeding
 - CDR in Muskegon (2 hatcheries)
 - Suspect forage fish transfer
- **Broodstock Surveillance**
 - Testing of cultured and wild broodstock used for spawning (K.S. CVFA)
 - Pathogens of note:
 - *F1 psychrophilum/columnare_A* salmonicide (Dept for VA)
 - EEDv (1/4 pools broodstock, 3/8 pools OVFA)

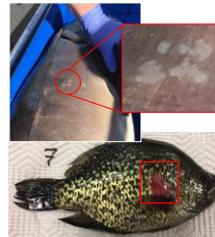


WILD FISH HEALTH

- **VHS Surveillance**
 - 3 hatcheries, surface water fed from 4 lakes
 - For stocking, lakes & hatchery fish need to be VHS – (150 fish/lake tested)
 - Results: NSF
- **Forage Fish**
 - Purchased from vendors to feed MUE/WAE
 - 8 lots tested (7 FHM, 1 GSH)
 - Results: GSV in 3 lots (3 facilities), Unknown replicating agent in 1 lot (1 facility)
- **Wild Fish Transfers**
 - 4 transfers
 - Special exemption granted to forgo testing of LMB



WILD FISH HEALTH (CONTINUED)



- **Unusual Morbidities/Morbidities**
 - BLC (3): lesions, suspect for black crappie sarcoma (BCS)
 - WAE (1): mass, spindle cell tumour (sarcoma)
 - SMB (1): suspect VHS, negative for viruses
- **On-going Cases**
 - **AcIHV1:**
 - Cutaneous lesions continue (spring) testing + in new locations
 - Sex link possible (4x more females affected)
 - 2020: test OVFL
 - **BCS**
 - Red focal proliferative lesions, ulcerating into the underlying cutaneous layers
 - Definitive cause unknown (possible retrovirus, papillomavirus)
 - 2020: PCR, TEM, ISH testing

AADAP-INAD



Smooth Sailing

- Successful treatments
- No reports of toxicity

GOALS FOR 2020

- **Fish Health Database Development**
 - We need things in one place
- **Meetings/Conferences**
 - Northeast Fish Health Committee/AFS-FHS
 - Eastern Fish Health Workshop
- **Biosecurity Audits**
 - Part of regular inspection most likely rather than 'surprise' visits
- **Case reports/Publications on New and Emerging Fish Disease**
 - Fingers crossed



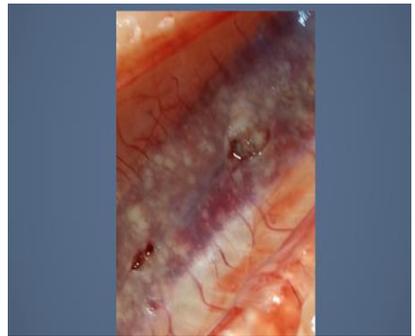




Crystal Springs Hatchery

- 3 year old Brook Trout
- Lesions next to eye
- Most only to males
- *Vibrio vulnificus* was isolated from eye lesions of 4 fish (3, 4,5,6)
- *Aeromonas hydrophila* was isolated from eye lesions of 2 fish (1,2,7)
- *Pseudomonas* was isolated from kidney (1)





Kidney issue in Cisco from Lake of the Woods

- Initial Lesions reported by two fishermen
 - Lake Survey found 50% of gill net Cisco displayed similar condition in the kidney
 - MN DNR lab no R. sal detected
 - MI state lab detected low R. sal antigen in one of two fish sample
 - USGS lab initial diagnosis as Lymphosarcoma or Lymphoid tumor
 - Histopathology result pending
- First three slides are from fishermen
 - Last two pics are from lake survey

Ministry of Natural Resources and Forestry

Ontario Update to the GLFHC

**Fish and Wildlife Services Branch
Provincial Services Division**

Prepared by Kevin Loftus, Fish Culture Section

February, 2020

Ontario 

Update on Work Aimed at Enhancing Fitness

- Updates on...
 - Grad student project at Normandale
 - Expansion of that work by Section staff
 - Emerging collaboration
- Why relevant?
 - Return on Investment
 - Traditional focus on Outcomes vs outputs
 - Emerging concerns about post-stocking survival (e.g., Larocque, Johnson and Fisk (2019))

Why Explore In-hatchery Strategies to Enhance Post-stocking Survival?

- Why is it that captive-reared fish generally have lower fitness (and survival) in natural environments after stocking than their wild counterparts?
- The answer stems, in part, from
 - the fact that hatchery environments are homogeneous and impoverished compared to natural environments (Johnsson et al. 2014)
 - and the fact that fish reared in such environments often exhibit behavioural deficits (Brown and Day, 2002)

Pekka Hyvarinen presentation & paper



3

Ongoing 'Enhancing Fitness' Investigations

Atlantic Salmon Stocked as Fall Fingerlings

- Enriched vs Unenriched (8 reps)
 - 6 foot circular tanks - RAS
 - In-tank structure
 - Periodic exposure to live prey
 - Periodic exposure to predator
- Stocking
 - E Duffins – 2 locations / 1 pair
 - W Duffins – 8 locations / 4 pairs
 - Ganaraska – 8 locations / 4 pairs
- Assessment Metrics
 - In-hatchery behavioural work
 - Survival to d/s migration (pit tags)
 - Other



Presentation Name

4

Ongoing 'Enhancing Fitness' Investigations

Atlantic Salmon Stocked as Spring Smolts

- Enriched vs Unenriched
 - 15 m³ raceways (flow-through)
 - Structure
 - Periodic exposure to live prey
 - Periodic exposure to predator
 - Enhanced flow regime
- Stocking
 - Ganaraska – 8 locations / 4 pairs
- Assessment
 - In-hatchery Behavioural work
 - Survival to d/s migration



Presentation Name

5

Enhancing Fitness - An Emerging Collaboration

Algoma U

- Istvan Irme
- William Dew

Concordia U

- Grant Brown

Lake Superior State U

- Kevin Kapuscinski

Roger Greil

MNRF

- Fish Culture Section

Proposal to GLFC was rejected, but good feedback.

- Concern with ecological relevance of behavioural responses from the lab
- Recommended stronger linkages with work in Ontario

Interest in trying again

- May involve a second facility

Broaden team

- MNRF – Dr Chris Wilson
- Fisk & Pitcher – U Windsor

6 Presentation Name



Update on Bloater Program

Performance of In-house Broodstocks
Performance/Behaviour after Stocking

Securing a Reliable Gamete Source = Developing a Broodstock =

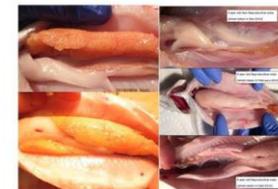
History

Began in 2011

- 7 year classes have been created
- 2011 YC built from < 300 survivors
- Better approach in subsequent years

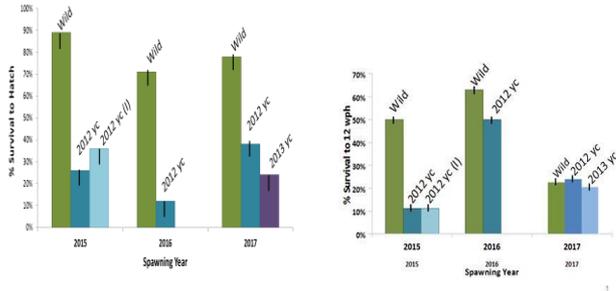
Progress & Research Needs

- Excellent progress over past 7 years
 - Good growth / high survival of brood fish
 - Gonads developing well by age 3
 - Began viable collecting gametes in 2014
 - But asynchrony/incomplete maturation
- GLFWRA-supported research / U Windsor



8

Securing a Reliable Gamete Source = Performance of Broodstock Gametes =



Bloater Program Performance of In-house Broodstocks

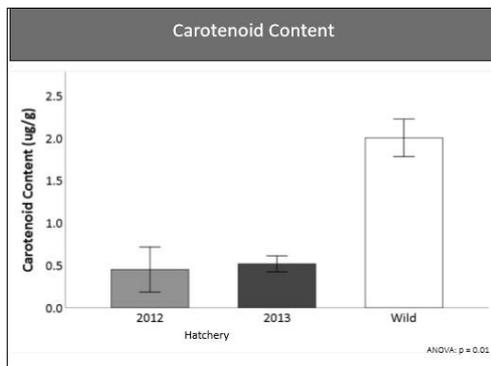
Differences in egg quality between hatchery-reared and wild-origin Bloater (*Coregonus hoyi*) eggs

Celine Lajoie, Tim Drew, Kevin Loftus, Michael Arts, Ryan Wehse, and Trevor Pitcher*

University of Windsor | Ontario | RYERSON UNIVERSITY



Bloater Program Performance of In-house Broodstocks



Bloater Program Performance of In-house Broodstocks

Polyunsaturated Fatty Acids			
Fatty Acids	Hatchery Eggs	Wild Eggs	Role
ARA	0.97±0.11	6.6±2.2	Hatching Success
EPA	9.12±1.5	18.2±5.4	Immune System
DHA	22.6±5.6	22.5±5.8	Brain/Retinal Development
Σ n-3	36.5±8.24	53.7±15.1	
Σ n-6	8.1±1.7	15.4±4.9	
Σ PUFA	44.7±9.8	69.1±20.0	

(Hazel 1989) | (Sargent et al. 1995) | (Haring et al. 2018)



Bloater Program

Performance of In-house broodstocks

Action to Date

- Since 2018, we worked with feed supplier and fish nutritionists to try to improve diet.

What's Next?

- Fall 2019, prepared GLFWRA proposal in collaboration with NYSDEC, USFWS, USGS and GLFC
- If funded, brood diet investigations will be initiated at several facilities.

13 Presentation Name

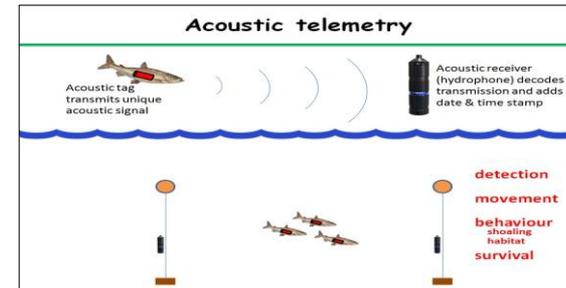


Bloater Program

What Happens after Stocking?

Overview

- Used acoustic telemetry to assess post-stocking behaviour, habitat use, and survival of hatchery-reared bloater – Aaron Fisk, Tim Johnson, Natalie Klinard



14 Presentation Name



Bloater Program

What Happens after Stocking?

Key Findings

- Some bloater travel large distances and are detectable for a long time
- Mortality rates over time are higher than anticipated
- Most fish descend to 50 m or more within 2 minutes of stocking.
- Some die immediately after descent – rapid compression effect?

Implications/Questions

- Can rapid compression cause mortality – Johnson, Fisk, Gorman
- Are we stocking the right locations (e.g., deep, offshore)
- Are we stocking the right life stages?
- Can we produce fit that are more fit (both behaviourally and physically)?

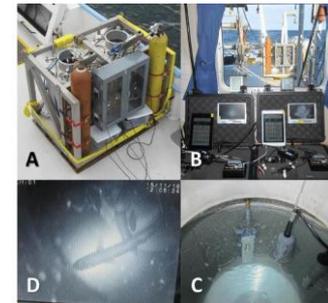
15 Presentation Name



Bloater Program

What Happens after Stocking?

- Effects of rapid decompression on fish are well-known
- Can the opposite – rapid compression – cause mortality?
- If so, what are the implications for rehabilitation stocking programs?
- Issue is not discussed in the literature - recently discovery
- Expert opinion.
- Next steps – test it!



USGS Barometric Chamber,
Courtesy Owen Gorman

16 Presentation Name



Other Stuff

1. U of Guelph Monitoring Contracts
2. Fishing License Revenues
 - New marketing plan
 - urban/near urban fisheries
3. Effluent Phosphorus
 - Ontario guidelines are 30 years old
 - Current approach has flaws
 - MNRF is supporting research to characterize effluents
4. Feed registration
5. OAHN – fish committee
6. Commercial Industry
 7. Planet Shrimp / IHNV
 8. Apparent interest in ISA testing