GREAT LAKES FISH HEALTH COMMITTEE

2019 Summer Meeting
Sault Ste. Marie, ON
July 31 – August 1, 2019

Minutes
(with attachments)

Submitted By:

Erin Bertram

Great Lakes Fishery Commission

GREAT LAKES FISHERY COMMISSION
2200 Commonwealth Blvd Suite 100,
Ann Arbor, MI 48105
Great Lakes Fish Health Committee

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List of Attendees

Kerry Hobden	Ontario Ministry of Natural Resources and Forestry
Danielle Godard	Wisconsin Department of Natural Resources
Nicole Nietlisbach	Wisconsin Department of Natural Resources
Gary Whelan	Michigan Department of Natural Resources
Ken Phillips	U.S. Fish and Wildlife Service- La Crosse
Andrew Noyes	New York State Department of Environmental Conservation
Kevin Loftus	Ontario Ministry of Natural Resources and Forestry
Dave Meuninck	Indiana Department of Natural Resources
Sunita Khatkar	Department of Fisheries and Oceans
Tom Loch	Michigan State University
Paula Phelps	Minnesota Department of Natural Resources

Invited Guests:

Chris Wilson	Ontario Ministry of Natural Resources and Forestry

Great Lakes Fish Health Committee Meeting
Sault Ste. Marie, ON
July 31 – August 1, 2019

Tuesday, July 30th, 2019

Check-in — Delta Hotel - Sault Ste. Marie Waterfront
208 St. Mary's River Drive, Sault Ste. Marie, ON P6A 5V4
1-800-268-1133
https://www.marriott.com/hotels/travel/yamds-delta-hotels-sault-ste-marie-waterfront/

Wednesday, July 31st, 2019

8:00-8:15	Welcome and Introductions (Hobden) - lunch & dinner options - Hatchery Tour information
8:15-8:30	GLFC Update (Dettmers)
8:30-8:45	Baitfish Statement (Dettmers)
8:45-9:15	EEDv update (Whelan)
9:15-9:45	In-hatchery strategies for enhancing post-stocking fitness (Loftus)
9:45-10:00	ONMNRF Fish Culture Section update (Loftus)
10:00-10:15	**Break**
10:15-10:45	Mussel culture update (C. Wilson, OMNRF)
10:45-11:15	Radon monitoring in ONMNRF Fish Culture Stations (Loftus)
11:15-11:30	Asian carp health assessment project (Phillips)
11:30-12:00	Guests from Fish Disease Laboratory at the Heilongjiang River Fisheries Research Institute (HRFRI), Chinese Academy of Fishery Sciences re: research projects
12:00-1:30	**Lunch**
1:30-2:15	Update – *Vagococcus salmoninarum* (Phillips)
2:15-3:00	KHv research discussion & Position Statement (Dettmers & All)
3:00-3:15	**Break**
3:15-4:30	Casework & Research Update (Loch)
6:00	**Dinner**

Thursday, August 1st, 2019

8:00-8:15	Reconvene and introductions (Hobden)
8:15-8:30	Technical Advisors list review (Hobden)
8:30-10:00	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 (Hobden) - Winter 2020 meeting location - Summer 2020 meeting location
12:00	**Adjourn**
1:00	**Tour** – OMNRF Tarentorus Fish Culture Station Hatchery Road, Sault Ste. Marie

Summary of Action Items & Decisions

ACTION ITEM: The Commission will work with the CLC chair to draft a letter on behalf of the CLC to USGS to request to add GLFHC to the list of groups to share Thiamine Deficiency results.

DECISION: GLFHC will add Jacques Rinchard (SUNY- Brockport) and Don Tillitt (USGS) as Technical Advisors to the GLFHC.

ACTION ITEM: Kevin Loftus will send his PowerPoint presentation and the digital version of the research paper on enriching the captive environment of fish
Completed January 2020

ACTION ITEM: The chair will ask the list of suggested people if they are interested in serving as technical advisors and inquire about others that may be retiring.

ACTION ITEM: In relation to splake mortalities related to handling, Paula Phelps & Ling Shen will create a summary of what type of analyses and water quality testing that has been done at the hatchery, when this phenomenon first appeared, and what has changed at the hatchery over the years, to discuss at the next meeting.

ACTION ITEM: A case history of working with private entities from MI DNR will be given to Danielle and her Fish Chief, Justine.

DECISION: Consensus from the committee is that CTv in the Rome strain brown trout is not a concern from a fish health perspective

ACTION ITEM: John Dettmers will email Coja with a consensus statement on CTv from the GLFHC.

ACTION ITEM: Danielle Godard knows a shrimp veterinarian and will get them in contact with Sunita regarding the shrimp virus.

Welcome and Introductions

The chair of the Great Lakes Fish Health Committee (GLFHC), Kerry Hobden of OMNRF, welcomed all attendees and guests.

GLFC Update

Discussion at the spring Council of Lake Committees (CLC) meeting determined that more information about Thiamine Deficiency and its status was needed in the upper lakes. The U.S. Geological Survey (USGS) has been taking care of samples during the last several years but has not been very prompt in getting results back to the lake committees. Lake committees want this data in a more timely way. Dettmers suggested it would be good if USGS would share those results with GLFHC. Dave Meuninck noted that Jacques Rinchard could take on this role if USGS was no longer interested and could do the testing but he does not have a data base of information to determine baseline or optimum thiamine levels. Are there any lakes without alewife to be able to get a baseline level of thiamine in salmonids? Gary Whelan observed that Lake Huron has mostly wild fish and there are barely any alewife left. There has also been natural recruitment of lake trout and walleye since alewife crashed. Lake Superior also has self-sustaining lake trout populations since terminating stocking of salmonids and rarely had observable impacts from alewife. Andy Noyes wondered whether the GLFHC should include a technical advisor for thiamine. The committee thought this was a good idea.

ACTION ITEM: The Commission will work with the CLC chair to draft a letter on behalf of the CLC to USGS to request to add GLFHC to the list of groups to share Thiamine Deficiency results.

DECISION: GLFHC will add Jacques Rinchard (SUNY- Brockport) and Don Tillitt (USGS) as Technical Advisors to the GLFHC.

GLFHC Draft Baitfish Statement. The GLFHC generally liked the draft position statement but thought that the statement should list the specific pathogens it wants to target. The goal would be to get all Great Lakes agencies to start testing baitfish annually. However, funding to do this will remain an issue. Could annual testing be conducted as part of the National Fish Health Survey (NFHS)? Ken Phillips thought that this sort of testing may not fall under the NFHS. Tom Loch noted that with frozen fish coming in from the Pacific North West it would be easy for IHNv to come into the basin. Could the committee recommend that baitfish be preserved? Gary Whelan believed that most anglers would not be willing to preserved bait. Minnesota allows people to re-freeze their fish after preserving them. There are dehydration, salting, and heating methods. The only preservation method people did not prefer was using mineral oil. After discussion, the GLFHC decided to focus on live baitfish in the position statement. It also agreed to focus on these pathogens: VHSv, FHMv, GSv, Asian fish tapeworm,

heterosporis, and *Pleistophora ovariae.* The committee will plan to discuss this in more detail at the winter meeting.

EEDv update

MI DNR has been investigating what causes EEDv to erupt in its hatcheries, where the pathogen is located in the basin, and what the virus does physiologically. It is likely the virus has been around a lot longer than was first detected in the 1980s.There have been patterns of mortality associated with a trigger event or conditions to create this pattern of mortality. The Marquette State Fish Hatchery has 45 years of data needed to answer these questions. A genetic test for EEDv was created in 2008 by Hedrick. However, the test is not specific to EEDv and detects other salmonid herpes viruses. Broodstock were tested in 2009, and 100% of them were positive for EEDv using Hedrick's genetic test. Gary believes that fish harvested from Marquette harbor and brought into hatcheries brought the pathogen with them. Fish from the Saratoga National Fish Hatchery were used as back-up broodstock for the Great Lakes around the same time in the 1980s and may have also brought the virus with them. The virus may be historical to the Great Lakes.

<u>EEDv 2012 to 2013 cumulative mortality by raceway and LAT strain:</u>
MDNR lost 90,656 production lake trout. Although mortalities differed among each raceway, the raceways were right next to each other. It is a mystery how EEDv was transmitted from broodstock to production fish. It's uncertain if it is vertically transmitted or not. Based on a rain event, Michigan hypothesized that turbidity could trigger the virus to show up.

<u>EEDv in 2017 and 2018:</u>
There were no unusual mortalities but samples were still taken to examine for stocking the Seneca Lake strain and Lake Superior strain fish. Several positives occurred for both strains. The two strains respond differently to the virus.

<u>2018 Cumulative Mortality:</u>
Michigan lost 35,779 production lake trout. The trigger was a high intensity rainfall event in late September. Michigan hypothesized that temperature might not be directly correlated to the trigger event as temperature fluctuates but not drastically enough even on a surface water system.

Discussion

What is the land use in the area? Could any of it be contributing to these water quality related trigger events? Land use is mostly forested, deep sand, and a bit of urbanization with houses upstream but in low density. The water drains away from the hatchery. A few streams feed the source creek. Delayed mortality is something to be aware of.

Michigan did strain susceptibility to EEDv testing for Seneca Lake strain fish in controlled lab conditions and we could never kill any of them even though we could find high virus levels. They maintained the virus for a long period of time. There are definitely strain susceptibility differences. Michigan controls the disease like BKD and uses similar procedures. Eventually Gary intends to get a publication of the entire history of EEDv. Loch has some ideas for vaccines. Wisconsin did EEDv testing at its Bayfield hatchery on the fry and fingerlings, and all were negative. Andy Noyes noted that there is no fish herpes virus that has shown to be vertically transmitted as far as we know. It may be that if you disinfect the eggs you should be able to kill the virus. Tom Loch clarified that with reproductive fluids yes, there can be vertical transmission, but otherwise there is not vertical transmission of fish herpes viruses. Even though it is not inside the egg, it will behave as if it is vertically transmitted.

5. In-hatchery strategies for enhancing post-stocking fitness

Kevin Loftus explored the potential value of in-hatchery strategies to enhance post-stocking survival.

The premise is that fish destined for stocking in the wild should be trained for life in the wild while in the hatchery and should, to the extent feasible, have the behavioral repertoire of wild fish at the time of stocking. This rarely occurs for most stocking programs as we as managers continue to see captive-reared fish with lower fitness than wild fish. Ontario has been stocking Atlantic salmon for restoration into Lake Ontario for more than 13 years and has stocked 9M fish, still without satisfactory returns. This could be due to the inadequate methodologies used for assessing and measuring these returns. Alternatively, the results could be possible because they are still not surviving well after stocking or perhaps they are and this will be the year they show up.

Atlantic salmon at the Normandale Fish Culture Station were used to test in-hatchery strategies to enhance post-stocking fitness. Three strains and three life stages of Atlantic salmon were used in this study. The fish were reared in normal densities used in the hatcheries.

Initial returns were not sufficient to warrant continued investment in the program. OMNRF conducted an Atlantic salmon culture workshop for more insight and collaboration. Larger fish should be produced for each of the three life stages as science has shown this will increase post-stocking survival. The larger life stages just started returning last year and this summer and there has been an improvement in post-stocking survival but will likely need to continue enhancing in-hatchery environment.

Hatchery managers will need to decide if this program is working or not while OMNRF will continue doing what they can to produce post-stocking returns on Atlantic salmon.

Results from a study entitled “Enriching the captive environment of fish – why and how” has initiated changes to the hatchery rearing program for Atlantic salmon. This study reported fish in the wild that came from the enriched environment had two times the

survival rate as control fish. These fish also had higher feeding rates on food in the wild and enhanced disease resistance. The workload necessary for this study was only a 5% increase from former methodologies that used and thus could be manageable to adopt.

ACTION ITEM: Loftus will send the PowerPoint and the digital version of the research paper
Completed January 2020

Ontario is now adopting some of these enrichment methods and using live prey such as black soldier fly larvae to introduce the fish to finding food in the wild. The longer hatcheries wait to begin adding live prey, the slower the fish are going to learn or it will be less likely they will learn to find prey. There is also conditioning for predatory presence. The results should be available by May 2020.

Discussion

Wisconsin noted that one of its facilities is using artificial turf within tanks as extra enrichment and has proved to be beneficial.

How early are you introducing the fish to live feed and predators and what species of predator was used? If stocked as spring fry, they are trained ahead of time. Otherwise, at 45 grams in weight, they are stocked with the probability of reaching smolt stage the next spring so the time to expose them to predators or live feed will depend on the life stage they are stocked at. Colin Brown says it needs to be reinforced frequently or the fish will forget. The appropriate frequency is still being figured out. Rainbow trout were used as predators in our studies.

Some enrichment happens passively when wild water sources are used to introduce some live prey items. Fish are being reared on 100% well water so it will be interesting to see how well they do compared to those reared with wild water sources and live prey.

How does OMNRF evaluate returns, by creel survey? There are some in-stream stock assessments and some attempts to capture adult returns. A fish counter was recently put in so every fish that goes up, it is seen. There is also a pit tag array built in to identify the tagged fish.

Do you know what percent of fish are hatchery vs wild in your returns? There is definitely some natural spawning occurring and occasionally New York fish are found. It is known exactly what parents are used to cross and exactly where those fish are stocked so one can typically determine which are hatchery vs wild.

This approach has been used for at-risk or listed salmonid stocks in the Pacific North West (PNW) with really mixed results. They were enhancing raceways and other things. Interested to see how this works in a very small environment. Those on the west coast are not convinced they controlled the right variables correctly in their initial attempts.

6. OMNRF Fish Culture Section update

Walleye OMNRF is in year five or six of efforts to learn how to culture newly hatched Walleye at White Lake Fish Culture Station and Blue Jay Creek Fish Culture Station.

OMNRF is trying the clay systems and a green water system, where some are flow through. OMNRF is communicating with our U.S. contacts but it is a slow process with setbacks resulting in large losses at times, despite intensive time investment by staff.

Bloaters

OMNRF and the USGS lab at Tunison have been working to develop culture for bloaters for the past eight years with decent success. Gamete collection occurred in January and February from Lake Michigan. Agencies are now establishing brood stocks as the future source of bloater gametes. Some of these broodstock year classes are now mature. In a direct comparison between wild gametes from Lake Michigan and gametes from broodstock, wild gametes did better upon eye-up. During the last two years, no wild collections occurred. All gametes were from in-house broodstock that resulted in a much lower eye up rate than desired. Survival post-hatch has drastically decreased, potentially related to nutrition in the broodstock.

Gametes from the wild and from hatchery broodstock were cultured at the same time, and both sources were sent in for fatty acid analysis to compare. There was a difference between the wild fish and captive fish. Dr. Dominic Bureau of the University of Guelph is working with us to determine if it is a nutritional issue. It is likely an essential fatty acid issue. Custom diets alongside commercial diets will be investigated. Krill Canada has blocks of frozen Antarctic krill or Pacific krill that might be used as an option for diet enhancement. Genetics of the broodstock are pure bloater. Ontario is transferring some of these to the U.S. in an effort to get more than one agency looking into this shortcoming.

Dr. Tim Johnson of OMNRF used acoustic telemetry tags in bloaters in fall yearlings (30g) and some in older larger fish. At the time of stocking, OMNRF tracked them. The fish went straight to the bottom of the lake and spread out. Some of the newly stocked fish are going to the bottom of the lake and dying. One million bloater were stocked in the last seven years. So far, about six or seven fish have been captured, which is in the ballpark for the level of stocking and expected survival.

7. Mussel culture update

Chris Wilson, the production-planning biologist of the fish culture section presented on “Sharing Ontario’s Experience Developing Expertise in the Culture of At Risk Mussels”

Methods:

Host-fish are collected where the mussels do not occur for manual infestation in the lab. Wild gravid female mussels are also collected from the wild. There is a source population available to do this. Mussels are pre-treated before entering the lab and getting them on feed. The glochidia are extracted from the female mussels via flushing

the marsupial gills with a syringe of water. Some species like the Kidney Shell have conglutinates of glochidia. Conglutinates mean that it is a more tedious process is needed to harvest glochidia. Glochidia collection is time sensitive and is usually completed within an hour. Glochidia are checked for viability by testing a portion of the glochidia with a salt treatment. When the glochidia clasp shut in response to the salt, they are considered viable. To infest fish hosts, four host-fish species are placed in water buckets with intense aeration of oxygen while the glochidia are poured into the bucket. This is done for 30 to 60 minutes with about 4000 glochidia per fish. The infestation rate is calculated by manually counting the number of attached glochidia on each gill arch of each fish. There are typically 100-300 glochidia per fish depending on the size of the host. Occasionally an immune response can occur with too dense of an infestation or when fish have scar tissue or damage to their gills.

Plankton nets collect metamorphosed juveniles that have dropped off fish. Approximately 90% of juvenile mussels are lost before they get to 5mm in length but then mortality drastically drops. “Mucket Buckets” are used to collect and hold onto juveniles.

The snuffbox and kidney shell mussels do not do well in the Mucket Buckets so they are put in sediment pans. Sediment grain sizes that are smaller than the size of juveniles are used.

Snuffbox are on no water circulation in the static sediment tanks. Sediment directly from the river in the wild is used but that also means everything else from the wild comes with the sediment and has caused fungal growth issues. Sterilized sand could be used but then there is a loss of natural food sources from the wild. Advanced rearing begins with a Tubweller and larger PVC-made forage tubes with feed for grow out of the mussels. “River Troughs” are then used. They are filled with sand and gravel and have flowing water. At times, mussels over wintered in these troughs however, it was difficult to get the feed in effectively and the growth rates are compromised even though there was still survival. Then “Pond Buckets” are placed in an old walleye pond. It is comprised of a large bucket with water drawing from the bottom and up through the top. Mussels overwinter in the pond this way just feeding off of what is in the pond. Growth rates are much better than the river troughs. Dr. Dave Zanatta at Central Michigan University is working on propagation genetics.

The Ministry has been testing multiple females on a single fish. Some females infest really well, whereas others do not. Using a single female per fish produced highly variable results as the survival of offspring was very inconsistent across individual female mussels.

The culture facility is potentially developing a propagation guide that reflects the most recent findings in mussel culture as methods advance. The Ministry plans to share these techniques soon.

Discussion

How much is being spent on this effort, as the states are willing to be involved in this. Between the two stations is it is about $25K a year. In the US, this initiative could be quite easy to pick up with this if the Recovering America's Wildlife Act goes through.

Do we know about mussel pathogens at this point?Very little is known about pathogens and viruses in unionids. We work in quarantine and in watersheds where mussel pathogens do not currently exist. Regardless, no mussels have left the facilities or been stocked out yet.

If we are trying to teach fish hatchery staff to culture and/or stock out mussels, we need to train them soon because it is not a quick process and when we have an emergency of potentially losing a population of mussels, we will need to be prepared.

8. Guests from Fish Disease Laboratory at the Heilongjiang River Fisheries Research Institute (HRFRI), Chinese Academy of Fishery Sciences research projects

Scientists from the Fish Disease Laboratory at the Heilongjiang River Fisheries Research Institute (HRFRI) joined the meeting by phone. They primarily work with cold-water aquatic species in culture. They discussed some of the fish health challenges that China faces with flavobacterium being a common pathogen of interest. Their lab has been in contact and has made visits to Dr. Loch's lab at Michigan State University regarding this pathogenic bacterium.
Dr. Shaowu Li presented "Current Status of Occurrence and Management of Diseases in Farmed Salmonids in China". At present, HRFRI is the leading institute in China in the research fields of fish breeding and salmonid aquaculture. Culture production of salmonids in China is very small compared to the rest of the world. In China, aquaculture production accounts for about 77% while fishing production accounts for 23% of the country's total aquatic food output. Rainbow trout is the main culture species in China. Experimental aquaculture for rainbow trout began in 1959-1965. Other cultured salmonid species include Atlantic salmon, chinook salmon, coho salmon, and *Hucho taimen*.

<u>Disease Outbreaks</u>
Infectious diseases of concern to HRFRI include IHN/IPN, bacterial diseases such as *furunculosis*, fungal diseases, and parasitic diseases. Non-infectious diseases occur related to nutrition, environmental stressors, and management, sometimes leading to co-infections. China has a lack of commercial vaccines. There are no commercial vaccines available for salmonids. Prevention and control methods include using antibiotics, disinfectants, immunostimulants, and herbal medicines.

<u>HRFRI Research</u>
Epidemiology investigation, etiology research, and prevention and control techniques

Discussion

What do you think is the pathogen of greatest concern in your salmonids? IHN is the pathogen of greatest concern.

What is the vaccine or drug approval process in China? How long would it take to be able to apply a vaccine or drug if it was known to be effective? There are vaccines that are known to be effective so could technically use them right now, but must first apply for certification (or the license) for the application of vaccines.

9. **Radon monitoring in OMNRF Fish Culture Stations**

Radon 101: Radon is a Naturally-Occurring Radioactive Material (NORM) that is produced by decay of uranium in soil, rock, water. It is an invisible, odorless and tasteless gas. Radon released outdoors is diluted and not a concern. Radon released indoors can be a concern. The only known health effect from radon exposure indoors is the risk of lung cancer. The Canadian Nuclear Safety Commission has legislative control over man-made radionuclides and nuclear fuels. Provinces have jurisdiction over the use of, and exposure to, NORM.

Radon Regulation in Ontario: There is no legislation in Ontario governing radon. Health Canada introduced NORM Guidelines in 1988, setting the reference level at < 800 Bq/m^3. The Ontario Ministry of Labour (MOL) website did not clearly indicate that it had adopted the Health Canada guidelines. Health Canada revised NORM Guidelines in 2007, lowering the reference level to 200 Bq/m^3. This change was published in Health Canada's 2011 Guidelines. The MOL website did not clearly indicate it had endorsed the change in standard (as of 2014).MOL's website now clearly endorses the new standard.

Discussion

What do agencies need to abate to keep workers safe from exposure levels? Agencies would need to know the concentrations in each area per year and then multiply the amount of time workers are there. Agencies are encouraged to monitor radon levels in their hatcheries and take appropriate remediation measures if necessary.

10. ***Vagococcus salmoninarum*** **update**

V. salmoninarum (V. sal) has not been detected inside eggs. However, bacteria can be cultured from the outside of eggs that were disinfected. With the continued presence of *V.sal* in lake trout at Iron River National Fish Hatchery (IRNF), even after multiple attempts to clear the pathogen with medicated feed, USFWS decided to cull all brood and future brood stock at IRNF and disinfect the facility in March 2019.

Isaac Standish has taken a lead on this testing and data alongside Tom Loch training him. He has also developed a qPCR and is testing against some of the *Carnobacterium*. USFWS is collecting isolates from different locations. There has been a lot of feedback and interest on this data from a conference. The USFWS is planning to compare all the different isolates. Some isolates appeared similar to *V. sal* but molecular testing has

shown they are almost all *Carnobacterium*. Investigating if there is a symbiotic relationship potential with *V. sal* and *Carnobacterium*.

V. sal was found at the Red Cliff Fish Hatchery in brook trout broodstock but are not seeing mortality associated with it. However, there was not much of an egg take this year so there are low fry numbers.

Discussion

Was it correct that you thought it got to Iron River through surface water and then Iron River supplied these three hatcheries? Yes, they are all in the same watershed and two did get eggs from Iron River. Keewenaw Bay has two different water supplies/buildings. Fish from Iron River never had direct contact with the fish that were positive at Keewenaw Bay. Red Cliff did have mixing of Iron River fish and Red Cliff fish, but they are also not that far apart from each other.

You said you were still able to isolate the *V. sal* from outside the eggs even after disinfection of eggs upon coming in, so maybe that is also a cause. Did you do a culture sensitive test of *V. sal* with the Aquaflor? Yes, and we determined it is marginal sensitivity to Aquaflor. Mortalities did drop down but once treatment stopped, it would come back. It is potentially in the brain and Aquaflor possibly cannot reach the brain effectively to get rid of it.

11. KHv research discussion & Position Statement

The CLC was supportive of the committee's position statement on Koi Herpes Virus but wanted to include the possibility for appropriately controlled research on the virus in isolation in a laboratory. After discussion, the committee agreed that it will accept the suggested changes to the position statement that were offered by the CLC and re-word some of the language about the virus in lake sturgeon. The Committee will bring this revision back to the CLC for final approval in October 2019.

12. Casework & Research Update from Michigan State University

<u>New Students/Research</u>

Loch has a new MS student in the lab looking at infectious diseases as a potential bottleneck to the recruitment of lake whitefish. The committee should expect to see her present her findings at the next meeting or two. An undergraduate student is working on *Flavobacterium* and an MS student is working on a grant from the Great Lakes Fish and Wildlife Research Act on diseases of lake sturgeon which will also be presented in the next meeting or two.

<u>Study 1</u>

"Is *Flavobacterium psychrophilum* intraspecific genetic diversity associated with host species affinity?" Across the world, genetic diversity data exists for *F. psychrophilum.* The Colonial Complex originated from one ancestral strain. MSU is the most recent university to add to this worldwide understanding of the pathogen's genome. Genetic

differences seem to matter for a variety of different things that this lab cares about. Some isolates are far more virulent than other isolates. There is strong evidence that *F. psychrophilum* originated in North America and then was imported into Europe and other parts of the world. Movement has reciprocated and some European origins have slipped in back to the U.S. There is also evidence that it is transmitted intra-ova. Individual genotypes appear to be correlated with its host species preference.

Research Question:

Do the different isolates really have a preference for different host species?

Methods:

Dying fish in the wild shed 10^8 cfu/ml per fish per hour, reflecting the concentration the fish were exposed to in this study. Coho Salmon are the only species used in this study. Coho salmon are immersed in the Coho Salmon Strain and then in the potential Rainbow Trout Strain to see if there are differences.

ST13= Coho Salmon Strain (only by immersion not injection).

ST78 = Rainbow Trout Strain

Results:

ST78 RTS

There were no adverse external gross pathology results and no internal damage. The Rainbow Trout strain may not be as virulent but still able to kill rainbow trout.

ST13 CSS

External Gross Pathology: adipose fin is gone or degraded. Similar to cold water disease effects. Internal Gross Pathology: hemorrhaging

Mortality:

Coho salmon + ST13 CSS in the highest dose= 100% cumulative mortality.

After day 11 mortality stopped.

ST78 RTS generated no mortality in Coho Salmon and none in the negative controls either. Thus, a lethal dose of the rainbow trout strain could not be determined since it did not kill any of the fish. The Coho Strain in the Coho Strain survivors was still recovered. However, the rainbow trout strain was not recovered or detected it at all even at the highest dose.

Histopathological Findings:

Coho Salmon at time zero did not have a big difference in tissue sections between the two strains. At 48 hours, with the ST13 CSS, the adipose fin is ulcerating and causes an edema. With the rainbow trout strain (ST78 RTS), the adipose fin still intact. At 72

hours, you see more pathology in ST13 CSS fish tissue with many inflammatory cells trying to eat up the bacteria. The Rainbow Trout Strain of *Flavobacterium* does not infect or damage Coho Salmon or different species than what it comes from.

Immuno-histochemistry testing:

Immuno-histochemistry (IHC) was then used to see if there were differences of tissue distribution of the bacteria. Brown tissue stained indicates presence of the bacteria.

Results:

The results were interesting and semi-unanswered. The ST78 RTS in Coho shows that the bacterium may not be able to infiltrate into the internal organs, which may lean more towards the assumption of it having fish host affinity. At future meetings, I plan to discuss our results after the tissues of a Coho Salmon individuals are injected with ST13 CSS and let it go for 24 hours (then euthanize the fish) and fix that tissue loaded with bacterium. Then, IHC will be used to confirm that the antibody used does not hit this strain (which 99% of the time is the case). Otherwise will have to develop a new antibody.

Conclusions:

There is *in vivo* evidence supporting host species "preference" of the Coho salmon *F. psychrophilum* strain. There are implications for BCWD prevention and control.

Discussion

Could an immune response clear up the infection in the upper most tissues eventually, then if a Coho individual gets the ST78 RTS? It may depend on the host preference if again that does exist, and the immune ability of the fish.

Study 2

"Elucidation and prevention of EEDv (Salmonid Herpesvirus-3) contagion in hatchery-reared lake trout."

A Masters student did the wet lab work and a post doc did most of the analysis. In the initial studies of EEDv, a cell culture was developed to use *in vitro*, but was not successful. However, a lot about its recent emergence was discovered.

Research Questions:

1.) Mostly interested in how the virus is shed. What is the temporal trend? How long do host fish shed the virus, does it come and go, how many particles are they shedding?
2.) Can the particles be transmitted by inanimate objects and, if so, can they be treated with a disinfectant?

Methods of question 1:

Thirty-five two-year-old Lake Superior Strain-Lake Trout from Marquette, MI were used. All 35 fish were PIT tagged to track individual fish. Two weeks later, 29 fish were injected with the virus (six were used as controls). Every week after injection, each fish is collected, each fishes' PIT tag is read, and then they are placed in an aquarium for eight hours. After that exposure, the fish were removed and the water was tested to determine how much EEDv was shed during that eight hours using qPCR. This was done for three months.

Results of question 1:

Induction of clinical EEDv.

Fish started to die around week six, with 80% cumulative mortality of the 29 infected fish. Detectable levels of the virus were in the water after the eight hour time period. There was a difference among individuals in the levels of shedding. Some fish would go in and out of shedding or go through "shedding periods". Some would shed all the way through and die, or some would shed, stop, shed, and stop and then recover or die.

Results of Question 2:

Fomite Challenge Group (80% mortality) vs Virkon Treatment Group (3% mortality)

Infected nets can lead to contagion. If hatchery personnel use Virkon, that procedure offers some level of risk reduction for spreading it from nets and buckets and other objects.

Discussion

Is there a time line of infection and peak clinical signs? It varies by age and route of infection. Mortality occurs about four weeks post challenge by immersion and injection. Two-year olds took longer to die. There are some strain differences in susceptibility as well. At six months, the Lake Superior strain died but Seneca strains did not.

You said you never saw any dead Seneca strain fish in your study but then why did we see dead positive Senecas at Marquette SFH?

Senecas were shedding a lot more than the Lake Superior strain. Under lab conditions, EEDv might not be able to kill without a trigger. Field conditions like turbidity or density could be a trigger that we don't manipulate in the lab and could occur in the wild for Senecas, causing their death. It is also possible they have secondary infections in the wild.

Salt induces sloughing risk but it could induce shedding. We do know whether the virus does go systemic? Assuming we could identify the trigger even if it doesn't intend to cause infection we could intercept the problem right there.

Do symptoms go away for the Seneca strain when they are intermittently shedding? Fish held on to those clinical signs and still lived through the study. Some individuals

come out of the infection and end up recovering, which might speak to the fact that some of the fish are better shedders than others.

Study 3

“Wild Fish Kills”.

Gary Whalen (MI DNR) gave a case overview of several fish kills that were observed or reported near the Hydro Dam at 1 atm = 30 ft at Belleville Lake.

Photographic results:

At some point more recently, the eye was acutely bulging out and then retracted. Across species, there are substantial gastric protrusions out of their mouth and cloacal hemorrhaging.

Internal analysis:

The tunica externa was ruptured and the tunica interna was pushing into the body cavity. There were big pockets of air that looked as though fish very rapidly experienced it and acutely burst that affected tissue. Swim bladders of walleye have thick walls but theirs’ were perforated and caused a hole. Their gills also had a lot of gas emboli. It’s like an acute pressure change that these fish went through.

Additional Lab Findings:

Assorted protozoan and helminth parasites. Other bacteria *Shewanella* sp. (few cfu) from minority of fish. No viruses detected via cell culture. No gross signs consistent with systemic infection.

Summary:

Reports of elevated DO. Gas emboli observed across affected fish species. The dam had switched from mid-draw to bottom draw just prior/during the rapid ice melt (where deep water may have contained high N2). Gastric eversion, ocular emphysema, hyperinflated and/or perforated swim bladders, cloacal hemorrhage and prolapse. Hypothesized the mortalities were due to gas supersaturation & barotrauma from acute pressure change.

13. Update on Asian Carp

There has been multi-agency screening of Asian carp for VHSivb in the Illinois River. VHS has been detected in Illinois waters of Lake Michigan (near Chicago).There is a proposal to use Asian carp from the Illinois River as lobster bait out east either in Maine or the Canadian side, as their usual supply of lobster bait is much less available. The concern is VHS being present farther downriver near Peoria, where they plan to harvest Asian Carp for lobster bait.

USFWS sampled and collected Asian carp along the southern reach of the Illinois River where commercial fishing is allowed in April, May, and June. the USFWS did molecular

screening and cell culture for VHS and Asian fish tapeworm. No positives were detected on the three sampling dates. Most fish collected were silver carp (around 150/per sampling trip), as well as common carp and bighead carp. From the May sampling there was no CPE in the viral samples. EPC at 20 for SVCv. April and June screenings with qPCR had very week positives for VHS and could not confirm with conventional PCR. The samples were pooled from Starved Rock and Marseilles pools from April, May, and June and took a subsample for testing. It is not confirmed whether these species are susceptible for VHS so a wider sample of species and numbers of fish to be screened is needed.

Discussion

Why did they choose those upstream locations to collect and screen? Illinois would have to go further south in the river to get the half million pounds.

Probably because it's closer to Lake Michigan. They would have to ask Kevin for sure. It might have to do with where they are doing contract work.

They already have koi herpes virus in Maine so they are not concerned about that? Correct, they are just interested in SVCv and VHS.

14. Technical Advisors list review

Per discussion earlier in the meeting, the committee will invite two people to advise on Thiamine Deficiency: Jacques Rinchard (SUNY Brockport) and Don Tillitt (USGS). The committee was pleased to add a new advisor for bacteriology – Hui-Min Hsu. Diane Elliot may be ready to retire. The committee can possibly replace with Maureen Purcell. Jim Winton is now retired. Gael Kurath is a possible replacement for Virology. Parasitology- Matt Griffin from Mississippi State is in question.

ACTION ITEM: The chair will ask the list of suggested people if they are interested in serving as technical advisors and inquire about others that may be retiring.

15. Agency Updates

<u>IN DNR</u>

A spring kill occurred late March after a harsh winter at Geist Reservoir northeast of Indianapolis. The kill was predominantly redear sunfish. Nine redear and two largemouth bass were submitted to Purdue's ADDL April 3, 2019. Results were *Aeromonis* sp., *Yersinia ruckeri* and fungus were cultured. Nothing more specific than that was detected. All fish had gill congestion. A bluegill virus report was distributed last year and are wondering if that could be a possibility. However, Purdue didn't have the cell lines to screen for it.

Phillips mentioned that Erich has worked on a bluegill virus. BF1's were created and it works also on BF2's but they are hard to grow. Hope to know more about this by the next meeting

The East Fork hatchery had a problem with *Columnaris* in walleye. Migrating Skamania steelhead captured from a USFWS sea lamprey barrier on Trail Creek in Michigan city were exhibiting high mortalities after returning to the Bodine hatchery. *Aeromonis hydrophylla* was detected which was resistance to tetracycline. Trail Creek and Lake Michigan harbor temperatures were notably warmer this year. Initial handling mortality was normal. However, mortality would spike about two week after each new batch of fish were brought into the hatchery. Losses so far have been 50-60%. Symptoms seen were eroded tails and fungus that was not resent last year. Only one death as of yesterday (July 31st). More mortalities may still be coming within the next week as it was about 10 to 15 days before there was a spike in mortalities, after which they would drop off.

MI DNR

Eyes in the field system are still being used to report fish kills and other fish related events. Michigan has had 133 reports since last March. DNR is required to answer every one, which becomes time consuming. The department is also getting a lot of input from Facebook, which requires even faster response times with one or two a week. By the time kills are reported, only old carcasses are available for diagnostics. There have been several *Flavobacterium* issues in Michigan hatcheries but not a lot else. Seven diagnostic cases have been going on since April, which is a normal load of cases.

MDNR is concerned about LMBv spreading into the northern part of the lower peninsula around Alpena. Unusual LMBv lesions were found on fish. There are reduced numbers of SMB in fisheries that used to be very good. MDNR still allows catch and release fishing year-round, with harvest later in the year now.

Grand River in Lansing had a large carp kill but nothing else to report there. There is a concern that it is koi herpes virus.

There were mud puppy die offs that can be somewhat normal when there are harsh winters. Several individuals were sent for diagnosis to USGS but didn't come back with any interesting or diagnostic.

There was a large die off of Black Crappies only. The samples were not provided soon enough to investigate and there were no useful photos to see if there were lesions. Single species kills typically mean something odd is going on.

There was a very large Gizzard Shad kill on River Rouge in downtown Detroit next to the Marathon Oil Plant. There was a 10 degree temp shift in a day. Many of the fish were densely packed into the lower river in April. The event turned into a case of trying to close the oil plant because of the fish kill with influence from the media. The

temperature rise was the cause and not uncommon to kill the shad. Jim Francis commented that it was nothing to worry about. There was no analysis done to confirm the fact that it was not from an oil spill or contamination from the plant.

Discussion

It is a rare instance that one would find VHS in Gizzard Shad but that wasn't driving the mortality. MSU also collected *Flavobacterium* and *Columnaris* from internal organs of fish. It is not common to isolate *Columnaris* and *Flavobacterium* at the same time. There we no external signs on the fish.

Tom Loch- MSU

Surveillance from Michigan Hatcheries:

A yeast-like organism was found in the gastrointestinal tract of fish. The yeast may have been coming in with food. The feeders were cleaned last year. This year a similar issue arose and two different yeasts were recovered. Still unsure what is driving the mortality issues but it could be density related. Yeast is still in the gastrointestinal tract of the fish. No immune response seen in response to the yeast. So hatchery staff are just increasing flows and making sure it's not a feed related issue.

Case-Update:

"Shiawassee National Wildlife Refuge: Shiawassee River Black Bullhead Mortality". A grad student at University of Michigan was in the field doing work at Shiawassee National Wildlife Refuge and started to see fish kills of just Black Bullhead in the area. They were not large scale kills. There are also common carp present and the refuge gets its water from the Shiawassee River and probably some from the Saginaw River. It is a seasonal marsh system and drags a lot of fish in. It is a classic lower warm water river complex. Mortalities were inside one of the ponds. Water was around 21 C during that period. Fish had external ulcerations. There are suspicions it was c*olumnaris*. Freshly collected fish that MSU received showed multi-focal shallow ulcerations of the epithelium and in some indivduals the ulceration penetrated deeper with some discoloration. Scrapes of the epithelium showed dense long rods highly suspicious of a *Flavobacterium*. Kidney and Brain samples were *columnaris* and *Flavobacteirum* positive. Water temperature was favorable for *columnaris* to live and is usually the driving force of catfish mortalities. No viruses were detected. No gross signs were present consistent with systemic infection. It was convincingly largely driven by *columnaris*.

Discussion

Prior to the water getting pumped in, are these ponds emptied? Are they dredging? Wet versus an intermittent aquatic system could be introduced to something totally different during those draw down times. These ponds are not drained entirely and no they don't dredge.

MN DNR

There have been reports of winter kills this spring. There have been minnow shortages from producers within the state. Another bill to allow the importation of minnows was introduced during the 2019 legislative session. MN DNR does not support the importation of live minnows. There was a report of tilapia lake virus in Idaho. Based on this report, MN DNR has changed testing requirements for tilapia that is imported into Minnesota. Testing now follows OIE protocols. Common carp kills continued in Minnesota, but at reduced levels from last year. KHV and CEV have been detected but common carp populations are not impacted. Crystal Springs Hatchery was depopulated because of a furunculosis outbreak that couldn't be controlled. There are plans to start stocking again soon. Minnesota is in the process of developing a new strain of Brook Trout that will be genetically native to Minnesota called the "Minnesota Heritage Strain". The only space to culture it would be Peterson Hatchery, which is a class A hatchery. MN DNR will wait for three years of disease free testing there before the project can begin. The hatchery was almost cleared for year three, but then a test of the ovarian fluid was positive for BKD. However, only one fish was BKD positive this time around so bringing in wild fish to make this strain is still possible. Lanesboro Hatchery has a new nursery office. During construction, contaminated soil of diesel oil was found. There is a rearing pond right behind the hatchery and it is not lined. Hatchery staff have never seen any oil slicks so it is not likely that there is contamination but now that the soil was disturbed, it will be monitored closely. A new sinkhole has opened there so now there is a surface water connection. Previously, there were two others that opened up and were dealt with by either digging down and filling it in with different sized rocks or re-routing the streambed. The other one built a large dyke to move the stream as well. This sinkhole has a stream that enters it and goes into the ground it may not be possible to reroute the water. There is also a cow pasture near by the stream.

An update from last summer about splake mortalities related to handling; it is still continuing with no pathogens found or detected. It remains unresolved. The lake trout come from a wild strain to make the splake.

Discussion

What signs of disease if any do you see with the splake? We don't see any signs of disease. They are completely healthy but when we touch them and move them, they die at all ages. We're trying to just not touch them until it's time to stock and keep them in low densities for now.

No one in management is suggesting to make some changes to fix this issue? No, it is currently not a concern.

ACTION ITEM: In relation to splake mortalities related to handling, Paula Phelps & Ling Shen will create a summary of what type of analyses and water quality testing that has

been done at the hatchery, when this phenomenon first appeared, and what has changed at the hatchery over the years, to discuss at the next meeting.

WI DNR

Wisconsin DNR has been conducting fish health inspections at the state fish hatcheries, broodstock OVFL surveillance, VHS surveillance, and baitfish testing and surveillance from private vendors. There is an issue with having to monitor the fish management database for fish kills, as these are not being reported in a timely matter from biologists in the field. There will be a large renovation of the re-circulation system at the Kettle Moraine State Fish Hatchery, to be completed by spring of 2021. The state is supplementing fish production through a partnership with private entities but that is proving to be very difficult.

ACTION ITEM: A case history of working with private entities from MI DNR will be given to Danielle and her Fish Chief, Justine.

Acipenserid Herpesvirus 1 research ccontinues as WDNR evaluates the prevalence of herpes-lesions specific to lake sturgeon around the state. This year sampling kits were developed and sent out across the state. It is a non-invasive process using skin scrapes that are then sent to Florida for analysis and results. There were positive fish from last year. Electromicroscopy analysis revealed the herpes virus. Map of + and – detections shows that it is linked between the basins. There was a plan to take Wolf River Lake Sturgeon to repopulate the Fox River but it is on hold until this virus is investigated further. It is a little more ubiquitous across the state so it wouldn't be suddenly introduced into the Fox River basin.

Black Crappie sarcoma has been happening since the late 1980s mostly angler identified and in north west WI now, but has been detected more south since the 80s.

Hatchery-reared Northern Pike continue to show 20% incidence of shortened peduncles. The plan is to look at vitamins A, C, and K in the feed. They are continually the same source of fish from the wild. There are early signs always in the same section of the vertebrae. The fish are then re-stocked back in the same place. Inbreeding is suspected.

Discussion

Regarding putting sturgeon into the Fox River, are there concerns about positive sturgeon populations throughout the basin now? The next level of investigation should ask, if this virus is more broadly seen in lake sturgeon across the state.

At this point, we want to investigate this further but managers want a decision now. We would definitely want to carry this out further and test more of the basins around the state. Decision makers are much more inclined to make a decision before we get the larger picture.

It may be a good idea to look at the genetics of the fish. These results could be a classic result of inbreeding. Degenerative issues can also express themselves with flavobacterium .

WDNR has never detected *Flavobacterium* during site inspections. We have to do testing on Saturdays because we get samples on Friday and need to be able to send it out by Saturday. Is there anywhere that can do testing on Saturdays? In Minnesota, when we send samples out, we would just note in the report that we did the testing outside of the 72 hour time period and just did the sampling the following days to get it done. Samples would show up on Monday or Tuesday and then we would note how many days later we tested the samples. Michigan freezes our ovarian fluids at -20 and then send them as we have the time.

For those of the committee that work with Great Lakes Spotted Musky, has anyone ever seen green liver or kidneys in fingerlings?

It can be attributed to bile from having large meals. Sometimes it extends into the musculature and that is probably not normal. Otherwise, green liver is definitely normal.

PFBC

How concerned is the committee about cutthroat trout virus (CTv) if Rome strain brown trout that are intended to go into Lake Erie are positive? Fish were positive through next-gen-sequencing but did not pick it up with cell culture so what should PFBC do with these fish? They are supposed to go into Lake Erie and tributaries at a location the PFBC has been stocking for years. Pennsylvania has not run into this before. Approximately 120,000 fish are infected that are scheduled to go into tributaries of Lake Erie.

CTv is not known to be pathogenic to fish but these fish are positive and destined for the Great Lakes. The PFBC did another risk assessment and it came out as a low risk.

In addition, the FDA are doing some inspections so make sure you keep all of your records for inspections. Authorities have the right to come in and do these inspections.

Pennsylvania has had an Enteric Redmouth disease (ERM) detection for the first time. The PFBC is inspecting any moribund fish.

DECISION: Consensus from the committee is that CTv in the Rome strain brown trout is not a concern from a fish health perspective

ACTION ITEM: John Dettmers will email Coja with a consensus statement from the GLFHC.

WI USFWS

Todd Turner retired yesterday as regional assistant director for fisheries.

USFWS is working on bringing in new lines of Huron Perry Sound stock from the wild. This was last done in the early 2000s. Ontario was able to breed them and bring them over but are not sure that is possible now. If the parents are tested then Ontario can hold the eggs until getting the Title 50 permit to get them into the US.

NYSDEC

Discussion

How many hatcheries or agencies have had to do the Homeland Security permitting for peroxide? Minnesota and Wisconsin did.

How many hatcheries did you abate? Minnesota did have to abate any hatcheries. Michigan stopped using it at the Marquette SFH because of the requirements. New York does not have concerns with ongoing diseases in state hatcheries. There is persistent chrysobacterium that is similar to flavobacterium. It was found in rainbow trout that were brought into the hatchery with them. Chrysobacterium does respond to Aquaflor-T.

Wild Fish Disease Issues:

New York is seeing LMBv in small mouth bass. An LMBv outbreak did occur with a combination of *E. tarda* and *columnaris* and then LMBv showed up. Oneida Lake discontinued recreational fishing because of it.

New York has persistent infrastructure issues for some time with hatcheries that are decaying, which in turn lead to some fish health issues. The Randolph Hatchery produces 80% of salmonid eggs. A spring pond feeds into the hatchery. It went from 8ft to 4 inches deep. So, when a rain event occurs, all the sediment goes into the eggs and broodstock causing issues.

In 2010, the state reduced the cost of state services including reduced cost for fishing licenses, which put the state's conservation fund into deficit. This mistake was realized and resulted in a lot of funding being provided by the administration. NYSDEC is planning to use it for gas super saturation, radon abatement, and well water and other water issues, using about $2-4M a year.

Romiskany Fish:

New York has used Rome strain brown trout and lake trout that were resistant to *furunculosis* for many decades. These strains are now inbred and do not do well in the wild. A population of wild Rome strain fish was found and were crossed with domestic females in the hatchery and made a hybrid fish called the Romiskany strain. They are going to be broodstock this year and will be used in the hatchery system. Last summer, the first stream study was done for the Rome strain inbred fish compared to the new Romiskany strain fish. There was much better survival of the Romiskany fish. New York will cull the inbred Rome strain fish, replacing them with Romiskany strain fish in the future.

DFO

No unusual detections occurred with recent inspections. KHv was detected in a closed system, but no one of this virus was detected from samples collected in the wild. Lake Tilapia virus has been detected in the U.S. that may affect Canada in the future. There are no samples for testing yet but it is underway. Florida has detected shrimp virus, which also has been detected in Alberta and British Columbia. DFO is working with CFIA now for investigation of the pathogen. There was 350 shrimp used and are still figuring out how and what tissues to test, etc. but DFO is making headway on the virus.

ACTION ITEM: Godard knows a shrimp veterinarian and will get them in contact with Sunita regarding the shrimp virus.

OMNRF

Ontario has detected gas bubble disease (GBD) and some cold water disease. Since the winter meeting, inspection of younger bloater brood lots at White Lake found ERM. The Ministry separately inspects stations in the Atlantic and Hudson Bay watersheds, because VHSv has been detected in the Atlantic drainage but in the Hudson Bay drainage.

Wild walleye collection from the Bay of Quinte and Manitou Island are all cleared for bacteriology and virology. Reports are coming in of die offs but they seem to be focused on a few specific locations including Manitoulin Island for whitefish, with no signs of infectious disease.The Ottawa River has seen die-offs of multiple species due to a spill of some sort on the Quebec side. SVC, KHv, and VHSv, were all negative from the pooled samples from Manitoulin Island Lake.

Creative Dx/Rx innovations/Interesting Disease Cases (All)

Lake Erie Common Carp –Tom Loch

Field crews found common carp that have tremendously extended abdomens. They are finding them throughout the year (about two to three at a time).

MSU got a fresh fish to analyze. Necropsy results showed extremely large amounts of fluid in the abdomen. The lab tested for carp edema virus, koi herpes virus, and spirulina. There were no true positives. Gills were not necrotic. Nothing too significant for bacteria. Loch will plan to present on more testing at the next meeting.

16. 2020 and 2021 meeting dates and location

DECISION: It was decided that since Hobden is the Chair and international travel will be difficult for her, the next few meetings would be held in Canada.

Winter 2020 meeting location

Ottawa, February 4-5. Sunita will host

Summer 2020 meeting location

Thunder Bay, August 5-6. Kerry will Host

Winter 2021

Windsor, TBD. Kerry will Host

ACTION ITEM: Generate a summary page of the meeting minutes to send to the Chair and Vice Chair.
Completed: September 2019.

GREAT LAKES FISH HEALTH COMMITTEE
TECHNICAL ADVISORS

August 2019

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)

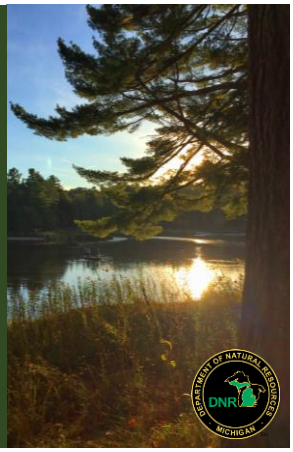
Parasitology

David J. Marcogliese (Environment Canada)

Thiamine Deficiency

Jacques Rinchard (SUNY Brockport)
Don Tillitt (USGS)

EEDv Case History – The pathogen that just keeps giving (Preliminary Analysis)



Gary Whelan
GLFHC Meeting
August 2019

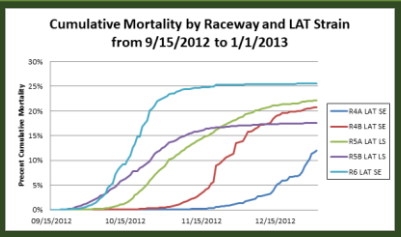
History of EEDv Detections/Outbreaks at MSFH

- 1980s – Initial mortality events
- 2009-2010 - Detection
- 2012-2013 - Mortality
- 2017-2018 - Detection
- 2018-2019 - Mortality

EEDv 2009 Detections

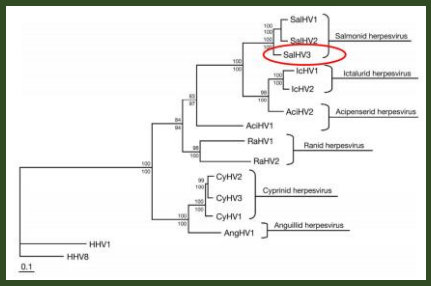
- Hendrick develops genetic test
  - CA SFH positive
  - WI Testing – Les Voigt - Bayfield SFH Strains positive 30/30 fish
- MI Testing
  - Ovarian Fluid – Kurobe (U-Cal Davis) – 9/30
    - 2001 Broodstock – 15/41 positive
    - 2004 Broodstock – 11/45 positive
  - Ovarian Fluid – Kurobe (U-Cal Davis) - 10/21
    - 2001 Broodstock – 3/10 positive
    - 2004 Broodstock – 4/10 positive
  - Similar to Bayfield SFH Strains
  - Primer questions on specificity
- WY Testing – Story SFH Positive
  - Fish were from Jenny Lake
    - Hatchery received some Lewis Lake fish in 1980s from Saratoga NFH
  - with no introductions since 1967
  - 98% match with Bayfield SFH fish

EEDv 2012-13



- 90,656 LAT Production Fish Lost

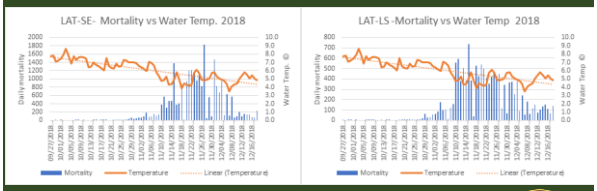
EEDv 2012-13 Strain Map



EEDv 2017-2018 Detection

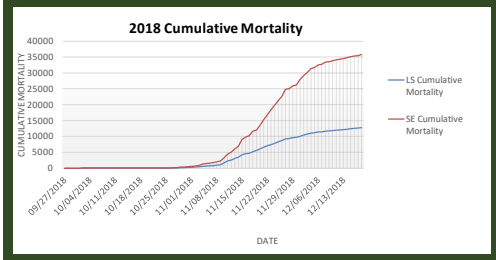
- No unusual mortalities seen
- Samples run to examine for stocking
  - SE
    - February 52/60 positive – Ct mean = 27.1
    - March 44/60 positive – Ct mean = 29.3
    - April 18/60 positive – Ct mean = 32.2
    - May 33/60 positive – Ct mean = 32.5
  - LS
    - February 59/60 positive – Ct mean =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

EEDv 2018



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

Wild EEDv Surveillance

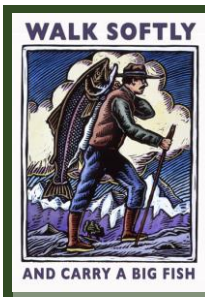
- 2010 - USFWS
  - Lake Michigan
    - Wisconsin waters, 20 LAT (4/20 positive via PCR, no sequencing)
    - Illinois waters, 20 LAT (6/20 positive via PCR, no sequencing)
    - Sturgeon Bay, WI, 20 LAT (7/20 positive via PCR, no sequencing; 3 of the positives were analyzed via Gavin Glenney's realtime 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

Wild EEDv Surveillance

- 2018 – MI DNR
  - Lake Huron
    - Northern Lake Huron – 0/10
    - Southern Lake Huron – 1/90 (low titers)
  - Lake Michigan
    - Northern Lake Michigan – 2/60 positive
  - Lake Superior
    - Marquette Harbor - 58/60 positive
      - Ct values ranged from 22.8 – 34.3, average Ct=31.4; 2/60 w/ Ct >35
- 2019 MI DNR
  - Northern Lake Huron
  - Lake Superior
    - Isle Royale - Lean – 0/30
    - Big Rock
      - Fat – 0/30
      - Lean – In progress

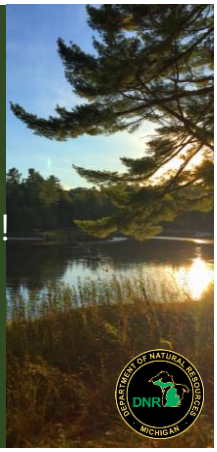
Disease Control Direction

- 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

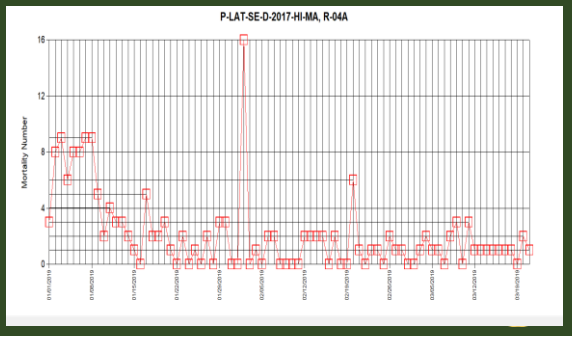


Thank You!

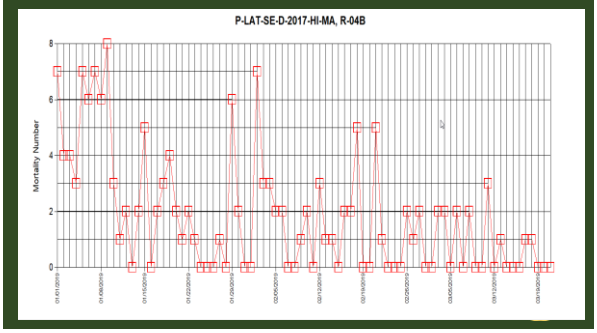
Gary E. Whelan
Michigan DNR
whelang@michigan.gov
517-284-5840



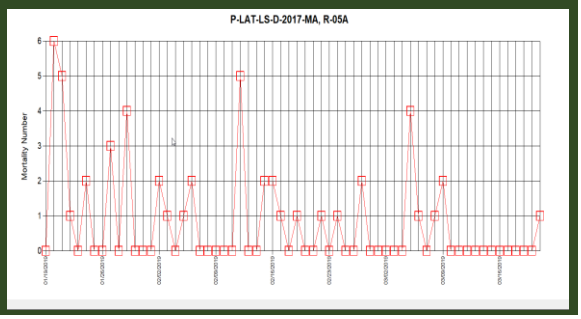
EEDv 2019



EEDv 2019



EEDv 2019



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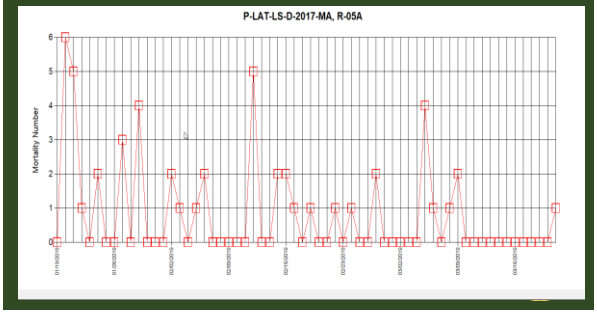


Ministry of Natural Resources and Forestry

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

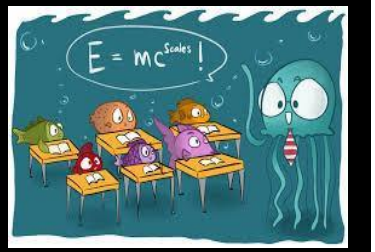
Prepared by Kevin Loftus, Manager, Fish Culture Section

July 2019

Ontario

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).



3

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)

2

Findings from Other Research

Enriching the captive environment of fish- why and how

6.11.2018, Oslo

Pekka Hyvärinen
Natural Resources Institute Finland (Luke)
Kainuu Fisheries Research Station
Paltamo, Finland
www.ktrs.fi, www.luke.fi

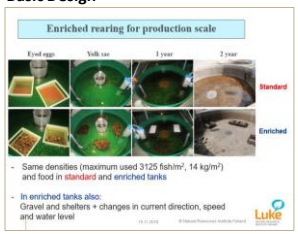
Luke

Ontario

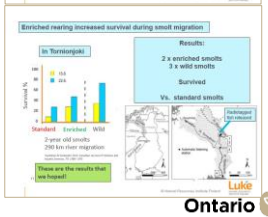
Findings from Other Research

Objectives

Basic Design



Results



5

Ontario

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

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

6

Ontario

Ministry of Natural Resources and Forestry

Sharing Ontario's Experience Developing Expertise in the Culture of At Risk Mussels

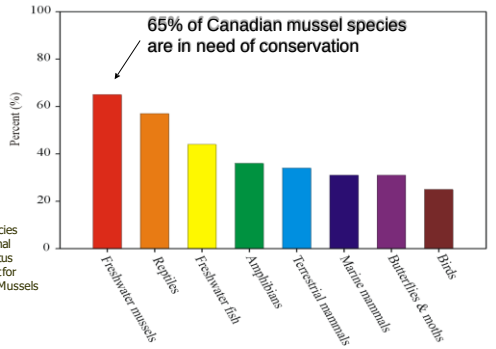
Fish and Wildlife Services Branch
Provincial Services Division

Chris Wilson, Production Planning Biologist,
Fish Culture Section

July 31, 2019

Ontario

COMPARATIVE RISK STATUS - CANADA



Source: Wild Species 2000; National General Status Assessment for Freshwater Mussels (2004)

Ontario

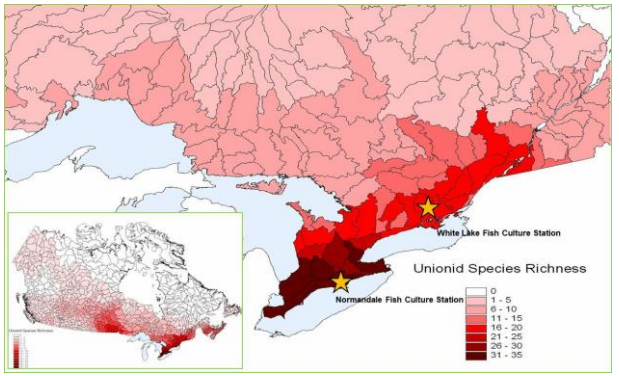
Why Culture 'at risk' Mussels

To support recovery efforts (in a manner consistent with recovery plans and government response statements) by

- Providing animals to support restoration (i.e., stocking) initiatives if and when required
- Providing animals to support research that, in turn, will support recovery efforts and/or help establish water quality standards
  - Life history
  - Toxicity
  - Genetics
- Enabling the development of 'ark' populations if and when required.

Ontario

SOUTHERN ONTARIO IS A MUSSEL HOT-SPOT



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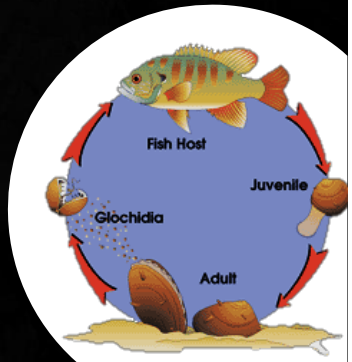
FRESH WATER MUSSELS: TAXONOMY

- Phylum Mollusca
- Class Bivalvia
- Order Unionoida



GLOCHIDA HARVEST
"Free Glochidia"

Ontario

GLOCHIDA HARVEST "Conglutinates"

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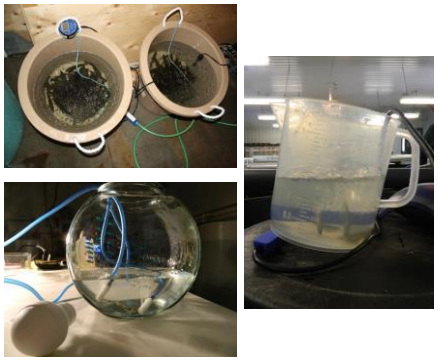
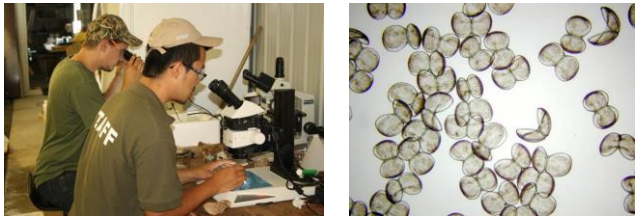
INFESTATION



- Swim fish in stirred glochidia suspension
- 4,000 glochidia/fish (+/-)
- 30-60 minutes
- Fish inhale glochidia, which attach to gills

Ontario

VIABILITY TESTING & ENUMERATION



- Glochidia and newly metamorphosed juveniles and are usually 60-300 microns - *smaller than a grain of sand* - depending on species.
- Good microscopes and lighting are essential
- Handling requires involves suitable sieves, volumetric pipettes, and glassware

Ontario

ASSESSING INFESTATION RATE

- Manual count of attached glochidia on each gill arch
- Ideally 100-300 glochidia per fish depending on the size of the host

Ontario

HOST FISH HOLDING & RECOVERY OF JUVENILES

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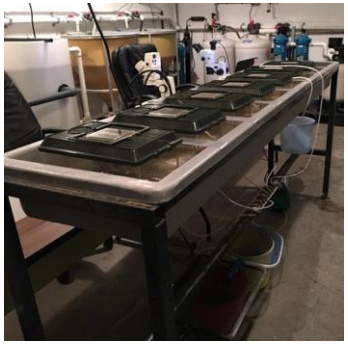
EARLY REARING
"Sediment Pans"

Ontario

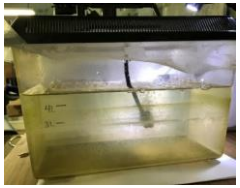
EARLY REARING
"Mucket Buckets"

Ontario

EARLY REARING
"Static Sediment Tanks"



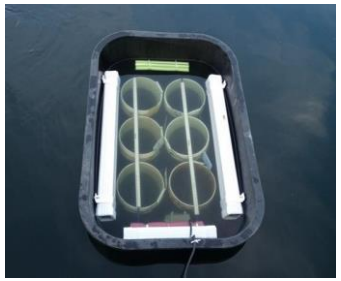
- Dusting of "wild" sediment
- Filtered hatchery water
- Gentle aeration from an air stone
- 2,000 newly transformed juveniles/tank
- 100% water and sediment change every 7-14 days



**Designs by Megan Bradley, USFWS, and Dr. Chris Barnhart, Missouri State U.*

Ontario

ADVANCED REARING
"Tubweller" & "Flupsy"



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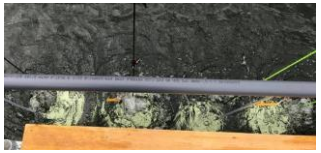
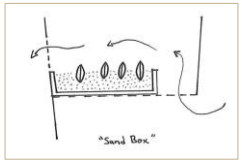
ADVANCED REARING
"Pond Buckets"



Ontario

ADVANCED REARING
"River Trough"




2013 Wavy Rayed Lamp Mussel

Ontario

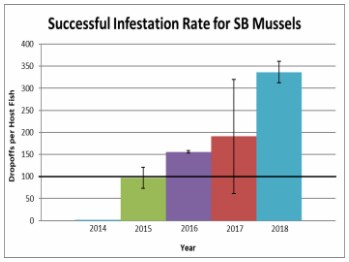
PROGRESS OVERVIEW

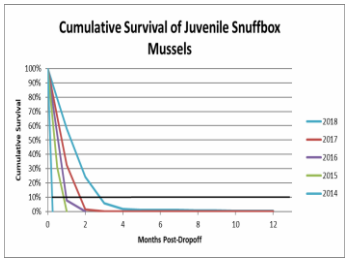
2012 – 2018

- Small program funded using consolidated revenue funds
- Experience working with 4 SAR species
- Experience collecting, treating and caring for host fish
- Constructed juvenile recovery systems
- Constructed multiple types of early and advanced rearing units
- Regular communications with the mussel community
- No new culture efforts after fall 2018

Ontario

PROGRESS - SNUFFBOX AND LOGPERCH





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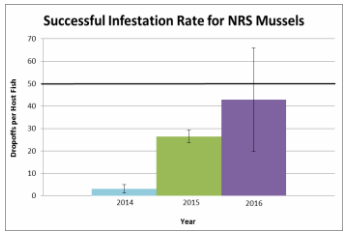
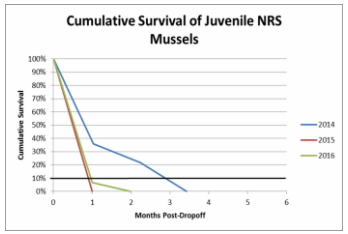
PROGRESS - NORTHERN RIFFLESHELL & IOWA DARTER





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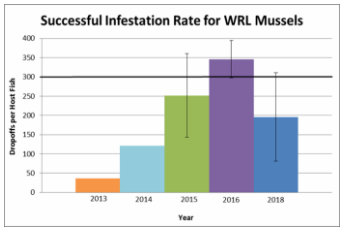
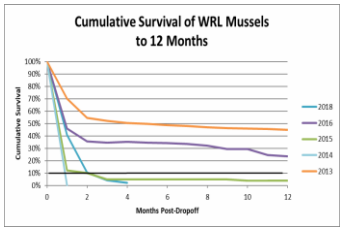
PROGRESS - WAVY-RAYED LAMPMUSSEL AND SMALLMOUTH BASS





- Year-to-year increases in infestation rate from 2013 to 2016 are the result of improved procedures.
- The decline between 2016 and 2018 coincides with a switch from using glochidia from multiple females during infestation to glochidia from a single female.

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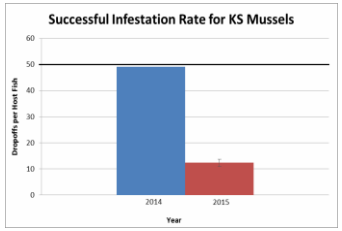
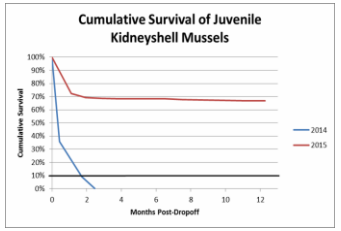
PROGRESS - KIDNEYSHELL AND BLACKSIDE DARTER OR JOHNNY DARTER





- 2014 is based on using the Kidneyshell's primary host, the Blackside Darter.
- 2015 is based on using the Kidneyshell's secondary host, the Johnny Darter.

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CURRENT SITUATION

- To date, no requests for mussels for stocking
- However, significant interest from research community
  - Environment Canada & University of Guelph – Chloride Toxicity Testing
  - Central Michigan University – Genetic Variation in Propagated Mussels
  - University of Guelph – Flow Chamber Feeding Trials
  - MNRF Aquatic Research & Monitoring Section – Mussel eDNA Testing
  - DFO / National SAR Network – Potential for Experimental Introductions
- Many of the research needs identified in recovery plans require organisms to work with…but they are endangered!
- Revenue from of sales over last two years – $10.6 K
- Does not include value of donations to other research – estimated at $5K

Ontario

FUTURE

- Responsibility for the SAR program has been transferred to MECP
  - Fish Culture Section's efforts on are hold – no new efforts
- Fish Culture Section's will
  - Continue to care for our current inventory to see if they become reproductive (<1 hour/week)
  - Support MECP if requested
- Complete our propagation guide
- Continue to share what we have learned

Ontario

LEARNINGS

- Culturing 'at risk' mussels is very difficult
- Developing the skills and achieving success, consistently, takes time
- Attention to detail is huge!
- Consistent & experienced staff, with the right training, are critical
- Access to a network of experts is critical - reach out for guidance
- Success requires more than science

Ontario

ACKNOWLEDGEMENTS

To Our Partners

- Dr. Chris Barnhart, Missouri State University
- Dr. Paul Johnson, Alabama Conservation & Natural Resources
- Dr. Todd Morris and team, Fisheries and Oceans Canada
- Dr. Joe Ackerman and team, University of Guelph
- Megan Bradley, U.S. Fish & Wildlife Service
- Nathan Eckert, U.S. Fish & Wildlife Service
- Dr. Cynthia Lee and team, Toronto Zoo
- JR Shute, Conservation Fisheries Inc., Tennessee
- Daelyn Woolnough, Central Michigan University

And to

- Dr. Al Dextrase, Dr. Chris Wilson, Scott Gibson (MNRF)

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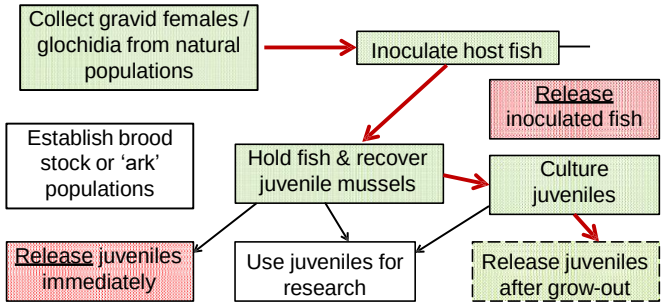
QUESTIONS

CULTURE & RELEASE STRATEGIES



Ontario

Ministry of Natural Resources and Forestry

Recent Experience with Radon at MNRF Fish Culture Stations

Fish and Wildlife Services Branch

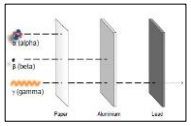
Provincial Services Division

Prepared by Chris Weaver, Radiation Safety Officer, FWSB and Policy and Program Coordinator, Fish Culture Section

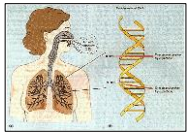
July 2019

Ontario

Health Risk



- Radon gas, itself, is an extremely low health risk
  - Emits alpha particles - cannot penetrate skin/paper
  - Breathe in and breathe right back out
- Primary risk are radon progeny (i.e., decay products / daughter particles) which attach to dust particles in the air
  - Unlike radon gas which is breathed right back out, these dust particles (radon progeny) can stick to lungs
  - These particles can decay in the lung and can damage cells
  - Cell damage can lead to increased risk of lung cancer

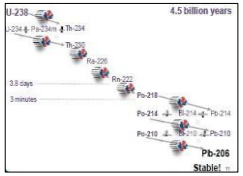


- Everyone is exposed to background levels of radiation

Ontario

Radon 101

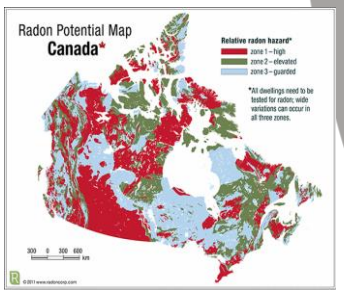
- A **N**aturally-**O**ccurring **R**adioactive **M**aterial (NORM)
- Produced by decay of uranium in soil, rock, water
- An invisible, odourless and tasteless gas
- Radon released outdoors is diluted / not a concern
- Radon released indoors can be a concern
- Only known health effect – ⇧ risk of lung cancer
- Canadian Nuclear Safety Commission has legislative control over man-made radionuclides and nuclear fuels.
- Provinces have jurisdiction over the use of, and exposure to, NORM.
- Health Canada provides guidelines and advice, including the "*Canadian Guidelines for the Management of Naturally Occurring Radioactive Materials (NORM) (2011)*"
- In Ontario, the MOL has endorsed Health Canada's Guidelines.

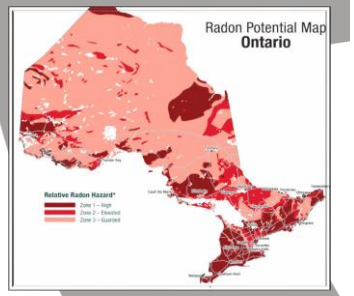


The World Health Organization estimates that around 14% plus of all lung cancer deaths globally are radon induced.

Ontario

Geographic Variation in Radon Potential



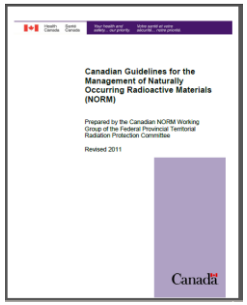


Health Risk

- The health risk associated with exposure to radon gas increases as the dose increase.
- Dose is a function of time of exposure and concentration during exposure.
- Someone who spent 8 hours in a room at 200 Bq/m^3 would incur the same dose (or risk) as someone who spent 1 hour in a room at 1600 Bq/m^3.
- This relationship is different than with some chemicals where risk increases with concentration, irrespective of the duration of exposure
- An analogy. A risk of driving is that we might have an accident. The more we drive, the more this risk goes up. The more we drive in high risk areas, the more this risk goes up. However, there is no guarantee that we will have an accident even if we drive in high risk areas for long periods of time.

Radon "Regulation" in Ontario

- No legislation in Ontario governing radon
- Health Canada introduced NORM Guidelines in 1988 setting reference level at < 800 Bq/m^3
- MOL website did not clearly indicate that it had had adopted the Guidelines
- Health Canada revised NORM Guidelines in 2007 lowering the reference level to 200 Bq/m^3
- This change was published in Health Canada's 2011 Guidelines
- MOL website did not clearly indicate it had endorsed the change in standard (as of 2014)
- MOL's website now clearly endorses the new standard.



Worker Dose and NORM Management

(Health Canada's 2011 NORM Guidelines)

Annual Effective Dose Limit

- 1.4 mSv/year – Dosage limit based on working 2000 hours/year at an average radon level of 200 Bq/m^3 (mSv = millisieverts).

Classification of Workplaces under the NORM Guidelines

- If all of work spaces have annual average concentrations < 200 Bq/m^3, then **Unrestricted** category applies. No further action is required.
- Where annual average concentration in one or more work space is > 200 and < 800 Bq/m^3. **NORM Management** applies, and the following steps should be taken:
  - Introduction of public and incidentally exposed worker access controls
  - Changes in work practices; and
  - Reducing the radon concentration levels to below 200 Bq/m^3 if feasible (ALARA applies)
- Where 1 or more spaces have annual average concentrations > 200 Bq/m^3 can still have an **Unrestricted** classification if we can demonstrate that annual estimated dose is < 1 mSv.
  - This is accomplished by either tracking time spent in different work spaces or by managers and workers agreeing upon reasonable estimates of time spent in different workspaces.

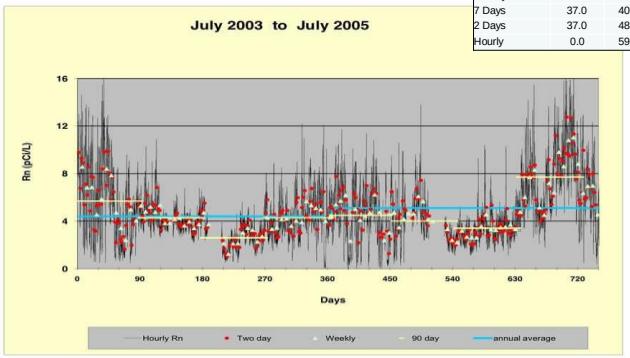
Radon Measurement Accuracy

- 200 Bq/m^3 of radon is equivalent to only 4 parts per quadrillion. Therefore, measurement accuracy over the short term is poor.
- Historically, as recommended by MOL, Fish Culture Section utilized short term, 2-7 day cannister devices to monitor radon. It is now recognized that infrequent, short duration testing does not provide a reliable estimate of average annual radon concentration.
- Health Canada and MOL no longer support short term testing and recommend a minimum of 91 day testing x 4 quarters or a 365 day testing period.
- Radon is a gas and is a heavy molecule. Being a heavy gas, it can accumulate near the floor if air circulation is poor; however, it will mix well if there is sufficient air movement.

  The weight of 1 gallon of milk is 8.6 pounds. The weight of 1 gallon of lead is 95.08 pounds. The weight of one gallon of Radon (if it was a solid) would be 160 pounds
- The Radon concentration in hatchery water is actually quite low (and difficult to accurately measure); however, because we use millions of litres of water per day, significant amounts of radon can be released into work spaces. Where this water is aerated matters a lot!

Radon Measurement Accuracy

Range of Test Results Bq/M³		
Length of Test	Low Value	High Value
Year-Long	166.5	185.0
90 Days	111.0	281.2
7 Days	37.0	407.0
2 Days	37.0	481.0
Hourly	0.0	592.0



From St. John's University, MN. Compares short-term and long-term radon test results in a house over a two year period. 5.4 pC=200 Bq

Ontario

How Did We Get Here?

- Fish Culture Section had been monitoring radon levels in its facilities on a periodic basis for years.
- In 2014, an employee concerned about elevated radon levels at one station, despite the recent installation of a ventilation system to reduce radon levels.
- Management and the Ministry of Labour MOL investigated.
- Management contracted the services of the Radiation Safety Institute of Canada's senior scientist to guide our efforts.
- Led MOL to announce official adoption of Health Canada's updated guidelines
- Outcomes
  - Heightened awareness of radon issue
  - Implemented a robust training and monitoring program at all fish culture stations
  - Made infrastructure changes at several stations to reduce radon levels
  - Learning curve for both MOL & MNRF
  - We are operating in full compliance with the guidelines
  - All staff are safe

How to Measure Radon?

Corentium.ca
$275 or $241
for 10 or more

To be used for investigational purposes. Can be moved around to test for hot spots or to determine if most areas of a building measure similarly.

Alpha Track Detector

Recommended 91-365 day exposure period. This is the only MOL approved device

Radon concentration [Bq/m3]	20% uncertainty	10% uncertainty
100	4 days	3 weeks
200	2 days	10 days
300	2 days	7 days
500	1 day	4 days
1000	1 day	2 days
2000	1 day	1 day

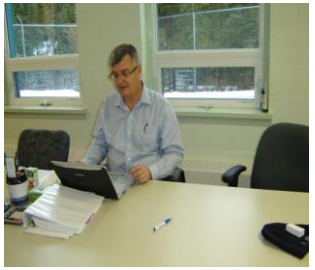
Cost $25-55
Available from:

Radiationsafety.ca
Accustarcanada.ca
Radondetect.ca

Ontario

Ministry of Labour (MOL) Direction

- Follow 2011 HC NORM Guidelines
- Create plan re how to comply with HC NORM Guidelines
- Designate a Radiation Safety Officer
- Implement long-term monitoring plan
- Inform workers
- Provide training
- Minimize radon exposure – worker access control restrictions
- Work with JHSC on interim measures



MOL Radiation Protection Officer preparing his report at Dorion FCS

Ontario

Where are we Now?

- RSIC reviewed historical data for all stations and provided summary reports.
- RSIC developed a generic Radon Monitoring Program which we, with RSIC's help, customized to and implemented at each Fish Culture Station.
- RSIC developed a Radiation Protection Program.
- RSIC developed a Radiation Protection Program Implementation Plan for each station.
- RSIC developed and delivered a radiation safety training program to all FCS staff.
- RSIC trained one FCS employee as a Radiation Safety Officer who was then designated as the RSO for Fish and Wildlife Services Branch.
- RSIC provided reports for each station interpreting the results of each 91 day monitoring period, rolling up the results over the course of a year, and providing advice on future sampling needs.
- RSIC's services included participating in calls with staff to review monitoring results, provided advice on requirements for the protection of workers and/or interpretation of Health Canada's guidelines, provided advice on radon mitigation strategies, and participated in discussions with MOL.
- Completed radon reduction projects at DN, BJC, TT & CW
- Most stations are now on a reduced radon monitoring plan (reducing costs)

Video – The Most Radioactive Places on Earth

(or How 20 Million [illegible])

https://www.youtube.com/watch?v=TRL7o2kPqw0

This is an educational video.

The host of this video uses microsieverts.
Health Canada uses millisieverts.
One millisieverts is equivalent to 1000 microsieverts.

Results of Four Years of Monitoring

- Six of nine stations fall within NORM's "unrestricted" category (i.e., all spaces have an annual average radon concentrations < 200 Bq/m^3)
- Three of nine stations have at least one space > 200 Bq/m^3 but <800 Bq/m^3 and, as such, fall within the "NORM Management" classification.
- In general, the spaces that are > 200 Bq/m^3 are spaces where staff spend little time.
- Because staff spend so little time in these areas, we are managing the risk to staff, as allowed by the guidelines, by monitoring the time spent and radon levels in these (and all other) areas and calculating exposure levels.
- This is being done to ensure that worker doses remain below the guideline's recommended dose limit for members of the public, which is 1 mSv per year.
- All of the staff at these three stations have a calculated radiation exposure level which falls below 1 mSv, therefore, we are successfully managing risk to the **same level** as that of the Unrestricted category.

中国水产科学研究院 黑龙江水产研究所 HEILONGJIANG FISHERIES RESEARCH INSTITUTE

中国水产科学研究院 Chinese Academy of Fishery Sciences

Current Status of Occurrence and Management of Diseases in Farmed Salmonids in China

Shaowu Li, PhD

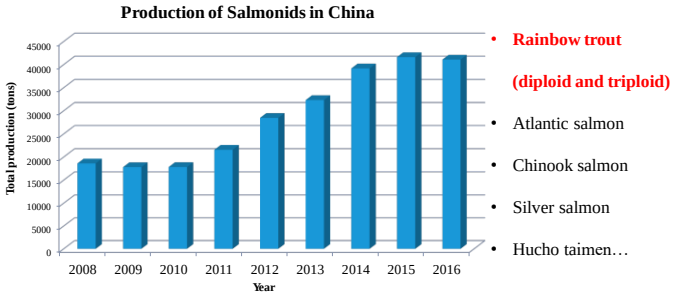
Laboratory for Fish Diseases, Dept. of Aquaculture, Heilongjiang River Fisheries Research Institute (HRFRI), Chinese Academy of Fishery Sciences (CAFS), Harbin, China

E-mail: lishaowu@hrfri.ac.cn

Aug 1, 2019

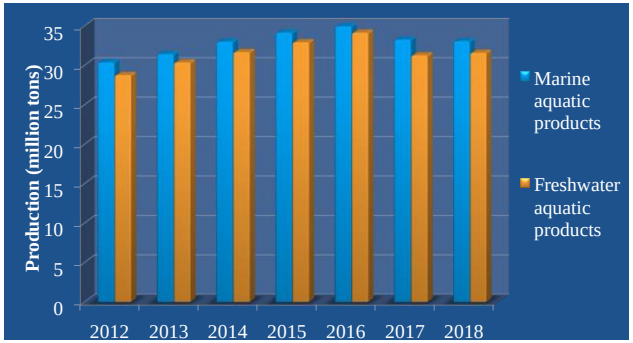
Production and Cultured Species of Salmonids

- **The total production of salmonids in China is about 40 000 tons in 2016.**
- **The production of rainbow trouts and other salmonids in 2017 is about 41 460 tons and 3089 tons, respectively.**



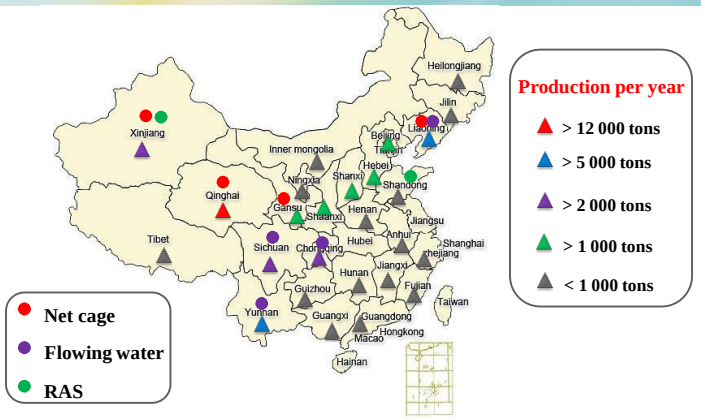
Data from《 China Fisheries Yearbook》

Total Output of Aquatic Products in China



- **Aquaculture production accounts for about 77%, while fishing production accounts for 23%.**

Distribution of Salmonids Aquaculture in 2017



Disease Outbreaks—the major obstacle

Infectious Disease

- Viral Diseases **IHN/IPN**
- Bacterial Diseases **Furunculosis/ERM/BCWD/BGD/CD/BKD**
- Fungal Diseases **Oomycete infections**
- Parasitic Diseases **White spot disease**

Non-infectious Disease

- Nutrition
- Environmental Stress
- Management

Co-infection

Lack of Commercial Vaccines

- Grass carp hemorrhage virus
- *Aeromonas hydrophila*
- *Edwardsiella tarda*
- *Vibrio anguillarum*

Certificate of New Veterinary Medicine

- ***No commercial vaccines available for salmonids***

- Antibiotics
- Disinfectants
- Immunostimulants
- Herbal medicines

MERCK Animal Health PHARMAQ part of zoetis

Differences in aquaculture scale, species and models

Our Institute (HRFRI)

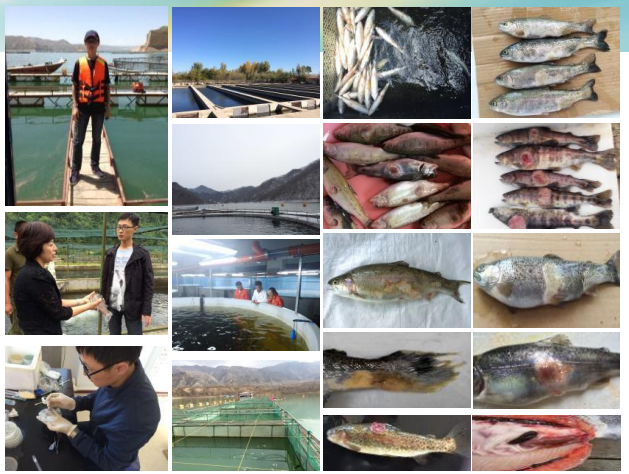
中国水产科学研究院 黑龙江水产研究所 HEILONGJIANG FISHERIES RESEARCH INSTITUTE

- Founded in 1950, Heilongjiang River Fisheries Research Institute (HRFRI) of Chinese Academy of Fishery Sciences is the oldest fresh-water fishes research institute in China.
- At present, HRFRI is the leading institute in the research fields of fish breeding and salmonids aquaculture in China.

Website: http://www.hrfri.ac.cn

Works in Our Laboratory

- **Epidemiology investigation**
  - *Prevalence of fish diseases*
- **Etiology research**
  - *Detection method/Genotype/Serotype/Virulence/Drug-resistance*
- **Prevention and control techniques**
  - *Interaction between host and pathogen/Vaccine/Probiotics/Herbal medicine*



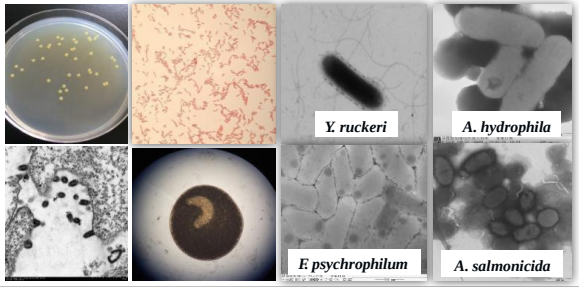
Works in Our Laboratory

- **Epidemiology investigation**

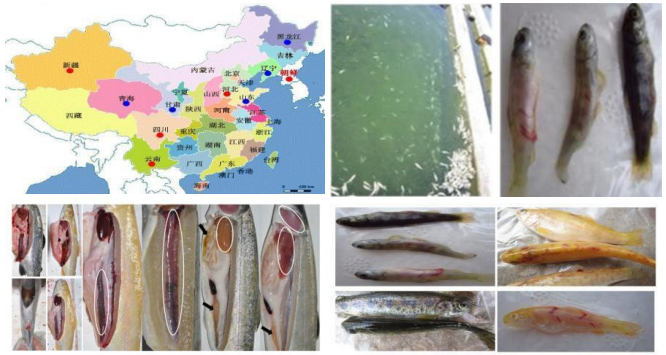
- **>10 provinces**
- **>100 fish farms**
- **Rainbow trout, Atlantic salmon, brown trout, golden trout, king salmon…**

Establishment of Microbiological Resource Pool

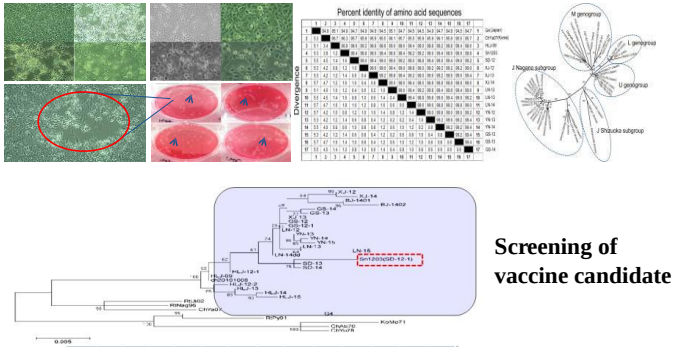
- ***IHNV, IPNV, A. salmonicida, Y. ruckeri, F. psychrophilum, F. branchiophila, F. columnare, Chryseobacterium* spp., *P. fluorescens, A. sobria, A. hydrophila*, etc.**



Infectious haematopoietic necrosis (IHN)



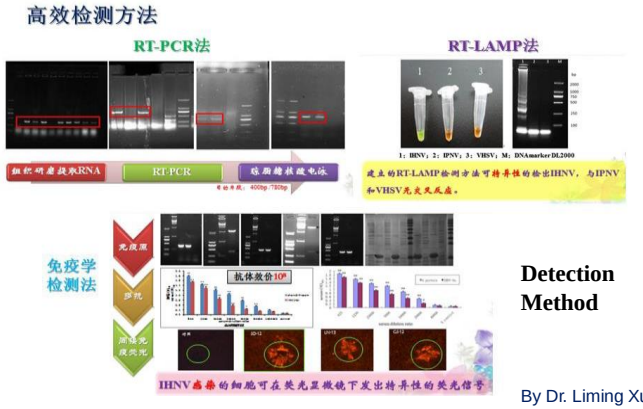
Infectious Haematopoietic Necrosis (IHN)



Screening of vaccine candidate

By Dr. Liming Xu

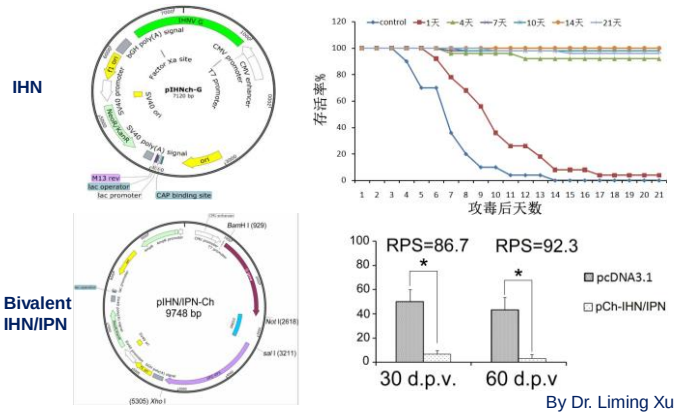
Infectious haematopoietic necrosis (IHN)



Detection Method

By Dr. Liming Xu

Construction of DNA vaccine for IHN

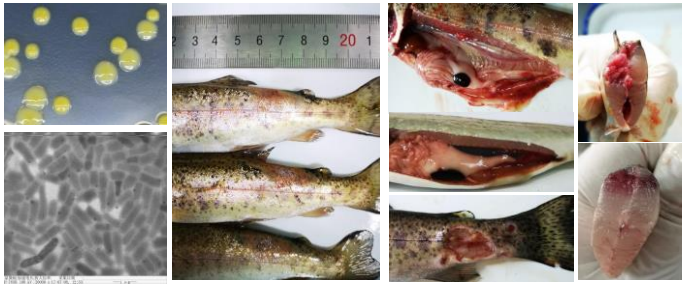


By Dr. Liming Xu

Appendix 6.

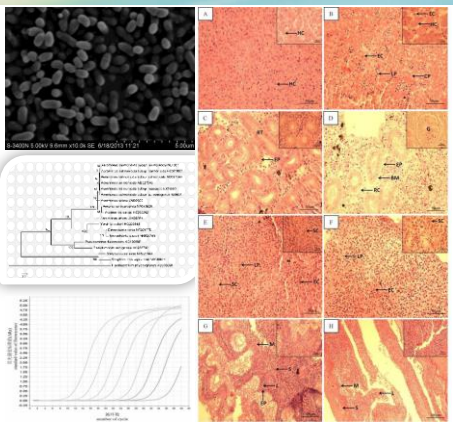
Flavobacterium psychrophilum

- **The causitive pathogen of BCWD/RTFS**



Aermonas salmonicida ssp.

- **Morphological observation**
- **Virulence determination**
- **Biochemical characterization**
- **16S rRNA analysis**
- **Antimicrobial susceptibility**
- **Histopathology**
- **qPCR detection**



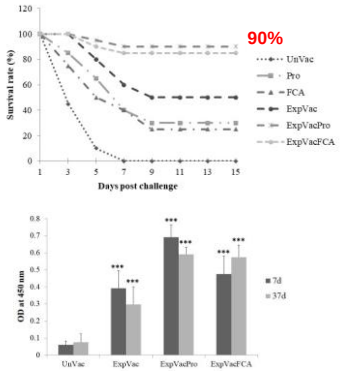
Flavobacterium psychrophilum

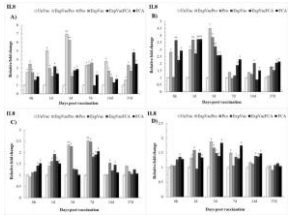
- **MLST analysis**
- **ST-12 and ST-78**
- **The fertilized eggs from USA/Denmark/Norway/Canada…**

Isolates	atpA	dnaK	fumC	gyrB	murG	trpB	tuf	ST
LN38	2	2	2	8	2	2	7	ST-12
LN40	2	2	2	8	2	2	7	ST-12
LN41	2	2	2	8	2	2	7	ST-12
LN44	2	2	2	8	2	2	7	ST-12
GS45	2	2	2	8	2	2	7	ST-12
GS50	2	2	2	8	2	2	7	ST-12
LN55	2	2	2	8	2	2	41	ST-78
QH58	2	2	2	8	2	2	7	ST-12

Aermonas salmonicida ssp.

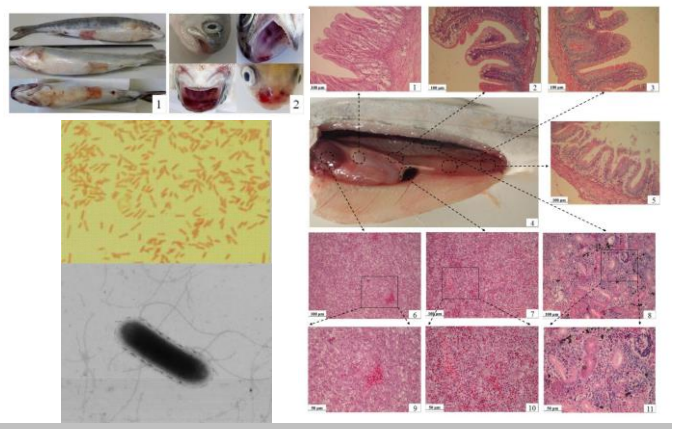
- **Development of inactivated vaccines against furunculosis**





- Propolis adjuvant highly enhances the vaccine immune effect.

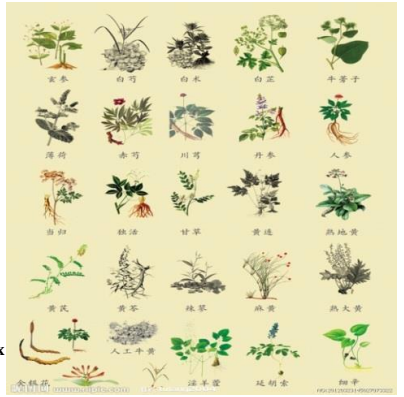
Yersinia ruckeri



Chinese herbal medicine—Additives/Drugs

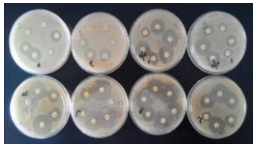
- antioxidation
- Anti-stress
- Anti-bacterium
- Anti-parasites
- Immuno-stimulants

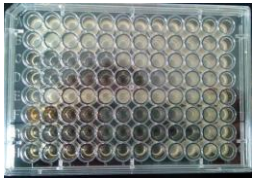
- Rhubarb
- Astragalus mongholicus
- Rhus chinensis Mill
- Artemisia carvifolia Buch
- Reynoutria japonica Houtt
- Phellodendri Chinensis Cortex
- ……



Antimicrobial susceptibility of *Aeromonas* ssp.

- *A. salmonicida, A. sobria, A. hydrophila, A. veronii, A. caviae.*



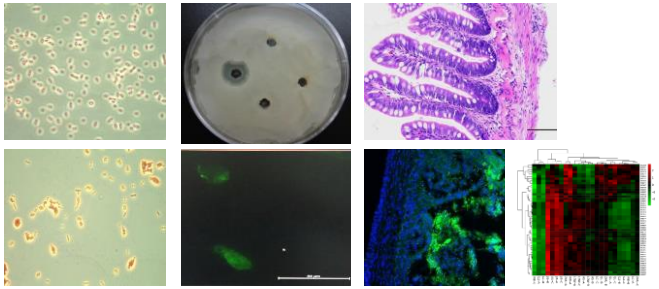


Antibiotics	Drug resistance rate (%)	Antibiotics	Drug resistance rate (%)
Levofloxacin	5.5	Tetracycline	38.9
Ciprofloxacin	25	Deoxytetracycline	16.7
Norfloxacin	33.3	Furazolidone	100
Enrofloxacin	30.5	Furantoin	100
Ofloxacin	30.5	Ampicillin	100
Streptomycin	58.3	Peillin G	100
Kanamycin	38.9	Amoxicillin	83.3
Amikacin	44.4	Chloramphenicol	36.1
Gentamicin	50	Florfenicol	19.4
Neomycin	30.5	Thiamphenical	44.4
Oxytetracycline	38.9	Rifompicin	38.9

- **The isolated strains were highly resistant to nitrofurans, β-lactams and aminoglycosides drugs, while sensitive to fluoroquinolones and chloramphenicol drugs.**

Protobiotics application in rainbow trout

- ***Bacillus ssp.***



Control measures

- Vaccine development and application
- Biosecurity
- Disease surveillance
- Laboratory diagnostics
- Scientific management

Many Thanks!

- Michigan State University
- Aquatic Animal Health Laboratory
- Dr. Thomas P. Loch
- Great Lakes Fish Health Committee

Our Team

GREAT LAKES FISHERY COMMISSION
GREAT LAKES FISH HEALTH COMMITTEE

POSITION STATEMENT REGARDING THE USE OF *Cyprinid Herpesvirus-3(CyHV-3)* AS A BIOLOGICAL CONTROL AGENT FOR COMMON CARP

The Great Lakes Fishery Commission - Fish Health Committee (GLFC-GLFHC) recognizes that various governmental entities throughout the world have considered the use of cyprinid herpesvirus-3 (CyHV-3), commonly called koi herpesvirus (KHV), as a control agent for nuisance cyprinid populations including for Common Carp (*Cyprinus carpio*). CyHV-3 is listed by, and reportable to, the World Organization for Animal Health (OIE). According to the OIE, common carp, koi carp, and several carp hybrids are susceptible to the virus (OIE, 2009). Both Silver (*Hypophthalmichthys molitrix*) and Grass Carp (*Ctenopharyngodon Idella*) can serve as asymptomatic carriers, but do not develop clinical disease (Bergmann et al. 2009). No information is available about the susceptibility of Black Carp (*Mylopharyngodon piceus*) or Bighead Carp (*Hypophthalmichthys nobilis*) to CyHV-3. The limited number of hosts susceptible to CyHV-3 is consistent with other herpes viruses, which co-evolve with a specific host. When considering the effectiveness of KHV as a control agent in the wild, additional information relevant to the GLFHC's deliberations include:

- CyHV-3 has caused numerous common carp mortality events in the United States, including in the Mississippi River drainage, making it likely that the Asian carps (Black, Silver, Bighead and Grass Carps) present in the basin have been exposed to the virus. No mortality events of Asian carps have been traced to CyHV-3.
- There is no information about the susceptibility of native cyprinids to the virus. Native cyprinids are important prey fish in lakes and streams, and serve as important sources of baitfish throughout the Great Lakes. In Michigan, between 25,000 to 38,000 gallons of minnows are harvested annually with about 85 to 90 percent coming from the Great Lakes or their tributaries (Kinnunen, 2016). In addition to their value as bait fish, some native cyprinids are listed as endangered species by state, provincial, and federal agencies such as the Topeka Shiner native to Minnesota that is listed as endangered by the U.S. Fish and Wildlife Service (2018).
- Russian sturgeon (*Acipenser gueldenstaedtii*) and Atlantic sturgeon (*Acipenser oxyrinchus*) are suspected carriers of CyHV-3, indicating a potential risk to North American sturgeon populations (Kempter et al. 2009). Some of which are endangered or are being considered for listing, or have ongoing rehabilitation efforts including Lake Sturgeon (*Acipenser fulvescens*).
- Boutier et al. (2015) suggests that Siluriformes may also be carriers of CyHV-3.
- Possible use of CyHV-3 for biocontrol purposes could have unintended negative impacts on the ornamental aquaculture industry in the U. S., as koi farms and businesses, as well as koi raised by hobbyists in outdoor ponds, may be inadvertently exposed to the virus through common water supplies that are being treated with CyHV-3.
- Although biocontrol efforts employing CyHV-3 almost certainly will kill large numbers of Common Carp, it is unlikely that this control agent will completely eliminate entire populations. Survivors will develop immunity, making them less susceptible to re-exposure to the virus.

  - If resistance is a genetically driven trait, resistance to the pathogen will be passed on to offspring, reducing the efficacy of CyHV-3 for biocontrol.
  - If resistance is due to exposure, the cyclic latency/reactivation nature of herpesviruses may also lead to increased resistance over the long-term.
- Direct transmission of CyHV-3 occurs in the water or by direct contact with infected fish. Indirect transmission can occur due to the persistence of the virus in the environment. Because multiple virus reservoirs are present in most watersheds, virus eradication would be very difficult or impossible and once conducted, would be an irreversible management action.
- The GLFHC also notes that the social acceptance of using CyHV-3, or other pathogens, for biocontrol is not well understood, and should not be contemplated without extensive public engagement before a final decision is made.

Given the above listed concerns and unaddressed data and knowledge gaps, the GLFHC's consensus recommendation is that using CyHV-3 for biocontrol in the wild is not an appropriate control strategy at this time.

The GLFHC also recognizes that filling knowledge gaps about the biology of CyHV-3 and the susceptibility of other species to CyHV-3 could be useful for fishery managers. The GLFHC appreciates that further investigation of CyHV-3 that includes experiments in isolation facilities and of retrospective analyses of past outbreaks could provide additional information useful for fishery managers. The GLFHC does not recommend conducting research on CyHV-3 in the wild.

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MSU-AAHL Fish Health & Research Updates

Thomas Loch

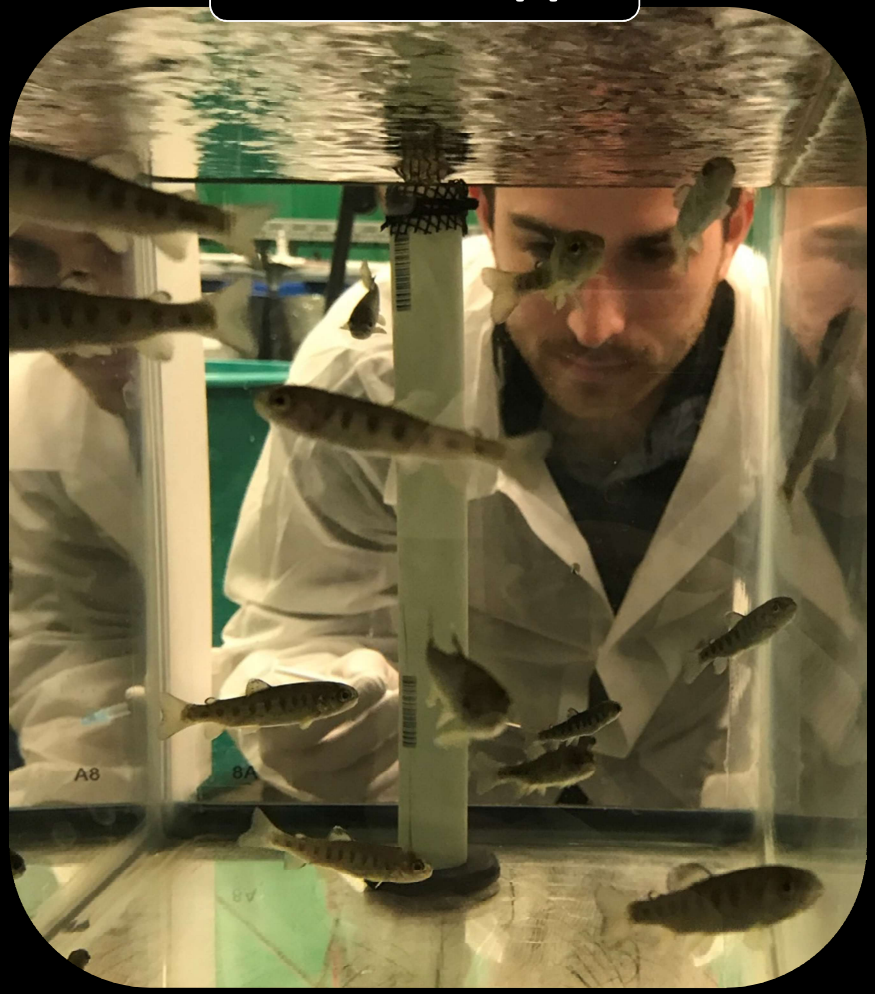
Department of Fisheries & Wildlife, College of Agriculture & Natural Resources, Michigan State University;
Department of Pathobiology & Diagnostic Investigation, College of Veterinary Medicine, Michigan State University

Is *Flavobacterium psychrophilum* Intraspecific Genetic Diversity Associated With Host Species Affinity?

Christopher K. Knupp[1], Matti Kiupel[2], Eileen E. Henderson[1,2], Mohamed Faisal[1,2], Thomas P. Loch[1,2]

[1]Department of Fisheries and Wildlife, College of Agriculture and Natural Resources, Michigan State University (MSU); [2]Department of Pathobiology & Diagnostic Investigation, College of Veterinary Medicine, MSU.

Chris Knupp



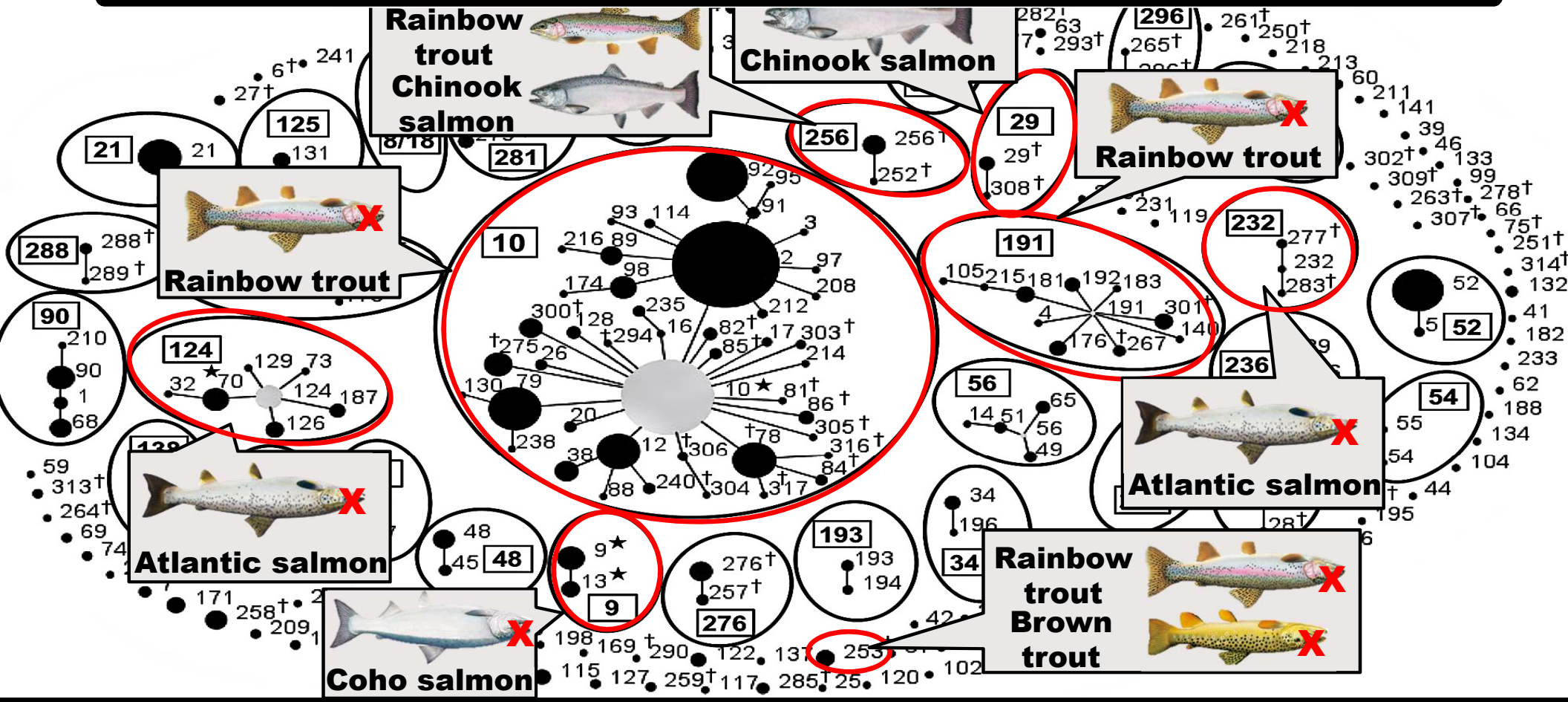
Flavobacterial Diseases in Fish

Leading pathogen-associated impediments to hatchery conservation & fish culture worldwide


Flavobacterium
psychrophilum

Global *F. psychrophilum* Genetic Diversity (MLST)

Exploring relation to: virulence, AMR, diagnosis, geographical range, more



Knupp, C., Wiens, G.D., Faisal, M., Call, D.R., Cain, K.D., Nicolas, P., Van Vliet, D., Yamashita, C., Ferguson, J.A., Meuninck, D., Hsu, H.M., Baker, B.B., Shen, L., Loch, T.P., 2019. Large-Scale analysis of *F. psychrophilum* multilocus sequence typing genotypes recovered from North American salmonids indicates that both newly identified and recurrent clonal complexes are associated with disease. Appl. Env. Microbiol. 85.

Is there evidence for host species affinity according to *F. psychrophilum* MLST genotype under *in vivo* laboratory conditions?

Methods: Challenge Strains

ST13 strain

- **CC-ST9 (coho salmon - "specific")**
- **Feral spawning coho salmon**
- **Systemically infected**
- **CC causes BCWD in MI (& IN) hatcheries (at least 2010 – 2016…)**

ST78 strain

- **CC-ST10 (*O. mykiss* – "specific")**
- **Hatchery juvenile steelhead (BCWD)**
- **CC causes BCWD across the USA (& globally)**

Methods: *F. psychrophilum* Challenge

Bacterial Preparation

Cultured ST13 & ST78 in mTYES broth for 48-hrs at 15°C

Concentrated to 10^8 & diluted to:

- ~10^7 cfu/ml
- ~10^6 cfu/ml
- ~10^5 cfu/ml

Immersion Challenge

Adipose fin-clipped coho salmon (n=22/Tx)

Immersed coho in 4 doses of either ST13 or ST78

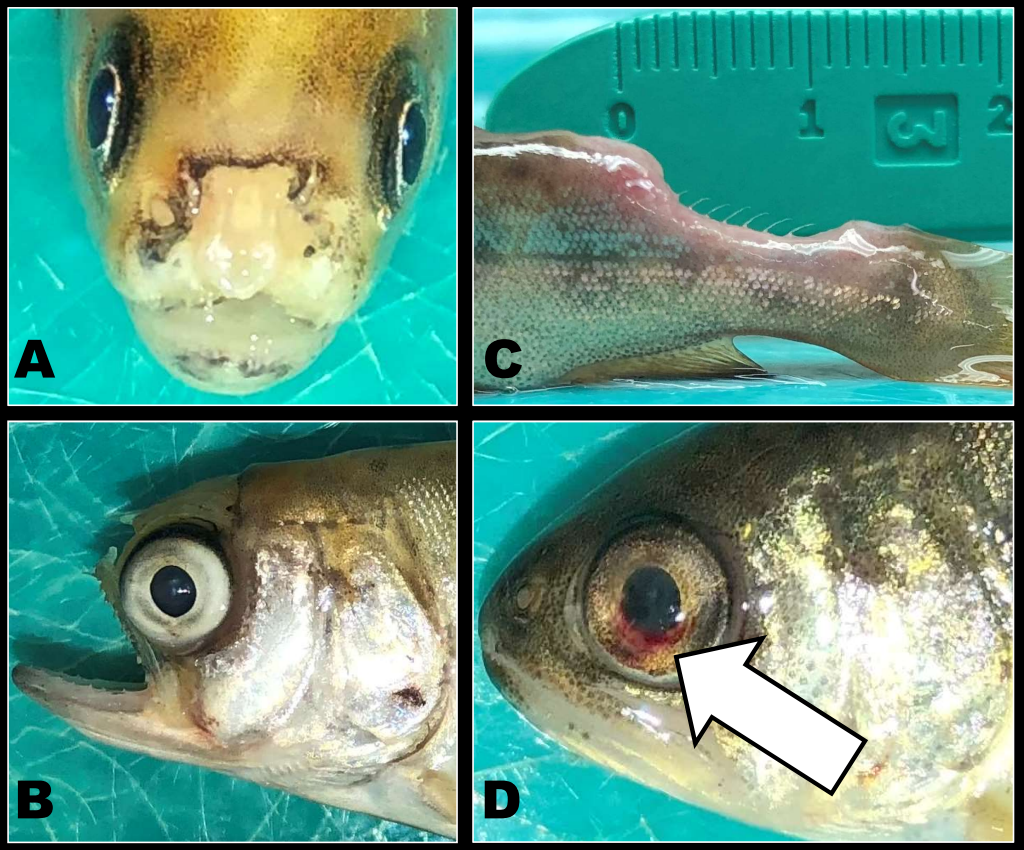
Placed into duplicate tanks

Challenge Sampling

Euthanized 2 coho per dose for H&E/IHC at regular intervals

Dose 1 (High) : Every 12 hrs
Dose 2: Every 24 hrs
Dose 3: Every 48 hrs
Dose 4 (Low) : Every 72 hrs

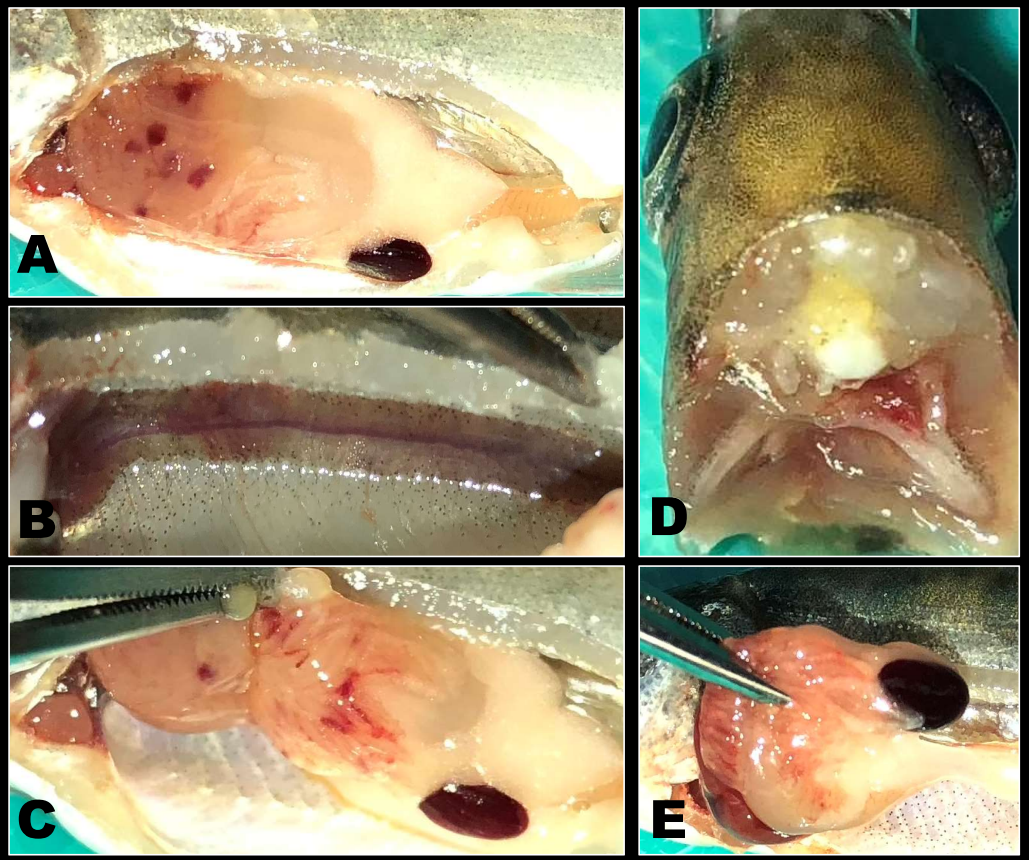
Results: External Gross Pathology



ST13

ST78

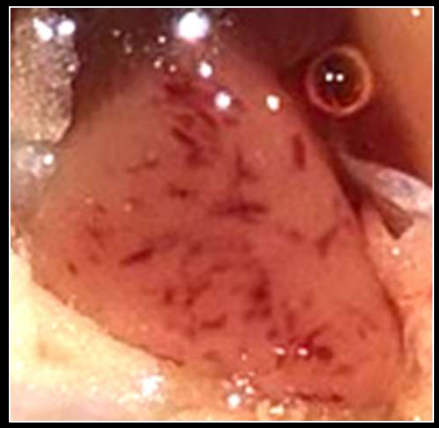
Internal Gross Pathology



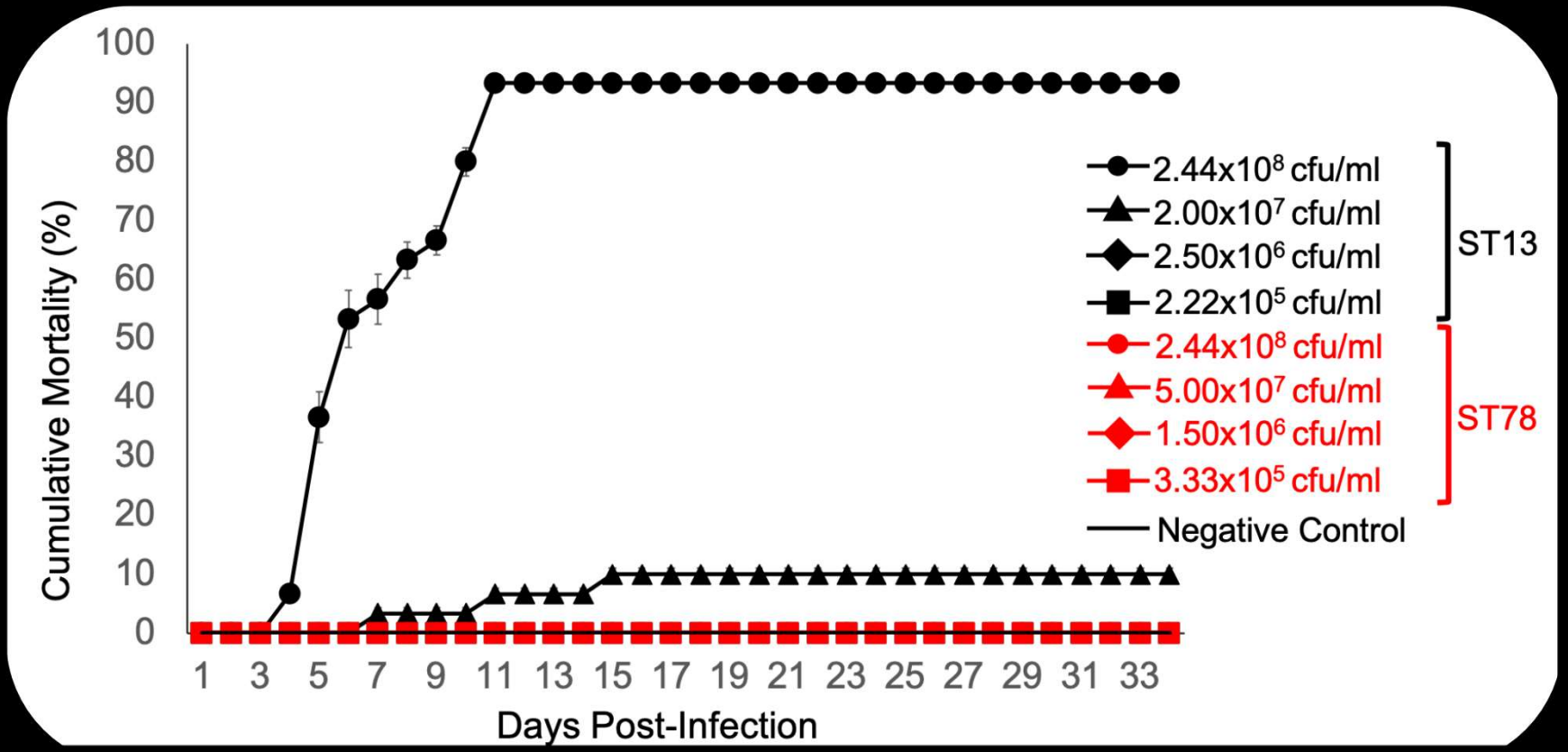
ST13

ST78

Gross Pathology in ST78-Infected Rainbow Trout (previous experiment)

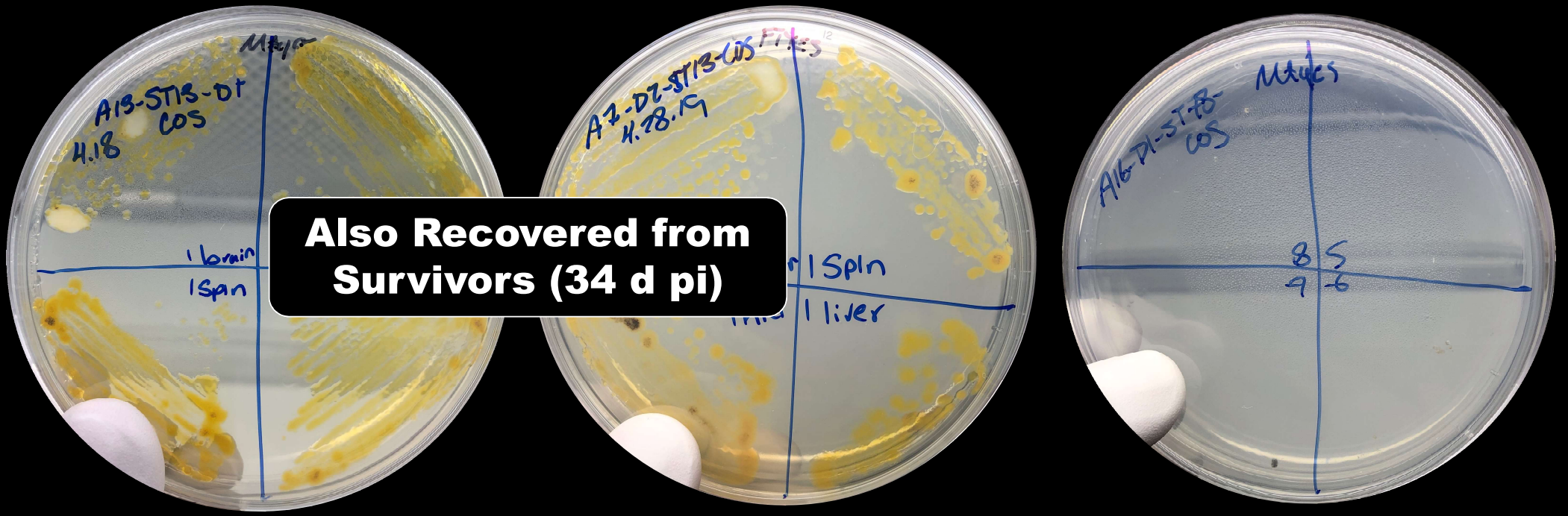


Results: Cumulative Mortality

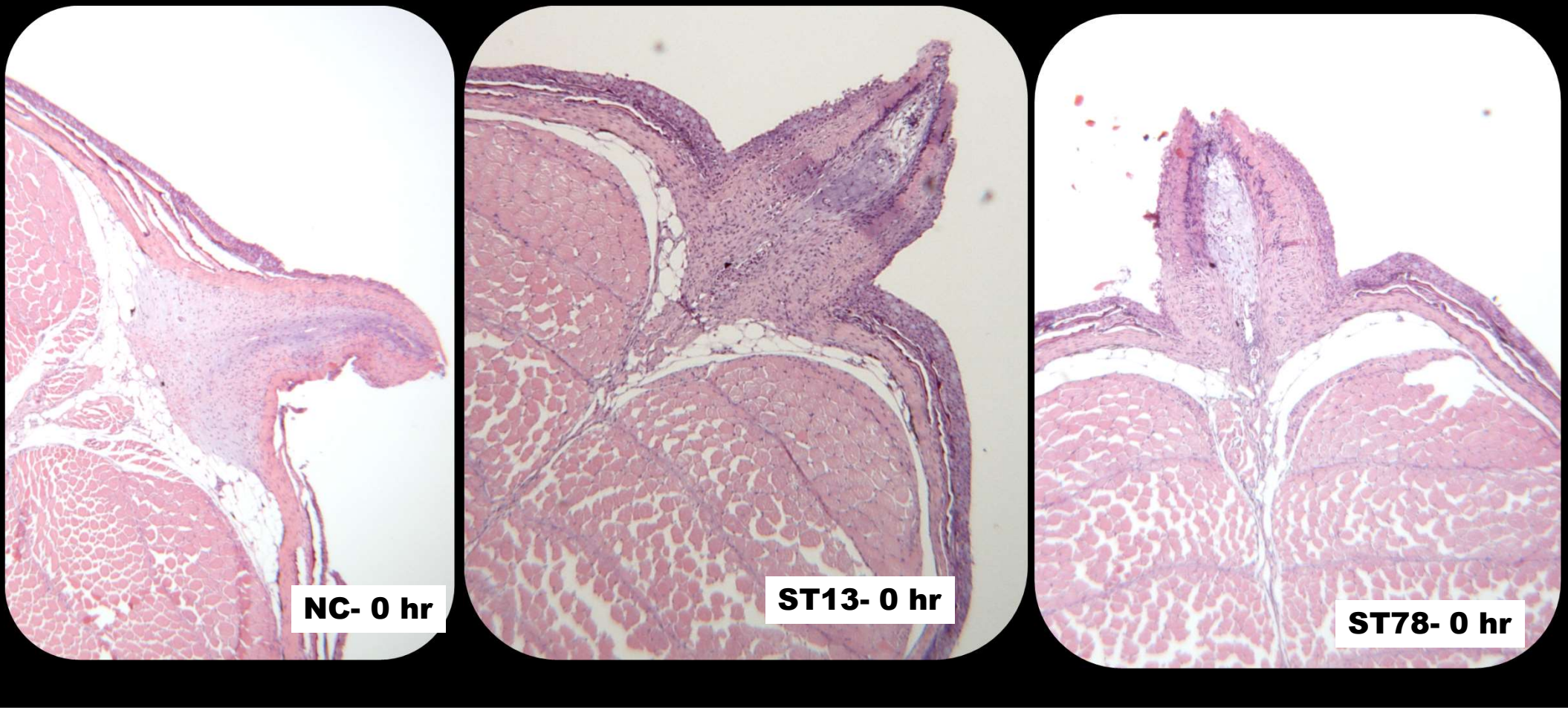


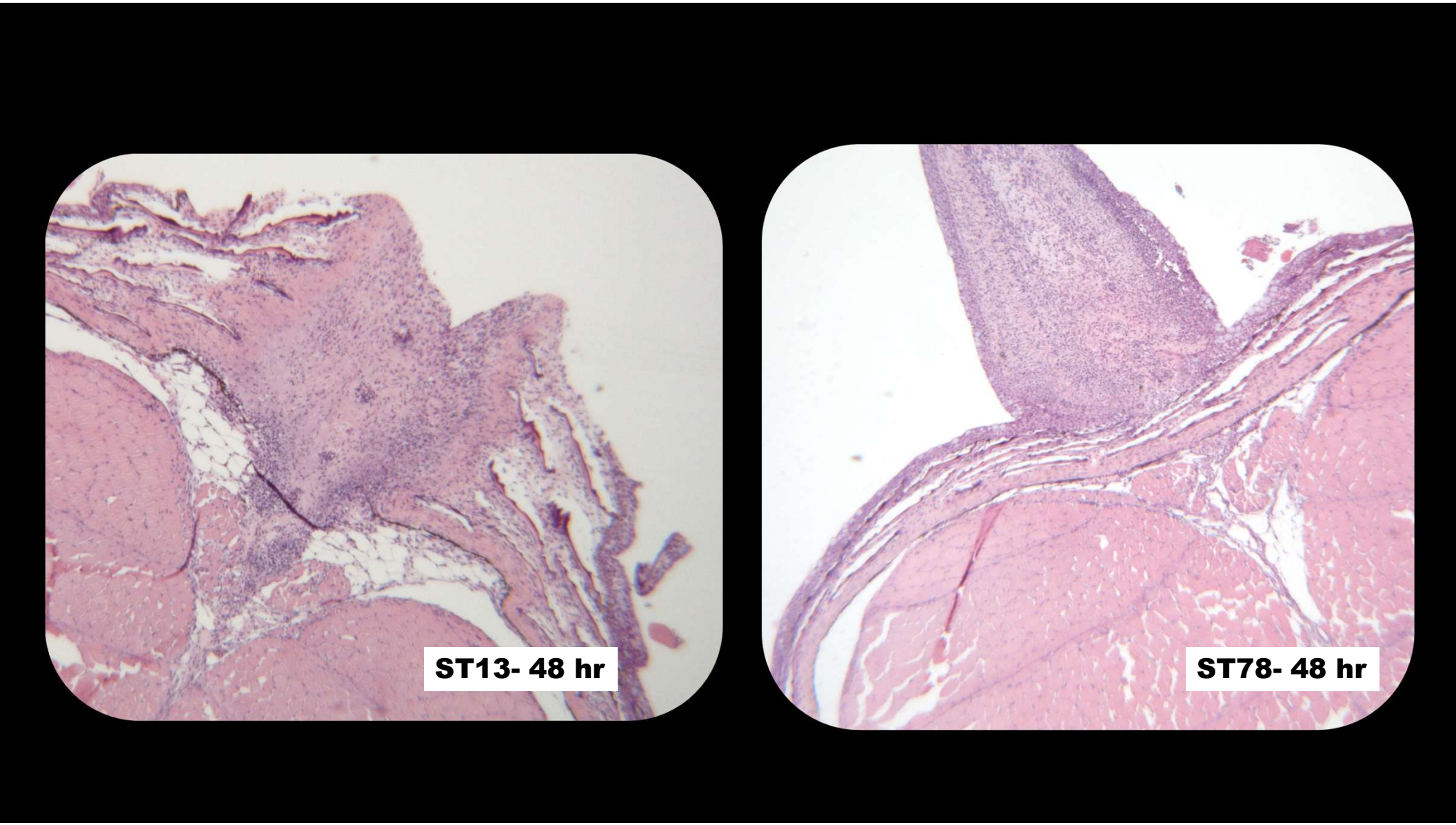
LD_{50} ST13 = $6.6x10^7$ CFU **LD_{50} ST78 >$2.44x10^8$ CFU**

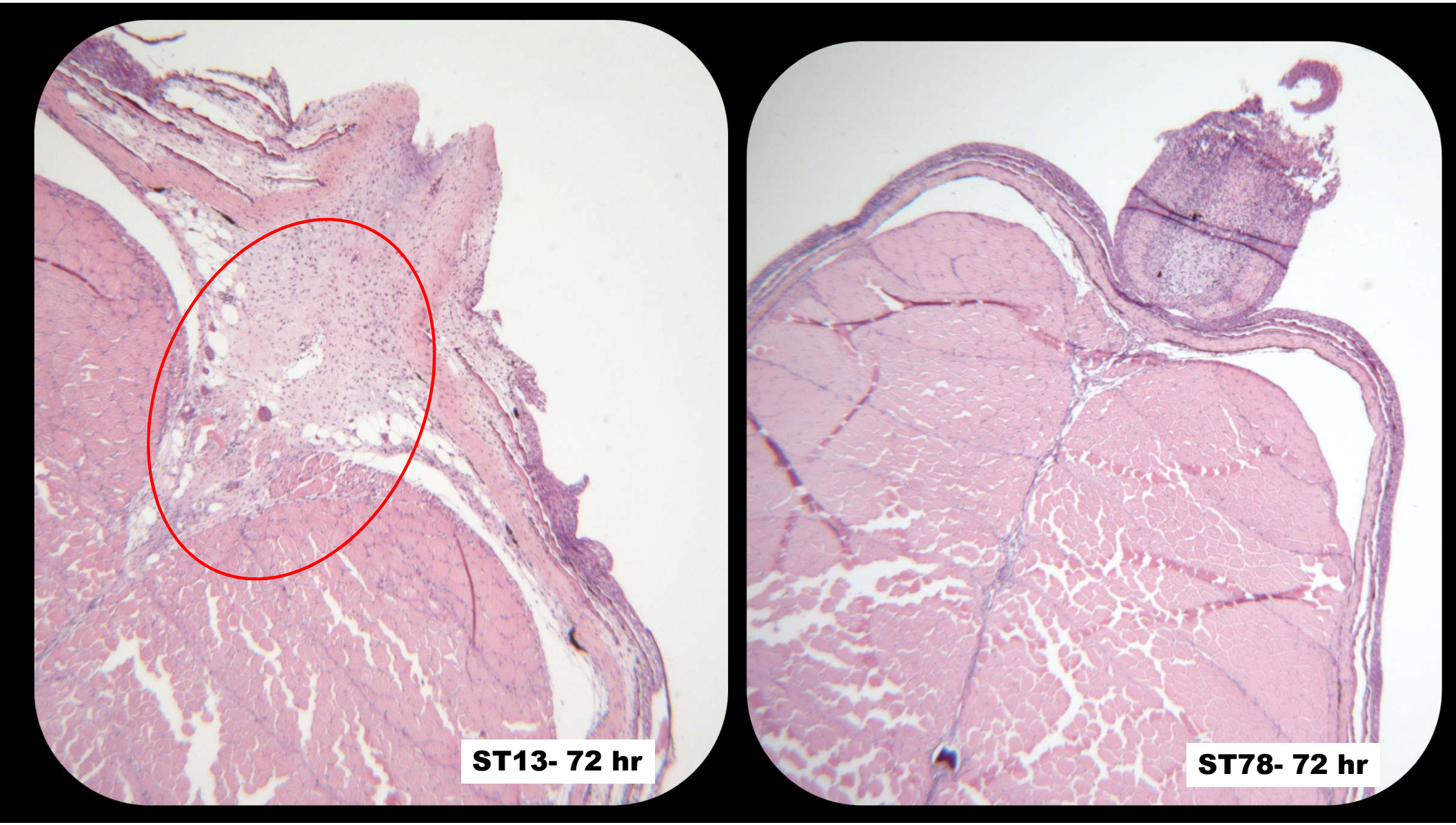
F. psychrophilum Re-Isolation

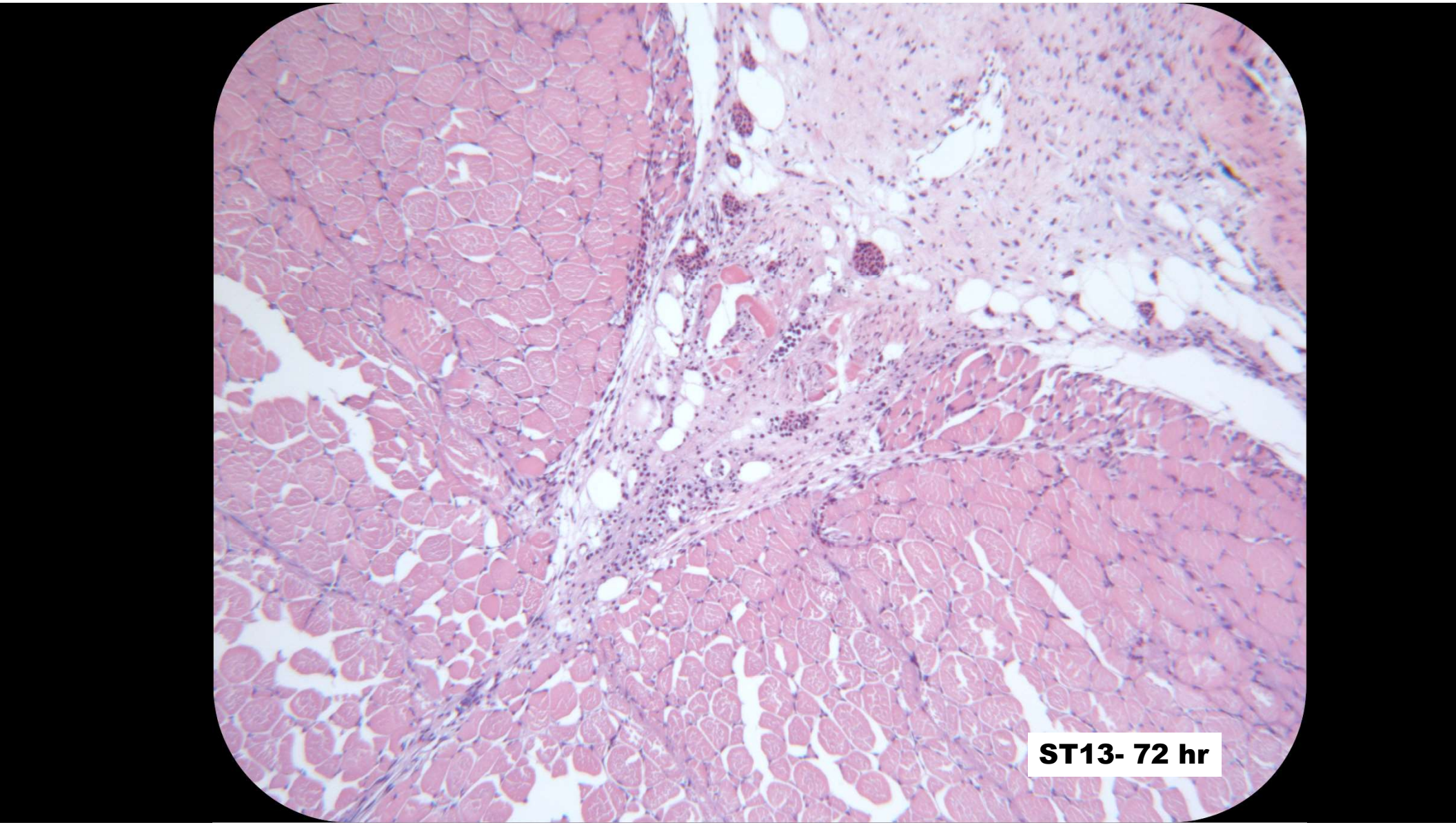


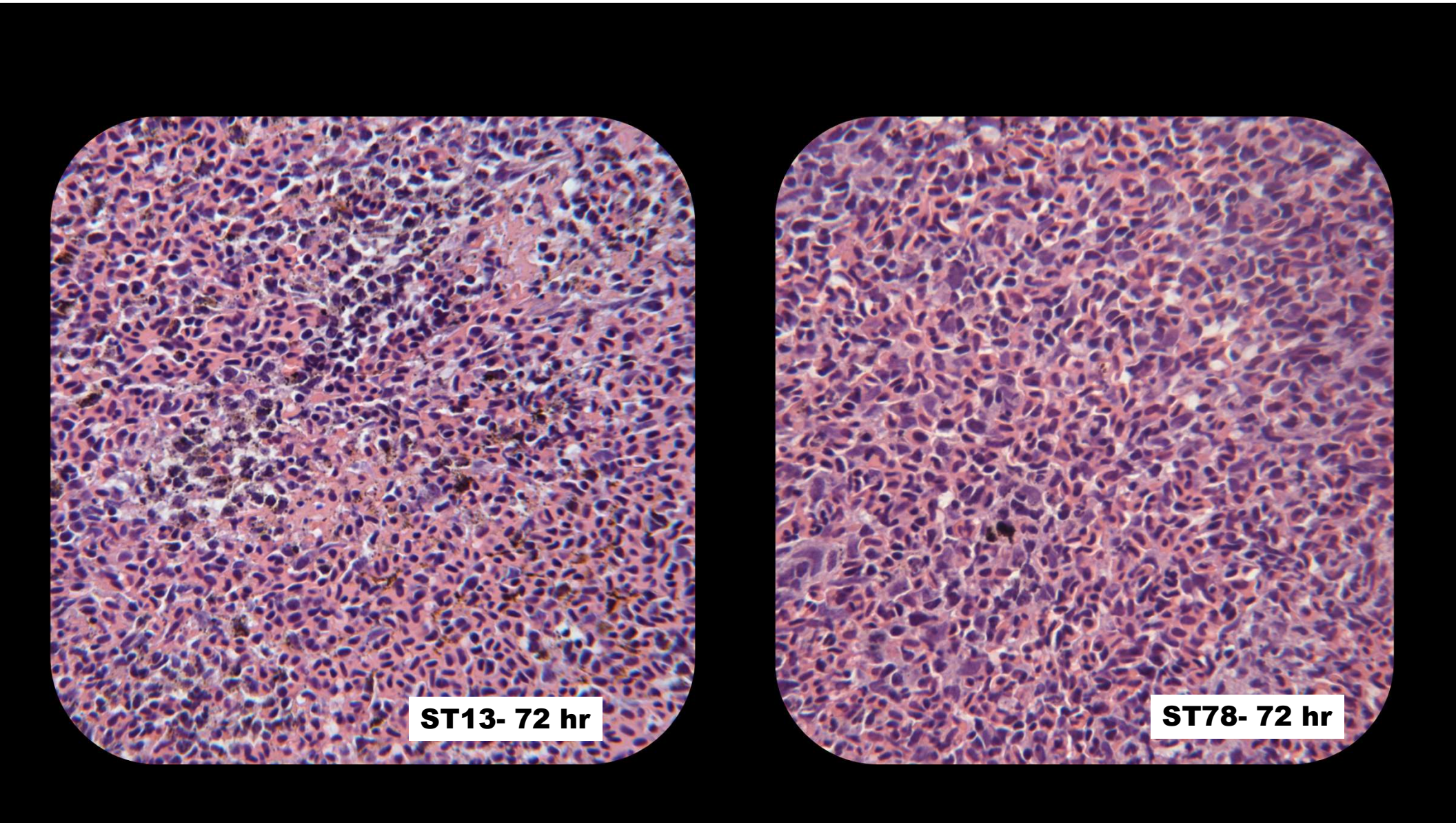
Preliminary Histopathological Findings



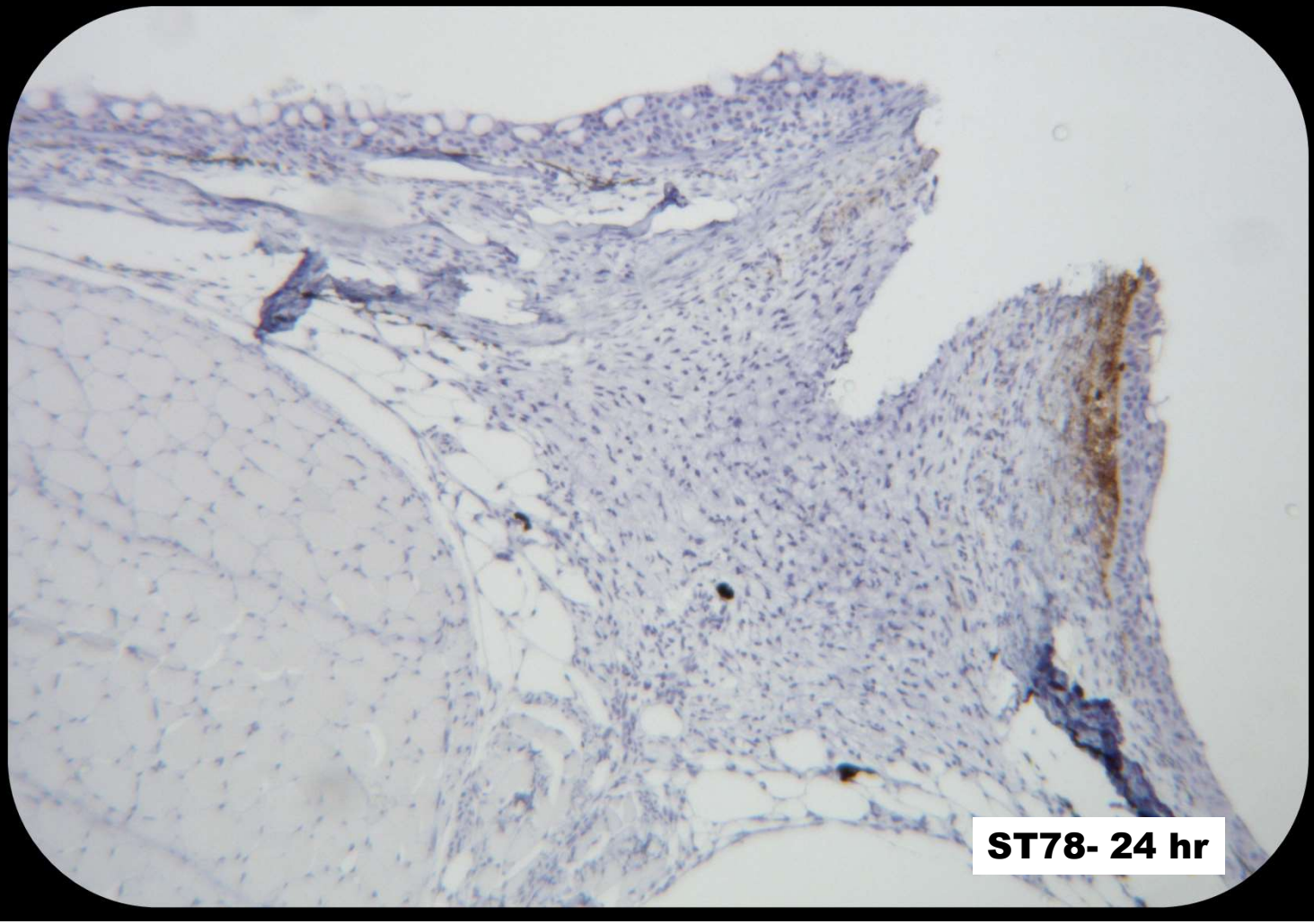

ST13- 48 hr
ST78- 48 hr


ST13- 72 hr
ST78- 72 hr


ST13- 72 hr


ST13- 72 hr
ST78- 72 hr

Preliminary IHC Results



Preliminary IHC Results

Preliminary IHC Results

No positive labelling in internal organs

Preliminary IHC Results

ST13...

Conclusions (Thus Far)

- ***In vivo*** **evidence supporting host species "preference" of coho salmon *F. psychrophilum* strain**

- **Implications for:**
  - **BCWD prevention and control**
  - **Historically variable experimental challenge results**

Acknowledgements

Funding agency

- **USDA-NIFA**
  - **Grant #: 2016-67015-24891**
  - **Grant #: 2016-70007-25756**

- **MSU- Veterinary Diagnostic Laboratory**

- **Michigan Department of Natural Resources**

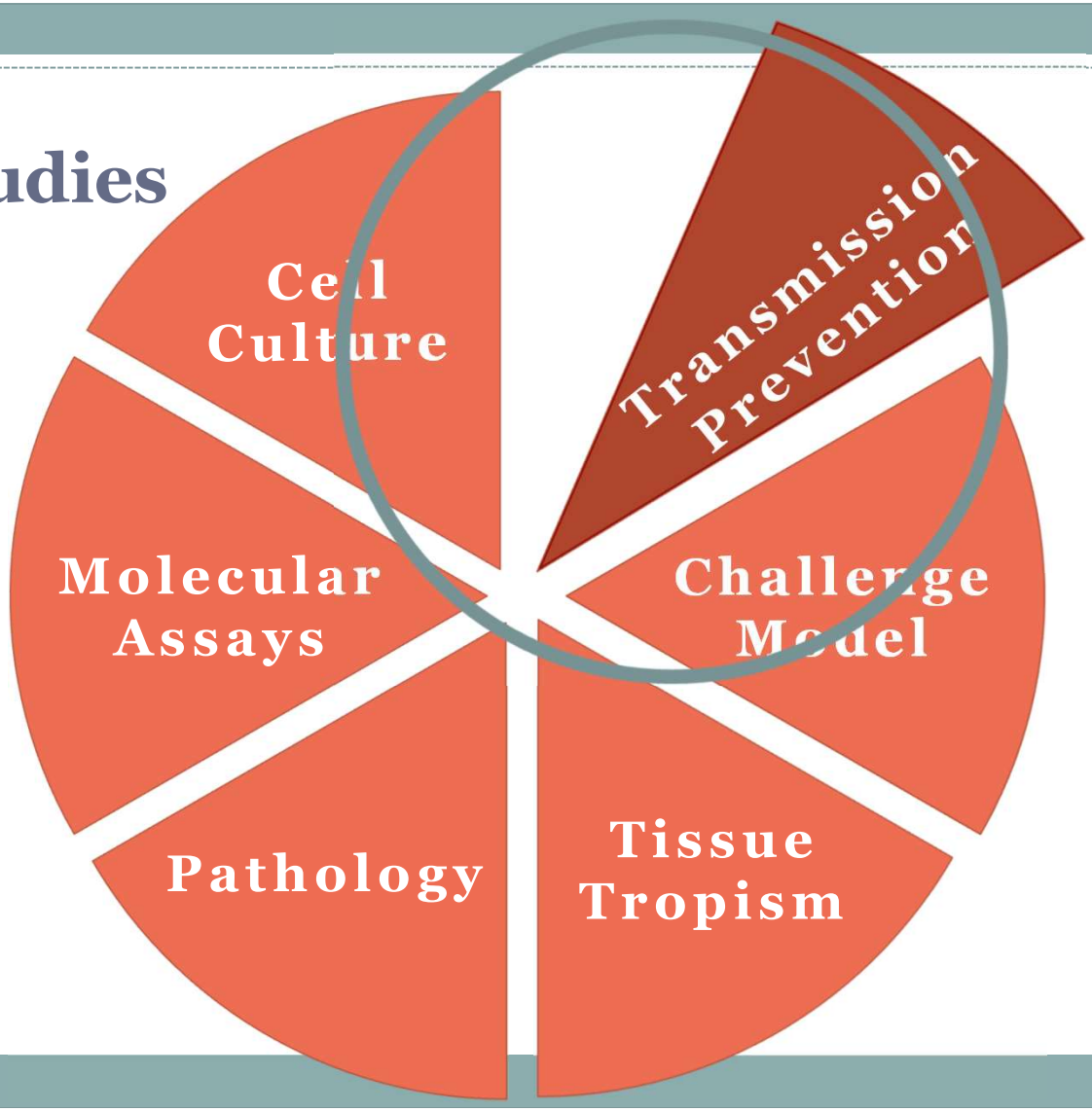
- **MSU-AAHL Colleagues**

Elucidation and Prevention of Epizootic Epitheliotropic Disease virus (Salmonid Herpesvirus-3) Contagion in Hatchery-Reared Lake Trout (*Salvelinus namaycush*)

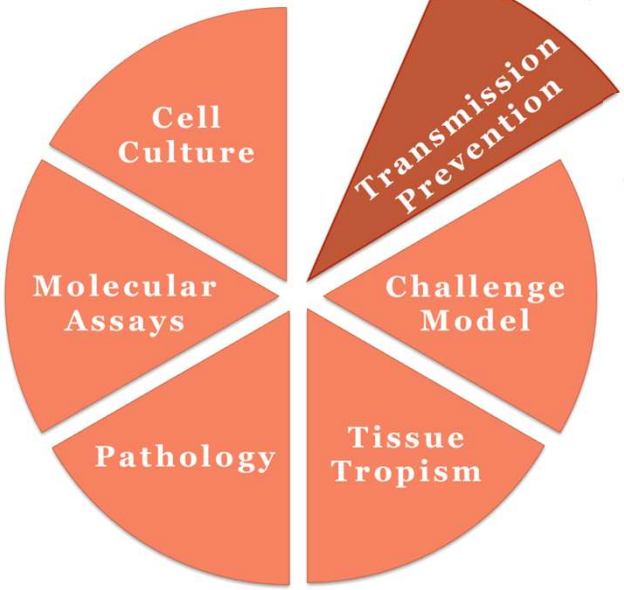
[1,2]MEGAN A. SHAVALIER, [1,3]MOCHAMAD A. PURBAYU,
[1,2,3]MOHAMED FAISAL, [1,2,3]THOMAS P. LOCH

[1]Aquatic Animal Health Laboratory
[2]College of Agriculture and Natural Resources
[3]College of Veterinary Medicine
Michigan State University

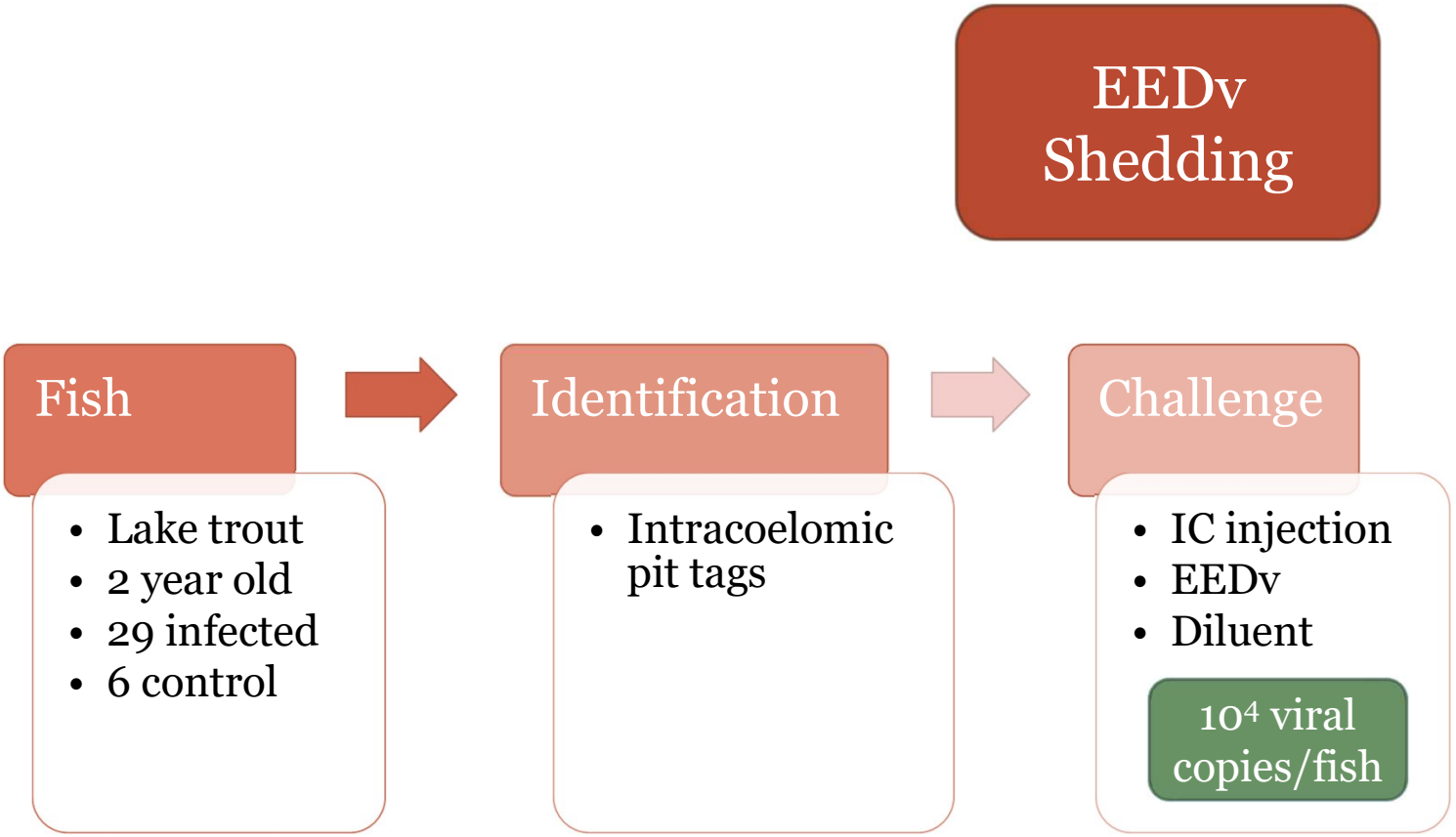
Initial Studies

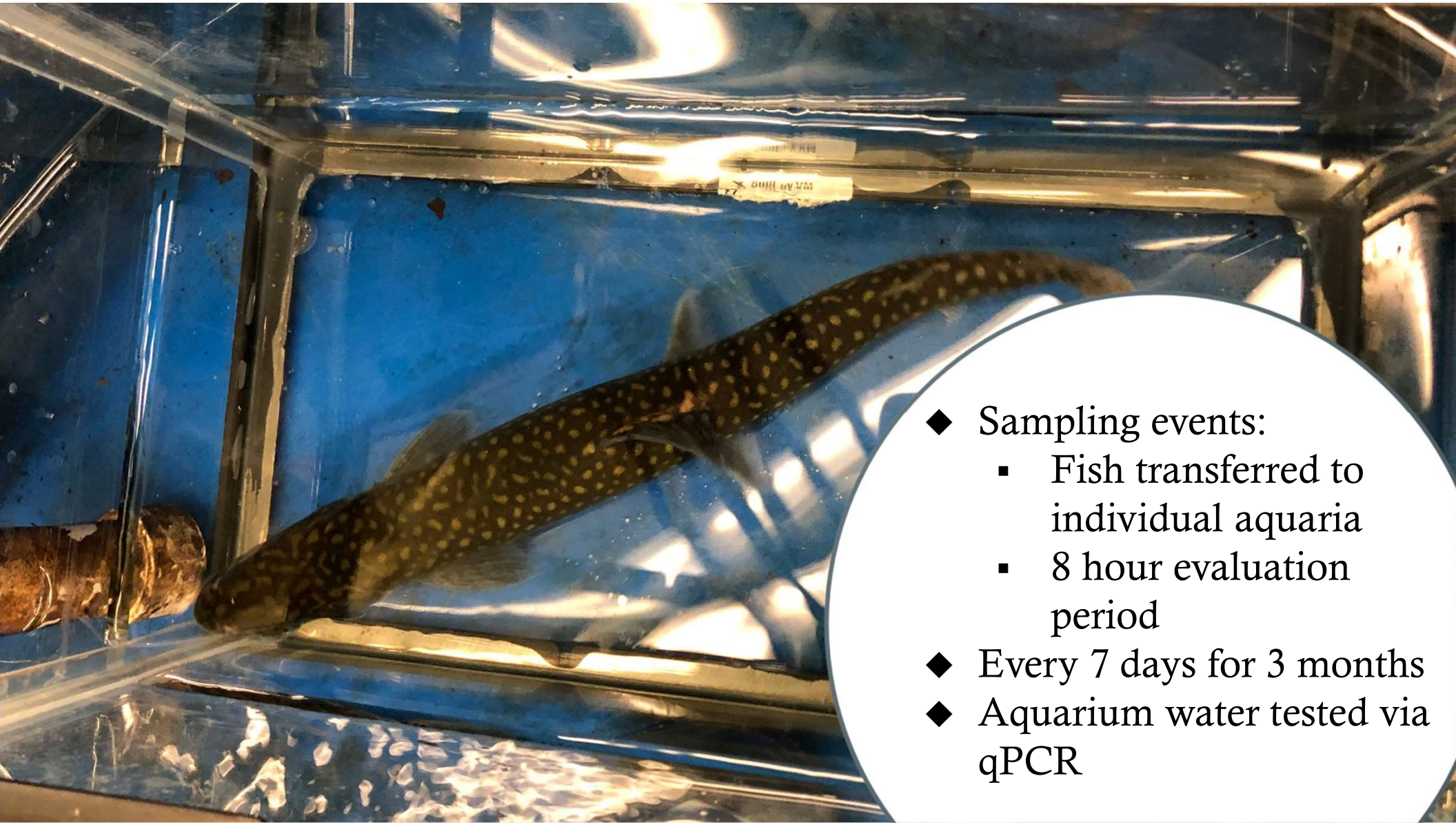


Current Studies



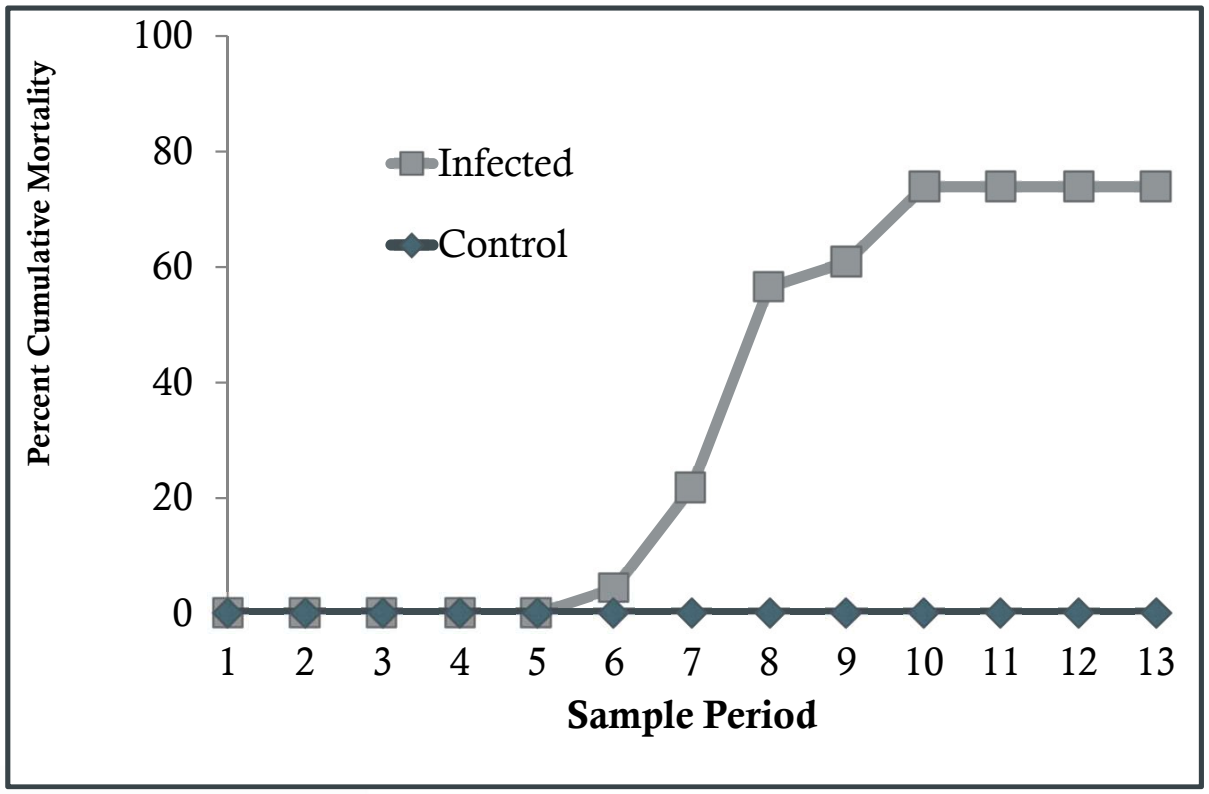
- EEDv Shedding
- Contagion via Fomites
- Disinfectant Efficacy

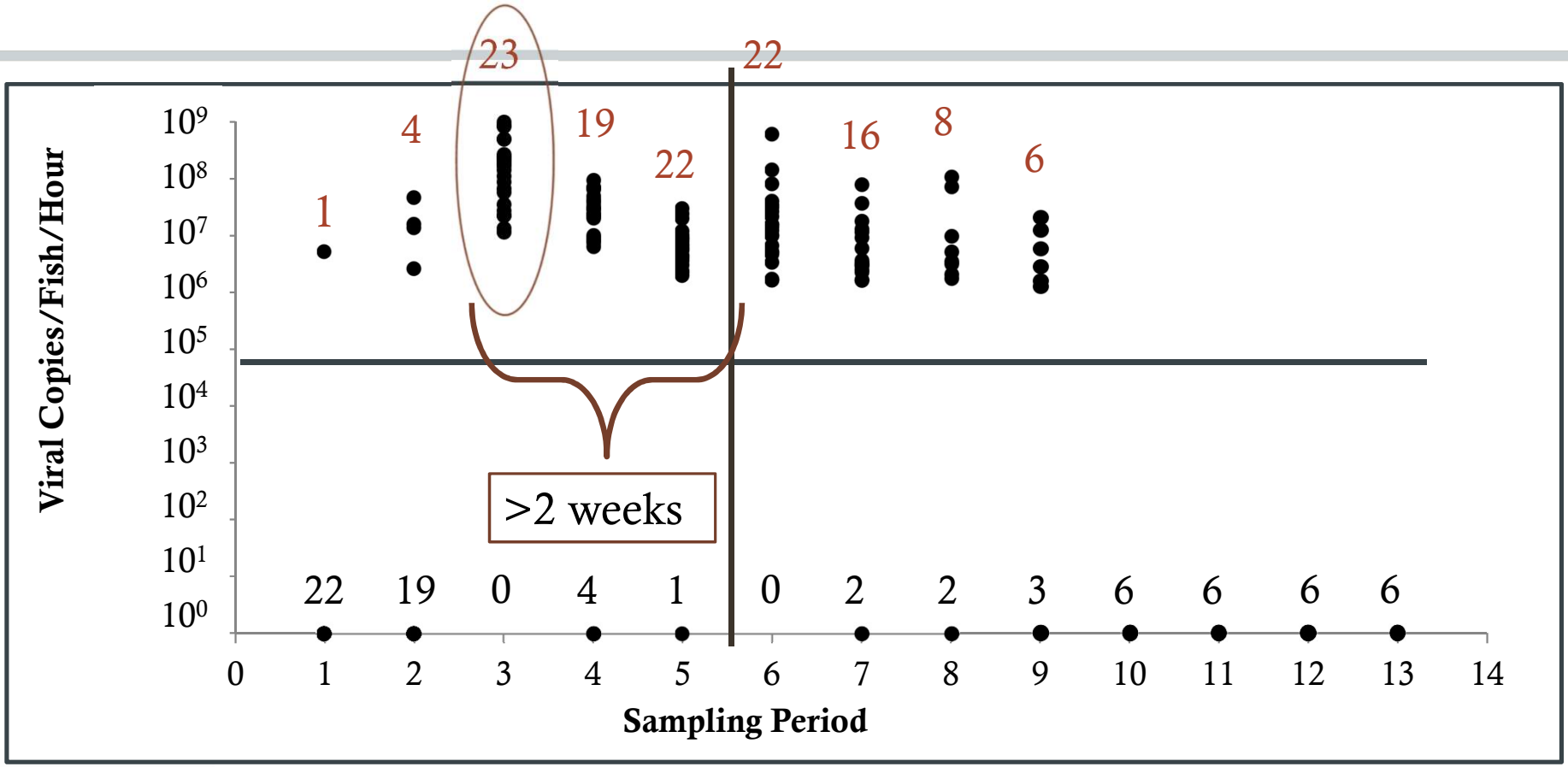

EEDv Shedding
Fish
Lake trout
2 year old
29 infected
6 control
Identification
Intracoelomic pit tags
Challenge
IC injection
EEDv
Diluent
10⁴ viral copies/fish



- Sampling events:
  - Fish transferred to individual aquaria
  - 8 hour evaluation period
- Every 7 days for 3 months
- Aquarium water tested via qPCR

Induction of Clinical EED




EEDV Shedding
Viral Copies/Fish/Hour
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
1
4
23
19
22
22
16
8
6
>2 weeks
22 19 0 4 1 0 2 2 3 6 6 6 6
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Sampling Period

Chronic Shedders?

58 Weeks

6 Survivors

4 Shedders

10^5 viral copies/fish/hour

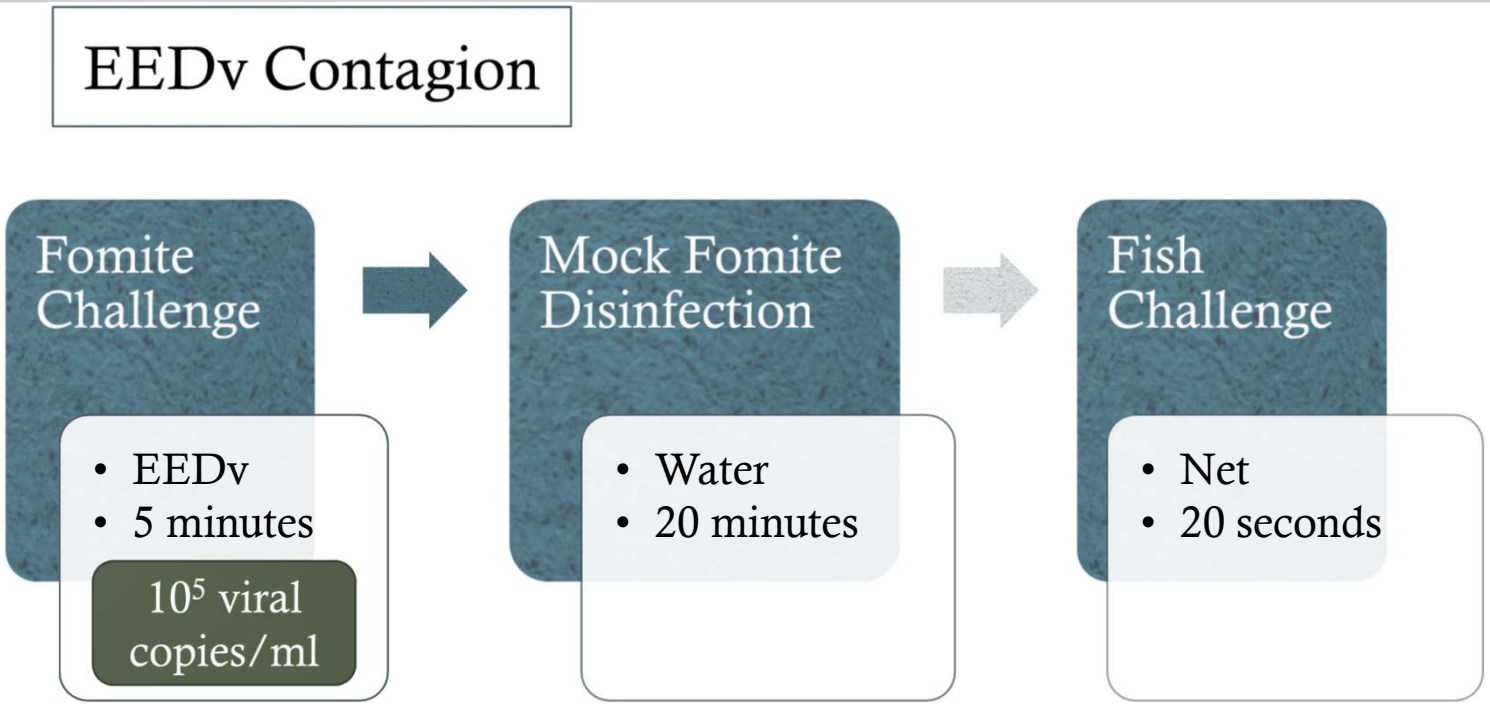
EEDV Shedding – Conclusions

EEDv transmission via fomites?

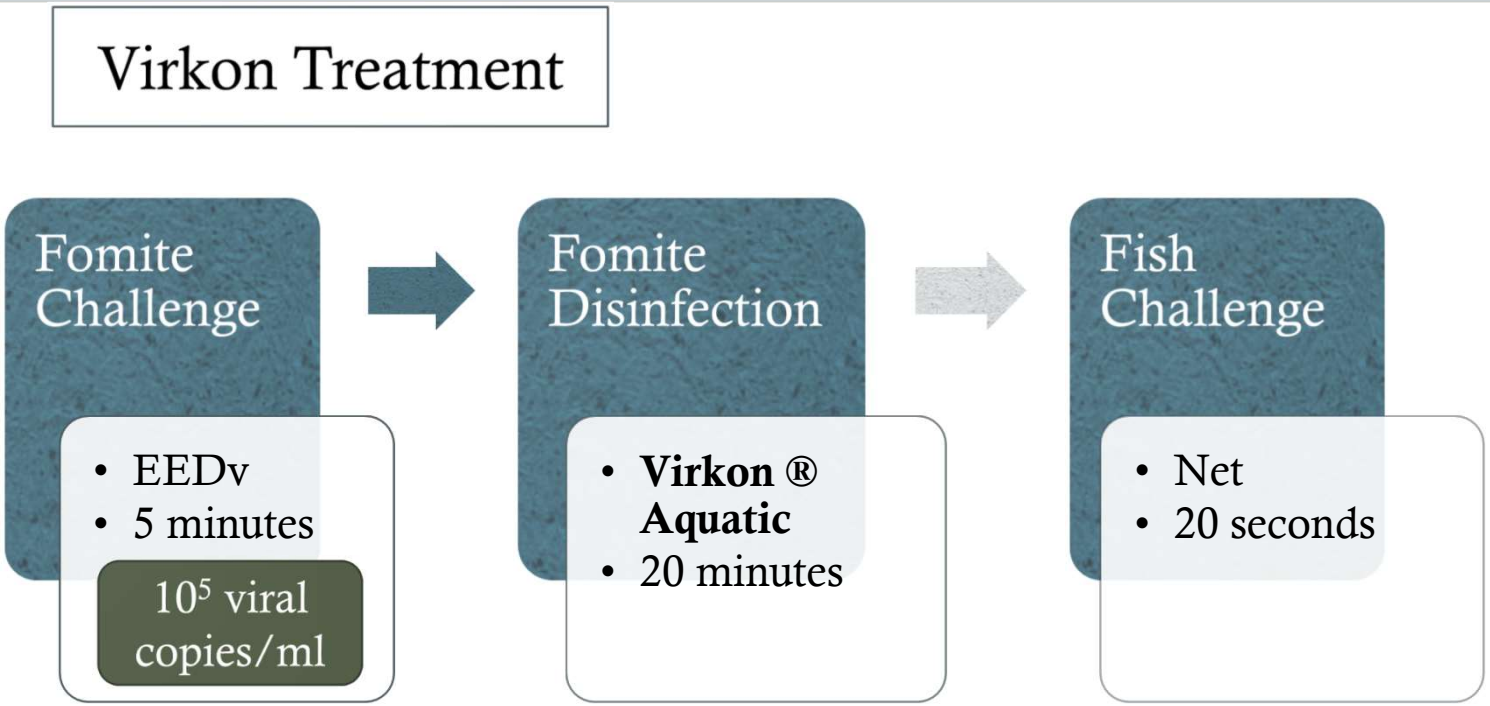
Limit contagion, prevent spread

- Between rearing units within infected facilities
- Between facilities and bodies of water

Disinfectant Efficacy



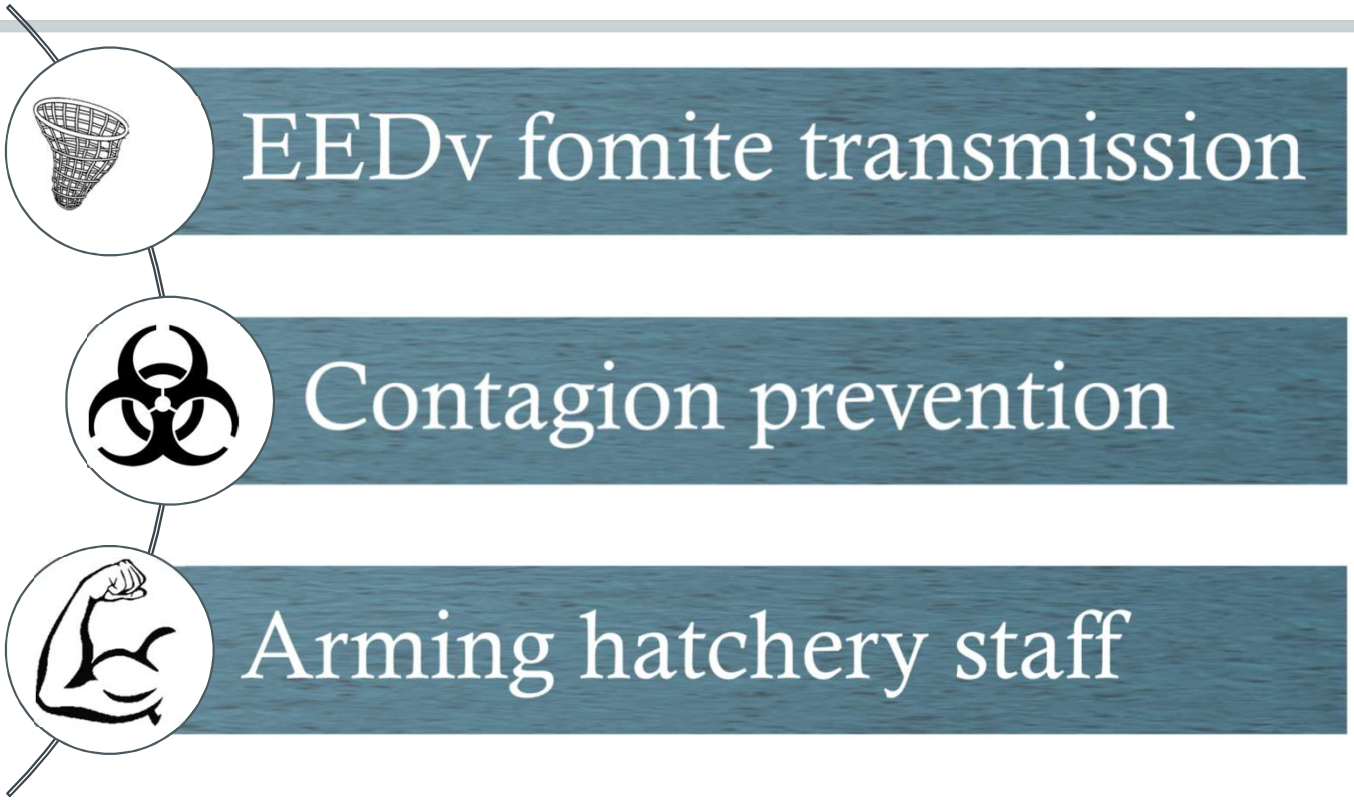
Disinfectant Efficacy



Disinfectant Efficacy - Results

Fomite Challenge Group	Virkon Treatment Group
• 80% mortality	• 3% mortality
• Clinical EED	• No clinical disease
• 18/20 EEDv positive (qPCR)	• 0/60 EEDv positive (qPCR)

Disinfectant Efficacy – Conclusions



Acknowledgements

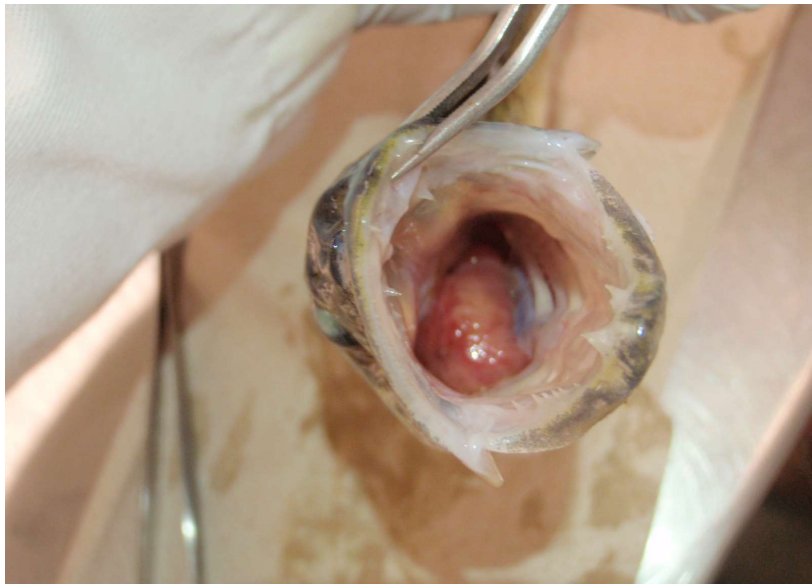
- Great Lakes Fishery Trust Grant # 2014.1455
- Michigan Department of Natural Resources
- MSU-AAHL Colleagues

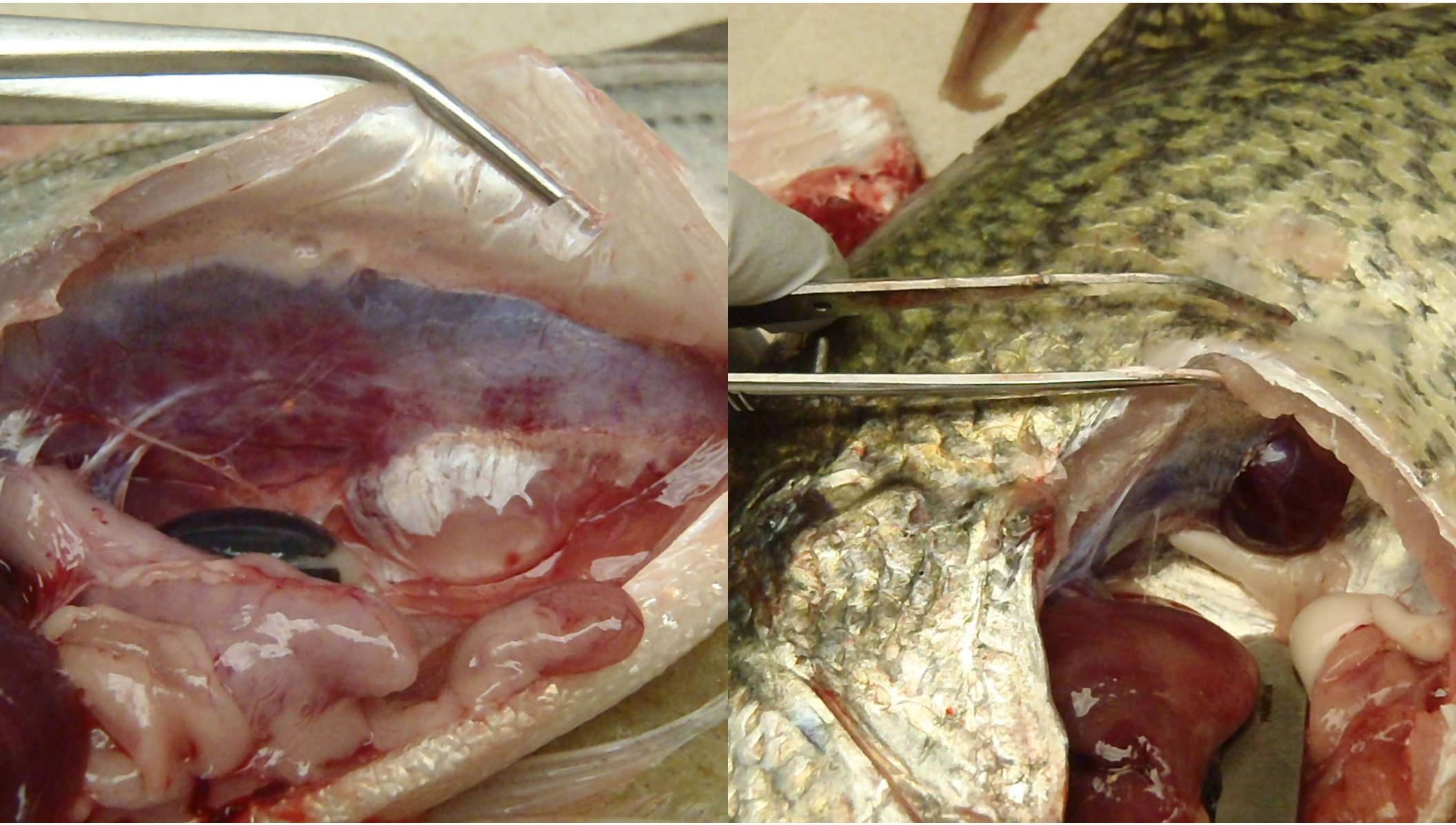
Wild Fish Kills (selected)

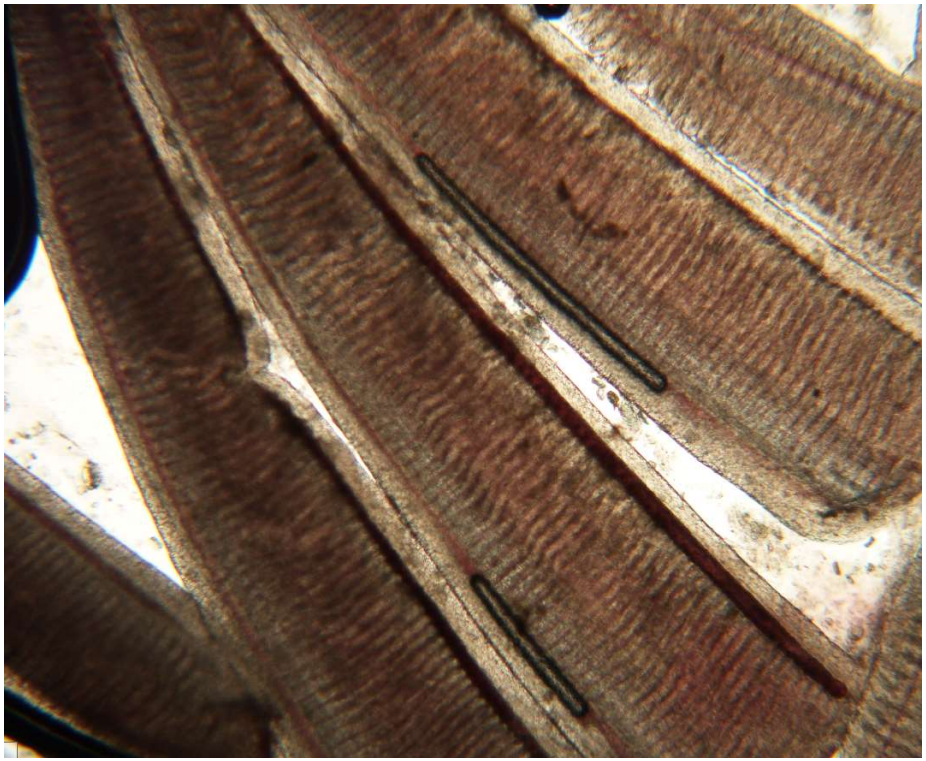
Belleville Lake (WHB, BCR, WHP, WAE, BLG, SMB)

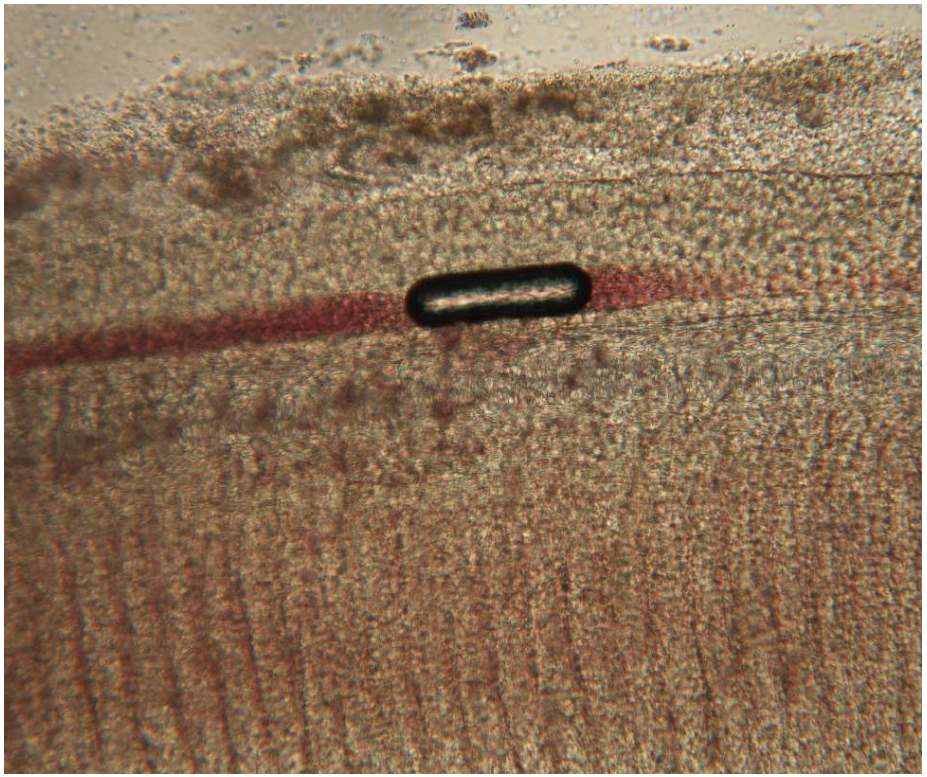
Case History

Gary









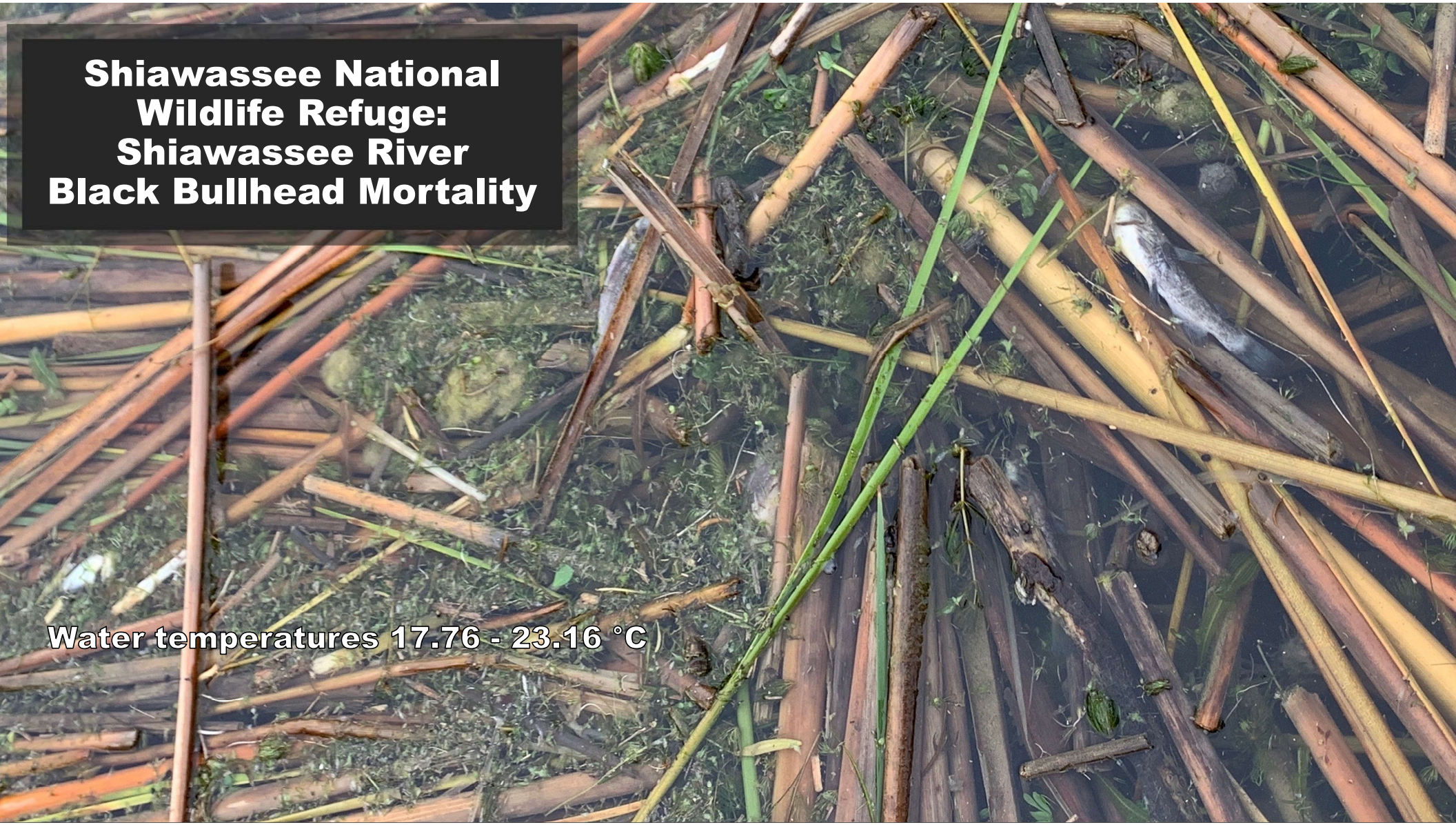
Additional Laboratory Findings

- **Assorted protozoan and helminth parasites**
- **Other bacteria *Shewanella* sp. (few cfu) from minority of fish**
- **No viruses detected via cell culture**
- **No gross signs consistent with systemic infection**

Summary

- **Reports of elevated DO**
- **Gas emboli observed across affected fish species**
- **Dam switched from mid-draw to bottom draw just prior/during the rapid ice melt (where deep water may have contained high N2)**
- **Gastric eversion, ocular emphysema, hyperinflated and/or perforated swim bladders, cloacal hemorrhage and prolapse**

Gas supersaturation & barotrauma from acute pressure change


Shiawassee National Wildlife Refuge: Shiawassee River Black Bullhead Mortality
Water temperatures 17.76 - 23.16 °C


Skin ulcers, kidney, brain

Additional Laboratory Findings

- **Water mold (pres. *Saprolegnia*)- skin ulcers**
- **Protozoa, trematodes (muscle; pres. *Clinostomum* sp.)**
- **Motile *Aeromonas* sp.**
- **No viruses detected via cell culture to date**

Summary

- **Columnaris disease**
- **Also other factors?**

Thanks for your attention!

Wisconsin DNR

Agency Update (Feb-July 2019)

Location	Supplier/Grade	Species	Sample type	# samples	Sample date	Results
Wild Rose	Robinson's (Pillow Fish Farm) / 1	FHM	Whole Fish (heads/tails removed) Virology	60 fish/12 tubes	06.28.2018	Negative
Wild Rose	Dakota Wholesale / 11	FHM	Whole Fish(less heads/tails) Virology	60 fish/12 tubes	08.23.2018	Negative
Governor Thompson	Hwy 10 Bait / 11	FHM	Whole Fish Virology	60 fish/12 tubes	07.03.2018	Negative
Governor Thompson	Hwy 10 Bait / 11-18	FHM	Whole Viscera Virology	60 fish/12 tubes	07.31.2018	Negative
Governor Thompson	Hwy 10 Bait / 11-18	FHM	Whole Viscera Virology	60 fish/12 tubes	08.30.2018	Negative
Art Oehmcke Forage	Robinson's / 10	FHM	Whole Fish Virology	60 fish/12 tubes	07.11.2018	Golden Shiner Virus +
Art Oehmcke Forage	Robinson's /10	FHM	Whole Viscera Virology	60 fish/12 tubes	08.09.2018	Negative
Art Oehmcke Forage	Robinson's /10	FHM	Whole Viscera Virology	60 fish/12 tubes	09.17.2018	Unviable
Art Oehmcke Forage	Robinson's / 10	FHM	Whole Viscera Virology	60 fish/12 tubes	09.17.2018	Negative
BAFF	Gollon's / 11-18	FHM	Whole Fish Virology	60 fish/12 tubes	07.19.2018	Golden Shiner Virus (+)
BAFF	Gollon's / 11-18	FHM	Whole Fish(less heads/tails)Virology	60 fish/12 tubes	09.05.2018	Unknown Replicating Agent Found(+)
Northfield Lake	Robinson's /10	FHM	Whole Viscera Virology	60 fish/12 tubes	07.26.2018	Negative
Northfield Lake	Robinson's /10	FHM	Whole Fish(less heads/tails) Virology	60 fish/12 tubes	09.10.2018	Negative

The Everyday…

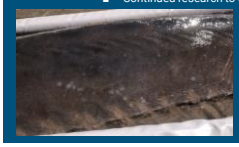
- Fish health inspections at state hatcheries
  - NSF
- Broodstock/OVFL surveillance
  - WSK Bunyavirus detected; paucity of literature
- VHS surveillance
  - 4 lakes supplying 3 hatcheries
  - Test 150 fish from supply lakes as part of WI DNR Policies of fish
  - NSF
- Baitfish testing
  - Surveillance of baitfish from private vendors; monitoring activity

- Wild Fish Kills
  - Having to monitor Fish Management Database

- Large renovation to begin at KMS State Fish hatchery
  - Re-circ system
  - Slated to be ready for fish Spring 2021
- Continue to develop and evolve partnerships with private entities
  - Supplement fish production needs for the state

The Research…

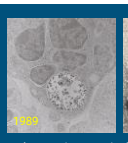
- **Acipenserid Herpesvirus 1**: We continue to evaluate prevalence of herpes-lesions specific to lake sturgeon around the state
  - Now have general idea of ubiquity and TEM pictures!
  - Moving forward
    - May rear Lk STN at WR SFH for Fox River Restoration project (previously on hold)
    - Continued research to evaluate vertical transmission in reproductive fluid



- **Black Crappie Sarcoma**: Reopening the investigation into the etiology of suspected sarcomas in black crappies
- History
  - Black crappies from the lakes around Madison presented with large open raised sores as early as the late 1980's
  - Originally thought to be related to a rickettsial organism, but this was never confirmed
  - Incidence: cycles between 1-2% to as high as 20-30% per lake at a given time

- Clinical Signs:
  - lesions that develop into large open raised sores that penetrate deep into the muscle eventually resulting in liquefactive necrosis of tissue
  - can appear anywhere externally on black crappies; often located along the operculum, mid-body, dorsal, anal, and pelvic fins.

- Moving Forward
  - Continue to collect samples around the state to better ID the etiology
  - TEM with new samples, In-situ hybridization



- Testing
  - Virology
    - In 2005/6 an unknown virus was isolated from 1 sample, but the sample was subsequently damaged during shipping and could not be analyzed
  - Bacteriology
    - 2019-aeromonas sp, pseudomonas
  - Histology
    - 2016 (dermal round cell sarcoma)
    - 2019 (highly suggestive of neoplasia, such as a sarcoma)
  - TEM
    - 2016 (suggestive of papillomavirus)
  - Next-Generation Sequencing
    - 2016 (recovered the nearly complete genome of a novel retrovius)

The 'What Is Going On Here???'

- Northern Pike raised at Wild Rose State Fish Hatchery continue to show a 20% incidence of stunted peduncles
  - Multiple etiologies possible, current focus is on vitamin (A, C, K) in feed

- Still have yearly unexplained mortality events in lake sturgeon at Milwaukee Sturgeon Trailer.
  - Virology and bacteriology results -> NSF
  - Histology results pending
  - Suspect husbandry/environmental issue
    - Lack of regular culling -> overcrowding
    - Other facility stricter culling schedule; do not show same mortality events

Questions for the Committee

- Can you recommend labs that do testing on Saturdays?
- For those of you who work with Great Lakes Spotted Musky, do you normally see green viscera (liver, kidney) in fingerlings?