GREAT LAKES FISH HEALTH COMMITTEE

2015 Winter Meeting
West Lafayette, Indiana
February 3-4, 2015

Minutes
(with attachments)

Submitted By:

Christina Haska
Great Lakes Fishery Commission

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

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List of Attendees

Bridget Baker	Wisconsin Department of Natural Resources
John Coll	U.S. Fish and Wildlife Service- Pennsylvania
John Dettmers	Great Lakes Fishery Commission
Mohamed Faisal	Michigan State University
Megan Finley	Wisconsin Department of Natural Resources
Christina Haska	Great Lakes Fishery Commission
Andy Jarrett	Ohio Department of Natural Resources
Dave Meuninck	Indiana Department of Natural Resources
Andy Noyes	New York State Department of Environmental Conservation
Paula Phelps	Minnesota Department of Natural Resources
Ken Phillips	U.S. Fish and Wildlife Service- Wisconsin
Ling Shen	Minnesota Department of Natural Resources
Gary Whelan	Michigan Department of Natural Resources
Coja Yamashita	Pennsylvania Fish and Boat Commission

Other attendees included:

Jennifer Strasser	Indiana State Board of Animal Health
Andy Richards	Indiana Department of Natural Resources

Great Lakes Fish Health Committee Meeting

Feb 3-4, 2015
Stewart Center, Purdue University
West Lafayette, IN

Draft Agenda

Tuesday, February 3, 2015

8:30 am – 8:40 am	Welcome & introductions (Shen)
8:40 am – 8:50 am	Technical advisors
8:50 am – 9:00 am	Approval of meeting minutes (Shen)
9:00 am – 9:05 am	CLC update (Dettmers)
9:05 am – 9:10 am	Model Program/Risk Assessment update (Shen/Dettmers)
9:10 am – 9:35 am	Surveillance for Pathogens Demonstrated Absent – When Can We Stop? (Gustafson)(Webinar)
9:35 am – 10:00 am	Unknown virus discovered in WI hatchery (Waltzek)(Webinar)
10:00 am – 10:30 am	VHS Discovery in WI Coho Salmon eggs (Baker)
10:30 am – 10:45 am	Break
10:45 am – 11:05 am	Grass Carp/Black Carp risk assessment (Haska)
11:05 am – 11:30 pm	Research at Purdue's Aquatic Lab— (Sepulveda)
11:30 am – 12:00 pm	Agency updates— (All)
12:00 pm – 1:30 pm	Lunch
1:30 pm – 2:30 pm	MI State University research updates (Faisal/Loch)
2:30 pm – 3:00 pm	BKD research: non-lethal sampling study (Elliot)(Webinar)
3:00 pm – 5:30 pm	Tour of the Purdue Aquaculture Research Lab

Wednesday, February 4, 2015

8:30 am – 9:00 am	Pathogen descriptions (Shen)
9:00 am – 9:40 am	Handling foreign import requests and African Longfin Eel risk assessment (Meuninck)
9:40 am – 9:50 am	NY DEC update on dead/sick steelhead in Salmon River (Noyes)
9:50 am - 10:30 am	Agency updates— (All)
10:30 am – 10:45 am	Break
10:45 am – 12:00 pm	Agency updates— (All)
12:00 pm – 1:30 pm	Lunch
1:30 pm – 1:50 pm	Vice-Chair selection
1:50 pm – 2:00 pm	Future meetings (Shen) -Dates/location for summer 2015 meeting
2:00 pm – 4:00 pm	Tour of the Animal Disease Diagnostic Lab

1. Welcome and introductions (Shen)

Ling welcomed committee members and guests to the meeting.

2. Technical advisors (Shen)

All of the people contacted to be technical advisors to the committee accepted the invitation. The list (Appendix 1) will be posted to the GLFHC website and be revisited every two years. Dale Honeyfield is on the verge of retirement, so committee members are urged to consider who could be a potential replacement.

3. Approval of meeting minutes (Shen)

The August 2014 minutes were approved pending changes.

Draft meeting minutes will now be sent immediately following each meeting, instead of prior to the next meeting. Committee members will approve minutes via email.

4. CLC update (Dettmers)

1. At the lake committee meetings in March of this year, the common session will be dedicated to celebrating 50 years of the lake committee process.
2. Managers are interested in re-introducing coregonids to the Great Lakes. Eventually, hatcheries will be needed, and protocols for disease/pathogen management will have to be in place.
3. At the CLC meeting in October 2014, members learned about a new chemical called Zequanox which can be used to control invasive mussels. It may be useful around water intakes, etc, but could also be used lakewide. There are a lot of unknowns about its use, but it has been approved by the EPA for use in open waters.

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

Both documents are online. The CLC is working on protocols for publishing documents, and John will meet with the Science Director this spring to finalize the publication of the Model Program.

6. Surveillance for Pathogens Demonstrated Absent – When Can We Stop? (Gustafson)

See Appendix 2 for the presentation.

Discussion points:

- The VHS rule from APHIS was an emergency rule, and by its nature, could not be kept indefinitely.
- Most states have regulations adapted for VHS management, and they need to recognize there is still a risk even though APHIS has rescinded its rules.
- The susceptible species list needs to be updated, both with OIE and APHIS. OIE has plans to update soon, and it is likely USDA will remove their guidelines so there are no conflicting documents. States will have to refer to OIE from then on.
    - Some communication about this has already been released to the states. Action Item: Lori will contact Ling about where to find this information so it can be forwarded.

- For states which are considering relaxing regulations, APHIS could support reducing surveillance where VHS has never been detected; however, import regulations should not be relaxed.
- Lori will remain the GLFHC's contact person from APHIS.

7. VHS discovery in Wisconsin Coho Salmon eggs (Baker)

See Appendix 3 for the presentation.

Action Item: Christina will work with Bridget and Megan to create a spreadsheet aimed at gathering information about how the member agencies would react to a similar situation in their jurisdiction. Overall, the agencies present at the meeting agreed the situation was handled well.

8. Grass Carp/Black Carp Risk Assessment (Haska)

In 2014, DFO's Asian Carp Program initiated two bi-national Asian Carp risk assessments – one for Grass Carp and one for Black Carp. At a meeting in December 2014, a knowledge gap was recognized about what pathogens/diseases these species may carry that are not already found in the Great Lakes (or, alternatively, which pathogens/diseases are in the Great Lakes which could affect them). The authors of the risk assessment were hoping the GLFHC could provide information towards these knowledge gaps. The following suggestions were made:

1. Potential contacts for information include Andy Goodwin (USFWS, Portland, OR [formerly with University of Arkansas – Pine Bluff]) and Norm Heil (USFWS, Warm Springs, GA). Both have given good advice to committee members in the past.
2. The National Fish Health Survey Database for Region 4 may have information about pathogens/diseases of Black Carp in culture ponds.
3. Action Item: Ling will look into what pathogens affect Black Carp in China (she has contacts overseas).

9. Research at Purdue's Aquatic Lab (Sepulveda)

See Appendix 4 for the presentation.

Discussion points:

- All tests were done with *in vitro* cultures and not with diseased fish.
- The water quality/alkalinity for the TFM study involving sturgeon was about 260 mg/ml.
- The tanks used for the sea lamprey wounding tests were 700 L and Class I-IV sturgeon were used (400mm and larger). There was one sea lamprey per fish per tank.
- The Shovelnose Sturgeon data are from the wild, not laboratory experiments.
- There are a lot of unknowns about how sex is determined – temperature, exposure to hormones, etc., all play a role. A potential cause of intersex animals is the presence of BCPs in the water and a release of estrogens. This was shown to happen experimentally with adult bass.
- What could cause sex reversal in summer run steelhead from Lake Michigan?
    - There might be a differential survival. These compounds do not bioaccumulate – they would dilute quickly in a large lake.

10. Michigan State University update (Faisal)

See Appendix 5 for the presentation.

11. African Longfin Eel (Meuninck)

Indiana was contacted by Emergent Aquaculture LLC (Bell Aquaculture) last year, which requested an aquaculture permit to import and produce African longfin eel. The source fish would be from Madagascar. A new production facility would be built with UV disinfection in a recirculating system. There would not be any outfall treatment. The discharge would go into the White River watershed (Wabash then Ohio River drainage).

Dave contacted John Coll, Mohamed and Ling for information and guidance. There are no Federal restrictions for this species, but it does have a known history of air bladder parasite. A risk assessment was done in-house resulting in a high score.

Discussion points:

- The committee recommended against importing this species.
- The GLFHC could draft a letter for Indiana, if needed.
- Other agencies deal with these sorts of issues by having lists of approved species. Creating a list of Prohibited species would be nearly impossible, and tradesmen would likely find species which were not listed to legally import.
- Getting a Federal restriction would be difficult in light of the aquarium trade.

12. BKD research: Non-lethal sampling study (Elliot)

See Appendix 6 for the presentation.

Discussion points:

- Before using this non-lethal sampling, she would like to test naturally-infected fish to see if the results translate.
- Without doing a lethal test on the same fish, it would be hard to determine if the same results would occur.
- Future research would need to pair lethal and non-lethal tests on the same fish.

13. Tour of the Purdue Aquaculture Research Lab (All)

Meeting attendees were given a tour of the lab and told synopses of current research.

14. Pathogen Descriptions (Shen)

The committee members were given two formatting options for a Pathogen Description document which will go online (Appendix 7). The group decided to move forward with finalizing the table format. Action Item: The table should be edited to include the current distribution of the pathogen, citations, and links to OIE descriptions.

[LMBv and Koi herpesvirus have been found in Indiana – be sure those are included in the table]

15. NYSDEC update on dead/sick steelhead in the Salmon River (Noyes)

Anglers in the Salmon River reported drifting lethargic fish. A biologist was sent to investigate and did not witness any such fish, but spoke to anglers who said the fish looked good but just had no energy. Another team of biologists attempted to collect fish for sampling and realized they were difficult to capture. The fish were seen belly up, but they disappeared underwater when anyone tried to net them. Eventually, the team was able to capture several fish, which were sent to Cornell and Dale Honeyfield for evaluation.

The Salmon River hatchery is an endpoint of migration. Six fish were collected by hatchery staff which underwent thiamine testing – 3 received thiamine injections and 3 received saline injections. They were watched for effects.

Glycogen storage is in the liver, and PAS testing showed a decline in thiamine and glucose in the liver. Dale came back with results of very low thiamine levels in liver and muscle (80% decrease in the muscle). The next step at the hatchery was to inject fish at the end of the spawning run on a weekly basis. To date, staff have tagged and moved 1100 fish from the ladder to a holding pond, with a 30% mortality rate.

Steve LaPan believes the cause of this thiamine deficiency is the result of strong classes of alewives in recent years. This condition has not yet been seen in cohos or chinooks, and it's unclear why not. The good news is thiaminase will be gone from a predator in 4-5 days, and the Salmon River is a refuge from alewives, so fish are likely to recover if they spend enough time in the river.

Discussion points:

- Various viruses could also be involved, including alpha virus and toga virus. It was recommended the fish be injected with vitamins and see what happens.
- No lesions were found in the brain.
- There are very little data out there about adults with thiamine deficiency. Andy will be buying a glucose meter (at a drug store – used by diabetics) and see what that shows. This may not be accurate for fish but it could show trends.

16. Unknown virus discovered in a Wisconsin hatchery (Finley/Baker)

Chambers Creek steelhead at Besadny had ovarian fluids sent to Tom Waltzek for next-gen sequencing on a virus. It was found to be an orthomyxovirus virus but not ISAv, as it was only 40% similar. Tom had seen something similar in Washington State which was 90+% similar. The eggs from the affected spawning date were depopulated. The mortality rate at the hatchery increased shortly after this time, but it was due to bacterial gill disease.

Megan and Bridget will continue to update the committee as results become available.

17. Michigan State University update, cont. (Loch)

See Appendix 8 for the presentation.

Discussion points:

- Egg disinfection improvements included a rinsing cycle and double-checking the treatment concentrations.
- The West Coast federal guidelines were followed along with OIE.
- Strain-to-strain comparisons were made, and the isolate was genotyped, but the 2009 samples were sequenced with a different set of primers and in a different area of the gene than recent samples. He is in the process of typing the strains.
- The testing/detection methods used were cell culture and PCR.

18. Agency updates (All)

New York State DEC: The statewide wild fish health survey collected fish from 20 locations and sent them out for analysis. EEDv was found in Lake Trout in Otsego Lake. Salmonid Herpesvirus was found in two lakes – Otsego and Lake Ontario. Research cooperators think it's a different strain of III or a new strain at V. NYSDEC contracts with Cornell to evaluate fish kills, and there were 16 different investigations, including VHS in gizzard shad and alewives in Lake Ontario. Fish health in the hatchery system is good with only low level problems. In 2012, at Rome SFH, about 800k brown trout from Virginia were destroyed because of an outbreak of furunculosis. After that, only Rome-strain brown/brook trout which are resistant to furunculosis remained. The hatchery classification was downgraded as a result, and after two years of inspections, it was upgraded to Class A. But then! Brown trout broodstock had furuncles (about 7 fish had the pathogen isolated from lesions and kidneys) and staff retested everything on site, but *A sal* wasn't found. The hatchery was downgraded again to Class B with a mitigation plan and will not transfer fish. The hatchery will likely quarantine non-Rome strain fish and see what happens. There was also an unusual variant of *Y. ruckerrii* in brook trout adults.

Michigan DNR: IPN was found at two hatcheries. BKD showed up in Marquette at low levels after being absent. Private aquaculture in Michigan wants to be a billion dollar business by 2025, with 80% of its rainbow trout cage culture in the Great Lakes. It is emulating Ontario's design model which has 10 facilities at a million pounds each. Two pre-proposals have been submitted but are unclear on details. The industry has gone around the standard process and courted personnel in the legislature who control the DNR budget. Lakes Michigan and Huron are proposed to house the cage cultures. State-owned property and private land are proposed, which could cause permitting issues. Escapement events could affect the whole lake. Theoretically, the fish would be triploid and female. This is very challenging for the DNR, and it is anticipating fish health issues.

Action Item: John will bring this up to the CLC in April and encourage them to act as they see fit, potentially contacting the governor and house/senate majority leaders with a letter.

Ohio DNR: Two employees are in the process of being certified as fish health inspectors. There were four whirling disease tests and 26 viral lots throughout 6 SFH on all species. They did not test the Blue Catfish broodfish, but are hoping for the first batch of fry production from those fish. Seven broodfish lakes were tested, and two had LMBv in Ohio River basin reservoirs. A GLRI project at St Marys fish hatchery on the eastern shore of Lake Erie is identified as potential Asian Carp pathway. The hatchery uses Grand Lake St Marys water and discharges it into the St Marys River, which then flows into Great Lakes basin. A consulting firm was contacted for developing a post-treatment water filtration option, and the project is expected to be completed in 2017. The DNR is working with the Department of Ag because they didn't

have anything in place when the APHIS rule was rescinded. The emerald shiner bait fish industry is looking at allowing importation from New York, but fish need to stay within the basin. It's a neat opportunity to look at other fish diseases that didn't have regulations.

Pennsylvania FBC: IPN is still an issue, and now it's at 11 hatcheries. The increase in detections could be due to more monitoring. The agency is looking for IPN-free broodstock or eggs. Whirling disease was found in the Bellefonte hatchery. BKD is in 4 hatcheries, but only one had a mortality event. Staff are working with Purchase College in New York to research potential reservoirs for the pathogen. There have not been any VHS detections. In 2012, CTv was detected at two hatcheries, and subsequent monitoring found it in Brown Trout at a third hatchery last week. There were not any mortalities. Pennsylvania has seven cooperative nurseries, none of which has any significant pathogens. Lake Erie winter steelhead had 6 pools positive for IPN, which is the first time since 2007. Coja will likely look to Vermont for eggs. There was a cestode infestation at the Benner Spring hatchery, and 70% of fish were infested in some raceways. No treatments are available, but staff used Prazi Quantel and only had limited mortalities.

Wisconsin DNR: CTv continues to be found in brown trout in 2 hatcheries and 1 egg collection facility. No symptoms or mortality have been noted, so staff are just trying to not spread it. Fathead minnows were found to have nidovirus and golden shiner virus in them. The DNR might be able to alter the contract with the supplier to make sure testing is done prior to importation. Vaccinations continue against furunculosis at two hatcheries, which are working well. One broodstock collection site had a high incidence of furunculosis in coho, and afterwards Seeforellen brown trout disappeared from that river system. Wild fish (brook trout) have gill lice in a number of rivers. Black crappies have been detected with a sarcoma, and they are looking into the cause. Diseased fish have subcutaneous tumors and necrosis. It's locally common in different areas but seems to becoming more prevalent.

USFWS- Lamar: Fish health surveys will be sent out in the next few months. A wild fish health survey looked at wild broodstock at 36 sites in New York and Pennsylvania, and did not find VHS but did detect EEDv and Salmonid Herpesvirus III and maybe V. Allegheny NFH is up and running. Last year had a late spawning because the enclosed facility changed the photoperiod from past years. This year, spawning did not happen late but the eyed eggs were bad. Testing will be done to see why. The inspection done in August made the hatchery Class A again. There is increased Lake Trout (Seneca) production at the Pittsburgh station. The Berkshire hatchery was converted to a NFH.

USFWS- LaCrosse: Jordan River continues to have coldwater disease with lesions around the dorsal fin. A prophylactic treatment with chloramine-T didn't help, but medicated feed with oxytetracycline did. Staff are looking for a control measure, such as an immune boosting diet. EEDv was not found, and samples will be sent to Tom Loch for analysis. Genoa found *Y. ruckerii* again in Lake Sturgeon. Iron River has been free of *A. salmonicida* since July, 2011, so it's back to Class A. The Fish Health Center is being restructured with an eDNA lab and FWCO, and it's new name is the Midwest Fisheries Center. They will be advertising for a Center Director in the next few months.

Minnesota DNR: The new VHS regulations, which were mentioned at the August meeting, have been implemented. There are two zones within Minnesota: the Lake Superior watershed and the rest of the state. The regulations for Lake Superior have not changed, but the rest of the state have new guidelines. Licensed aquaculture facilities can rear fish and move them within the state, with testing every 2 years instead of every year. Minnow dealers can choose between detection methods. The rules may be

modified again in the future, but the DNR wants to see how this works. State surveillance will test high-use recreational waterbodies and rivers. Fish health inspections continue at 5 hatcheries. French River had a low level of BKD in Kamloop and steelhead wild egg takes, but the others did not have any reportable pathogens. Staff used lethal and ovarian sampling. Crystal Springs had *A sal* last summer, and a mortality occurred after a terramycin treatment. Another fish died 3 months after a second treatment. Staff are now using a Florphenicol medicated feed, and so far things are okay. They are considering vaccinating the young fish. Approximately 13k fish were screened last year for VHS, and none was found. Minnesota had a few fish kill cases, but they were mostly due to flavobacteria.

Indiana DNR: BKD has been found in the northern hatcheries, and sometimes *A sal* will show up without disease outbreaks. Sensitivity testing is done whenever it's found, and occasionally it is resistant to treatments while other times it's sensitive to everything. Thiamine treatments for Cohos are successful, using injections. Broodstock egg takes went well this year (eye up was 92% and 88%). The DNR will begin stocking 9-inch Skamanias instead of 7 inch, and winter runs will be 8 inch. Trout Lodge lost 90k Rainbow Trout due to equipment failure. Bodine will be the incubation site from now on. The Mixawba hatchery had two processors go down that controlled alarms and wells, but it was luckily caught in time and no fish were lost. As a consequence, it is getting a new alarm system. Walleye and Muskie broodlakes were negative for VHS. A statewide bait dealer survey detected Golden Shiver virus. There were also lots of bacteria, but staff couldn't get clean isolates to identify.

19. Vice-Chair selection (All)

Andy Noyes was nominated and accepted.

20. Future meetings (Shen)

Summer 2015: July 28-29 in Lake Placid, NY.

Winter 2016: February 2-3, 2016 in Lansing, MI. The date may change to coincide with the Eastern Fish Health meeting.

Ron Bruck (WI) is interested in hosting a VHS symposium in 2016. More information will be relayed as it becomes available.

21. Tour of the Animal Disease Diagnostic Lab (All)

Meeting attendees were given a tour of the lab and told synopses of current research.

22. Adjourn

GREAT LAKES FISH HEALTH COMMITTEE
TECHNICAL ADVISORS

April 2015

Bacteriology
Diane Elliot (U.S. Geological Survey)
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
Dominique Bureau (University of Guelph)
Dale Honeyfield (U.S. Geological Survey)

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)

Surveillance for pathogens demonstrated absent – when can we stop?

Lori Gustafson[1], Ian Gardner[2], Marta Remmenga[1]
[1]USDA APHIS Veterinary Services
[2]University of Prince Edward Island
Great Lakes Fish Health Committee
2 February 2015

Photo: C. Zepeda

Veterinary Services

List of pathogens - and diagnostics – is extensive and rising

OIE (World Organization for Animal Health) List for Finfish

- Epizootic haematopoietic necrosis
- Epizootic ulcerative syndrome
- *Gyrodactylus salaris*
- HPR-deleted or HPR0 infectious salmon anaemia virus
- Salmonid alphavirus
- Infectious haematopoietic necrosis
- Koi herpesvirus disease
- Red sea bream iridoviral disease
- Spring viraemia of carp
- Viral haemorrhagic septicemia

Other Finfish Diseases of Concern

- Bacterial kidney disease
- Infectious pancreatic necrosis
- Piscine reovirus
- Totivirus

Some diagnostics cover multiple pathogens (e.g., cell culture), but with varying sensitivity

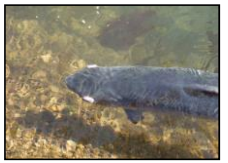


Photo: ISA Program Maine

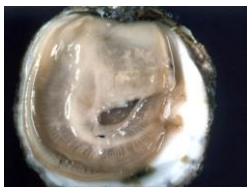
List of pathogens - and diagnostics – is extensive and rising

OIE List for Molluscs

- Abalone herpesvirus
- *Bonamia exitiosa*
- *Bonamia ostreae*
- *Marteilia refringens*
- *Perkinsus marinus*
- *Perkinsus olseni*
- *Xenohaliotis californiensis*

Other Mollusc Diseases of Concern

- *Haplosporidium nelsoni*
- *Marteiliodes chungmuensis*
- *Mikrocytos mackini*
- OsHV-1 µvar
- *Vibrio tapetis*



Bonamia ostreae, photo by Elston, R.

Surveillance design: we're good at the 'opening'; need to be just as good at the 'close'

Photo: ISA Program Maine

Not an easy task:
Sampling is a snapshot - a space/time slice - of a complex and dynamic system

Long-term accuracy depends not just on sample size, but on stability and homogeneity of the system

OIE provides guidelines: stopping rules ('maintaining freedom status') for highly clinical pathogens

Photo: A. Noyes, VHSV outbreak gizzard shad

- **Stop after demonstration**
  - e.g., 2 years of negative test results
  - IF conditions are conducive to clinical expression, and
  - IF early detection systems are in place
- OIE Code examples for country/zone/compartment
  - VHSV, IHNV
  - *B. existiosa, B. ostreae, Perkinsus marinus*

OIE guidelines: stopping rules ('maintaining freedom status') for non-clinical pathogens or conditions

Photo: ISA Program Maine

- Continue '**commensurate with risk**' ('according to likelihood of infection')
  - Still presuming early detection systems are in place
- OIE Code examples:
  - ISAV-free country/zone/compartment
  - Most pathogens, if local conditions are not conducive to clinical expression

On paper, this is sensible: In practice, there are nagging concerns

- Is 2, 3, 5 years long enough?
- How do we know a farmer will report clinical conditions?
- Observational surveillance won't detect non-clinical.
- What is 'commensurate with risk'?

A common default: Treadmill testing

Rationale: Traditional model forms basis for trade

Result: Resource exhaustion and limited reserves for emerging issues

Photo: ISA Program Maine

Where'd they put the pause button?!

- Our instruction manual should include
  - Further guidance on methods to retire or reduce surveillance
  - Example applications – for facilities, compartments and zones

Case-studies may help motivate solutions

Closed, land-based, systems

Hypothetical rainbow trout farm (akin to a compartment)

Open, managed, systems

Hypothetical pacific oyster system (akin to a zone)

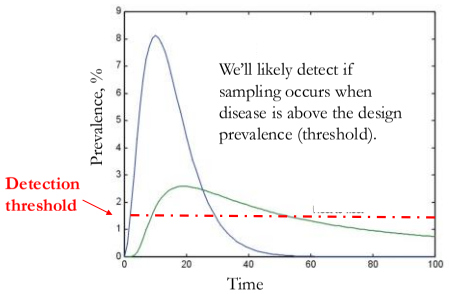
Closed systems *should* be easy

- Surveillance system components for our example farm
  - Active surveillance history, > 95% confidence
    - 2+ years for EHN, IHN, ISA, IPN, SAV, VHS
  - Early detection system in place
  - Negligible introduction risk per formal risk assessment

'Commensurate with risk' suggests it is safe to stop

But, we don't ….

What is the best duration for testing: Is 2 years enough?



A single year (multiple seasons) may be enough for most

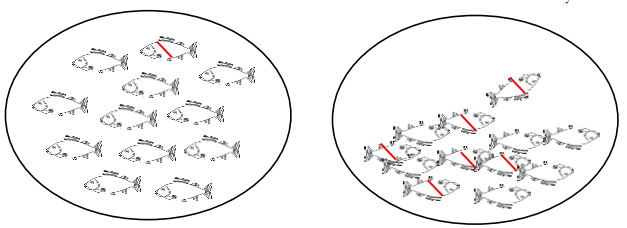
What constitutes a strong 'early detection system'?

- Here, this includes observational surveillance with triggers for veterinary investigation
- Concerns
  - Relies on farmer for clinical observations
  - Provides little assurance for non-clinical diseases

Routine moribund surveillance would address early detection concerns

- Periodic, high-value, sampling
  - Can incorporate veterinary visits
  - Can reduce sampling to very small number and retain assurance for most pathogens

60 fish or fewer may suffice



What about open systems?

Photo: Elston, R.

Where introduction risks are non-negligible or unclear

Zone borders follow natural boundaries, not facility walls

Some immediate benefits:

- Commercial populations may serve as sentinels for wild, and vice versa
- Data responsibilities are shared across stakeholders and sites

This calls for an approach 'commensurate with risk'

What are the pathways for disease introduction *into the zone*?
How do we adjust surveillance to compensate for those risks?

Methods available, few described examples

- Risk assessments
- Scenario trees
- Expert panel models

Expert panel identified VHSV risks

- Relationships with affected regions
  - Hydrologic connectivity
  - Close linear distance
  - Fomites via shared traffic or wastes
  - History of live bait transfers
  - History of live fish transfer for culture/stock
  - History of frozen fish transfer
  - Insufficient regulatory framework
- And, conducive environments
  - Presence of susceptible species
  - Cool to cold water environment

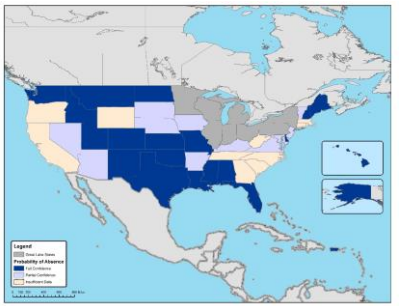
Emerald shiner

VHSV Expert Panel and Working Group. 2010. VHS risk factors and association measures derived by expert panel. Preventive Veterinary Medicine 94, 128-139.

And scored the risk factors

Risk Factor	Response Category	LR
Hydrologic connection	connected with fish movement	3.16
	downstream, no fish movement	1.41
Linear distance	< 100 km	2.50
Live bait transfers	yes, without testing	2.65
Other live fish transfers	yes, without testing	2.45
Frozen fish transfers	yes, without testing	2.45
Fomite exposure	shared traffic, equipment or wastes	2.24
Infrastructure for response	insufficient veterinary oversight	1.34

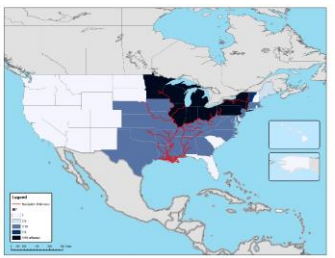
Risk scores dictate surveillance needed to achieve disease freedom status



- We credit context and surveillance using Bayes theorem
- Posterior Odds (VHS) = Prior Odds (VHS) * $LR_{(RF1)}$ * $LR_{(RF2)}$ …

Scores also direct surveillance needed to maintain disease freedom status

- Introduction Risk Score (IR)
  - Is the product of LRs representing open pathways
  - Example map (right) excludes anthropogenic factors. Displays distance and water connectivity only.
- To estimate ongoing sampling needs
  - Divide traditional sample by IR
  - This is the IR adjusted value of last year's surveillance
  - Traditional sample – IR adjusted value = new samples needed
- Note: Laboratory testing is not the only form of surveillance. Basic biosecurity, and observational surveillance, are alternate routes.



Gustafson et al. 2014. Viral hemorrhagic septicemia IVb status in the U.S.: Inferences from surveillance activities and regional context. Preventive Veterinary Medicine.

Examples and guidelines on the resolution of surveillance will ease implementation

Key benefits:

- Allocate resources according to information needs
- Improve decision support
- System refresh

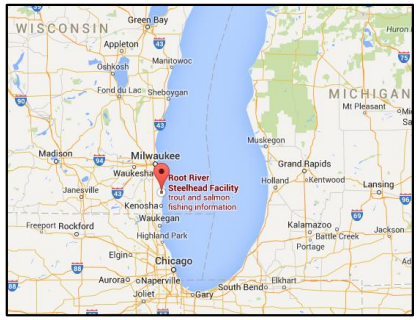
Key questions:

- What is sufficiency of early detection?
- What is 'commensurate with risk'?
- How long is long enough?
- How can we best use recovered funds?

VHSv in Feral Coho Broodstock from Lake Michigan

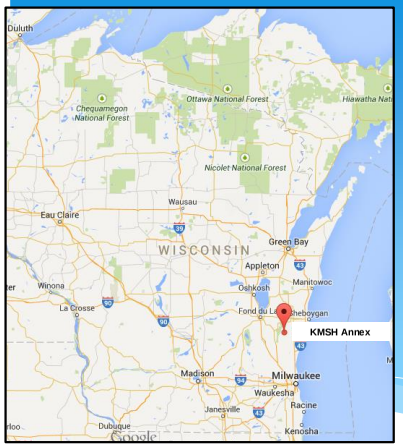
Bridget B. Baker, MS, DVM
Wisconsin DNR

Root River Egg Collection Facility



2014 Coho spawning dates

- October 20th – 150 ovarian fluids collected
- October 23rd – no samples collected
- October 27th – 60 tissue (k/s/h/l) samples collected
- November 3rd – no samples collected



Kettle Moraine State Fish Hatchery

- Eggs double disinfected
- Shared equipment w/ COS eggs from other spawn dates and CHS eggs
- H2O discharge into Mink Creek
- Transfer of eyed eggs to LVH

Response to positive VHSv results

1. Depopulate and incinerate fertilized eggs from October 20th, October 23rd, and November 3rd ☑
2. Quarantine, disinfection, biosecurity review at KMSH Annex and LVH ☑
3. Virology testing in COS and CHS fry at KMSH Annex ☑
4. Virology testing in LAT and splake fry at LVH ☑
5. Virology results from October 27th ☑
6. Virology testing in Mink Creek ☐

Questions for discussion…

* Egg disinfection protocols
  * # of disinfections
  * Concentration
  * Length
  * Use of thiamine
  * Shared equipment
* Broodstock assessment protocols
  * Ovarian fluids
  * Tissues
  * Milt
  * BB vs. OIE
  * Sampling schedule
* Different response in the future?

Appendix 3

Fish Health Research

Great Lakes Fish Health Committee Meeting

Maria S. Sepúlveda
DVM, PhD
February 03, 2015

PURDUE UNIVERSITY

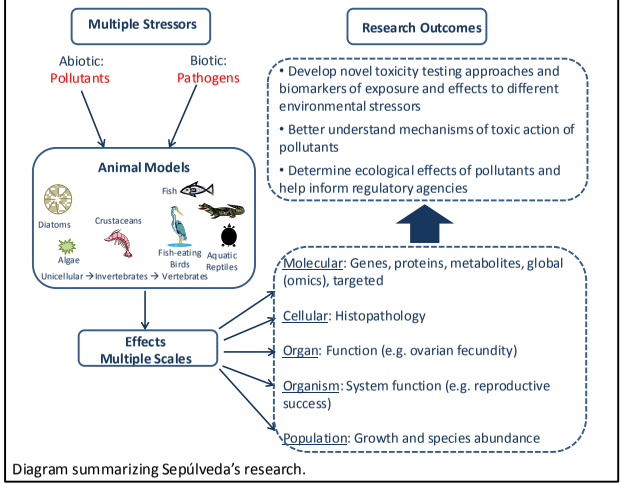
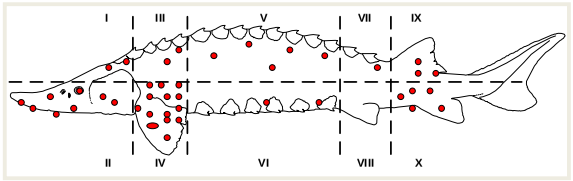


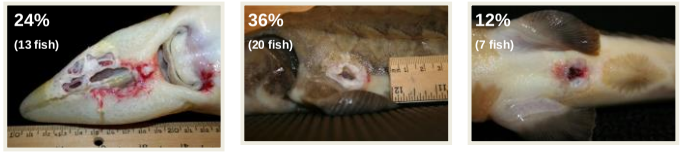
Diagram summarizing Sepúlveda's research.

Sea Lamprey effects on Lake Sturgeon

Sea lamprey parasitism negatively impacts survival of lake sturgeon (< 760 mm FL)



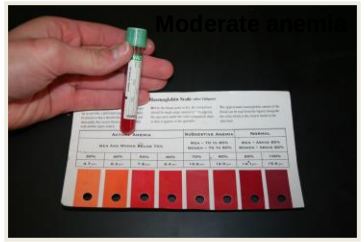
Region: II **IV** **X**



Patrick et al. 2009. Trans Am Fish Soc 138:1065-1075.

Sea Lamprey effects on Lake Sturgeon

A single lamprey attack causes acute anemia and mortality



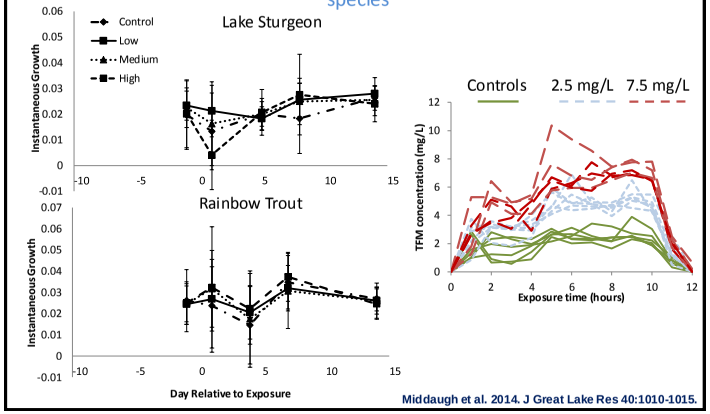
Hemoglobin - 50%
Hematocrit - 15%

Hemoglobin - 10%
Hematocrit - 0.5%

Sepúlveda et al. 2013. J Aquat Anim Health 24:91-99.

Lampricides: TFM

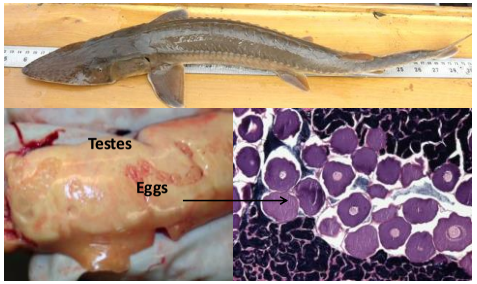
No effects of a typical TFM treatment on growth or behavior of non-target species



Middaugh et al. 2014. J Great Lake Res 40:1010-1015.

Gonadal Intersex

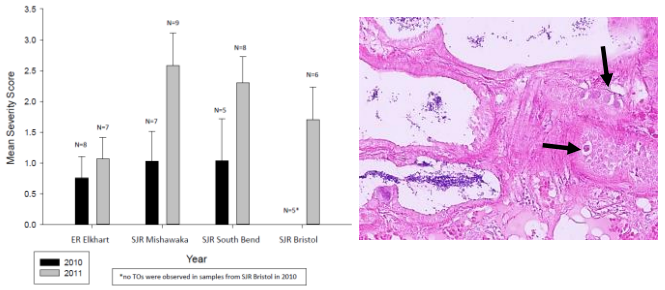
7% shovelnose sturgeon from the Wabash River are intersex. Cause(s) and ecological implications are unknown.



Amberg et al. 2010. Fish Physiol. Biochem. 36: 923-932.

Gonadal Intersex

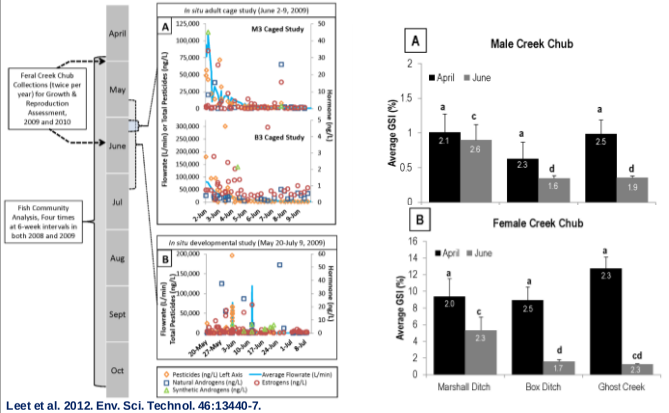
Up to 100% smallmouth bass from the St. Joseph and Elkhart Rivers are intersex. Cause(s) and ecological implications are unknown.



Abdel-Moneim et al. Unpublished data.

Emerging Contaminants

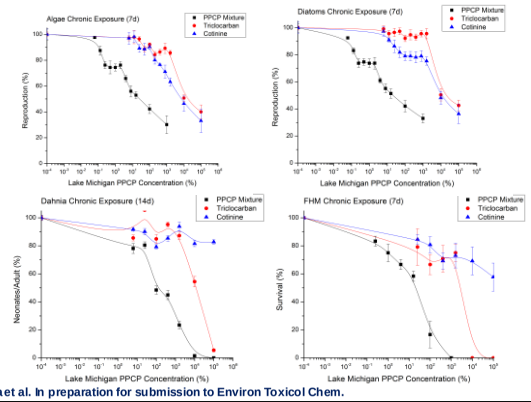
CAFOS are a source of hormones to streams. Effects on fish reproduction are unclear.



Leet et al. 2012. Env. Sci. Technol. 46:13440-7.

Emerging Contaminants

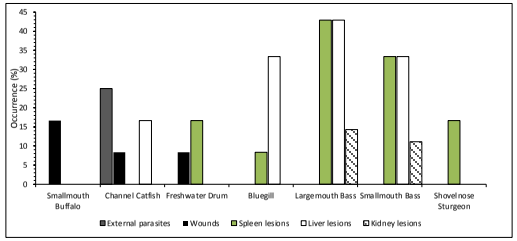
Pharmaceutical and Personal Care Products are a common group of emerging contaminants. Mixture effects are beginning to be addressed.



Mahapatra et al. In preparation for submission to Environ Toxicol Chem.

Diseases of Asian Carp

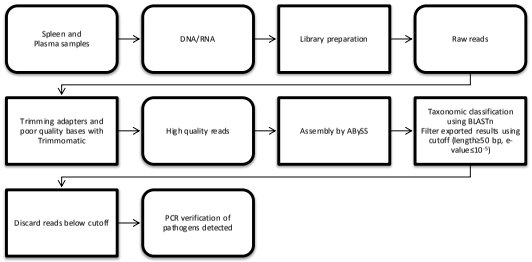
Asian carp from the upper Wabash River had no lesions, no ectoparasites and no gastrointestinal helminths



Thurner et al. In preparation for submission to J Fish Dis.

Diseases of Asian Carp

Metagenomic Workflow from Spleen and Plasma Samples



Thurner et al. In preparation for submission to J Fish Dis.

Diseases of Asian Carp

Read Statistics from MiSEq. *Number of contigs with blastn hits where length≥50 bp and e-value≤0.00001.

Species	Total Reads		# of Assembled Contigs (N50)		Bacteria*		Viruses*	
	Spleen	Plasma	Spleen	Plasma	Spleen	Plasma	Spleen	Plasma
Silver Carp	2,413,398	1,131,692	210,438 (578)	102,076 (630)	6	7	0	0
Bighead Carp	2,507,686	1,089,464	236,430 (579)	106,851 (642)	7	22	0	0
Common Carp	2,193,910	1,262,374	198,934 (575)	91,107 (682)	5	14	4	1,641
Bigmouth Buffalo	2,810,762	1,215,928	234,689 (659)	98,414 (674)	3	4	1,303	2
Channel Catfish	2,782,922	1,132,136	195,271 (585)	74,820 (639)	8	9	14	2
Flathead Catfish	2,340,522	796,410	204,459 (606)	77,659 (774)	5	4	17	1
Gizzard Shad	3,058,656	974,362	220,623 (591)	50,464 (746)	6	1	106	3
Freshwater Drum	2,262,574	1,462,810	145,404 (693)	121,067 (585)	1	12	3,381	2
Bluegill	4,106,104	-	323,410 (585)	-	8	-	381	-
Largemouth Bass	3,182,898	1,261,178	289,555 (586)	117,736 (662)	8	6	54	6
Smallmouth Bass	3,485,892	1,175,778	252,632 (594)	112,005 (665)	28	8	12	1
Shovelnose Sturgeon	2,524,750	1,182,856	227,246 (598)	80,167 (717)	4	5	9	0

Thurner et al. In preparation for submission to J Fish Dis.

Appendix 4

Diseases of Asian Carp

Pathogens detected by BLAST

Class	Order (Family)	Pathogen	SC	BC	CC	BF	CH	FC	GS	FD	BG	LB	SB	SS
Gamma Proteobacteria Gram –	Pseudomonadales	*Pseudomonas fluorescens*	X	X		X		X		X	X	X	X	X
		Pseudomonas putida	X	X	X	X	X	X		X	X	X	X	X
		Acinetobacter baumannii					X							
	Enterobacteriales (Enterobacteriaceae)	*Salmonella enterica*		X	X		X							
		Serratia marcescens			X									
		Serratia plymuthica			X									
		Klebsiella pneumoniae					X							
Bacilli Gram +	Lactobacillales	*Lactococcus lactis* subsp. *cremoris*							X					
		Lactococcus lactis subsp. *lactis*							X					
	Bacillales (Bacillaceae)	*Bacillus cereus*					X							
		Bacillus subtilis					X							
		Staphylococcus aureus subsp. *aureus*			X								X	
Copepoda	Siphonostomatoida (Caligidae)	*Caligus clemensi*			X									
		Caligus rogercresseyi	X	X	X	X		X		X		X	X	
		Lepeophtheirus salmonis		X	X					X				

Thurner et al. In preparation for submission to J Fish Dis.

Diseases of Asian Carp

Asian carp cell line virus sensitivity assays

Cell Line	SVCV	LMBV	BGV	CCV	IPNV	VHSV	GSV
Bighead Carp							
Skin	+	+	+	-	-	+	+
Gill	+	+	+	-	+	+	+
Fry	+	+	+	-	+	+	+
Fin	+	+	-	-	-	+	+
Silver Carp							
Skin	-	+	+	-	+	+	+
Gill	+	+	+	-	+	+	+
Fry	+	+	+	-	+	+	+
Fin	+	+	+	-	+	+	+

All cell lines tested (skin, gill, fin, fry) from both Asian carp species showed unusually high sensitivity to LMBV. In addition, all bigheaded carp cell lines were very sensitive to VHSV and GSV.

Thurner et al. In preparation for submission to J Fish Dis.

Acknowledgements

- Graduate Students/Technicians*:
  - Ahmed Abdel-Moneim
  - Christopher Klinkhamer*
  - Jessica Leet
  - Chris Middaugh*
  - Holly Patrick
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- Collaborators:
  - Daragh Deegan
  - Reuben Goforth
  - Tomas Höök
  - Craig Jansen
  - Linda Lee
  - Cecon Mahapatra
  - Tom Stefanovage
  - Trent Sutton

DNR Indiana Department of Natural Resources

Great Lakes Fishery Commission

Sea Grant Illinois - Indiana

City of Elkhart

Purdue University 1913 - 2013 Forestry & Natural Resources

This research is funded by U.S. EPA - Science To Achieve Results (STAR) Program Grant # RD-83184801-0

Appendix 4

VHSV: The tale continues....!!!

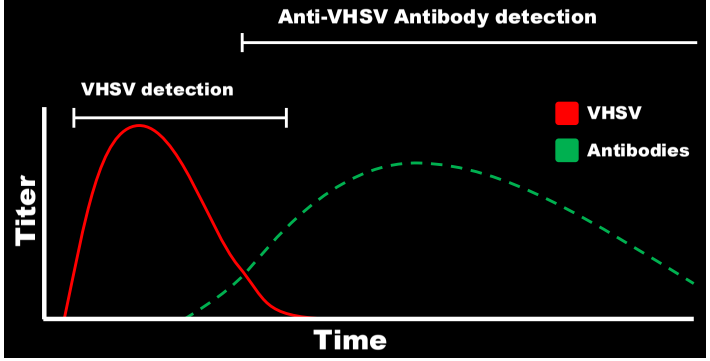
Sampling locations

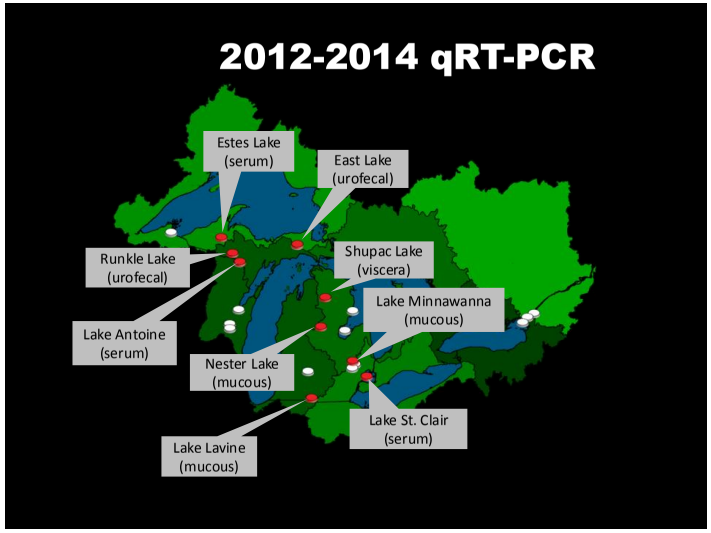
Sample Collection

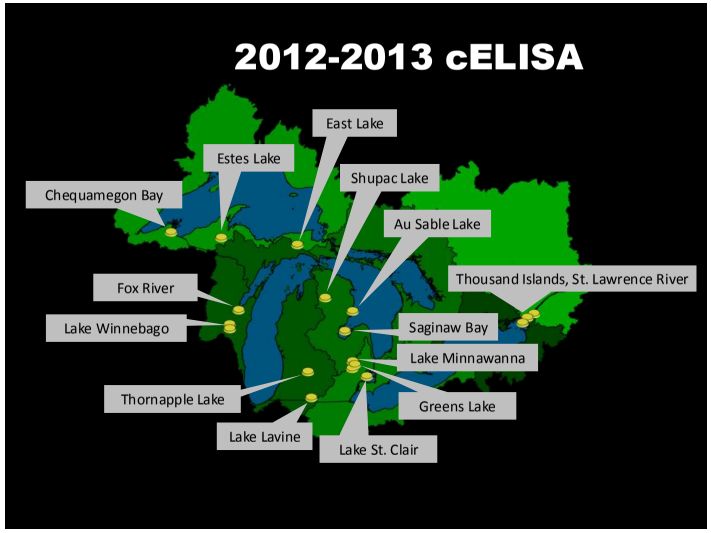
Tissue	Lake St Clair	All Other Locations
Serum	675	1313
Mucous	710	944
Urofecal	210	677
Visceral organs	0	1143
Gamete	0	13
Total	1595	4091

- qRT-PCR: Gamete, visceral organs, urofecal and mucous samples
- Serum: cELISA, and qPCR

- qRT-PCR (APHIS: +/-)
- SNT and cELISA

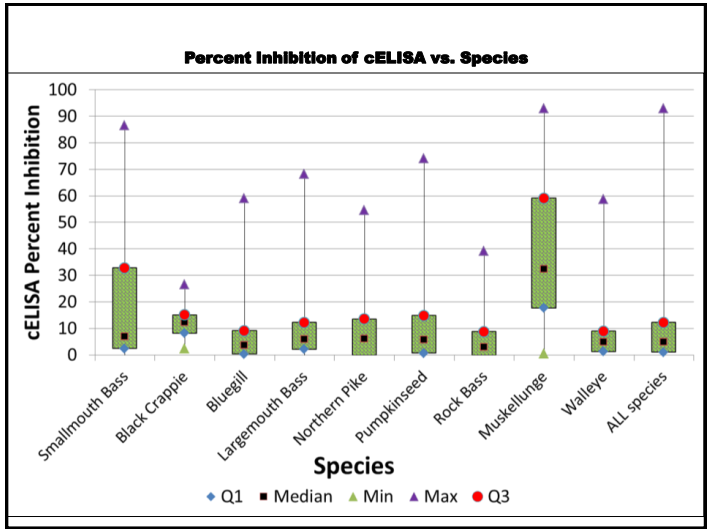




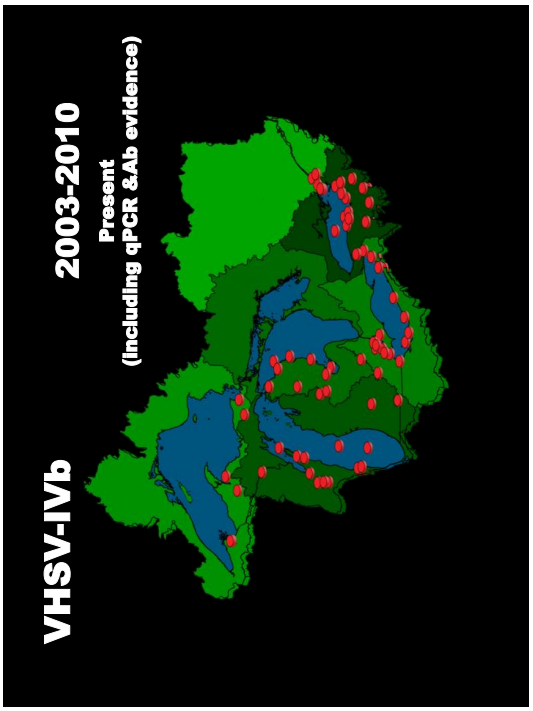


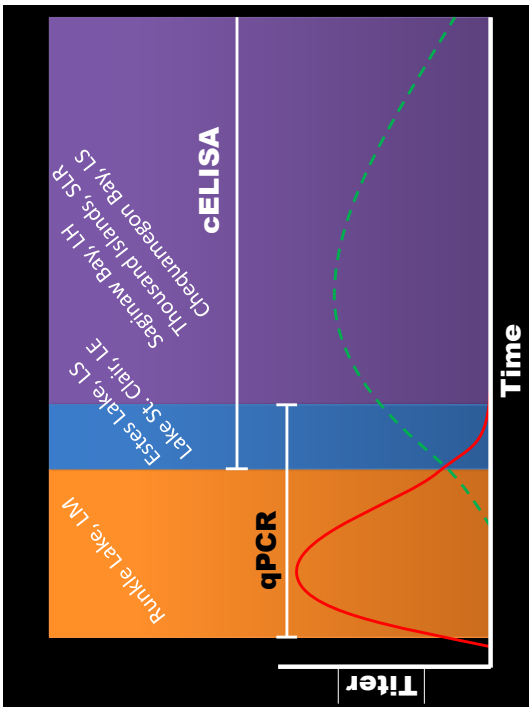
Results of Serological Testing

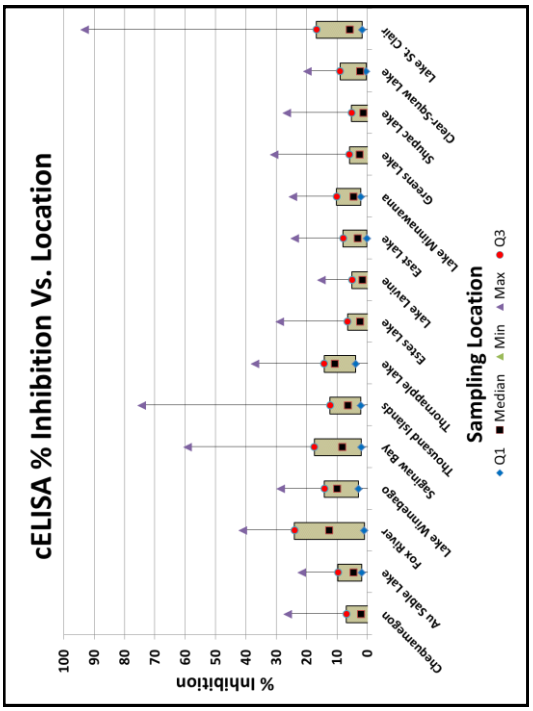
Site	Watershed	Serological	Molecular
Lake Lavine	Michigan	negative	negative
Runkle Lake	Michigan	positive	negative
Lake Antoine	Michigan	positive	positive
Thornapple Lake	Michigan	positive	negative
Greens Lake	Erie	positive	negative
Clear-Squaw Lake	Erie	positive	negative
Lake St. Clair	Erie	positive	positive
East Lake	Superior	positive	negative
Estes Lake	Superior	positive	positive
Lake Minnawanna	Huron	positive	positive
Shupac Lake	Huron	positive	positive
Nestor Lake	Huron	positive	negative
Au Sable	Huron	positive	negative
Saginaw Bay	Huron	positive	negative
Thousand Islands	St. Lawrence River	positive	negative

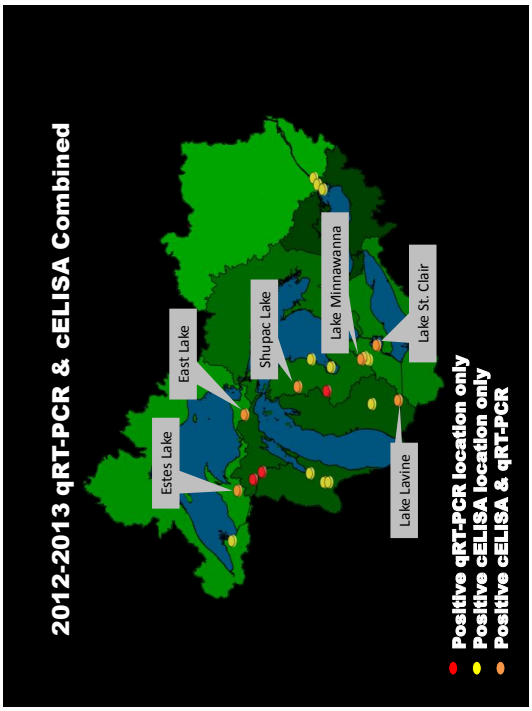


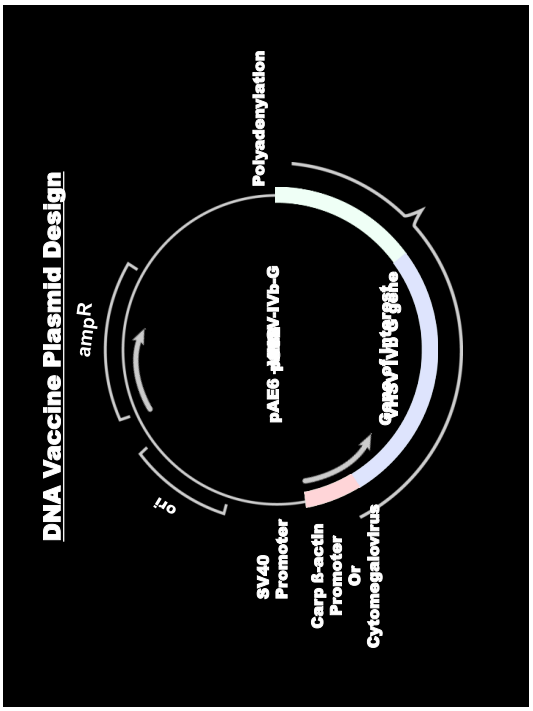
Appendix 5

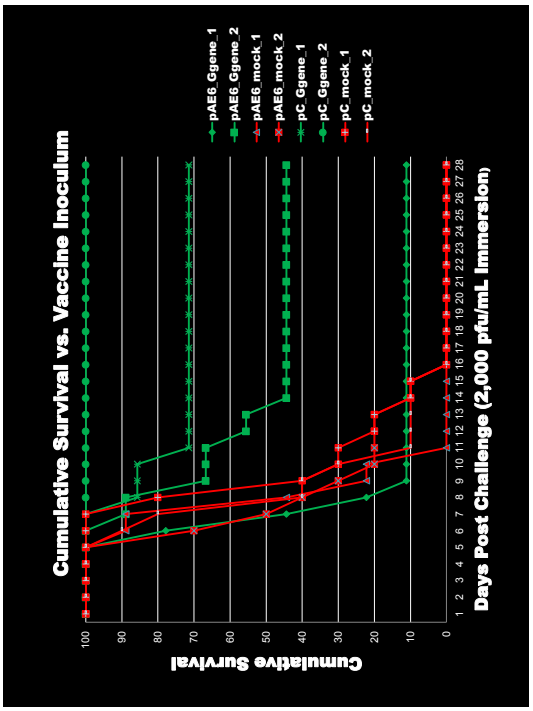

VHSV-IVb
2003-2010
Present
(Including qPCR &Ab evidence)

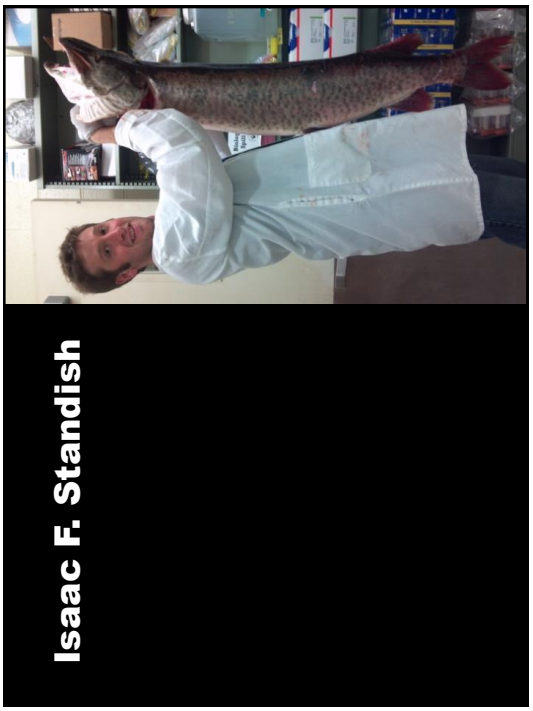

Runkle Lake, LM
Estes Lake, LS
Lake St. Clair, LE
Saginaw Bay, LH
Thousand Islands, SLR
Cheqauamegon Bay, LS
qPCR
cELISA
Titer
Time

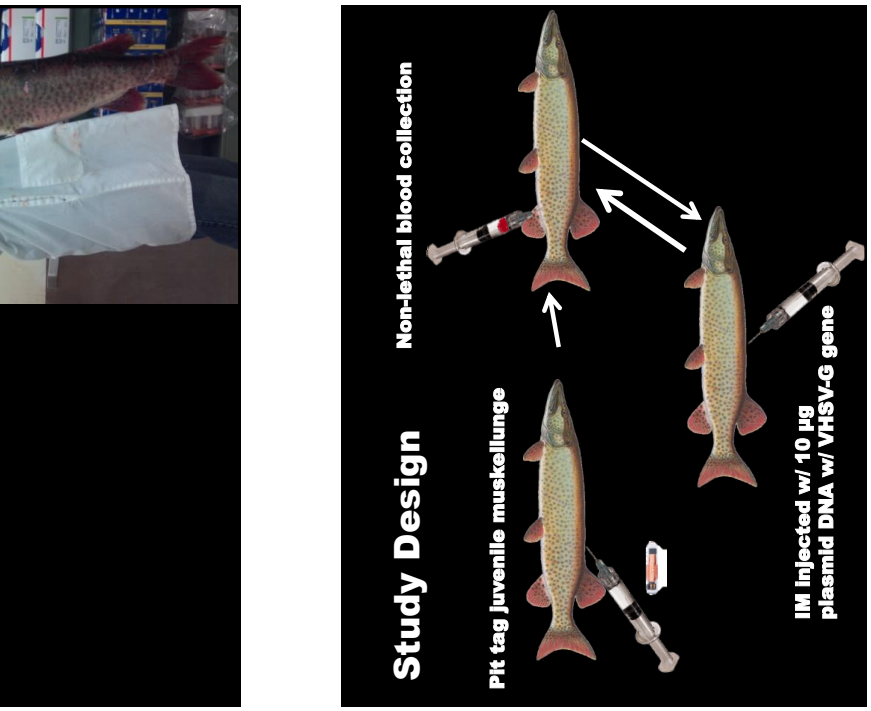

cELISA % Inhibition Vs. Location
% Inhibition
100
90
80
70
60
50
40
30
20
10
0
Sampling Location
Q1 Median Min Max Q3
Cheqauamegon
Au Sable Lake
Fox River
Lake Winnebago
Saginaw Bay
Thousand Islands
Thornapple Lake
Estes Lake
Lake Lavine
East Lake
Lake Minnawanna
Greens Lake
Shupac Lake
Clear-Squaw Lake
Lake St. Clair


2012-2013 qRT-PCR & cELISA Combined
Positive qRT-PCR location only
Positive cELISA location only
Positive cELISA & qRT-PCR
Estes Lake
East Lake
Shupac Lake
Lake Minnawanna
Lake St. Clair
Lake Lavine


DNA Vaccine Plasmid Design
ampR
ori
SV40
Promoter
Carp β-actin
Promoter
Or
Cytomegalovirus
Polyadenylation

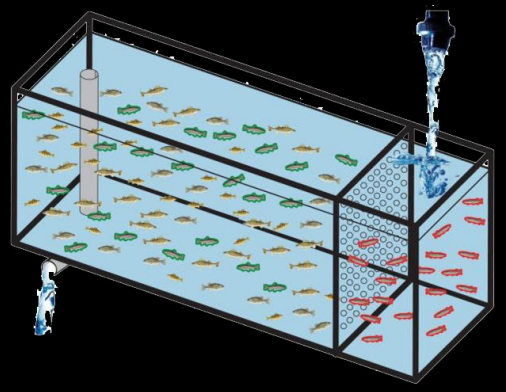

Cumulative Survival vs. Vaccine Inoculum
Cumulative Survival
Days Post Challenge (2,000 pfu/mL Immersion)
pAE6_Ggene_1
pAE6_Ggene_2
pAE6_mock_1
pAE6_mock_2
pC_Gene_1
pC_Gene_2
pC_mock_1
pC_mock_2


Isaac F. Standish


Study Design
Non-lethal blood collection
Pit tag juvenile muskellunge
IM injected w/ 10 µg
plasmid DNA w/ VHSV-G gene

- Vaccine elicits antibody production
- Muskellunge antibody levels are highly variable

Herd Immunity in Aquatic Systems

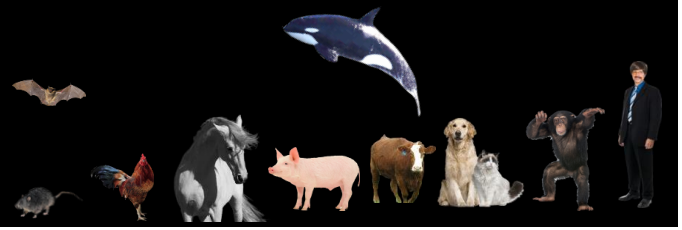


Ashley Baird

- B.S. in Lyman Briggs Zoology
- Current M.S. student in Fisheries & Wildlife

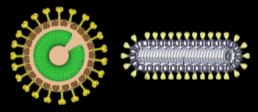
Order: *Nidovirales*

- Wide range of viruses
- Wide range of hosts



- **Family: Coronaviridae**

- **Genus: *Bafinivirus***



Nidoviruses in Fish

White Bream Virus (WBV)
(Routine health inspection of wild fish, Germany)
2001
Genus: *Bafinivirus*

→

Fathead Minnow Nidovirus
(Fathead minnows on a baitfish farm, Arkansas)
2001
Genus: *Bafinivirus*

→

Muskellunge Nidovirus (MNV)
(Muskellunge kidney from 2 state hatcheries)
- Wisconsin
- Michigan

2011

Are they the same virus?

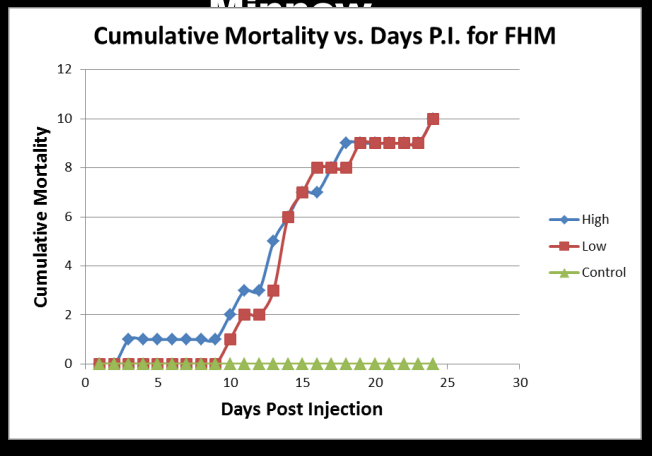
Cyprinids

↓

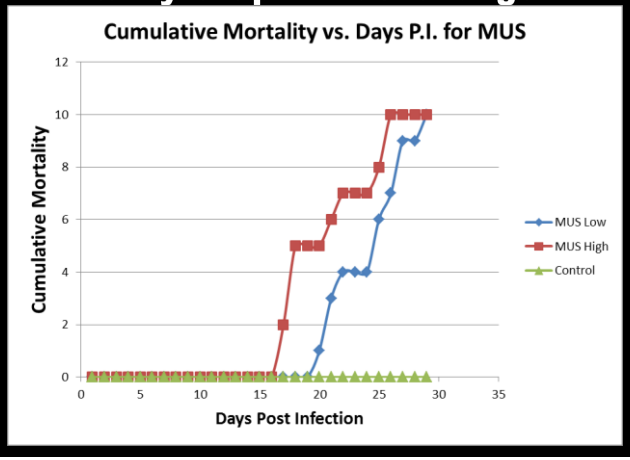
Esocids & other piscivorous fish

Experimental Infection through IP- Injection

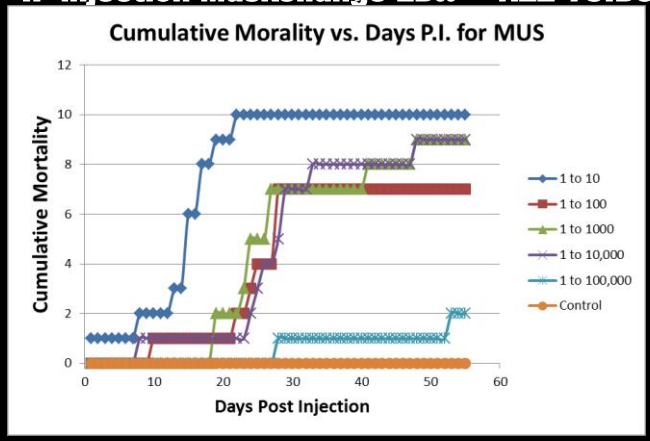
Mortality Graph – Fathead Minnow

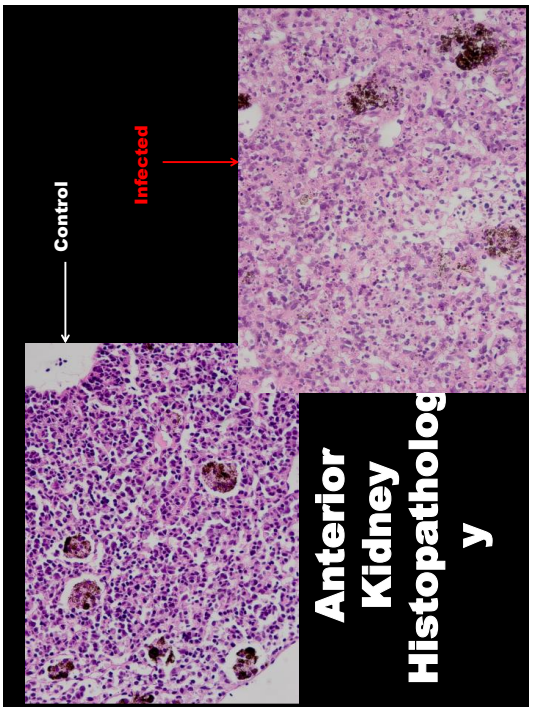


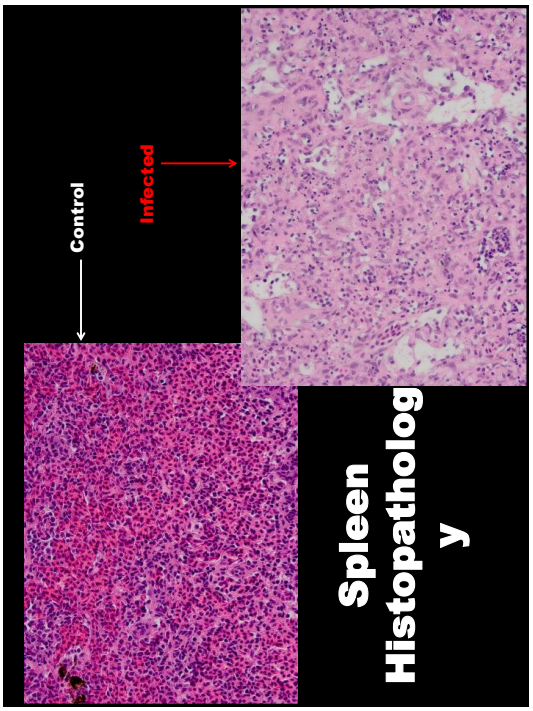
Mortality Graph - Muskellunge

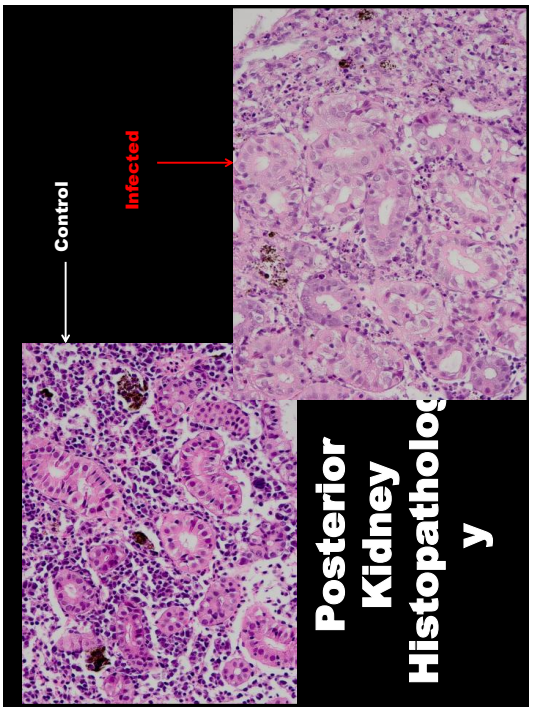


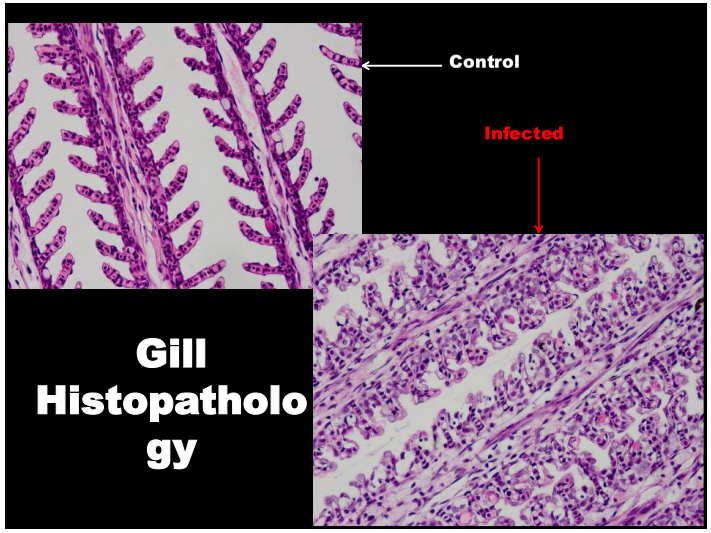
IP-injection Muskellunge LD_{50} = 1.22 TCID50




Control
Infected
Anterior Kidney Histopatholog y


Control
Infected
Spleen Histopatholog y


Control
Infected
Posterior Kidney Histopatholog y



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

Disease course in muskellunge

- Immersion challenge
- Experimental infection through ingestion of infected FHM
- Viral Shedding
- Virus Tropism

Risks Posed
by SVCV to
Representative Fish Species
Native to the Laurentian Great Lakes

Traimat Boonthai

B. Sc. (Microbiology) 2006

M. Sc. (Environmental Science 2009)

Ph. D. Student at Burapha University , Thailand

Joined AAHL in 2014

List of Fish Tested

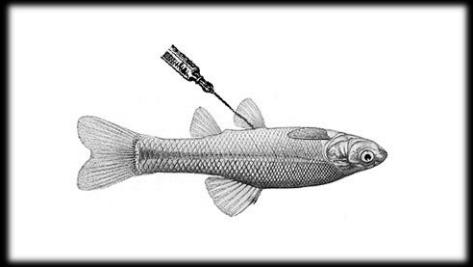
Cyprinid fish species

- Fathead minnows (*Pimephales promelas*)
- Golden shiners (*Notemigonus crysoleucas*)
- Spotfin shiners (*Cyprinella spiloptera*)
- Creek chub (*Semotilus atromaculatus*)

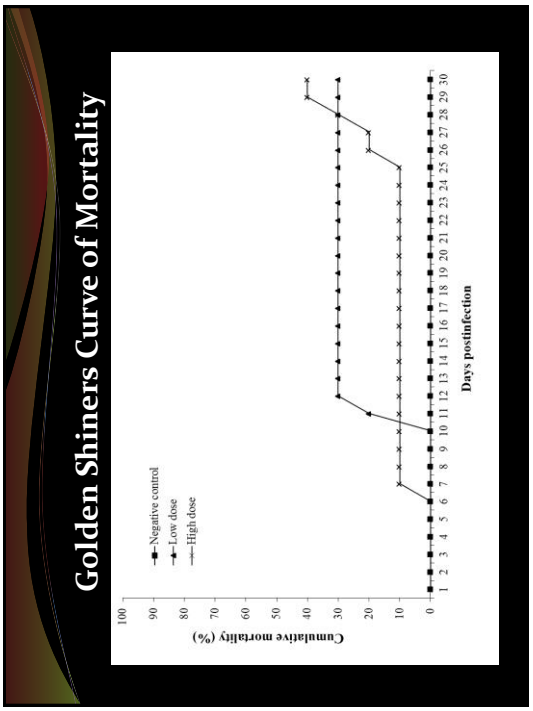
Non-cyprinid fish species

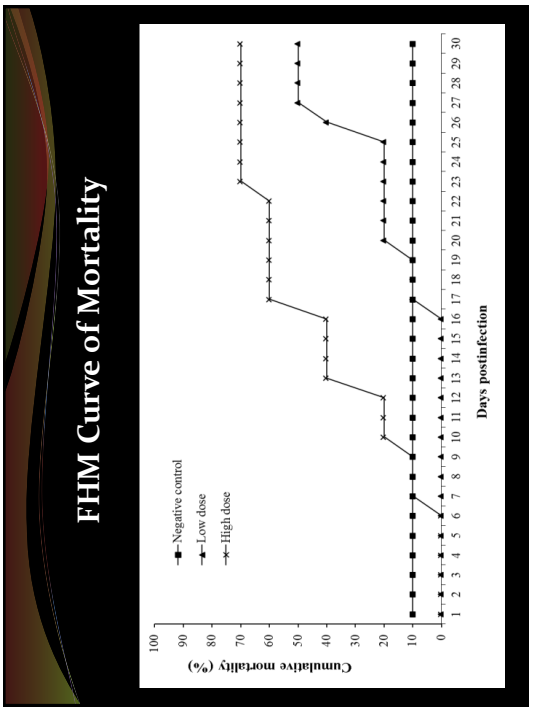
- Largemouth bass (*Micropterus salmoides*)
- Walleye (*Sander vitreus*)
- Rainbow trout (*Oncorhynchus mykiss*)
- Muskellunge (*Esox masquinongy*)

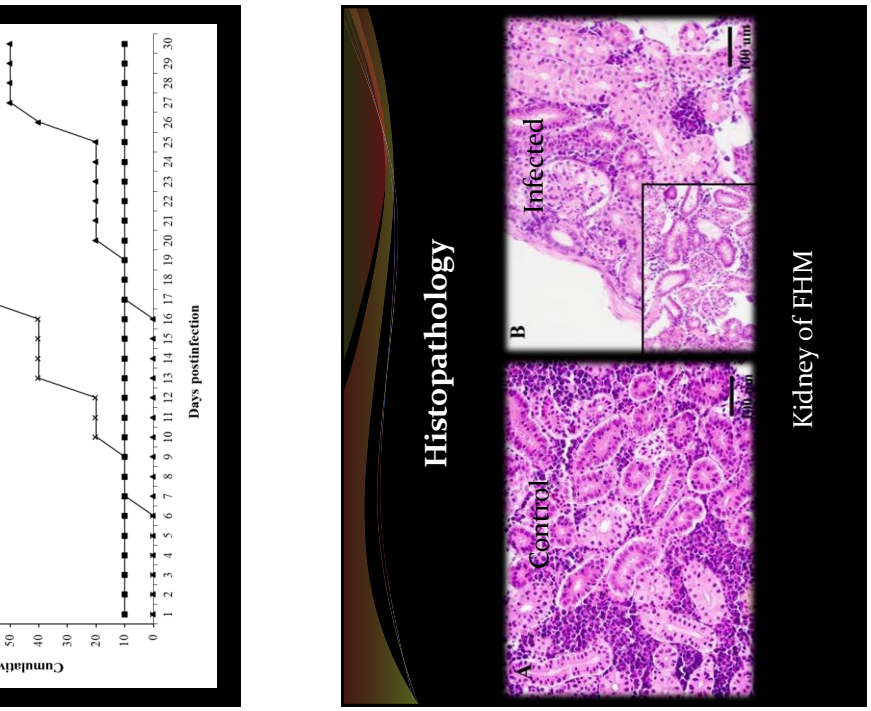
Infection by Intraperitoneal Injection




Clinical Signs of FHM
A
B

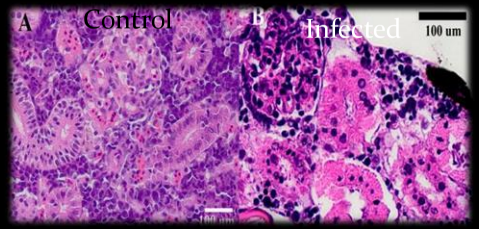

Golden Shiners Curve of Mortality
Negative control
Low dose
High dose
Cumulative mortality (%)
Days postinfection


FHM Curve of Mortality
Negative control
Low dose
High dose
Cumulative mortality (%)
Days postinfection


Histopathology
A
Control
B
Infected
100 um
Kidney of FHM

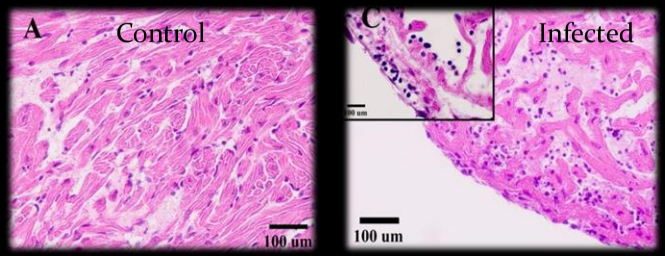
Clinical Signs of GOS

Histopathology



Kidney of GOS

Histopathology



Heart of GOS

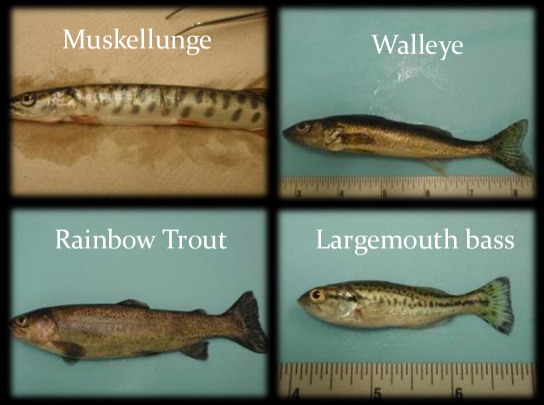
Other Cyprinid fish and All Non-cyprinid fish

No Mortality and absence of clinical signs

Spotfin shiners

Creek chub

Non-Cyprinid Fish



Megan Shavalier, DVM

- 2007 – Joined AAHL
- 2010 – B.S. in Zoology
- 2014 – DVM
- 2016 – PhD defense

GLFT-Funded

Re-emergence of
Epizootic Epitheliotropic Disease Virus:

Potential effects
&
development of improved diagnostics
&
control measures

Collaborator

- **LAMAR:**
  - Coll
  - Glenney
  - Barbash
- **LSSU:**
  - Li
- **U Florida:**
  - Waltzek
- **MSU: Basketball Champs of MI**
  - Loch
  - Faisal

- **Joint effort to:**
  - **Elucidate biological and pathological properties**
  - **Determine host range, disease course, transmission**
  - **Elucidate humoral immune mechanisms**
  - **Develop specific and sensitive diagnostic assays**
  - **Test efficacy of current biosecurity practices**

EEDV

- Objective 1 – Genomic characterization
  - Virus purification – **AAHL**
    - Using skin and gills of affected frozen fish from recent epizootics
  - Genomic sequence determination – **Dr. Waltzek**
  - Determination of Virulence factors – **Dr. Waltzek**
  - Phylogenetics – **Dr. Waltzek**

EEDV

- Objective 2 – Host range, disease course, transmission
  - Fish species choice and maintenance – **AAHL**
    - Lake trout (2 strains), brook trout, splake, Atlantic Salmon, brown trout, Chinook salmon, rainbow trout, lake herring, muskellunge, largemouth bass and mottled sculpin.
    - Housed at URCF, MSU
  - Viral screening to determine naivety and disease-free status - **AAHL**

EEDV

- Objective 2 – Host range, disease course, transmission
  - Experimental infections – **AAHL**
    - Susceptibility Screening, IP injections
      - high dose, low dose, negative control
    - Median lethal dose estimation
      - Two highly susceptible species
    - Development of immersion challenge model
      - Lake trout fingerling
    - Estimation of LD_{50} by immersion
      - Lake trout fingerling
    - Determination of disease course
      - Lake trout fingerling

EEDV

- Objective 2 – Host range, disease course, transmission
  - Determination of role of stressors – **AAHL**
    - Role of stressors in initiation of EEDV epizootics
      - Handling stress, fin clip, elevated water temperature, prednisolone injection
    - Assessment of survivor status on degree of protection

EEDV

- Objective 3 – Humoral immune mechanisms
  - Production of hyperimmune antibodies – **Dr. Li**
  - Assessment of local and systemic immune responses – **Dr. Li**

EEDV

- Objective 4 – Development of diagnostic assays for EEDV detection
  - Propagation of EEDV *in vitro* – **AAHL**, **Dr. Barbash**, **Dr. Coll**
    - Development of cell lines from lake trout
    - Virus propagation on established fish cell lines
    - Inoculation of cell cultures with EEDV infected samples

EEDV

- Objective 4 – Development of diagnostic assays for EEDV detection
  - Development of diagnostic assays
    - Real-time PCR assay – **Dr. Glenney**
    - Quantitative loop-mediated isothermal amplification (qLAMP) – **AAHL**
    - *In situ* hybridization technique – **AAHL**
    - ELISA-based assays – **Dr. Li**

EEDV

- Objective 5 – Test efficacy of current biosecurity practices
  - Efficacy of current disinfection techniques – **AAHL**, **Dr. Barbash**, **Dr. Coll**
    - Inactivation on surfaces
    - Egg disinfection
    - UV inactivation
  - Efficacy of stress test to detect carrier fish - **AAHL**, **Dr. Barbash**, **Dr. Coll**

Updates

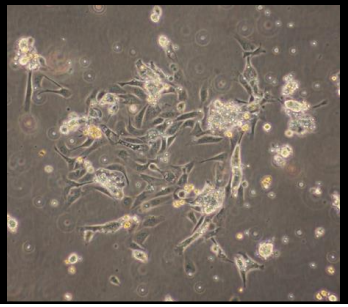
- Following EEDV outbreaks in 2012, surviving fish were transported to URCF.
  - Housed in individual raceways by lot
  - Additional disease outbreaks, mortalities
    - Summer 2013
    - Summer 2014

Updates

- Several aspects of the above project have been started:
  - A qLAMP assay has been finalized that discriminates between Salmonid Herpes Virus-3, -4 and -5.
    - Previous molecular assays were unable to

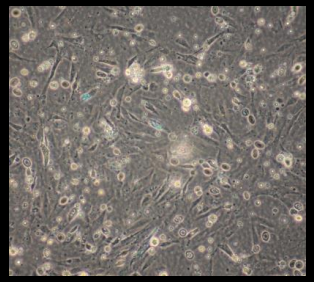
Updates

- **Propagation of lake trout cells *in vitro***
  - **Lamar and the AAHL at MSU**
  - **Current status:**
    - **Adult lake trout**
      - **Attempted skin, gill, fin, liver, testes, anterior kidney, posterior kidney**
      - **Initial success with liver, gill, and kidney**
      - **14 days post, surviving attached cells of liver**



Updates

- Sac fry
  - Two growth media (MEM and L-15)
  - 10 days post, attached cells forming coalescing monolayers

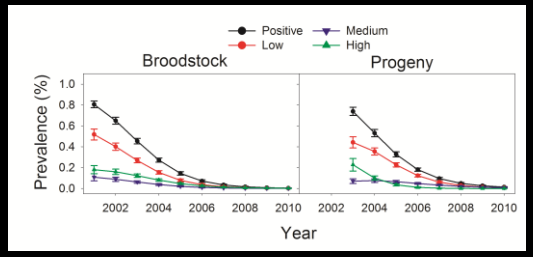


SVCV

- **Assessing its risk to representataive GL fish species**

Serosurveillance of *Renibacterium salmoninarum* in *Oncorhynchus* spp. in Michigan

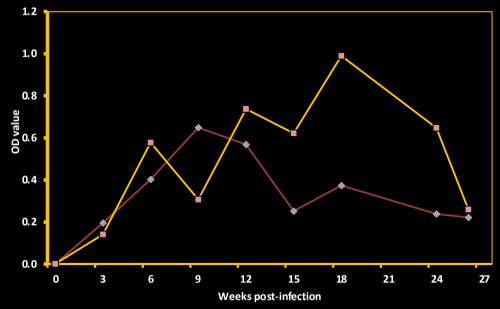
Overall prevalence of *Rs* in all *Oncorhynchus* spp.

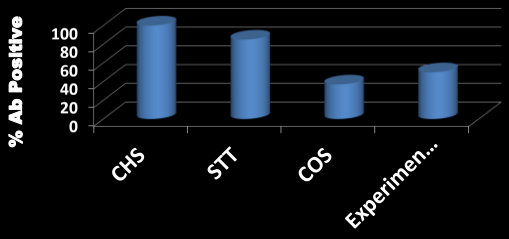


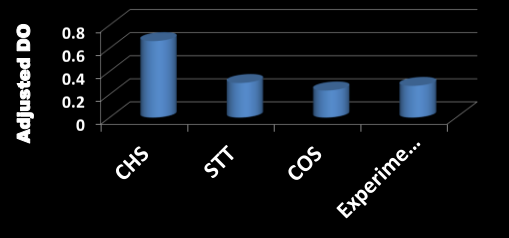
. A single-dilution indirect enzyme-linked immunosorbent assay (ELISA)

Cutoff value of OD=0.104

Experimentally Infected CHS & RBT







Conclusions

- Despite the low RS prevalence, exposure continues resulting in the production of antibodies.

USGS science for a changing world

Non-lethal Samples for Dectection of *Renibacterium salmoninarum* in Juvenile Chinook Salmon

DG Elliott, CL McKibben, CM Conway, DM Chase, MK Purcell, LJ Applegate

Western Fisheries Research Center
U.S. Geological Survey, Seattle, Washington USA

Renibacterium salmoninarum (Rs)

- Bacterial kidney disease (BKD) caused by Rs widespread in wild and cultured salmonids
  - BKD associated with high mortality of Chinook salmon in Lake Michigan 1988-92
  - Many Rs infections are chronic; subclinical infections common

USGS

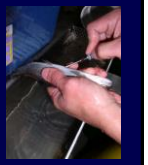
Renibacterium salmoninarum (Rs)

- Rs infections in juvenile fish usually detected by lethal sampling
  - Kidney is most common tissue sampled
- Most common Rs detection methods:
  - Culture
  - Enzyme-linked immonosorbent assay (ELISA)
  - Fluorescent antibody technique (FAT)
  - Polymerase chain reaction (PCR)

USGS

Why Non-lethal Sampling?

- Enables monitoring of performance and survival of fish after testing
  - Can be conducted in conjunction with tagging for studies of free-ranging fish populations
- Reduces numbers of fish sacrificed for pathogen monitoring
  - Important for threatened or endangered populations or valuable broodstock



USGS

Non-lethal Sampling Experiments

- Earlier experiments demonstrated that gill snips, fin clips, and mucus scrapings could be obtained non-lethally from fish as small as 3 g average weight
- Blood draws (caudal vessels) and kidney biopsy samples caused ≥5% mortality in fish 3-15 g in weight

USGS

Research Questions

- Can non-lethal samples be used to distinguish between Rs-exposed and non-exposed fish?
- Does non-lethal detection of Rs in surface samples (gill, fin, and mucus) reflect infection or passive association of bacteria shed into water by infected fish?
- Do prevalence and levels of Rs detected in non-lethal samples reflect changes in infection severity as determined by lethal (kidney) sample analysis?

USGS

Experimental Design

- Used Rs-free juvenile Chinook salmon (35 g) from two stocks: Strawberry Creek, Wisconsin (WI) and Soos Creek, Washington State (WA)
  - WA stock was progenitor for WI stock
  - GLFT-funded research determined that WI stock is now more resistant than WA stock to Rs injection challenge (Purcell et al 2008 JAAH 20:225; Metzger et al. 2010 DAO 90:31; Purcell et al 2014 JAAH 26:9)
    - Possible pathogen-driven selection for BKD resistance

USGS

Experimental Design

- Challenge: 24-h immersion in Rs (10^6 cfu/mL)
  - Both WI and WA stockschallenged
  - Equal numbers of mock-challenged controls
- Rs sampling: 3-week intervals over 5 months
  - Sampled 15 fish/tank (30/treatment) at each time point
  - Complete analysis of samples taken at 3, 12 and 21 weeks post-challenge

USGS

Assays Used (each fish)

- Lethal (kidney) samples:
  - Polyclonal ELISA (Pascho et al. 1991; Dis Aquat Org 12:25; ELISA II)
  - Quantitative bacteriological culture (Jansson et al. 1996; DAO 27:197)
  - Nested PCR (nPCR; Chase and Pascho 1998; DAO 34:223)

Assays Used (each fish)

- Lethal (kidney) samples continued:
  - Real-time quantitative PCR (qPCR; Chase et al. 2006 J Vet Diagn Invest 18:375, with modifications per Elliott et al 2013 J Fish Dis 36:779 and Elliott AFS-FHS Blue Book 2014)
  - Direct FAT (DFAT; Elliott et al 2013 J Fish Dis 36:779)
  - Histopathology/immunohistochemistry (Histo/IHC; Elliott et al DAO in press doi: 10.3354/dao02846)

Samples Tested (each fish)

- Candidate non-lethal samples (for nPCR and qPCR testing only):
  - Gill filament snip (2 mm x 3mm, 5-10 mg)
  - Ventral (pelvic) fin clip (5-10 mg)
  - Mucus scraping (anterior-to-posterior direction, 5-10 mg)
  - Blood (caudal vessels, 10 µL)

Kidney Testing Results

- Most (93%) of fish sampled during 5-month post-challenge period had no clinical signs of Rs
- Prevalence/intensity of Rs infections higher in weeks 3 and 12 than in week 21
- No consistent differences in Rs prevalence and severity between stocks
  - Previous studies showed significant differences in BKD mortality between WI and WA stocks after i.p. injection challenge

Analytical Approach

- Question: Can non-lethal samples be used to distinguish between Rs-exposed and non-exposed fish?
- Approach: For each sample/assay, examined:
  - Diagnostic sensitivity (true positive detection rate)
  - Diagnostic specificity (true negative detection rate)
  - Diagnostic odds ratio (DOR; odds of correct classification of fish as Rs-positive or Rs-negative vs misclassification)
- Compared results for Rs-challenged fish (true positives) vs mock-challenged fish (true negatives)

Kidney Assays

Assay	Sensitivity	Specificity	DOR*
ELISA	100%	99%	42,718
Culture	61%	100%	566
nPCR	50%	98%	51
qPCR	35%	100%	190
DFAT	20%	97%	9
Histo/IHC	10%	100%	41

*DOR >1 = higher odds of correct classification of fish as Rs-pos or Rs-neg vs misclassification

N=360 fish (180 Rs-challenged, 180 mock-challenged)

Non-lethal Assays

Assay	Sensitivity	Specificity	DOR
Gill nPCR	85%	98%	296
Gill qPCR	72%	99%	309
Fin nPCR	75%	99%	219
Fin qPCR	51%	100%	373
Mucus nPCR	98%	89%	397
Mucus qPCR	92%	98%	582
Blood nPCR	9%	62%	0.2
Blood qPCR	4%	100%	13

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Blood qPCR	4%	100%	13

Mucus qPCR showed best diagnostic performance for distinguishing between Rs-exposed and non-exposed fish

Analytical Approach

- Question: Does non-lethal detection of Rs in surface samples (gill, fin, and mucus) reflect infection or passive association of bacteria shed into water by infected fish?
  - Approach: Measured Rs concentrations in tank water at each sample time by solid-phase cytometry (SPC)
  - Results: Mean Rs concentrations (by qPCR) in gill and fin (>10^4/g) and mucus (>10^5/g) much higher than in water (≤10/mL by SPC)
    - Suggested concentration and/or proliferation of Rs in surface tissues and/or mucus

Analytical Approach

- Question: Do prevalence and levels of Rs detected in non-lethal samples reflect changes in infection severity as determined by lethal (kidney) sample analyses?
  - Approach: Devised composite reference standards based on results from multiple kidney sample assays to reflect changes in kidney Rs infection stage or severity
  - Compared results for Rs-challenged fish only

Analytical Approach

- Rationale for use of composite reference standards:
  - If a single gold standard assay does not exist, a composite reference standard combining several imperfect assays can provie a better perspective of infection status than a single imperfect assay.
    - No perfect gold standard assay exhibiting perfect diagnostic performance characteristics for Rs detection has been identified (Elliott et al 2013 J Fish Dis 36:779)

Analytical Approach

- No single "perfect" gold standard test for Rs detection in kidney samples
  - Culture detects only live Rs (sensitivity ~10^2 Rs/g) but Rs easily overgrown by contaminating organisms
  - ELISA can detect soluble Rs antigen in absence of live bacteria; antigen levels not precisely correlated with Rs numbers
  - nPCR and qPCR can detect DNA from dead bacteria (sensitivity ~10^3 Rs/g); small amount of tissue tested
  - DFAT detects morphologically intact live or dead bacteria (sensitivity ≥10^4 Rs/g); small amount of tissue tested
  - Histo/IHC not sensitive for Rs detection when distribution non-uniform (4-5 µm slice of tissue examined)

Analytical Approach

- Rationale: Use of results from more than one assay can improve evaluations of the presence and stage of infection
  - Kidney assays detect different Rs analytes, and occurrence and abundance of analytes may vary at different stages of infection
  - Because non-uniform distribution of Rs can affect detection by individual assays, use of results from multiple assays can improve accuracy of Rs evaluations
- Note: We also ran standard concordance and correlation assays between individual kidney assays and non-lethal assays, but results inconsistent (reported in Elliott et al. DAO in press)

Data Analysis

- Approach: Used number of Rs-positive kidney assays for each fish to define infection stage in that fish (mild = 1-2 assays positive; moderate = 3-4 assays positive; severe = 5-6 assays positive)
  - Approach based on relative assay sensitivity:
    - ELISA > culture > nPCR ≥ qPCR > DFAT > Histo/IHC
    - As infection severity increases in a fish, more kidney assays positive for Rs
    - If Rs detection by non-lethal test reflects changes in kidney infection stage, non-lethal detection rate should increase with increasing kidney infection severity (and approach 100% for "severe" infection stage category)

Rs Detection by Infection Stage Category

Infection stage (no. kidney assays pos)	Total fish in category	% of fish testing positive by non-lethal assay							
		Gill nPCR	Gill qPCR	Fin nPCR	Fin qPCR	Mucus nPCR	Mucus qPCR	Blood nPCR	Blood qPCR
Mild (1-2)	93	81%	67%	67%	35%	97%	90%	5%	1%
Mod (3-4)	61	91%	72%	84%	61%	100%	92%	5%	0%
Severe (5-6)	26	92%	92%	96%	81%	100%	100%	31%	15%

N=180 fish (Rs-challenged)

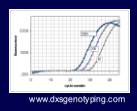
Rs Detection by Infection Stage Category

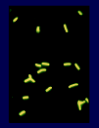
Infection stage (no. kidney assays pos)	Total fish in category	% of fish testing positive by non-lethal assay							
		Gill nPCR	Gill qPCR	Fin nPCR	Fin qPCR	Mucus nPCR	Mucus qPCR	Blood nPCR	Blood qPCR
Mild (1-2)	93	81%	67%	67%	35%	97%	90%	5%	1%
Mod (3-4)	61	91%	72%	84%	61%	100%	92%	5%	0%
Severe (5-6)	26	92%	92%	96%	81%	100%	100%	31%	15%

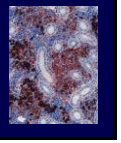
N=180 fish (Rs-challenged)

Analytical Approach

- Question: Do levels of Rs detected in non-lethal samples reflect changes in infection severity as determined by lethal (kidney) sample analyses?
  - Approach: Compared qPCR Rs quantity estimates from non-lethal samples with composite kidney infection intensity scores derived from results of quantitative and semi-quantitative kidney assays





Analytical Approach

- Intensity estimates by kidney Rs assays included:
  - ELISA: OD values (reflects amount of Rs soluble antigen)
  - Culture: Rs colony forming units/g tissue
  - qPCR: Rs/g tissue
  - DFAT: Rs cells/100 microscope fields
  - IHC: Distribution and intensity of Rs antigen staining
- Values for each assay added to produce kidney Rs infection intensity score (values for all assays except ELISA log-transformed)
  - Used Spearman's rank correlation analysis to test for correlation between kidney scores and non-lethal qPCR

Correlation Analysis

Sample time	Comparison-Kidney score and:	No. of points	Spearman r	P
3 wk	Gill qPCR	52	0.10	0.50
3 wk	Fin qPCR	26	0.46	0.02
3 wk	Mucus qPCR	50	0.56	<0.0001
12 wk	Gill qPCR	47	-0.07	0.65
12 wk	Fin qPCR	38	0.44	0.006
12 wk	Mucus qPCR	60	0.56	<0.0001
21 wk	Gill qPCR	31	0.17	0.37
21 wk	Fin qPCR	27	0.46	0.02
21 wk	Mucus qPCR	56	0.33	0.01
All	Gill qPCR	130	0.14	0.11
All	Fin qPCR	91	0.43	<0.0001
All	Mucus qPCR	166	0.36	<0.0001

Correlation Analysis

Sample time	Comparison-Kidney score and:	No. of points	Spearman r	P
3 wk	Gill qPCR	52	0.10	0.50
3 wk	Fin qPCR	26	0.46	0.02
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12 wk	Gill qPCR	47	-0.07	0.65
12 wk	Fin qPCR	38	0.44	0.006
12 wk	Mucus qPCR	60	0.56	<0.0001
21 wk	Gill qPCR	31	0.17	0.37
21 wk	Fin qPCR	27	0.46	0.02
21 wk	Mucus qPCR	56	0.33	0.01
All	Gill qPCR	130	0.14	0.11
All	Fin qPCR	91	0.43	<0.0001
All	Mucus qPCR	166	0.36	<0.0001

Correlation Analysis

Fish stock (all samples)	Comparison-Kidney score and:	No. of points	Spearman r	*P*
Wisconsin	Gill qPCR	60	0.14	0.28
Wisconsin	Fin qPCR	48	0.65	<0.0001
Wisconsin	Mucus qPCR	82	0.31	0.005
Washington	Gill qPCR	70	0.16	0.18
Washington	Fin qPCR	44	0.25	0.10
Washington	Mucus qPCR	84	0.40	0.0002

Correlation Analysis

Fish stock (all samples)	Comparison-Kidney score and:	No. of points	Spearman r	*P*
Wisconsin	Gill qPCR	60	0.14	0.28
Wisconsin	Fin qPCR	48	0.65	<0.0001
Wisconsin	Mucus qPCR	82	0.31	0.005
Washington	Gill qPCR	70	0.16	0.18
Washington	Fin qPCR	44	0.25	0.10
Washington	Mucus qPCR	84	0.40	0.0002

Summary and Conclusions

- Earlier experiments showed that gill, fin and mucus samples are suitable for non-lethal sampling of juvenile Chinook salmon as small as 3 g average weight
- Rs was detected by nPCR and qPCR in >50% of gill, fin, and mucus samples of fish up to 5 months after Rs immersion challenge
- Comparison of qPCR results from surface samples (gill, fin and mucus) with SPC testing of tank water samples suggested that concentration and/or proliferation of Rs occurred in surface samples

USGS

Summary and Conclusions

- Mucus qPCR showed best overall diagnostic performance characteristics among candidate non-lethal assays tested
  - Only assay with diagnostic sensitivity and specificity estimates >90% (and highest DOR) for distinguishing between Rs-exposed and non-exposed fish
  - Mucus qPCR Rs quantity estimates showed significant correlation ($P \leq 0.01$) with kidney Rs infection intensity scores at all sample times and in two different Chinook salmon stocks
- Mucus and other non-lethal samples should be tested for Rs detection in naturally infected fish and other salmonid species

USGS

Acknowledgements

Funding:

Great Lakes Fishery Trust

USGS science for a changing world

Technical Assistance:

Wisconsin DNR

Washington Dept. Fish and Wildlife

Western Fisheries Research Center Staff and Volunteers

Appendix 6

Aeromonas salmonicida salmonicida

Aeromonas salmonicida salmonicida infects numerous freshwater fish species. In salmonids this bacterium causes the disease furunculosis, and the bacterium can cause disease in other fish species. This bacterium is distributed worldwide, is enzootic throughout the Great Lakes basin. Clinical signs include boil-like lesions (furuncles) on the skin and in the muscle tissue, exophthalmia, bloody discharge from vent, and multifocal hemorrhages in the viscera and muscle.

Ceratomyxa shasta

Ceratomyxa shasta is a myxosporidian parasite that infects anadromous salmonids in the Pacific northwest of the United States and Canada causing the disease Ceratomyxosis. *C. shasta* initially infects the intestine but the infection generally becomes systemic over time. Ultimately, the spores displace functional tissue in the organs and the fish die. Clinical signs of disease include emaciation, lethargy, darkening of skin, ascites, and exophthalmia. The parasite requires an intermediate polychaete host (*Manayunkia speciosa*) (Willson et al. 2010) which has been reported from the Great Lakes basin (Hiltunen, 1965; Rolan, 1974; Spencer, 1976).

Epizootic Epitheliotropic Disease Virus

Epizootic epitheliotropic disease virus (EEDv) infects numerous salmonids, particularly lake trout in North America. This virus has been detected in Lake Superior and in hatcheries in California, Michigan, Pennsylvania, Wisconsin, and Wyoming. Clinical signs have only been reported in juvenile lake trout and include lethargy, riding high in the water, hemorrhages of the eye and gray-white mucoid blotches on the skin and fins.

***Heterosporis* sp.**

Heterosporis sp. is a microsporidan parasite that infects the muscle of yellow perch walleye, northern pike, ciscoe, rock bass and pumpkinseed. This parasite is known to occur in a limited number of Wisconsin, Michigan, and Minnesota lakes, the Canadian waters of lakes Ontario and Erie and the U.S. (Minnesota) waters of Lake Superior. It has not been reported from fish hatcheries. The parasite causes disease to infected host fish in the form of infected flesh has

patches of white, opaque muscle with the appearance of “freezer-burn” that is unpalatable to the public. Mortality has been induced in the laboratory but natural mortality has not been observed.

Infectious Hematopoietic Necrosis Virus

Infectious hematopoietic necrosis virus (IHNv) infects salmonids in fresh and salt water, in the wild and in hatcheries. This virus is a pathogen of international concern. IHNv is present in salmon and steelhead along the west coast of Canada and the United States. Clinical signs of disease include exophthalmia, darkening of skin, petechiae on the skin, in the mouth, pale gills, ascites, pale viscera with/without petechiae (including the swim bladder, body wall and mesenteries). The virus is most likely vertically transmitted.

Infectious Pancreatic Necrosis Virus

Infectious Pancreatic Necrosis virus (IPNv) has been isolated from wide range of fish species including salmonids, cyprinids and marine species. The pathogen has a wide geographic distribution, occurring in North and South America, Europe, Asia, and South Africa. In the Great Lakes basin IPNv has been found in Pennsylvania, Michigan and Wisconsin. Clinical signs include darkened body coloration, exophthalmia, petechiae on the skin, cessation of feeding and in the later stages show a loss of balance progressing to a corkscrew swimming motion.

Infectious Salmon Anemia Virus

Infectious salmon anemia virus (ISAv) infects primarily Atlantic salmon in wild and farmed fish in the North Atlantic waters of Canada, the United States, Norway, the Faroe Islands and the United Kingdom. This virus is a pathogen of international concern. Clinical signs of disease include anemia, ascites, petechiae in the body wall and eye. This virus is suspected to be vertically transmitted.

Koi Herpesvirus

Koi Herpesvirus (KHVv) infects carp, koi and goldfish causing the disease koi herpesvirus (KHV) in carp and koi. The virus has been found worldwide and in the Great Lakes basin in Michigan, New York State, Ontario. It is a pathogen of international concern. Clinical signs

include skin discoloration, increased respiratory frequency, skin lesions, appetite loss, erratic swimming, sunken eyes, notch on the nose, and swollen, pale, rotting gills.

Largemouth Bass Virus

Largemouth bass virus **(**LMBv) infects centrarchids east of the Rocky Mountains in the United States. In the Great Lakes basin, LMBv has been found in Lake St. Clair, western portion of Lake Erie. The virus also has been found in Illinois and Wisconsin hatcheries. Most fish with LMBv are carriers with no clinical signs. The mortality has only been found in largemouth bass. Clinical signs include difficulty swimming, bloated abdomen, loss of buoyancy regulation, hemorrhaging and discoloration of the swim bladder.

Lymphosarcoma

Lymphosarcoma is a malignancy of esocids in North America, the United Kingdom and Europe and is believed to be caused by a retrovirus (Wolf 1988). It may take up to a year for infected fish to show external signs of disease. Fish with lymphosarcoma do survive but the sores and growths associated with severe infections are unpalatable to the public.

Myxobolus cerebralis

Myxobolus cerebralis is a myxosporidean parasite of salmonids that causes whirling disease. It is found in Europe, North America, and South Africa. *M. cerebralis* has been found in Great Lakes tributary waters and in fish hatcheries in Michigan and inland waters in Pennsylvania, New York, and Michigan. *M. cerebralis* requires a tubificid oligochaete to complete its life cycle. Clinical signs include darkened tails, skeletal deformities, and “whirling” behavior in young fish.

Nucleospora salmonis

Nucleospora salmonis is an intracellular microsporidian parasite reported from salmonid species in Europe, South and North America. In the Great Lakes basin *N. salmonis* has been reported in National Fish Hatcheries in Michigan. *N. salmonis* infects blood leukocytes, hematopoietic tissues in the kidney and spleen, and tubular and glomerular epithelium in kidneys. Clinical signs include anemia and leukemia and can be associated with mortality.

***Piscirickettsia* – like organism**

A *Piscirickettsia*-like bacterium was isolated from adult muskellunge in Lake St. Clair during the 2003 spawning period and is a likely contributing factor in epizootic events. Clinical signs include quarter-sized rash-like skin lesions.

Renibacterium salmoninarum

Renibacterium salmoninarium infects salmonids, especially rainbow trout, brown trout, brook trout and coho salmon, Chinook salmon. This bacterium causes bacterial kidney disease (BKD). It occurs in virtually all areas where salmonids occur, except Australia, New Zealand and Russia. It is a serious problem in the northeast Pacific and Japan. *R. salmoninarum* is enzootic and broadly distributed within the Great Lakes basin. Clinical signs include dark coloration, exophthalmia, pale gills, ascites, skin lesions, white nodular masses in the kidney, abdominal distension or hemorrhages at the vent or base of the fins. This bacterium is vertically transmitted.

Spring Viremia of Carp Virus

Spring viremia of carp virus (SVCv) primarily affects carp and other species in the Cyprindidae family, but has also been found in a few species of other fish families such as Centrarchidae and Percidae. This virus causes the disease spring viremia of carp (SVC). It has been reported from Europe, Asia, North and South America. It is a disease of international concern. In the Great Lakes basin it has been found in healthy common carp from the Hamilton Harbor region of Lake Ontario and has been associated with die-offs in several inland waters in Illinois, Ohio, New York and Wisconsin. Clinical signs include darkened body coloration, pale gills, abdominal distension, exophthalmia, inflammation of the vent, petechial hemorrhages of skin, gills and eyes.

Tetracapsuloides bryosalmonae

Tetracapsuloides bryosalmonae is a myxosporidean parasite that infects salmonids in North America and Europe causing Proliferative Kidney Disease (PKD). The parasite infects the interstitial cells of the kidney and penetrates the lumen of the tubules. Clinical signs include

distended abdomen, enlargement of the kidney, exophthalmia and anemia. This parasite is not vertically transmitted in eggs.

Viral Hemorrhagic Septicemia (all genotypes except IVb)

The viral hemorrhagic septicemia virus (VHSv) infects wild and farmed freshwater and marine species of fish causing the disease viral hemorrhagic septicemia (VHS). There are four genotypes of VHSv: VHS genotypes I, II, and III occur in Europe, genotype IVa occurs in marine fish species in Japan and on the west coast of North America[1]. This virus is a pathogen of international concern. Clinical signs of disease include petechiae on the skin, in muscle, in and on the surface of the viscera, ascites, exophthalmia.

Viral Hemorrhagic Septicemia Virus (Genotype IVb)

Viral hemorrhagic septicemia virus genotype IVb (VHSv-IVb) infects a wide range of freshwater fish species including several species of Centrarchidae, Esocidae, Percidae, Salmonidae, Coregonidae, Cyprinidae, Sciaenidae. Some species, such as muskellunge are quite susceptible to disease and mortality however signs of disease have not been reported from other species such as emerald shiner. VHS-IVb is enzootic and has now been found in all Great Lakes. It has been detected inland in Michigan, New York, and Wisconsin and in the Ohio River basin in Ohio. It is a pathogen of international concern[2]. Clinical signs of disease include ascites, exophthalmia, enlarged spleen, and petechiae in skin, muscle, and viscera.

White Sturgeon Herpesvirus

Two strains of white sturgeon herpesvirus, WSHv-1 and WSHv-2, occur in white sturgeon in west coast of the United States. Both viruses cause moderate to high mortality in cultured fish. No specific external clinical signs of disease. Fish continue to feed until death. Internally, stomach and intestine filled with fluid, but other organs appear normal. Affected wild white sturgeon become listless and appeared to have stopped eating. Other species of sturgeon, including shovelnose and pallid sturgeon, are also susceptible to WSHv.

[1] VHS genotype IVb is present in the Great Lakes basin and therefore is listed as a restricted fish pathogen.
[2] VHS genotypes I, II, III, and IVa have not been detected in the Great Lakes basin and therefore are listed as emergency fish pathogens.

White Sturgeon Iridovirus

White sturgeon iridovirus (WSIv) is known to be pathogenic to the genus Acipenser in the Pacific northwestern United States and to both cultured and wild white sturgeon and has been detected in Russian sturgeon. The virus is also known to be mildly pathogenic to lake sturgeon.

Yersinia ruckeri

Yersinia ruckeri (serotype I and II) infects marine and freshwater fish in North America, Australia, Africa and Europe. Rainbow trout are especially susceptible. This bacterium causes the disease enteric redmouth (ERM) and is broadly distributed in the Great Lakes basin. Clinical signs include redness of the mouth, exophthalmia, pale liver, hemorrhages in the gills, skin and fins, swollen kidney and spleen. Chronic cases may demonstrate partial or total blindness, exophthalmia, distended abdomen, emaciation.

	Pathogen Type	Disease	Host Species	Classification	Clinical Signs	Transmission	OIE-reportable
Aeromonas salmonicida salmonicida	Bacterium	Furunculosis	Freshwater Fish	Level-1 Restricted	Boil-like lesions (furuncles) on the skin and in the muscle tissue, exophthalmia, bloody discharge from vent, and multifocal hemorrhages in the viscera and muscle		No
Bothriocephalus acheilognathi	Parasite	Asian Tapeworm	Cyprinids	Provisional			No
Ceratomyxa shasta	Parasite	Ceratomyxosis	Salmonids	Emergency	Emaciation, lethargy, darkening of skin, ascites, and exophthalmia		No
Epizootic Epitheliotropic Disease Virus	Virus	Epizootic Epitheliotropic Disease (EED)	Salmonids	Provisional	Lethargy, riding high in the water, hemorrhages of the eye and gray-white mucoid blotches on the skin and fins		No
***Heterosporis* sp.**	Parasite	N/A	Percids, esocids, centrarchids	Level-2 Restricted	Flesh with white/opaque muscle that is unpalatable		No
Infectious Hematopoietic Necrosis Virus	Virus	Infectious Hematopoietic Necrosis (IHN)	Salmonids	Emergency	Exophthalmia, darkening of skin, petechiae on the skin, in the mouth, pale gills, ascites, pale viscera with/without petechiae (including the swim bladder, body wall and mesenteries)	Unknown	Yes

Infectious Pancreatic Necrosis Virus	Virus	Infectious Pancreatic Necrosis (IPN)	Salmonids, cyprinids	Level-2 Restricted	Darkened body coloration, exophthalmia, petechiae on the skin, cessation of feeding, and eventuallyloss of balance amd corkscrew swimming motion		No
Infectious Salmon Anemia Virus	Virus	Infectious Salmon Anemia (ISA)	Salmonids	Emergency	Anemia, ascites, petechiae in the body wall and eye	Likely vertical	Yes
Koi Herpesvirus	Virus	Koi Herpesvirus Disease (KHV)	Cyprinids	Level-2 Restricted	Skin discoloration, increased respiratory frequency, skin lesions, appetite loss, erratic swimming, sunken eyes, notch on the nose, and swollen, pale, rotting gills		Yes
Largemouth Bass Virus	Virus	Largemouth Bass Disease (LMBV)	Centrarchids	Level-1 Restricted	Difficulty swimming, bloated abdomen, loss of buoyancy regulation, hemorrhaging and discoloration of the swim bladder		No
Lymphosarcome	Virus	N/A	Esocids	Provisional	Skin sores and lesions		No
Myxobolus cerebralis	Parasite	Salmonid Intranuclear Microsporidosis	Salmonids	Provisional	Anemia and leukemia		No
***Piscirickettsia* - like organism**	Bacterium	Musky Pox	Esocids	Provisional	Quarter-sized rash-like skin lesions		No

Renibacterium salmoninarum	Bacterium	Bacterial Kidney Disease (BKD)	Salmonids	Level-1 Restricted	Dark coloration, exophthalmia, pale gills, ascites, skin lesions, white nodular masses in the kidney, abdominal distension or hemorrhages at the vent or base of the fins	Vertical	No
Spring Viremia of Carp Virus	Virus	Spring Viremia of Carp Disease (SVCV)	Cyprinids, centrarchids, percids	Level-2 Restricted	Darkened body coloration, pale gills, abdominal distension, exophthalmia, inflammation of the vent, petechial hemorrhages of skin, gills and eyes		Yes
Tetracapsuloides bryosalmonae	Parasite	Proliferative Kidney Disease (PKD)	Salmonids	Emergency	Distended abdomen, enlargement of the kidney, exophthalmia and anemia	Horizontal	No
Viral Hemorrhagic Septicemia Virus (all genotypes except IVb)	Virus	Viral Hemorrhagic Septicemia (VHS)	Freshwater Fish	Emergency	Petechiae on the skin, in muscle, in and on the surface of the viscera, ascites, exophthalmia		Yes
Viral Hemorrhagic Septicemia Virus (IVb)	Virus	Viral Hemorrhagic Septicemia (VHS)	Freshwater Fish	Level-2 Restricted	Ascites, exophthalmia, enlarged spleen, and petechiae in skin, muscle, and viscera		Yes
White Sturgeon Herpesvirus (1-2)	Virus	White Sturgeon Herpesvirus Disease (WSH)	Acipenseridae	Emergency	Listlessness, cessation of feeding		No
White Sturgeon Iridovirus	Virus	White Sturgeon Iridovirus Disease (WSI)	Acipenseridae	Emergency	N/A		No

Yersinia ruckeri	Bacterium	Enteric Redmouth (ERM)	Freshwater Fish	Level-1 Restricted	Redness of the mouth, exophthalmia, pale liver, hemorrhages in the gills, skin and fins, swollen kidney and spleen; Chronic cases may demonstrate partial or total blindness, exophthalmia, distended abdomen, emaciation	No

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Flavobacterial Fish Diseases in Michigan: An Update

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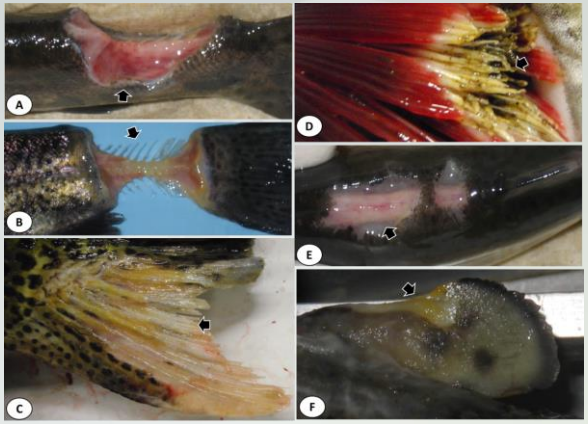

Loch TP, Faisal M. (2014) Emerging Flavobacterial Infections in Fish: A Review. *Journal of Advanced Research*. doi:10.1016/j.jare.2014.10.009

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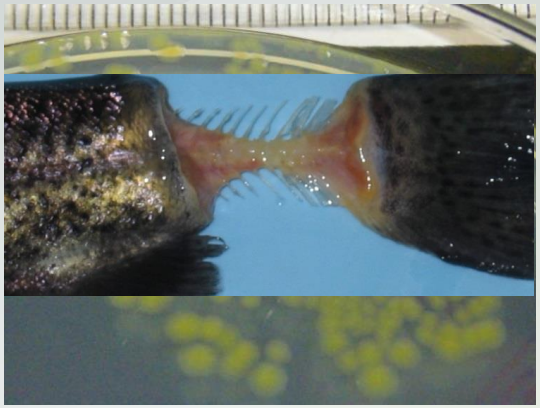
Flavobacterium psychrophilum



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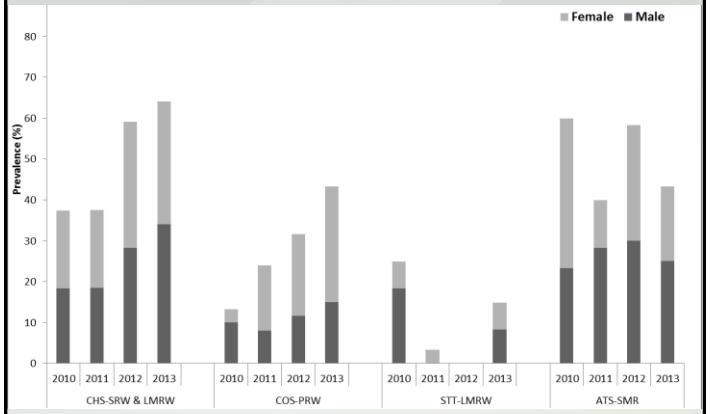
Prevalence of *F. psychrophilum* infections in *Oncorhynchus* spp. of the Great Lakes



Appendix 8

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Methods: Multilocus Sequence Typing

→7 housekeeping genes
→Highly polymorphic
→Single copy

- *atpA*
- *dnaK*
- *fumC*
- *gyrB*
- *murG*
- *trpB*
- *tuf*

Amplified → Purified → Sequenced

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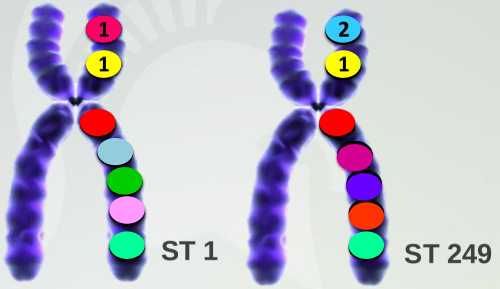
Methods: MLST Allele and Sequence Types

Allele type (AT): Specific allele observed at the loci



Sequence type (ST): Specific combination of AT

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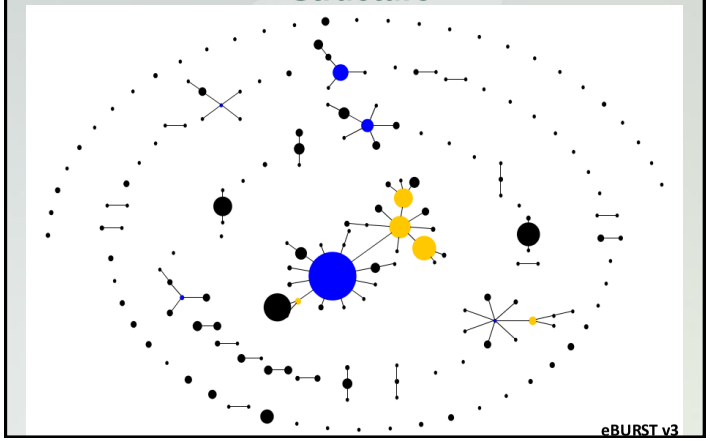
Clonal Complexes and Population Structure



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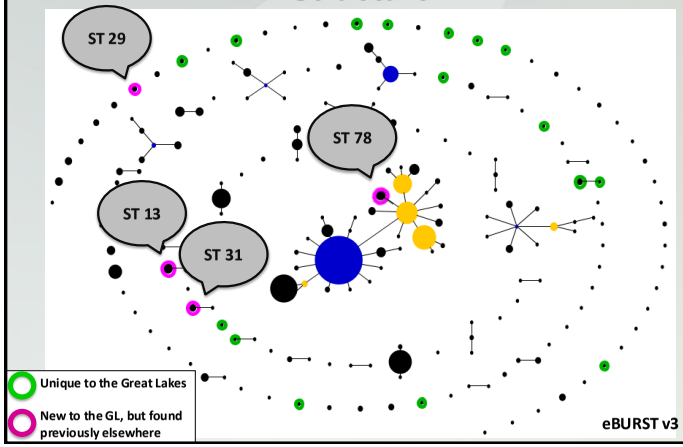
Clonal Complexes and Population Structure



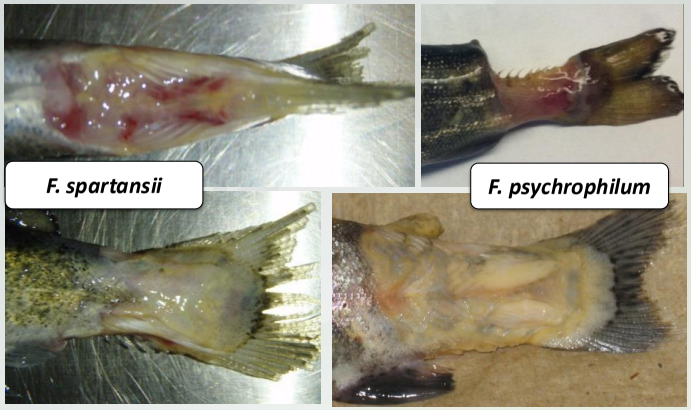
What Do the MLST Results Tell Us?

- Almost all *F. psychrophilum* isolates from disease outbreaks in MI SFHs belonged to one sequence type (ST78) that is known to be highly virulent elsewhere in the world, **but this ST was not detected in feral broodstock.**
- **Suggests there is a highly virulent Fpsy strain that is enzootic in at least 2 of our SFHs (WLSFH and TSFH).**
- **Efforts to eradicate this strain within hatcheries will likely minimize losses.**

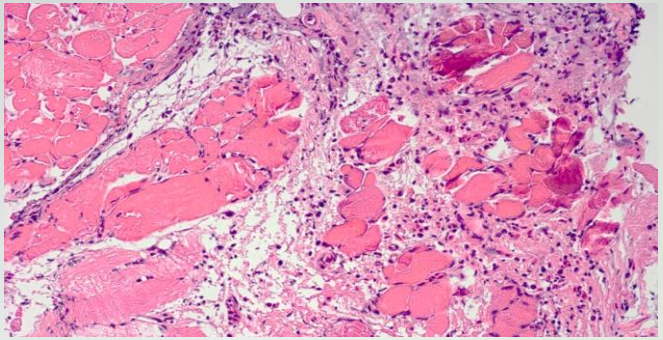
"Emergence" of Less-Typical Flavobacteria in MI

Loch TP, Faisal M. (2014) *Int Journal of Systematic and Evolutionary Microbiology*. 64, 1573-79.

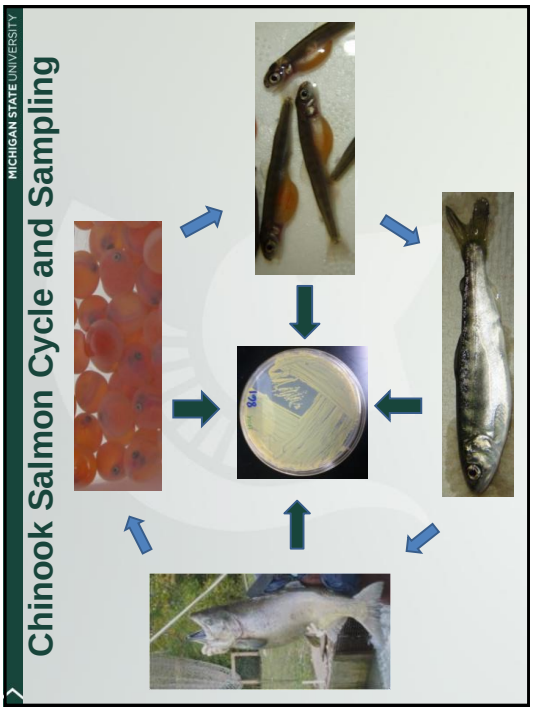
"Emergence" of Less-Typical Flavobacteria in MI



Loch TP, Faisal M. (2014). *International Journal of Systematic and Evolutionary Microbiology*. 64, 406-412.



Loch TP, Faisal M. (accepted 2015) *Journal of Fish Diseases*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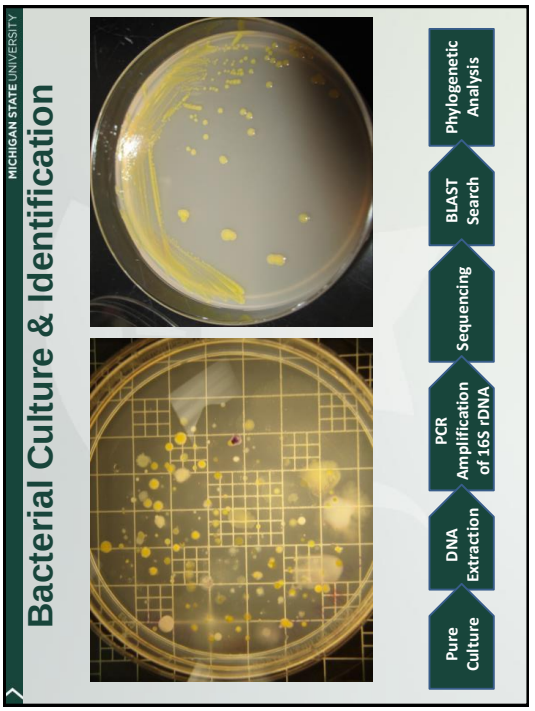
Chinook Salmon Cycle and Sampling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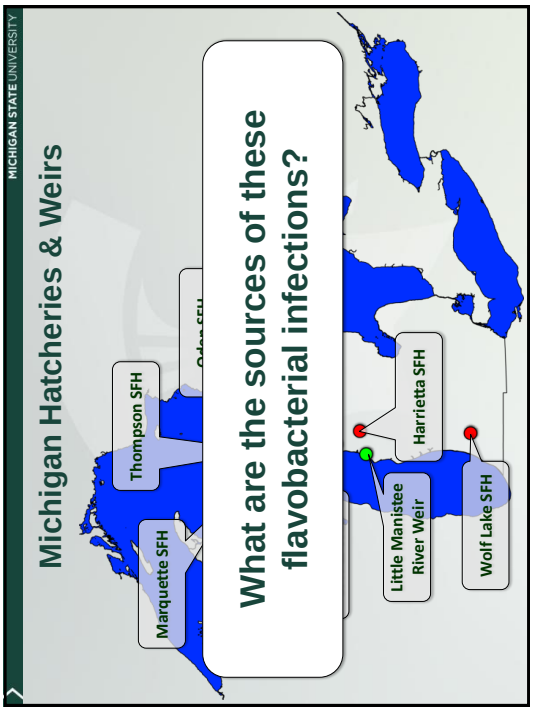
Bacterial Culture & Identification
Pure Culture
DNA Extraction
PCR Amplification of 16S rDNA
Sequencing
BLAST Search
Phylogenetic Analysis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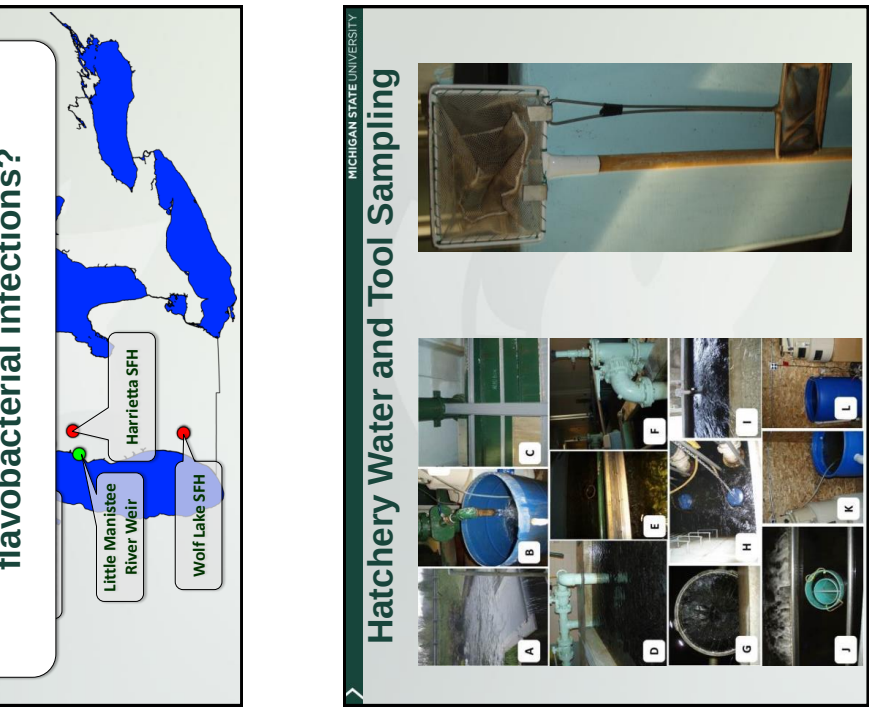
Michigan Hatcheries & Weirs
What are the sources of these flavobacterial infections?
Thompson SFH
Marquette SFH
Little Manistee River Weir
Harrietta SFH
Wolf Lake SFH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Hatchery Water and Tool Sampling
A
B
C
D
E
F
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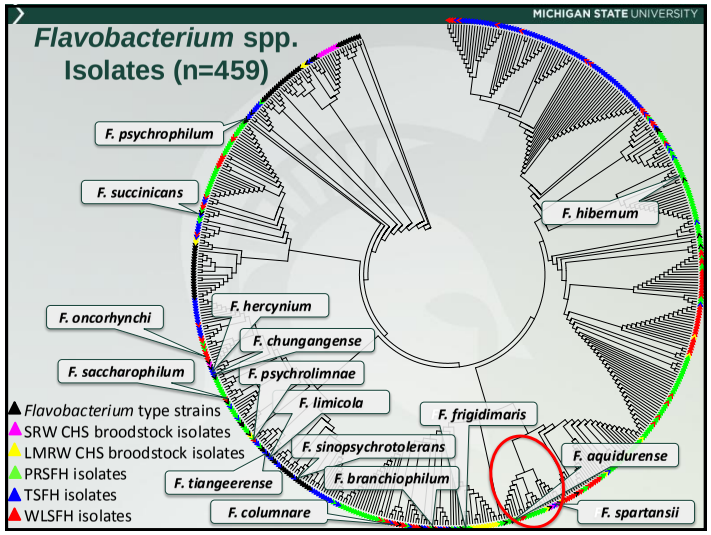
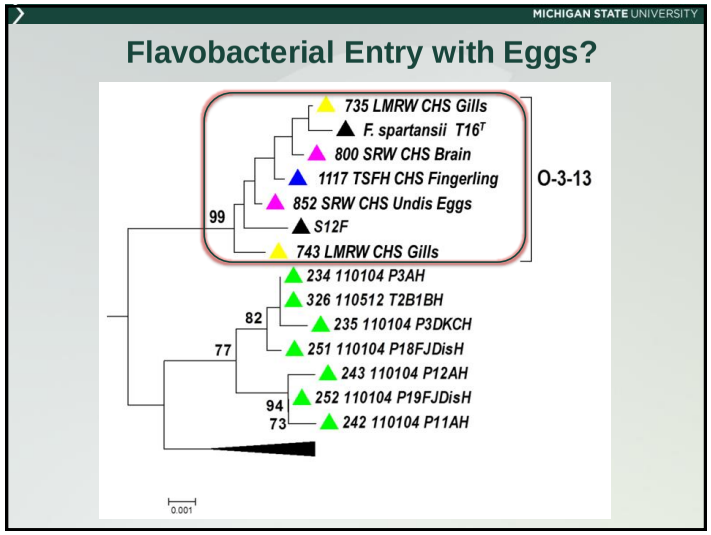
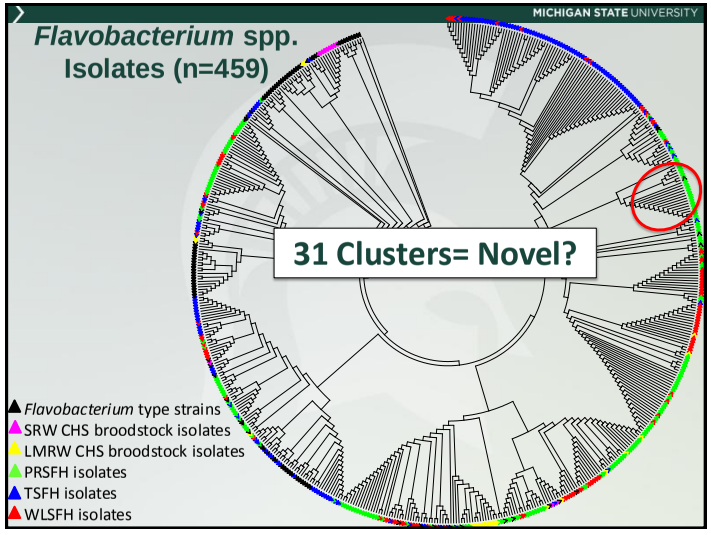
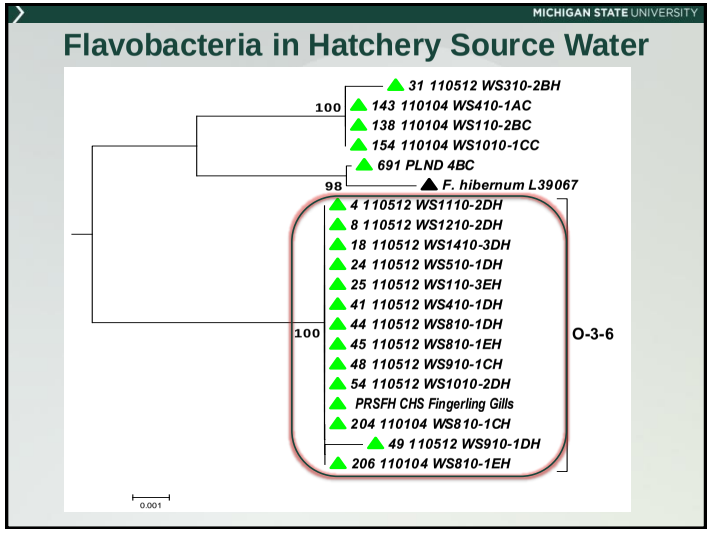
K
L

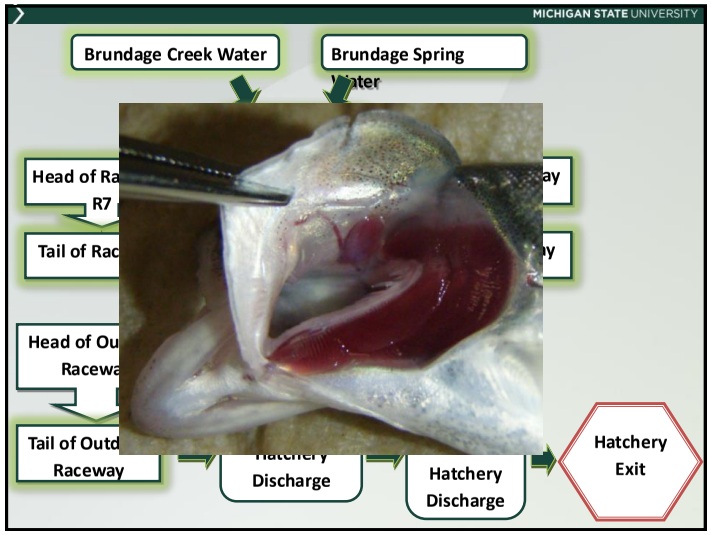

MICHIGAN STATE UNIVERSITY
Flavobacterium spp.
Isolates (n=459)
F. psychrophilum
F. succinicans
F. hibernum
F. hercynium
F. oncorhynchi
F. chungangense
F. saccharophilum
F. psychrolimnae
F. limicola
F. frigidimaris
F. sinopsychrotolerans
F. aquidurense
F. tiangeerense
F. branchiophilum
F. columnare
F. spartansii
Flavobacterium type strains
SRW CHS broodstock isolates
LMRW CHS broodstock isolates
PRSFH isolates
TSFH isolates
WLSFH isolates

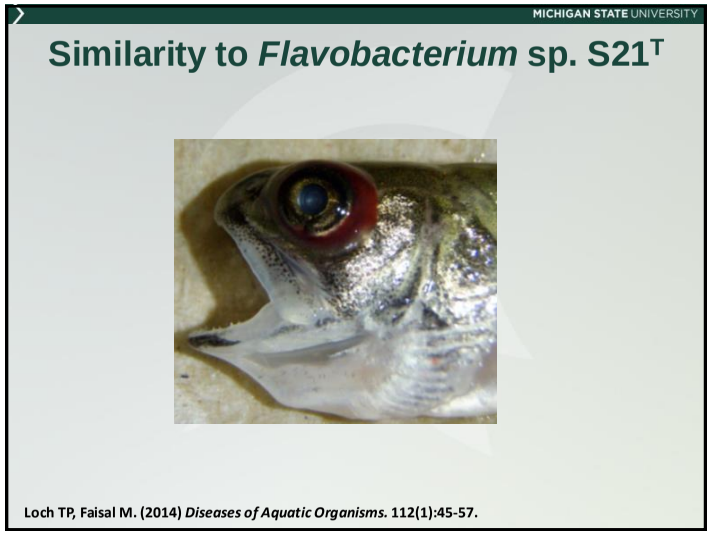

MICHIGAN STATE UNIVERSITY
Flavobacterial Entry with Eggs?
735 LMRW CHS Gills
F. spartansii T16T
800 SRW CHS Brain
1117 TSFH CHS Fingerling
852 SRW CHS Undis Eggs
S12F
743 LMRW CHS Gills
O-3-13
99
234 110104 P3AH
326 110512 T2B1BH
82
235 110104 P3DKCH
77
251 110104 P18FJDisH
243 110104 P12AH
94
252 110104 P19FJDisH
73
242 110104 P11AH
0.001

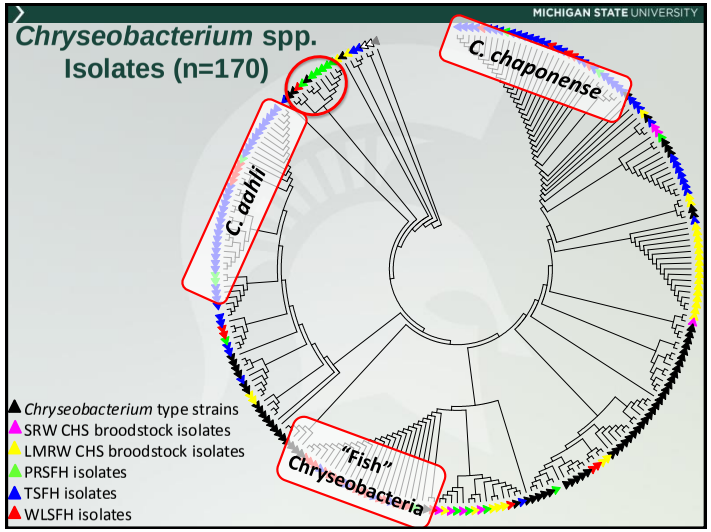

MICHIGAN STATE UNIVERSITY
Flavobacterium spp.
Isolates (n=459)
31 Clusters= Novel?
Flavobacterium type strains
SRW CHS broodstock isolates
LMRW CHS broodstock isolates
PRSFH isolates
TSFH isolates
WLSFH isolates

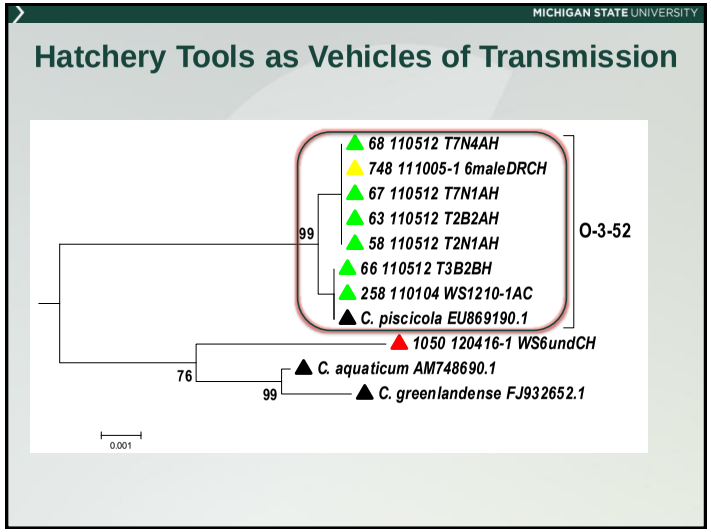

MICHIGAN STATE UNIVERSITY
Flavobacteria in Hatchery Source Water
31 110512 WS310-2BH
100
143 110104 WS410-1AC
138 110104 WS110-2BC
154 110104 WS1010-1CC
691 PLND 4BC
98
F. hibernum L39067
4 110512 WS1110-2DH
8 110512 WS1210-2DH
18 110512 WS1410-3DH
24 110512 WS510-1DH
25 110512 WS110-3EH
41 110512 WS410-1DH
100
44 110512 WS810-1DH
45 110512 WS810-1EH
48 110512 WS910-1CH
54 110512 WS1010-2DH
PRSFH CHS Fingerling Gills
204 110104 WS810-1CH
49 110512 WS910-1DH
206 110104 WS810-1EH
O-3-6
0.001

Appendix 8


MICHIGAN STATE UNIVERSITY
Brundage Creek Water
Brundage Spring
Head of Ra
R7
Tail of Rac
Head of Ou
Racewa
Tail of Outd
Raceway
Discharge
Hatchery
Discharge
Hatchery
Exit


MICHIGAN STATE UNIVERSITY
Similarity to *Flavobacterium* sp. S21^T
Loch TP, Faisal M. (2014) *Diseases of Aquatic Organisms.* 112(1):45-57.


MICHIGAN STATE UNIVERSITY
Chryseobacterium spp.
Isolates (n=170)
C. chaponense
C. aahli
"Fish"
Chryseobacteria
Chryseobacterium type strains
SRW CHS broodstock isolates
LMRW CHS broodstock isolates
PRSFH isolates
TSFH isolates
WLSFH isolates


MICHIGAN STATE UNIVERSITY
Hatchery Tools as Vehicles of Transmission
68 110512 T7N4AH
748 111005-1 6maleDRCH
67 110512 T7N1AH
63 110512 T2B2AH
58 110512 T2N1AH
66 110512 T3B2BH
258 110104 WS1210-1AC
C. piscicola EU869190.1
99
O-3-52
1050 120416-1 WS6undCH
C. aquaticum AM748690.1
76
99
C. greenlandense FJ932652.1
0.001

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Other Interesting Flavobacterial Findings

- Multiple clusters recovered from eggs/reproductive fluids both before and after iodophore disinfection.
- Multiple clusters recovered from deep well and spring water supplying hatcheries (some post-U.V. Tx).
- Multiple clusters with apparent tropism for Chinook salmon in their early-life stages.
- Multiple clusters of water-borne flavobateria without discernible pattern, suggesting possible infrastructure "hot-spots".

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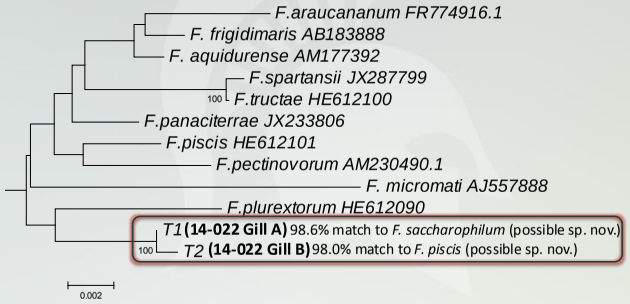
Flavobacterial Isolates from Ling



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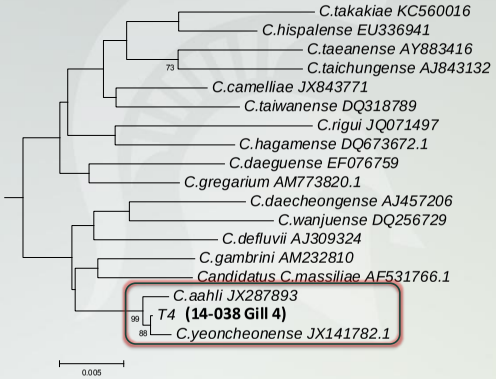
Flavobacterial Isolates from Ling



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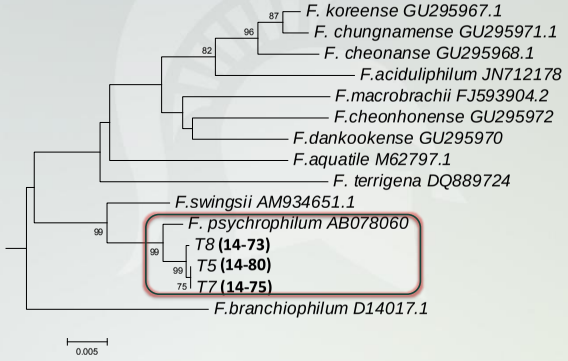
Flavobacterial Isolates from Coja



Appendix 8

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MI *Flavobacterium* spp. Isolates (n=211)

- Isolate S87- Isolated from Systemically Infected Largemouth Bass
- Biochemical, FAME, Phylogenetics = **Distinct**
- **Proposal as *F. micropteri* sp. nov. underway**

- Isolate T91- Isolated from Systemically Infected Channel Catfish
- Biochemical, FAME, Phylogenetics = **Distinct**
- **Proposal as *F. dolichovirga* sp. nov. underway**

▲ *Flavobacterium* Type Strains
▲ MI Isolates from "normal" fish
▲ MI Isolates from diseased fish

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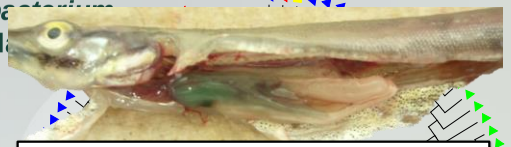
MI *Chryseobacterium* spp. Isol



- Isolate T86- Mortality Event in Muskellunge (H)
- Gill & Systemic Infection
- Biochemical, FAME, Phylogenetics = **Distinct**
- **Proposal as *C. esocidense* sp. nov. Underway**
- [illegible], FAME, Phylogenetics = **Distinct**
- **Proposal as *C. magnalucas* sp. nov. Underway**

▲ *Chryseobacterium* Type Strains
▲ MI Isolates from "normal" fish
▲ MI Isolates from diseased fish

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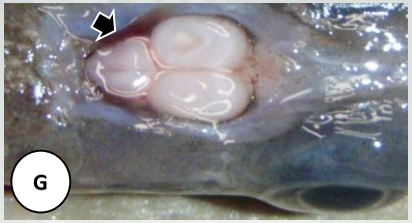
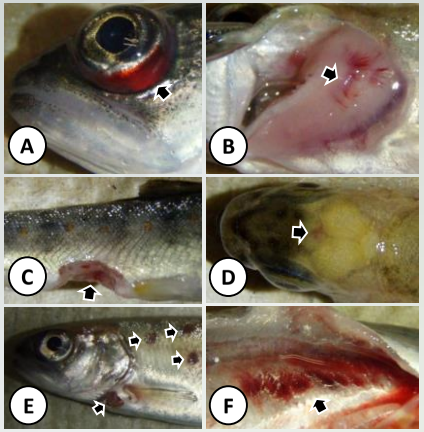
Gross Pathology in *C. esocidense* Challenged Fish



Loch TP, Faisal M. (In Press, 2015) *Diseases of Aquatic Organisms* (doi: 10.3354/dao02819)

MICHIGAN STATE UNIVERSITY

Gross Pathology in *C. magnalucas* Challenged Fish



Loch TP, Faisal M. (In Press, 2015) *Diseases of Aquatic Organisms* (doi: 10.3354/dao02819)

Appendix 8