GREAT LAKES FISH HEALTH COMMITTEE

2014 Summer Meeting
Winnipeg, Manitoba
August 6-7, 2014

Minutes
(with attachments)

Submitted By:

Christina Haska
Great Lakes Fishery Commission

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

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List of Attendees

John Dettmers	Great Lakes Fishery Commission
Christina Haska	Great Lakes Fishery Commission
Sunita Khatkar	Fisheries and Oceans Canada
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:

Jeff Long	Manitoba Fisheries Branch
Sharon Clouthier	Fisheries and Oceans Canada
Martha Wahlagamood	Michigan Department of Natural Resources

Great Lakes Fish Health Committee Meeting

August 6-7, 2014

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Draft Agenda

Wednesday, August 6

1:00 pm – 1:10 pm	Welcome & Introductions (Shen)
1:10 pm – 1:20 pm	Approval of Meeting Minutes (Shen)
1:20 pm – 1:30 pm	CLC update (Dettmers)
1:30 pm – 2:00 pm	Model Program/Risk Assessment Update (Shen/Dettmers)
2:00 pm- 2:45 pm	Lake Sturgeon nucleo-cytoplasmic large DNA virus in Manitoba (Dr. Clouthier)
2:45 pm – 3:05 pm	Manitoba Provincial Fish Health Overview (Jaff Long/Laureen Janusz)
3:05 pm – 3:20 pm	Break
3:20 pm – 4:15 pm	MI State University research updates (Dr. Faisal/Dr. Loch)
4:15 pm – 4:45 pm	Fish disease cases (Andy Noyes, Sunita Khatkar)
4:45 pm – 5:30 pm	Agency Updates— (All)

Thursday, August 7

8:30 am – 9:00 am	Research Priorities (Dettmers)
9:00 am – 10:00 am	Technical advisors (Shen and Subcommittee)
10:00 am – 10:30 am	Update on A.s. situation in Pequest, NJ (Dr. Lovy)
10:30 am – 10:45 am	Break
10:45 am – 11:05 am	Thiamine injection effect to EMS in Skamania steelhead (Dave Meuninck)
11:05 am – 11:50 am	Agency Updates— (All)
11:50 am – 12:00 pm	Future meetings (Shen)
12:00 am – 1:00 pm	Lunch
1:00 pm – 3:30 pm	Tour Freshwater Institute Aquatic Animal Health Lab

1. Welcome & introductions (Shen)

2. Approval of meeting minutes (Shen)

Minutes were approved pending changes.

3. CLC update (Dettmers)

Ling gave a presentation to the CLC in April about recommended changes to the risk assessment (see update below).

The lake committees are assisting with efforts related to increasing connectivity in watersheds (e.g., removing or modifying dams and barriers). This may affect fish health down the road. Outside the Great Lakes basin, Manitoba is removing small-head dams in agro-systems, and they are curious about lessons learned from the states (e.g., how to address pathogens, AIS, hydrology, etc). In the U.S., there's a recognition that this issues need to be incorporated upfront to make better decisions, but people are just starting to get involved. There's a lot of ecosystem benefits to having connectivity in Michigan's perspective, realizing they are on the edge of opinion.

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

Ling presented the risk assessment to the CLC, whose members had a number of questions. As a result, the writing subcommittee had a conference call with three members of the CLC to address their concerns. See Appendix 1 for more information. The new risk assessment was sent to the committee for final approval.

One potential change was to increase the degree of risk if a fish is infected with more than one pathogen. There were multiple ways to address this, including doing a risk assessment for each pathogen and using the one with highest risk. Instead, it was decided to take a precautionary approach and increase the risk to the next level if more than one pathogen is present.

This risk assessment is a living document and may be changed in the future after agencies use it. The committee reached consensus that it is ready to go online. Ken will check on the progress of automating the forms.

5. Lake Sturgeon nucleo-cytoplasmic large DNA virus in Manitoba (Clouthier)

See Appendix 2 for the presentation.

Discussion points:

- It is expected that some eggs have the virus on the surface, and most sturgeon management hatchery plans disinfect the eggs' surfaces to minimize risk of transmission. It is unknown if iodophore affects the virus.

- The virus is doubly encapsulated. Hendrick looked at cross species and there were some evidence the virus was retained, but there was no evidence of transmission between other salmonids.
- Without a massive die-off, it is hard to determine what the virus is doing. There are seasonal variations in virus load, and it is most likely endemic in Manitoba. Infections can be acute or chronic, and these events can be reduced by decreasing the viral load in the water. The fish's barbells can be affected, which prevent the fish from finding food, causing starvation and death.
- The expectation is the virus is widespread across the provinces. Future testing will determine that, but unfortunately only her lab is surveying the virus. Various stocking programs and sturgeon projects have helped with data collection.
    - USFWS in WI has a stocking program and has some histology information. They could collaborate on sample gathering and transfer.
- It is recommended that testing for the virus be required when moving fish between states. The outbreaks occur in the hatcheries.
- Sometimes coinciding with this virus are sturgeon herpesvirus and iridoviruses.

6. Manitoba provincial fish health overview (Long/Janusz)

See Appendix 3 for the presentation.

Discussion points:

- There is limited interaction with other provinces, more so with AIS managers than hatcheries, but there are no clear connections with other fish health people. Manitoba is at the bottom of a huge drainage basin, so things show up that are in other provinces, and the province is most similar to Saskatchewan.
- Alberta, Manitoba, and Saskatchewan do not have a very good fish health program. CFIA is coming into the picture, and it does not want DFO testing anything other than what is sent to them. The focus is on a limited number of pathogens and agriculture. As a result, Manitoba cannot go to them for guidance. With the ambiguous mandate for Manitoba, they end up doing things under the radar.
    - In the U.S., the GLFHC exists, and the Northeastern Fish Health Committee is following its footsteps. The committee recommendation is to network.
- The general consensus was the province is doing what it can. It's hard to justify testing for pathogens that have never shown up in the province BUT collecting that data is not a waste of time and provides a good data set. Never underestimate the power of private aquaculture being able to affect your work. There's always alternative vectors and routes for pathogens that are not being monitored. Improving biosecurity was recommended.
- There's a debate about whether or not disinfecting for VHS helps or hinders fish. The USFWS has found disinfecting walleye eggs for VHS can help improve overall health because it gets rid of other pathogens. In Manitoba, there are competing opinions over this, but the two experts have very different water supplies.

7. Michigan State University research update (Faisal/Loch)

Mohamed updated the committee about work being done in his lab at MSU.

8. Fish disease cases (Noyes/Khatkar)

See Appendix 4 for Andy's presentation.

Discussion points:

- The water supply was 48°F year-round. Water hardens around 1400 and is supplied by two springs.
- Mortality at its worst was 1% for about 5 days pre-treatment. There was a problem with medicated feed arrival, which extended mortality. After psycrophalum jumped in, there was some bacterial coldwater disease signs.

See Appendix 5 for Sunita's presentation.

Discussion points:

- CFIA says they have to grow the fungus to meet the demands of their and OIE protocols, but OIE wants issues reported but not investigated. They only care about certain pathogens.

9. Agency updates (All)

New York State DEC: In 2012, the Rome hatchery had furunculosis, and the hatchery lost about 800k fish. The hatchery classification was downgraded, and a two-year abatement plan included disinfection and depopulation of susceptible lots. The last inspection takes place next month. The hatchery does not plan to get fish outside of the state of NY again. CLT was approved for warmwater fish, walleye, and salmonids. Andy is working with Jim Balker to address targeted studies to move along the approval for esocids. There are promising data, but tests need to be repeated.

Pennsylvania FBC: CTv was not found; however, three hatcheries had large die-offs of Lake Trout finglerlings due to IPN. A vaccination program began for furunculosis, and they don't see larger die-offs like previously. There were no problems with BKD this year except for one hatchery that always has whirling disease. Eden City hatchery was Class A, but new broodstock were brought in for production of northern pike and furunculosis was found. The progegny were negative. The hatchery is changing policies so broodfish are not in same facility as production fish. Finally, in 2010 a picture was posted on Facebook of what looked to be a Bighead Carp at a pay lake in southwestern PA. In May 2014, another was posted. The lake was surveyed, and a diploid Grass Carp was found as well as some endangered species. The lake's owner received fish from a Lake Erie commercial fisherman and didn't know if the

fish were tested prior to stocking. The FBC sampled bluegill, carp, and largemouth bass for VHS and all were negative. Now, the lake owner has to seine out his lake (less than ½ acre in size) and keep the fish he wants, rotenone the lake, and then re-stock. Pennsylvania was not prepared for this type of situation.

Discussion: Should there be a new list to reference since APHIS has rescinded theirs? Now it's up to each state to create their own list, not the GLFHC. New York assumes all fish are susceptible to VHS, which takes care of testing requirements. CFIA has list of susceptible species on its website for Canadians to reference.

Action Item: Create fact sheets for major pathogens and post them on the GLFHC website. Refer to the Blue Book to reduce confusion. Pathogen descriptions should be written for the Model Program pathogens that are not included.

10. Research Priorities (Dettmers)

See Appendix 6 for the revised Research Priorities document.

11. Technical Advisors (Shen and Subcommittee)

A list of potential technical advisors was created and approved by the committee (Appendix 7). Ling will contact the scientists to see if they would be interested in serving the committee. This list will be revisited on a regular basis and may be revised in the future.

12. Continuation of VHS list of susceptible species

Minnesota changed its VHS rules:

1. Rearing ponds are being tested every two years instead of every year.
2. Fish are undergoing PCR testing.
3. The state is being divided into two districts: the Lake Superior watershed (regulations are staying the same) and rest of state (where VHS has not been found).
4. Sampling size has increased to 60 fish (from 30 right now).

The state is continuing active surveillance and analysis of fish kills.

Conversation regarding a potential place to house the original APHIS order

OIE still has a version of the list, and that could be referenced. The USFWS does not have regulatory authority and would not want to house the list, as they refer to state regulations. The Service is revising Title 50, which deals with the importation of salmonids. Pathogens make the fish potentially injurious.

The AFS Fish Health section also does not have regulatory authority, and its Blue Book only makes recommendations for families of fish, not specific species.

For now, it would be best to check with APHIS and see if they plan to update the list when a new species is found to be susceptible and what that process would be. Will they upkeep it or get rid of it eventually? Action Item: Ling will contact the agency and ask.

13. Thiamine injection effect to EMS in Skamania steelhead (Meuninck)

See Appendix 8 for the presentation.

Discussion points:

- The process of injecting 100 wild broodstock fish takes about 45 min.
- The fish also get an OTC injection to prevent furunculosis. The thiamine injection goes in the dorsal sinus.
- Inject every year? Just coming off the truck. Spawning females are sacrificed. Fresh broodstock is captured every year.
- There are tradeoffs between treating the fish or doing flow-through treatment of the eggs, such as expense costs and survival of the fish, likely.
- Fish get injected when coming into the facility and are held for a few months prior to spawning.

14. Update on furunculosis outbreak in Pequest, NJ (Lovy)

Discussion points:

- Andy offered to send Rome strain brook trout that are resistant to furunculosis. It would need to be worked out among the management folks.
    - A spread test of Rome-strain fish with A. sal showed the infection is gone after 3 weeks.
    - Coja has experience with the Rome strain, and they are resistant in PA.
- Jan did cultures of the fish that were not responding to treatment. Likely the outbreaks were occurring because some fish ate the medicated feed and other did not.

14. Agency updates, cont. (All)

U.S. Fish and Wildlife Service: Smallmouth Bass were infected with *Yersinia ruckerii* at Genoa this spring. The Iron River hatchery will be Class A if the inspection this month is clean. Eric Griese has made some Asian carp cell lines in conjunction with the USGS lab to screen potential chemical and viral controls. Construction should start soon at the Jordan River hatchery to

cover raceways and update the early-life stage rearing building. There will be room for coregonid culture as well – not sure what species yet.

Indiana DNR: There were no BKD detections in any coldwater units, but hatcheries still get occasional *A. sal* positive detections without any clinical signs of disease, which then disappear in the following inspection. EMS was found in Coho in the winter runs from Michigan this year. Treatment did not seem to help initially but the fish did recover. A local Department of Corrections has a walleye culture facility which grew Channel Catfish for children's fishing events. It began culturing Walleye but ran into problems and lost all of them. Analysis is being done at Purdue now. There was a large die-off of Gizzard Shad this winter in northern Indiana. There was concern about whether or not Muskie spawning would happen on time, but the ice melted and they had a good egg take. In southern Indiana, there is evidence that Gizzard Shad are causing reproductive failure in Largemouth Bass. A comparison study between a lake with and without Shad showed different thiamine levels in Bass between the reservoirs. Eagles around the area also eat Shad and are sometimes found to be lethargic. After receiving thiamine injections for a vet, their energy returns.

Fisheries and Oceans Canada: All hatcheries in Manitoba and Ontario are clean according to CFIA testing methods. A new process requires accreditation for labs, which is being pursued now. On the research side, SVCv is being looked for and an assay is being developed. CFIA is taking over domestic issues so DFO has to rescind their orders. There is concern this will affect the states – if anyone imports from Canada and has issues, let Sunita know. It's unclear what the issues will be as this transition occurs. It might be helpful to invite CFIA to the winter meeting and give an update. They could not be a formal member but could be invited regularly to ensure open communication. Action Item: Sunita will find a contact person. Might be helpful to find someone at APHIS to invite regularly as well.

Michigan DNR: There were limited mortalities in the wild this year except in shallow lakes. Oden hatchery had an IPN outbreak in broodstock and caused mortality in brown trout. It was initially detected in production fish but it disappeared, which is raising some concerns. The hatchery is taking an aggressive management approach: biosecurity, testing, culling out positives, and reducing stressors on fish to try to reduce the load. It's unclear why this is an issue this year. There was a trace of IPN in Marquette, which has the pathogen in the supply water, but it disappeared. A UV system needs to be installed for production fish (already have one for broodstock). Private aquaculture wants to be a bigger industry in Michigan. The strategic plan is vague and unspecific but wants an expansion from $5-10 M/year to a billion dollar agency by 2025. The plan is to culture 80% of fish in cages in the Great Lakes. This has created lots of work for the state with permitting and disease control. The DNR is unenthusiastic, as are constituent/commercial/angling groups.

Minnesota DNR: A few fish kills involving Crappies and other sunfish were due to *Columnaris*. The Lanesboro hatchery had Rainbow Trout yearling mortalities from *Aeromonas hydrophila*. The fish were treated with Oxytech medicated feed twice before moving them to an outdoor pond where they would have more space. No more mortalities were observed. A few weeks ago, Crystal Springs hatchery sent pictures of Lake Trout with clinical signs of disease. The fish are kept in covered raceways with panels for daylight to come through. Hatchery personnel do not think it's sunburn, but it may be frostbite. The issues seems to be strictly in the dermis and does not penetrate the body cavity. Histology has not been done yet.

15. Future meetings (Shen)

The Winter 2015 meeting will be in West Lafayette, IN from February 3-4, 2015.

The Summer 2015 meeting will be in Syracuse, NY from July 28-29, 2015 (including an Oneida Lake field trip).

The Winter 2016 will be in Lansing, MI.

16. Tour of the Freshwater Institute Aquatic Animal Health Lab

17. Adjourn

GLFHC Writing Subcommittee Responses to CLC Concerns Regarding the Risk Assessment
May 2014

Questions from S. LaPan's email (5/12/2014)

Form RA-2, question 5: Given that this criterion addresses pathogens not currently in the Great Lakes, and a necessary score above 733 is required to recommend against stocking, is the maximum, weighted value of this criterion (100) adequate?

- The value for Absence was increased to 30 points.

Form RA-2, questions 5, 6 and 7: Recognizing our ignorance of fish pathology, we know there are distinctions between these criteria, but would greatly benefit from a discussion (conference call) regarding their significance/weighting. Further to the previous question, the introduction of a new pathogen into the Great Lakes would receive a combined score of only 450 for criteria 4 through 8 and 12. Would we not need to recommend against stocking based on these 6 criteria alone?

- The scoring was changed for questions 5, 6, 7, and 12. The writing subcommittee is considering changing the wording of #12 as well. This can be discussed during the call.

Form RA-2, questions 6 and 7: Suggest revised wording: "Will introduction of these fish likely increase…."

- Done.

Form RA-2, question 10. Several of the criteria are auto-correlated and therefore appear unnecessary. For instance, if the "wild" receiving water is being considered for stocking, we can assume that there is fish stocking and likely recreational fishing in the receiving water. Also, are there any wild receiving waters that don't have the potential for predators?

- This question could potentially be eliminated.

Form RA-2, question 17. Under "Comprehensive/Continual…."/ "No other Model Program pathogen(s) detected," why would these selections generate a score other than "0"?

- In general, the value of '1' was used to show there is still a degree of risk. Several GLFHC committee members felt this distinction was important and disapproved initially of having zero values; however, the writing subcommittee decided to follow your suggestion and made this change.

Form RA-3. Once this form is signed by the GLFHC, who receives copies of the form?

- This is mentioned in the introduction of the document: "The summary report will be provided to all member agencies, the appropriate lake committee(s), and the CLC."

Comments raised during the CLC meeting

If a fish is infected with a pathogen, does that make it more susceptible to other pathogens?

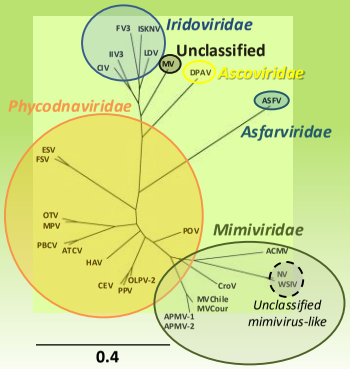
- This is very likely and will depend on the number of other active infections along with the virulence of the pathogens involved.

How does the risk assessment take into account if a fish is infected with more than one pathogen?

- The RA was not intended to take into account more than one pathogen because most cases are focused on single pathogens. Multiple pathogen cases would likely not get to this decision stage. If the CLC thinks it would be beneficial to have an additional risk assessment that would analyze multiple pathogens, this could be developed in the future.

NUCLEO-CYTOPLASMIC LARGE DNA VIRUS IN MANITOBA LAKE STURGEON

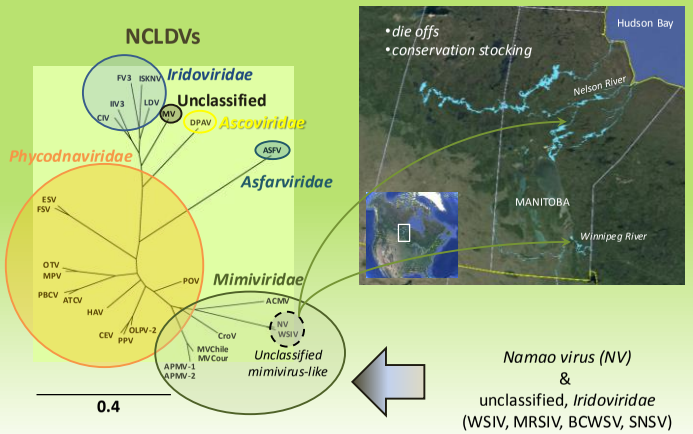
Sharon Clouthier*, DFO Freshwater Institute



Overview of presentation

- Virus discovery
- Current investigations
- Birthday Rapids case
- In vivo challenge study
- Diagnostic test development
- Disease management strategies

Namao virus (NV)



Namao virus (NV)
&
unclassified, *Iridoviridae*
(WSIV, MRSIV, BCWSV, SNSV)

Lake sturgeon (Acipenser fulvescens) in Manitoba, Canada

5 populations considered for SARA listing

- W. Hudson Bay – DU1
- Saskatchewan R – DU2
- Nelson R – DU3
- Red & Assiniboine R to Lk Winnipeg – DU4
- Winnipeg R to English R – DU5

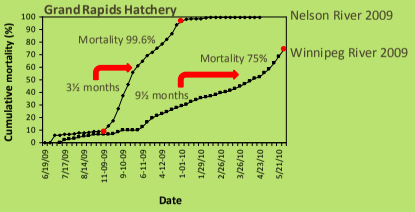
Conservation stocking, 1992-2014

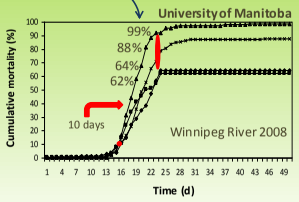
- Stakeholders:
  - First Nations
  - Provincial government
  - Manitoba Hydro
  - University of Manitoba
  - Federal government

Process

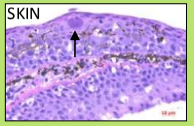
River	•Gametes collected from wild broodstock streamside
Hatchery	• Fertilization of eggs • Larval rearing
River	• Release of juveniles in summer and fall of hatch year • Release of juveniles in spring of following year

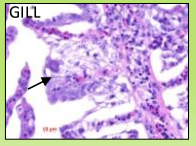
Virus-associated outbreaks in lake sturgeon

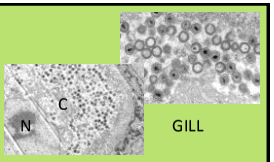




Namao virus diagnostic tests
histology, electron microscopy, PCR





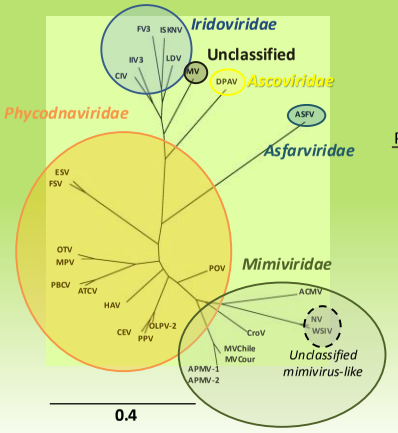


Molecular test method[a]	Diagnostic results			
	2008 YC	2009 YC		
	UMAAHF	Grand Rapids Hatchery		UMAAHF
	Winnipeg River	Nelson River	Winnipeg River	Winnipeg River
NV cPCR	7/8	3/3	35/37	14/15
WSIV qPCR	0/8	0/3	0/35	0/12
MRSIV qPCR	0/8	0/3	0/35	0/12

Diagnostic testing

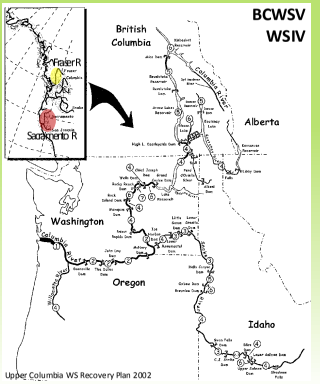
- Histology
  - epitheliotrophic (oral, nasal, barbels, skin, gill, fin)
  - pathognomonic changes
    - enlarged epithelial cells
    - cytoplasmic inclusion bodies
- Electron microscopy
  - icosahedral-shaped
  - 242 nm diameter
- Conventional PCR
  - Major Capsid Protein
  - 219 bp amplicon

NV taxonomy



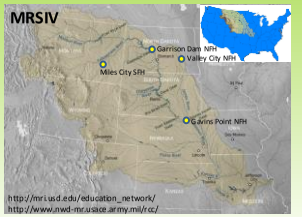
Phylogenetic analysis

- Major capsid protein
- 5 sNCLDVs & 25 other NCLDVs
- Common evolutionary past
- *Mimiviridae*
- sNCLDVs sub-species level



Sturgeon NCLDVs – geographic range

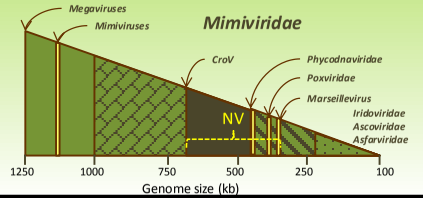
Current name	Geographic range	River drainage	First report
WSIV	California	Sacramento River	1990
	Oregon	Columbia River	1994
	Idaho – North	Kootenai River	
	Idaho - South	Snake River	
BCWSV	British Columbia	Fraser River	2003
MRSIV	North & South Dakota	Missouri River	2010
SNSV	New Brunswick	St John River	2014
NV	Manitoba	Nelson River	2013
		Winnipeg River	



Sturgeon NCLDVs – Biological characteristics

Characteristic	Sturgeon NCLDV			
	NV	WSIV	MRSIV	BCWSV
Host	lake sturgeon	white sturgeon	pallid & shovelnose	white sturgeon
Shape	hexagonal			
Size (side to side, nm)	242	262	254	261
Genome	dsDNA			
Tissue tropism	Integument & gill			
Cell tropism	epithelial cells (Malpighian & mucosal cells)			
Cell culture	no	Sometimes	no	N/A
Cell types	none identified[1]	white sturgeon spleen, gonad[1]	none identified[1]	N/A
Temperature (°C)	15	15[2]	15	N/A

1. LS gill or gonad primary cell lines & 2 WS cell lines; 8 WS cell lines established; PS/SS primary cell lines
2. Virus replication occurs at 10, 15 and 20°C but not at 5 or 25°C; optimum is 15°C



Overview of presentation

Virus discovery

Current investigations

Birthday Rapids case

In vivo challenge study

Diagnostic test development

Disease management strategies

Screening lake sturgeon broodstock, gametes & progeny for NV

Winnipeg River drainage conservation stocking program

Sample collection sites

NV diagnostic results: histology, qPCR

	Winnipeg River Drainage													
	Year and Origin of Broodstock													
	Sturgeon Falls		Slave Falls		Pointe du Bois		Pointe du Bois		Pointe du Bois		Pointe du Bois		Pointe du Bois	
	2005 to 2007		2008		2009		2010		2011		2012		2013	
Sample source	PCR	Histo	PCR	Histo	PCR	Histo	PCR	Histo	PCR	Histo	PCR	Histo	PCR	Histo
Male brookstock fin clip							0/5	0/5	0/3	0/3	0/9	0/9	1/7	0/7
Female broodstock fin clip							0/6	0/6	0/3	0/3	0/9	0/9	0/5	0/5
Milt									0/5		0/4		0/12	
Eggs									0/6		0/8		0/5	
Larvae							0/24	0/24	0/40	0/40	0/60	0/10	0/30	0/30
Juveniles fin clip	3/9		130/130		32/32		5/5	0/5			0/10			
Total number of samples	3/9		130/130		32/32		5/40	0/40	0/57	0/46	0/100	0/28	1/59	0/42

Screening lake sturgeon broodstock, gametes & progeny for NV

Nelson River drainage conservation stocking program

Sample collection sites

NV diagnostic results: histology, qPCR

	Nelson River Drainage													
	Year and Origin of Broodstock													
	Landing River near the Nelson River										Burntwood River		Birthday Rapids	
	2010		2011		2012		2013		2014		2013		2014	
Sample source	PCR	Histo	PCR	Histo	PCR	Histo	PCR	Histo	PCR	Histo	PCR	Histo	PCR	Histo
Male brookstock			0/2	0/2	0/2	0/2					0/2	0/2	1/2	
Female broodstock			0/2	0/2	2/2	0/2					1/2	0/2	1/1	
Milt					0/2		0/1				0/2		0/2	
Eggs					0/2		0/2				0/2		0/1	
Larvae			0/20	0/5					0/60				1/59	
Juveniles	0/94	0/79	0/60	0/3	0/60		0/120				0/60			
Total number of samples	0/94	0/79	0/84	0/12	2/68	0/4	0/123		0/60		1/68	0/4	3/62	

Overview of presentation

Virus discovery

Current investigations

Birthday Rapids case

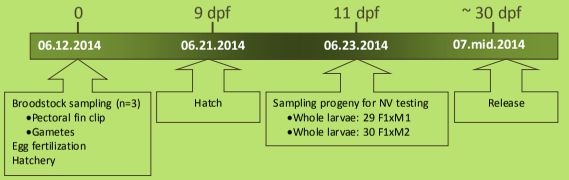
In vivo challenge study

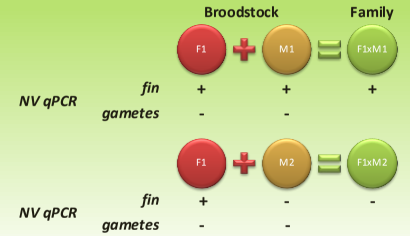
Diagnostic test development

Disease management strategies

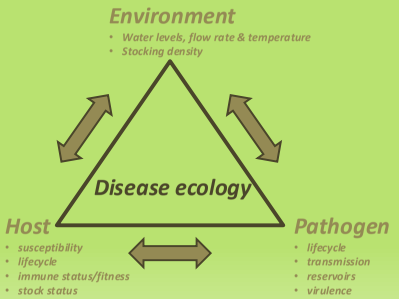
A case study – 2014 Birthday Rapids lake sturgeon

Nelson River conservation stocking program





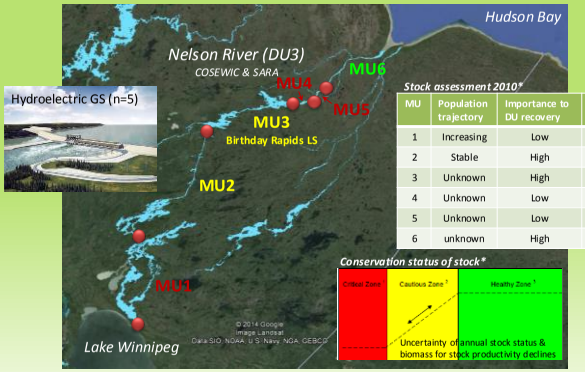
Factors to consider for disease management or control



Drivers of disease emergence

Risk Factors	2014YC Birthday Rapids
Increasing host populations	unknown
Changes in land/water use	√
Fish migration/movement	√
Rapid, large scale environmental changes	√
Rapid, long distance transport	√
Trade in wild animals and meat	X
Pathogen evolution	unknown

Host & Environment – Birthday Rapids lake sturgeon population



*From Cleator, H., K.A. Martin, T.C. Pratt and D. Macdonald. **2010**. Information relevant to a **recovery potential assessment** of Lake Sturgeon: Nelson River populations (DU3). **DFO** Can. Sci. Advis. Sec. Res, Doc. 2010/082. vi + 33 p.

Appendix 2

Pathogen - transmission

VIRUS TRANSMISSION PATTERNS

Virus → Fish density, Genetic susceptibility of host, Virus load, Stress → Onset & severity of disease

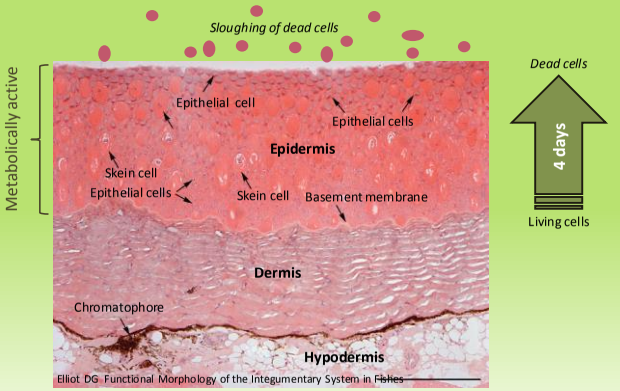
Transmission | Determinants | Disease

DISEASE MANAGEMENT STRATEGIES

Routes of transmission

- Horizontal – infected to naïve individuals
  - Direct contact with infected individuals
  - Exposure to water containing virus
- Vertical – adult to offspring via the egg
  - Egg surface
  - Intra-ovum

Pathogen - epitheliotropism & horizontal transmission of NV



Elliot DG. Functional Morphology of the Integumentary System in Fishes

Epithelial cell = malpighian cell, epidermal cell, filament-containing cell, filamentous cell, polygonal cell, polyhedral cell, keratocyte, keratinocyte, principal cell, and common cell.

Pathogen - Vertical transmission of NV?

Pointe du Bois

Winnipeg River	2010	2011	2012	2013
Broodstock	-	-	-	+
Gametes	n/a	-	-	-
Larvae/fry/yearlings	+	-	-	-

No evidence

Nelson River	Landing River 2011	Landing River 2012	Landing River 2013	Burntwood River 2013	Birthday Rapids 2014
Broodstock	-	+	n/a	+	+
Gametes	n/a	-	-	-	-
Larvae/fry/yearlings	-	-	-	-	+

Vertical or horizontal from unknown source

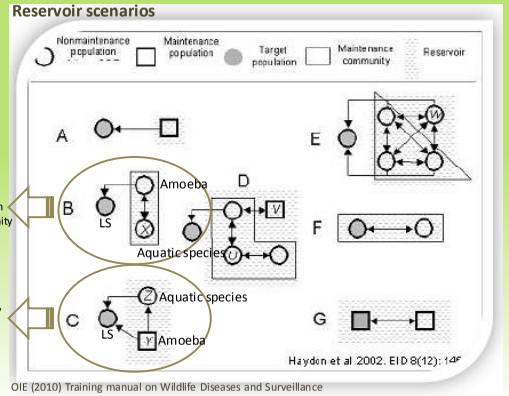
Pathogen - reservoirs

Reservoir = one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target population

NV reservoir - unknown

- 2 host pop
- Maintain pathogen by transmission
- Reservoir is maintenance community of two different species
- Transmission to target occurring through only one population

- 2 host pop
- Maintenance in one host pop only
- Transmission to target occurring through either population



OIE (2010) Training manual on Wildlife Diseases and Surveillance

Overview of presentation

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In vivo challenge study

Objectives
- Establish causal relationship between NV and disease
- Establish if horizontal virus transmission occurs
- Establish tissue tropism, virus load and infection dynamics

Control tanks

T3 Imm/naive n = 63/62 — Weekly sampling

T6 Imm/naive n = 63/62 — No sampling

Control tanks
- Mock immersion fish (n=63)
- Untreated fish (n=62)
- 1 tank: weekly sampling; diagnostic testing
- 1 tank: no sampling; cumulative percent mortality

Environmental conditions
- 70 L flow through aquaria
- Water temperature 15°C
- Flow rate 0.4 L/min
- Stocking density 15.5 g/L

Virus challenge tanks

T2 Imm/naive 63/62

T5 Imm/naive 63/62

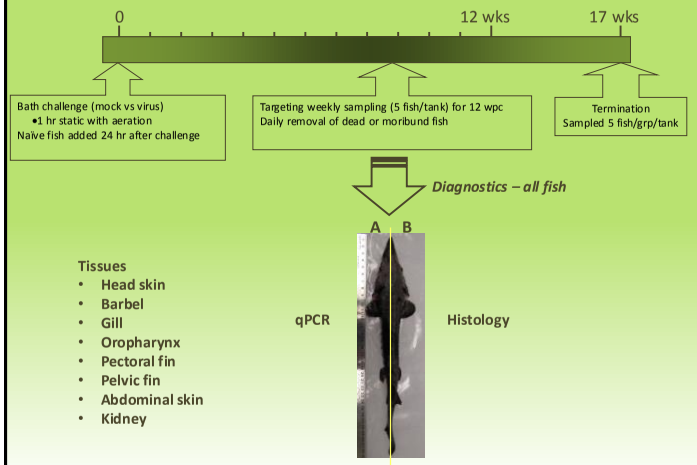
T1 Immersion 126 — Weekly sampling

T4 Immersion 126 — No sampling

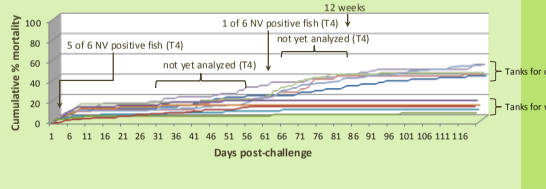
Virus exposed tanks
- 100% immersion challenge (n=126)
- Cohabitation (virus immersion challenged fish (n=63) & naïve fish (n=62))
- 1 set of tanks: weekly sampling; diagnostic testing
- 1 set of tanks: no sampling; cumulative percent mortality

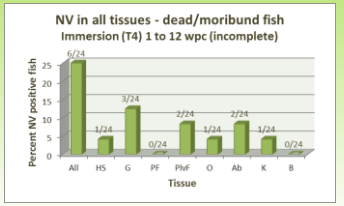
Virus challenge
- Source of virus: homogenate of whole fish from disease outbreak
- Challenge dose $3.2x10^9$ or 9.5 log10 plasmid copies per L water
- 1 hour static bath with aeration in 20 L suspension of homogenate

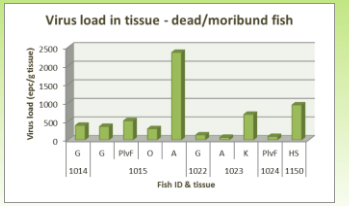
In vivo challenge study - Timeline



In vivo challenge study - preliminary NV qPCR results







Overview of presentation

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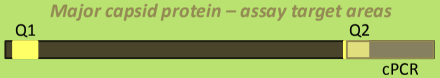
Diagnostic test development

Disease management strategies

Diagnostic assays - Virus isolation test method

Diagnostic assays - Molecular test development
sturgeon NCLDV

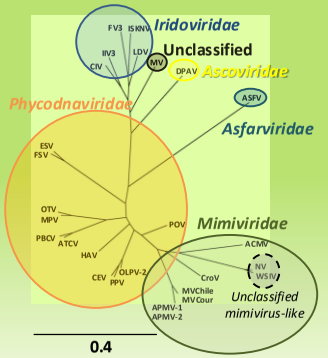
Major capsid protein – assay target areas



Analytical specificity and sensitivity

Virus	Diagnostic assays		
	cPCR	Q1	Q2
	specificity	specificity	specificity
WSIV	√	X	√
BCWSV	√	√	√
MRSIV	√	√	√
SNSIV	√	√	√
NV	√	√	√
	Analytical sensitivity (plasmid copies)		
NV	50	3	5

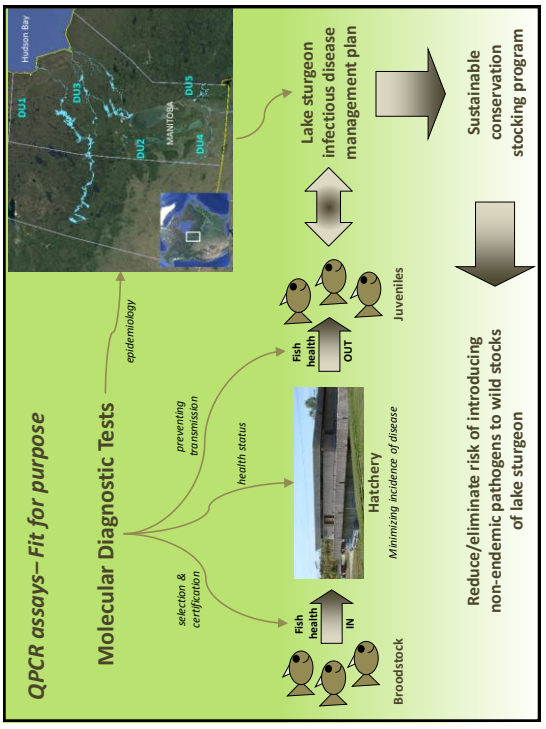
Diagnostic assays - Molecular test development
sturgeon NCLDV

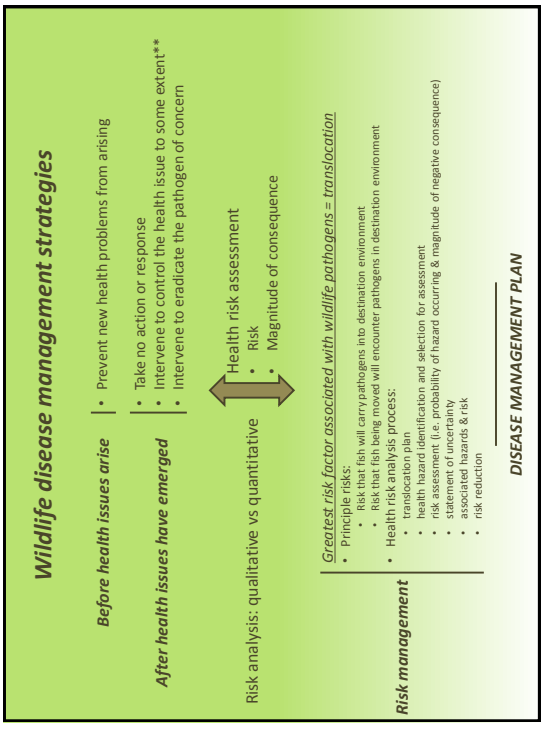


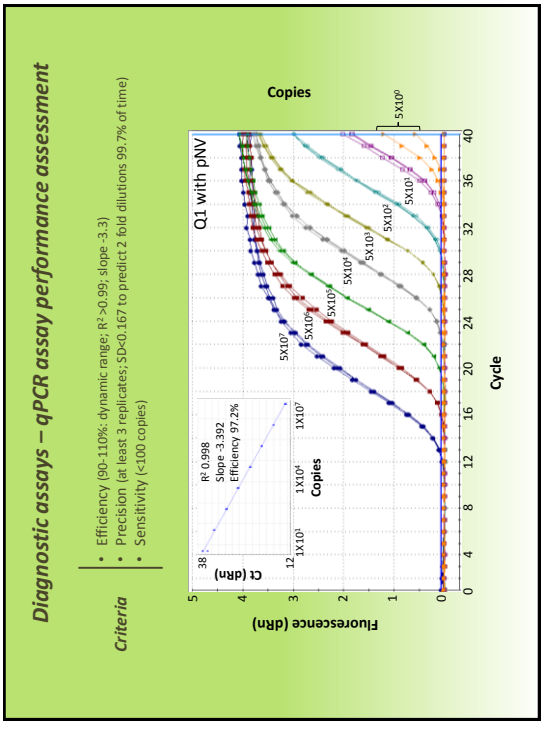
Analytical specificity - expanded

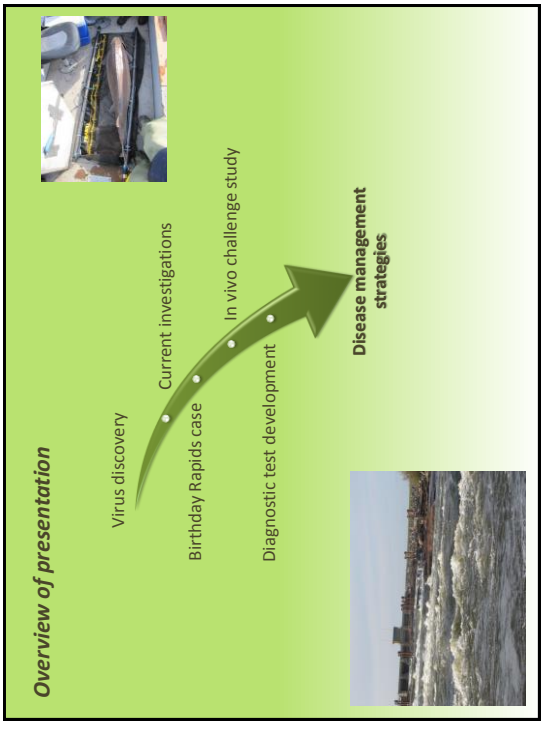
NCLDV	cPCR	Q1	Q2
Namao virus	√	√	√
BC white sturgeon virus	√	√	√
Missouri River sturgeon iridovirus	√	√	√
White sturgeon iridovirus	√	X	√
Shortnose sturgeon virus	√	√	√
Cafeteria roenbergensis virus (CroV)	√	tbt	tbt
Megavirus chiliensis (MVChile)	√	tbt	tbt
Megavirus courdo (MVCour)	√	tbt	tbt
Acanthoamoeba polyphaga mimivirus (APMV-2)	√	tbt	tbt
Organic lake phycodnavirus 2 (OLPV-2)	√	tbt	tbt
Chrysochromulina ercina virus (CEV)	√	tbt	tbt
Heterosigma akashiwo virus (HAV)	√	tbt	tbt
Osteococcus tauri virus (OTV)	√	tbt	tbt
Chile iridescent virus (CIV)	√	tbt	tbt

Appendix 2


QPCR assays– Fit for purpose
Molecular Diagnostic Tests
epidemiology
selection & certification
preventing transmission
health status
Fish health
IN
Broodstock
Hatchery
Minimizing incidence of disease
Fish health
OUT
Juveniles
Lake sturgeon infectious disease management plan
Sustainable conservation stocking program
Reduce/eliminate risk of introducing non-endemic pathogens to wild stocks of lake sturgeon
Hudson Bay
MANITOBA
DU1
DU2
DU3
DU4
DU5


Wildlife disease management strategies
Before health issues arise
Prevent new health problems from arising
After health issues have emerged
Take no action or response
Intervene to control the health issue to some extent**
Intervene to eradicate the pathogen of concern
Risk analysis: qualitative vs quantitative
Health risk assessment
Risk
Magnitude of consequence
Risk management
Greatest risk factor associated with wildlife pathogens = translocation
Principle risks:
Risk that fish will carry pathogens into destination environment
Risk that fish being moved will encounter pathogens in destination environment
Health risk analysis process:
translocation plan
health hazard identification and selection for assessment
risk assessment (i.e. probability of hazard occurring & magnitude of negative consequence)
statement of uncertainty
associated hazards & risk
risk reduction
DISEASE MANAGEMENT PLAN


Diagnostic assays – qPCR assay performance assessment
Criteria
Efficiency (90-110%; dynamic range; R²>0.99; slope -3.3)
Precision (at least 3 replicates; SD<0.167 to predict 2 fold dilutions 99.7% of time)
Sensitivity (<100 copies)
Q1 with pNV
Copies
Fluorescence (dRn)
Cycle
Ct (dRn)
R² 0.998
Slope -3.392
Efficiency 97.2%


Overview of presentation
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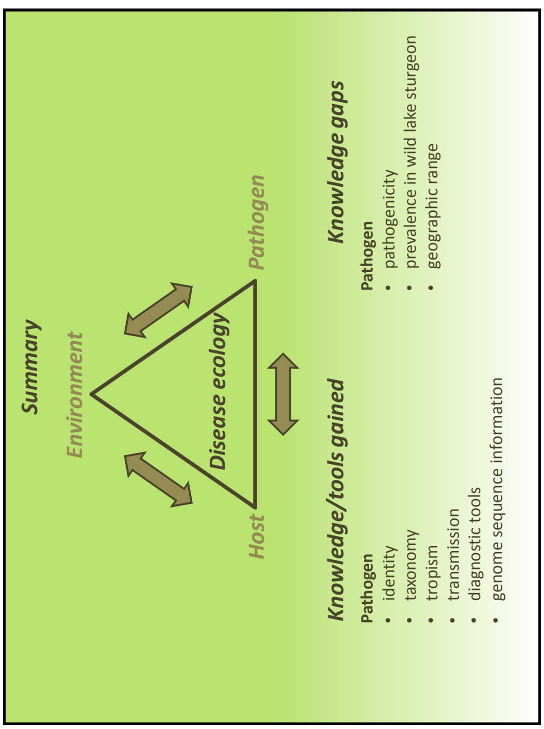
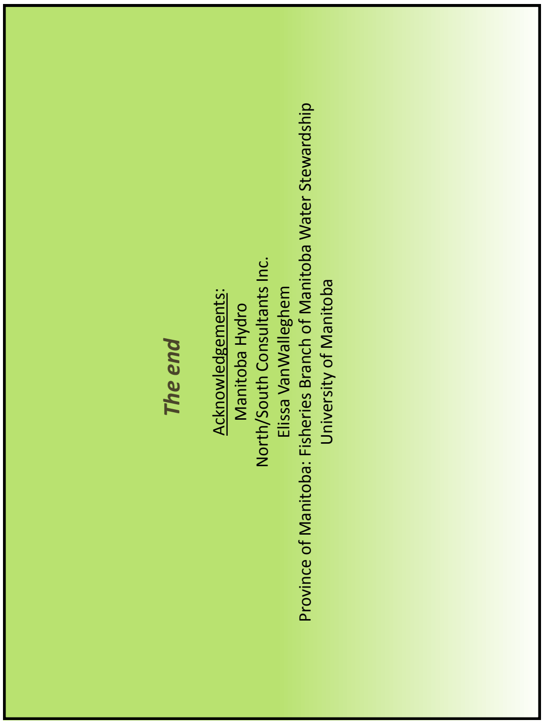

Summary
Environment
Host
Pathogen
Disease ecology
Knowledge/tools gained
Pathogen
• identity
• taxonomy
• tropism
• transmission
• diagnostic tools
• genome sequence information
Knowledge gaps
Pathogen
• pathogenicity
• prevalence in wild lake sturgeon
• geographic range


The end
Acknowledgements:
Manitoba Hydro
North/South Consultants Inc.
Elissa VanWalleghem
Province of Manitoba: Fisheries Branch of Manitoba Water Stewardship
University of Manitoba

Great Lakes Fish Health Committee Meeting

Manitoba: Fish Health Overview:

. . . . . .

Jeff Long / Laureen Janusz
MB Fisheries Branch
Ph: (204) 945-7792

Manitoba

Overview

1. Governance - MB's guiding documentation
2. Why does MB test wild fish and what stocks do we test?
3. Where & how we collect samples for testing
   - Challenges re: bio-security
4. What disease testing are we doing?
5. Results
6. Current Status
   - Q: are we on the right track?

Policy - Guidance

- MB Fisheries Branch is the management agency for the Province's fisheries resources. We have guiding policy for our Branch:

  "To ensure the orderly use of our fisheries resources."

  And among other things, we are:

  "*To provide a diversity of angling opportunities*".

- The Fish Culture program has provided stocked fishes since 1893. Our current guiding principles for the fish culture program include:
  1. To ensure human safety from biological harm;
  2. Conserve the health of aquatic ecosystems
  3. To protect fish stocks (source and receiving waters) from vertical and horizontal transmission of disease
  4. To produce high quality native and cultured fishes for stocking into MB waterbodies.

Why Does MB Test Wild Fish??

- At this point, there is no provincial (and only a transitional federal) legislated mandate for fish health.
- Notwithstanding, the MB Fisheries Branch Fish Culture program relies on wild brood stock to produce the following species for stocking purposes.

Percidae [25-80M fry / annum]
- Walleye
  - Sauger (new / renewed species for culturing)

Salmonidae [≈ 350,000 fingerlings / annum]
- Lake Trout
  - Splake
    - (Lake Trout X Brook Trout)
- Brook Trout (hatchery brood)
  - Tiger Trout
    - (Brown Trout X Brook Trout)
- Brown trout - hatchery brood stock
- Rainbow trout – imported egg

- Additionally, as the agency responsible for fish management, MB has an interest in understanding the disease profile of stocks that are subject to recovery / mitigation stocking.

Acipenseridae
- Lake Sturgeon (renewed species)

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WHERE DO WE COLLECT OUR SAMPLES?

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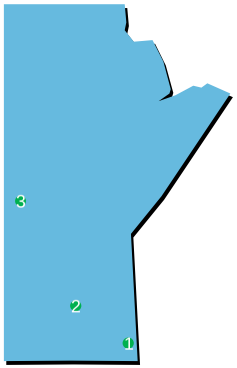
MANITOBA'S HATCHERIES



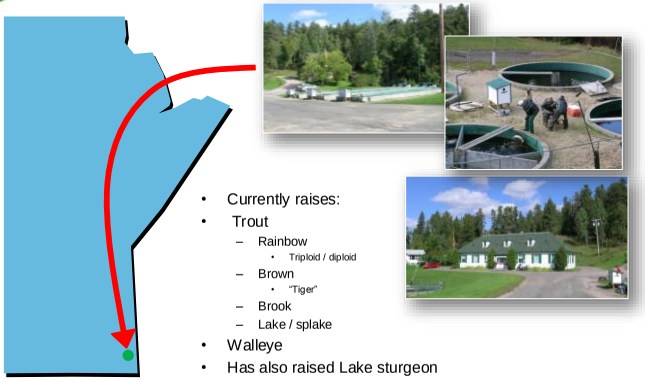
Manitoba Fisheries Branch

Fish Culture Hatcheries
& spawn camp locations

1. Whiteshell Hatchery
2. Swan Creek Spawn Camp
3. Clearwater Spawn Camp

Manitoba

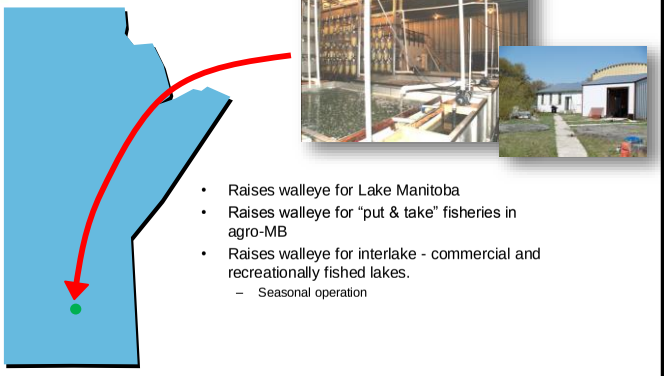
Whiteshell Hatchery



- Currently raises:
- Trout
  - Rainbow
    - Triploid / diploid
  - Brown
    - "Tiger"
  - Brook
  - Lake / splake
- Walleye
- Has also raised Lake sturgeon

Manitoba

Swan Creek Hatchery



- Raises walleye for Lake Manitoba
- Raises walleye for "put & take" fisheries in agro-MB
- Raises walleye for interlake - commercial and recreationally fished lakes.
  - Seasonal operation

Clear Water Lake Trout spawn Camp

Walleye

- Lake Manitoba – Swan Creek Spawn Camp – Lundar MB
  - Capacity 80M eggs
- Falcon Lake, MB –
  - Capacity 10-20M eggs
    - – Combined site with Salmonids

SWAN CREEK - WALLEYE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Manitoba
WHITESHELL - WALLEYE

Manitoba

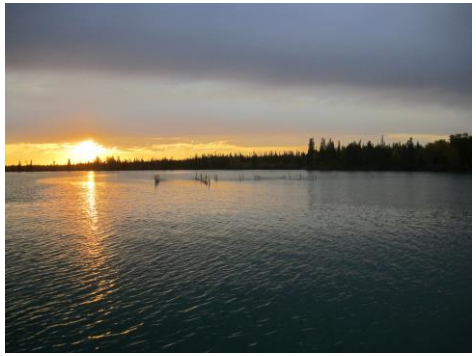
CLEARWATER – LAKE TROUT

Manitoba

Capturing Brood Stock
Pound Nets

Manitoba

Pound Nets



Manitoba

Electrofishing

Manitoba

Collecting Samples

- Ovarian Fluid
  - taken from running females AND /OR
  - Tissue taken from gravid females and necropsy done on site
- Tissue
  - Taken from running (usually) males
  - Necropsy done on site.
    - Intention is to take samples from those parents that produce eggs that go into the hatchery.
    - Conditions make sample collection “bio-security” challenging

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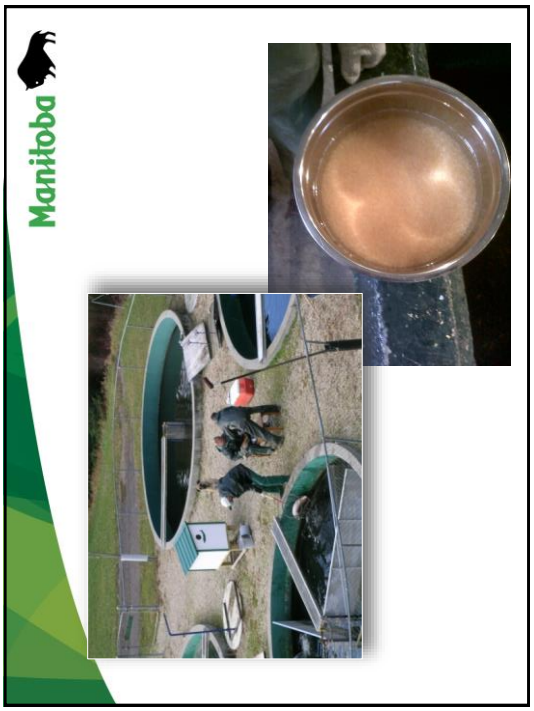
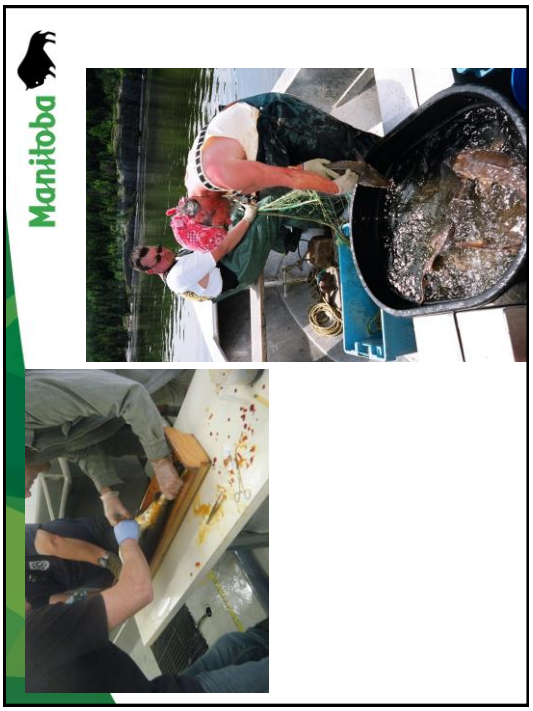
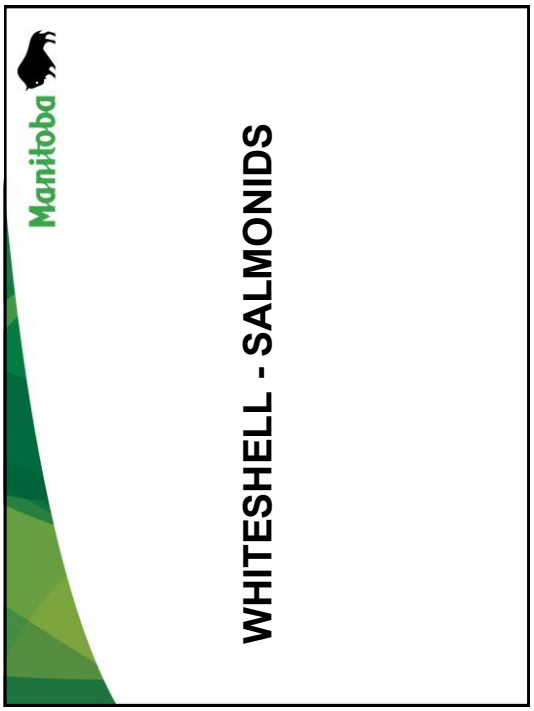

Manitoba


Manitoba


Manitoba
WHITESHELL - SALMONIDS


Manitoba
WHITESHELL - STURGEON

Diseases of Interest

- "Diseases of Interest" to MB are based on:
  - DFO Manual of Compliance AND
  - has morphed to meet CFIA Nat'l Aquatic Animal Health Program AND
  - Aims to meet fish culture and stock management needs.
- So, currently MB is interested in:
  - Percidae
    - VHS
  - Salmonidae
    - IPN
    - IHN
    - BKD
    - Whirling disease
    - ISA (potentially – based on UV hatchery treatment)
  - Acipenseridae
    - "Namao virus"
  - Various families – Lake Manitoba
    - Koi herpes Virus

Results

- VHS
  - No testing done to date by MB – testing pending for *Percidae* stocks
  - No known occurrence to date in MB
- IPN
  - Outbreak in Whiteshell in 1970-80s (but none since)
  - 2006 & 2012 in Grand Rapids – concern for future salmonid rearing
  - Clearwater – remains negative for IPN based on MB testing / CFIA maps MB as IPN positive.
- BKD
  - Seems to have outbreaks in brook trout – especially during crowding or other stressors across hatcheries and over time.
- Whirling disease
  - No known occurrences in MB
- KHv
  - Outbreak in Lake MB in 2009 – quiet ever since (except 1 case in Lk Wpg).
- Namao Virus
  - Endemic to DU 3 or MB or to LK St populations in general? Status uncertain.

Current Status

- Continue working with DFO on a wild fish surveillance program / protocol (despite no clear legislative mandate to do so).
- Have set up disease testing contract w/ RPC for hatchery testing – continue to follow our disinfection, testing and stocking BMPs.
- Use hatchery system as basis for testing based on the "hatchery health" driver.
- Aim to meet or beat CFIA compartmentalisation program re: IPN.

Thoughts/ Comments/ Questions

- So, given the ambiguous mandate under which we operate and the challenges of sample collection:
- Is MB on the "right track"??
  - Is the information we collect useful to other jurisdictions?
  - Are we collecting misleading information?

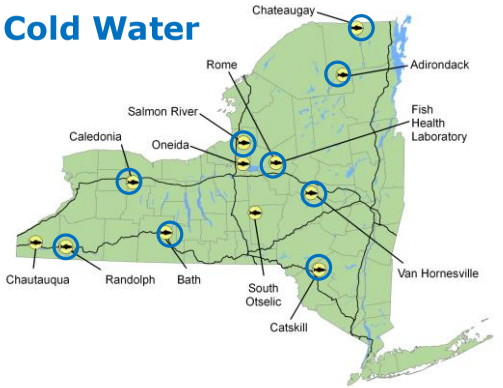
Van Hornesville SFH Summer '13

Andrew D. Noyes

Rome Field Station
New York State Department
of Environmental Conservation

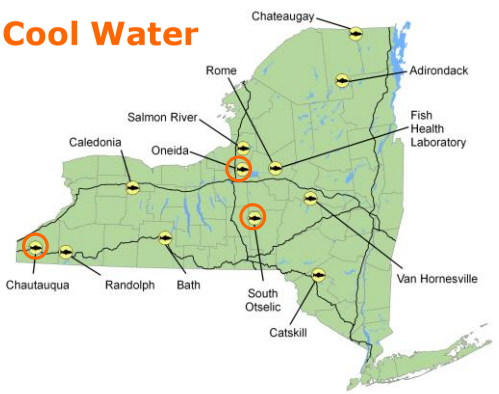
12 DEC Hatcheries

9 Cold Water



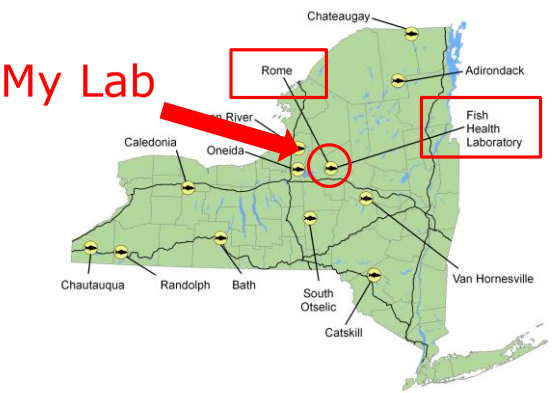
12 DEC Hatcheries

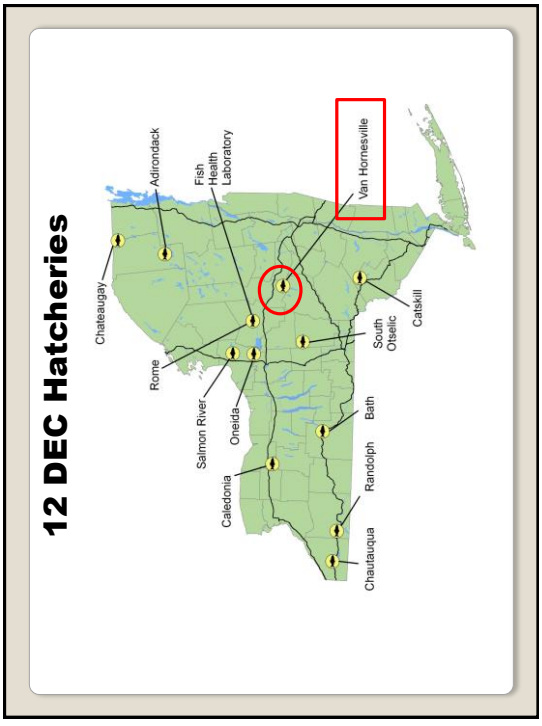
3 Cool Water



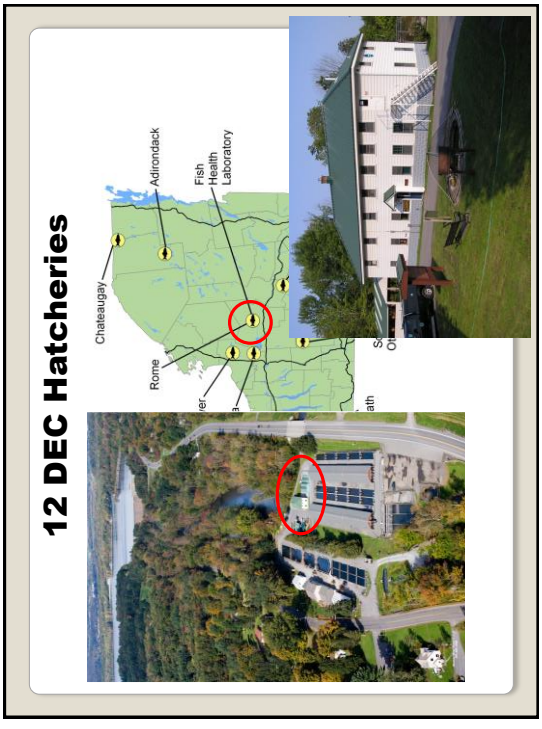
12 DEC Hatcheries

My Lab

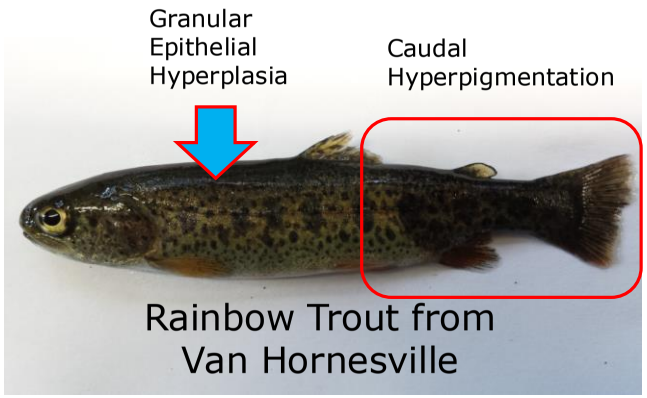


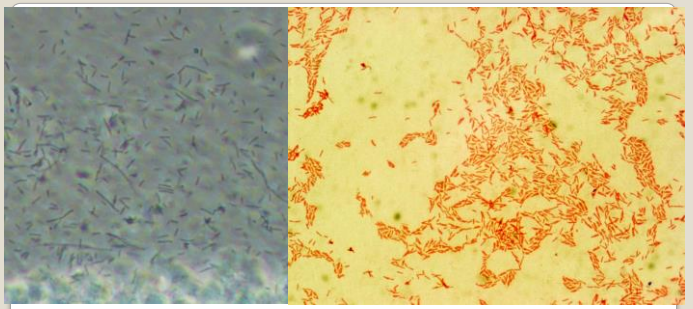

12 DEC Hatcheries
Chateaugay
Adirondack
Fish Health Laboratory
Van Hornesville
Catskill
South Otselic
Rome
Salmon River
Oneida
Bath
Caledonia
Randolph
Chautauqua


Rainbow Trout from Van Hornesville
July, 2013


12 DEC Hatcheries
Chateaugay
Adirondack
Fish Health Laboratory
Rome


Van Hornesville
-Raise 400K RT
400 gpm
08/10/2012



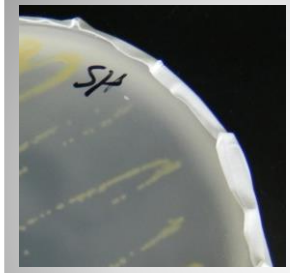


Wet mount-Skin

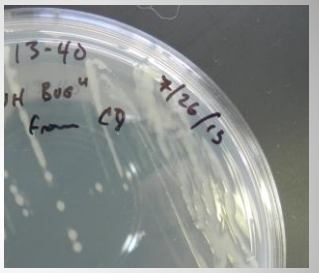
Gram Stain

What we did….

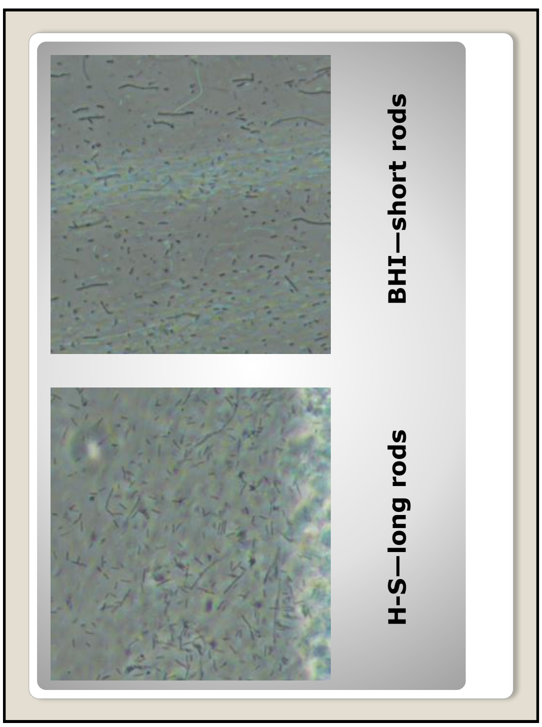
- Checked for WD **-NOPE**
- Virology **-NOPE**
- Bact—Skin/muscle, Gills, Liver
  - On BHI and H-S **-YES….**

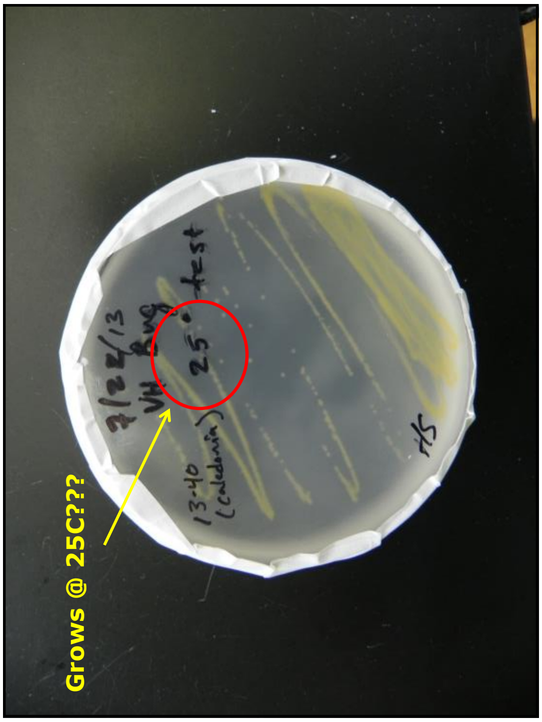


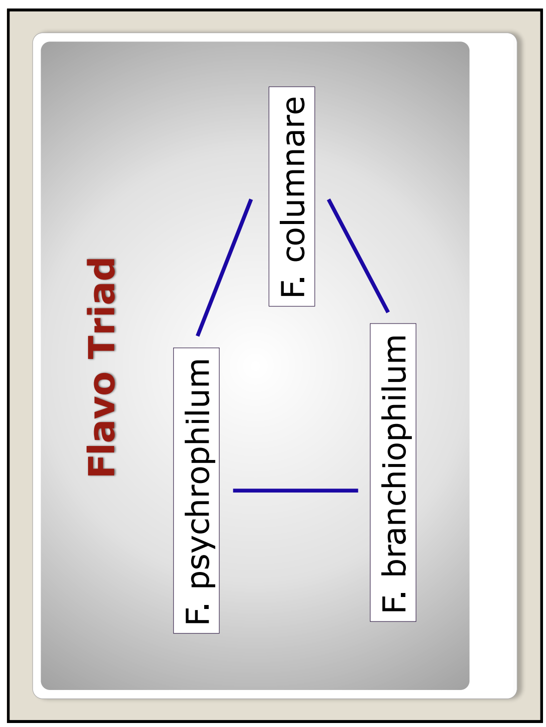
Grows on H-S

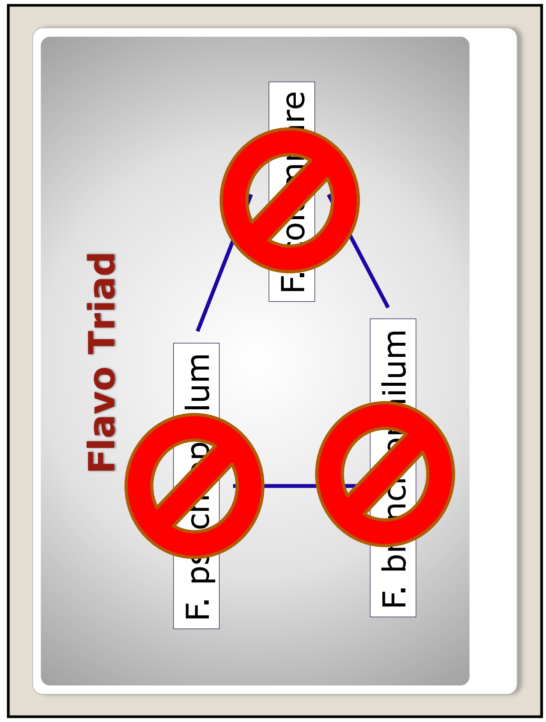


Also on BHI
-white colonies


H-S—long rods
BHI—short rods

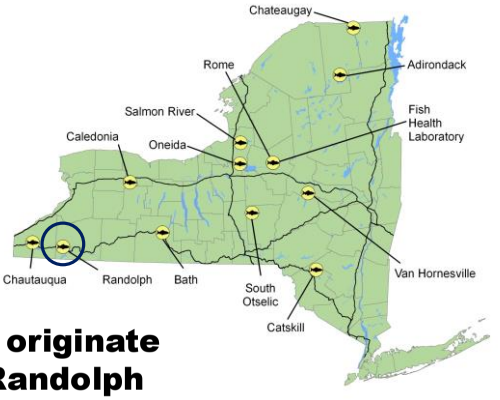

Grows @ 25C???


Flavo Triad
F. psychrophilum
F. columnare
F. branchiophilum

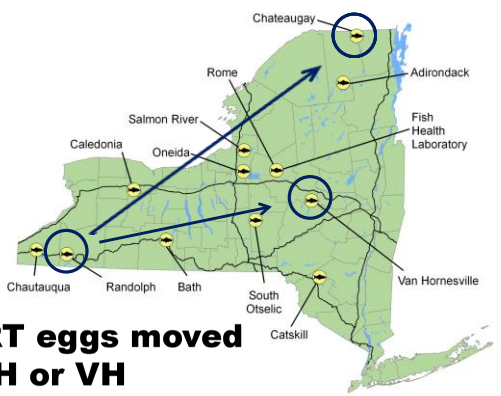

Flavo Triad

Sent to Kennebec River Biosciences….

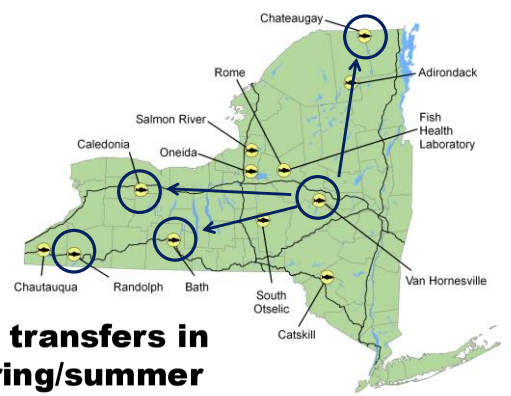
- For Sequencing, PCR, etc…
- ***Flavobacterium*** or ***Chryseobacterium***
  - Unknown species
- Flavo diversity also in Michigan
  - *(Loch et al.-JAAH-2013)*



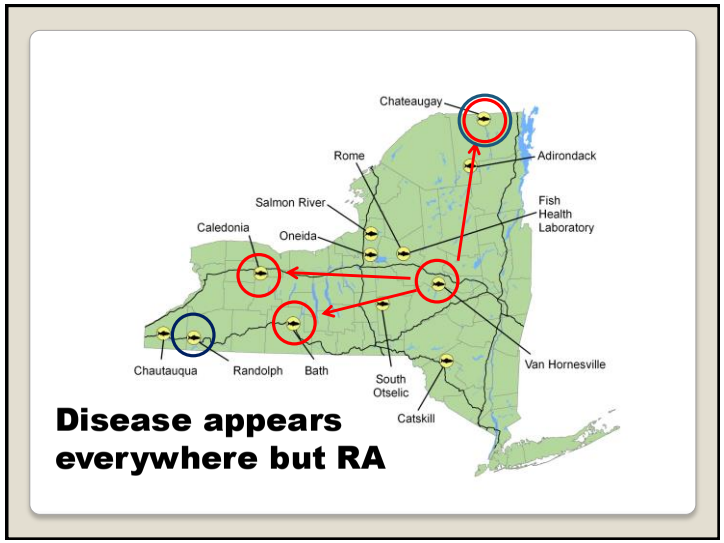
RT originate @ Randolph

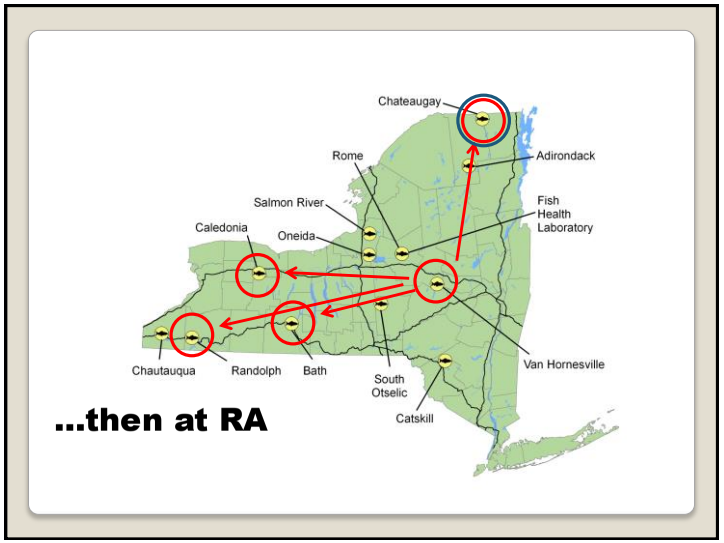


All RT eggs moved to CH or VH



VH transfers in spring/summer


Chateaugay
Rome
Adirondack
Salmon River
Fish
Health
Laboratory
Caledonia
Oneida
Chautauqua
Randolph
Bath
South
Otselic
Van Hornesville
Catskill
Disease appears
everywhere but RA


Chateaugay
Rome
Adirondack
Salmon River
Fish
Health
Laboratory
Caledonia
Oneida
Chautauqua
Randolph
Bath
South
Otselic
Van Hornesville
Catskill
...then at RA


• Started at VH
◦ moved from there
• Only infected RTs
◦ Did eventually see concurrent *F. psych*
• Grows up to 25C
• …on H-S and BHI
• Isolated from **skin**, **muscle*** and **liver**
• **Not seen in 2014**

Old Case from 2010

- Hamilton Harbour, ON
- 7 Brown bull head with lesions- Late Sep 2010
  - deep ulcers on: Head, face, base of fins, fins and belly
  - ulcers were deeply eroded with red centre and white rim
  - Some of the ulcers on body exposed the musculature and others had granulated texture
  - In some fish ulcers created hole through the tail fin or in some instances fins were completely eroded

???

- 2nd submission (Early November)
- 16 brown bull heads
  - Three fish had Similar lesions but in rest of the fish lesions were less severe (Possibly due to lower water temperature)

???

Suspect for *Edwardsiella tarda???*

Results

- Virology
  - EPC and CHSE at 15°C for 21 days
    - No CPE
- Bacteriology: Kidney and Lesions
  - TSA, BHIA and MacConkey at 20 and 25°C
    - *Pseudomonasa aeruginosa,*
    - *Aeromonas hydrophila,*
    - *Vibrio fluvialis and*
    - *Plesiomonas shigelloides.*

Results (Contd.)

- Histology/ Molecular testing*
  - Lesions
  - Spleen,
  - Kidney
  - Gills
  - Stomach

* No samples for molecular testing were collected from 1st submission

Summary of results

Sample ID	Lesion consistent with *A. invadans*	qPCR (DFO test) *A. invadans*	OIE PCR 1, 2 and 3	Histology Fungal hyphae consistent with *A. invadans*
2010-36-001	Yes (Severe)	Positive	Positive	Positive
2010-36-002	Yes (Severe)	Positive	Positive	Positive
2010-36-003	*Lesion at the base of fin and middle of tail fin was chewed up	Positive	Not tested	Positive
2010-36-004	Yes (Severe)	Negative	Not tested	Positive
2010-36-005	Yes (Severe)	Positive	Positive	Positive
2010-36-006	No lesions	Negative	Not tested	Negative
2010-36-007	Small haemorrhage around mouth – not sure	No sample	No sample	Positive
2010-36-008	No lesions	Negative	Not tested	Negative
2010-36-009	Small lesion on belly and operculum – not sure	Negative	Not tested	Not tested
2010-36-010	*Lesion at the base of pectoral fin	Negative	Not tested	Not tested
2010-36-011	No lesions	Negative	Not tested	Not tested
2010-36-012	Two small lesions on belly – not sure	Negative	Not tested	Not tested
2010-36-016	*Lesion on fins	Negative	Not tested	Not tested

Appendix 5

Summary of Results Contd.

Sample ID	Tissue	A. invadans qPCR Ct values	Positive for A. invadans
2010-36-001L-PCR	**Lesion**	21.07, 20.75	Pos
2010-36-001K-PCR	Kidney	29.72, 29.54	Pos
2010-36-001S-PCR	Spleen	32.58, 32.48	Pos
2010-36-001Lr-PCR	Liver	33.11, 32.6	Pos
2010-36-001G-PCR	Gill	28.69, 28.85	Pos
2010-36-002L-PCR	**Lesion**	19.47, 19.35	Pos
2010-36-002K-PCR	Kidney	31.57, 31.14	Pos
2010-36-002S-PCR	Spleen	33.2, 32.57	Pos
2010-36-002Lr-PCR	Liver	33.39, 33.67	Pos
2010-36-002G-PCR	Gill	31.23, 31.13	Pos
2010-36-003L-PCR	**Lesion**	No Ct, No Ct	Neg
2010-36-003K-PCR	Kidney	No Ct, No Ct	Neg
2010-36-003S-PCR	Spleen	35.93, 36.79	Pos
2010-36-003Lr-PCR	Liver	No Ct, No Ct	Neg
2010-36-003G-PCR	Gill	35.13, 34.37	Pos
2010-36-004L-PCR	**Lesion**	No Ct, No Ct	Neg
2010-36-004K-PCR	Kidney	No Ct, No Ct	Neg
2010-36-004S-PCR	Spleen	No Ct, No Ct	Neg
2010-36-004Lr-PCR	Liver	No Ct, No Ct	Neg
2010-36-004G-PCR	Gill	38.17, No Ct	Neg
2010-36-005L-PCR	**Lesion**	22.82, 22.87	Pos
2010-36-005K-PCR	Kidney	34.97, 34.92	Pos
2010-36-005S-PCR	Spleen	No Ct, No Ct	Neg
2010-36-005Lr-PCR	Liver	39.56, No Ct	Pos
2010-36-005G-PCR	Gill	No Ct, No Ct	Neg

2014 samples (Manitoba)

Results 2014

Bacteriology

Possibility of Vibrio fluvialis

Histology and molecular Biology

Results are still pending ???

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

Updated August 2014

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

Research Priorities

- What is the ecology of fish pathogens and diseases of concern in the Great Lakes Basin? Examples include (but are not limited to) viral hemorrhagic septicemia virus (VHSv) genotype IVb, Heterosporis sp., Epizootic Epitheliotropic Disease virus (EEDv), Flavobacterium sp., and emerging diseases.
- What non-lethal field sampling methods and tissue/fluid samples are equivalent to conventional lethal field sampling methods to determine fish pathogen and/or disease status?
- Develop and validate new methods to detect emerging fish pathogens or pathogens of concern in the Great Lakes Basin.

Additional Research Interests

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

(e) Does protein substitution in hatchery feeding formulations or extrusion manufacturing methods have a negative impact on survivorship, migratory behavior and reproductive success of hatchery-reared salmonids?

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

PROPOSED TECHNICAL ADVISORS

Bacteriology: Diane Elliot (USGS), Thomas Loch (MSU)

Virology: James Winton (USGS), Tom Waltzek

Molecular: Nick Phelps (University of Minnesota), Sharon Clouthier (DFO)

Nutrition: Dominique Bureau (University of Guelph), Dale Honeyfield

Quantitative Fish Health Data Analysis: Dominic Travis (University of Minnesota) Travis Brenden (Michigan State University)

Epidemiology: Lori Gustafson (USDA)

Parasitology: David J. Marcogliese (St. Lawrence Centre, Environment Canada)

Thiamine Injection of Skamania Steelhead Broodstock

Bodine State Fish Hatchery
Indiana Department of Natural Resources

Two Questions

- Can thiamine injected at harvest from the wild improve the survival of adult Skamania steelhead through spawning four to eight months later?
- How does treating broodstock with thiamine compare to treating sac-fry with regards to swim-up survival through the first 21 days on feed?

Injection Protocol – 2005/'06

- Half of all female broodstock received thiamine injection
- Numbered jaw tags were used to keep track of which fish were injected
- At first egg take, a 2 X 2 matrix will be used to establish four groups of females
- All males were thiamine injected
- Dose was 9.2 mg Thia-HCl/5 lbs

Female Study Groups

- Un-injected females
  - Sac fry not treated
  - Sac fry treated
- Injected Females
  - Sac fry not treated
  - Sac fry treated

Trial Period

- 21-Day mortality post first feeding

Implications

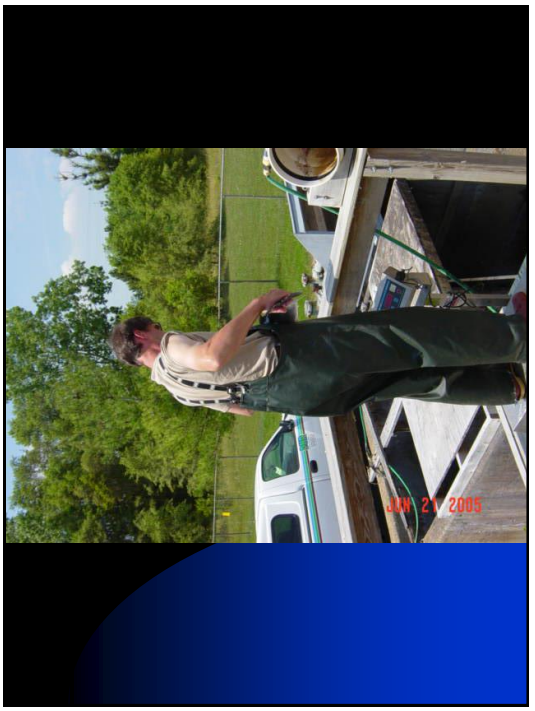
- Broodstock carrying capacity is limited
- Thiamine hydrochloride is expensive
- Best to get thiamine into egg prior to fertilization (Dale Honeyfield)

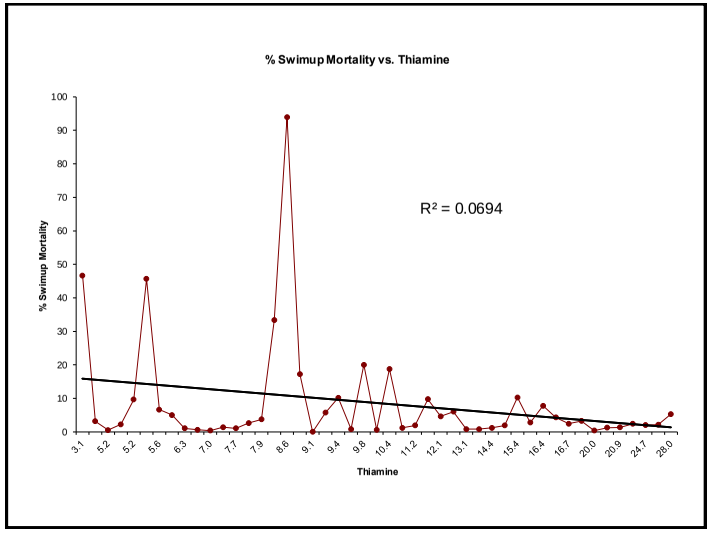
Appendix 8

Tag number of adult steelhead broodstock, type of treatment, and corresponding egg thiamine level (nmol/g of egg weight) prior to spawning.

Tag #	Injected	Thiamine Level
342	Yes	9.1
56	Yes	8.5
102	Yes	14.4
226	Yes	9.4
604	Yes	15.9
86	Yes	9.8
678	Yes	14.7
190	Yes	10.4
218	Yes	20.7
556	Yes	24.1
332	Yes	14.4
98	Yes	20.0
658	Yes	11.3
472	Yes	16.5
360	Yes	7.9
154	Yes	16.7
52	Yes	15.4
78	Yes	28.0
127	Yes	16.4
115	Yes	24.7
670	Yes	25.9
394	Yes	20.9
476	Yes	17.6
-	-	-
Mean		**16.2**

Tag #	Injected	Thiamine Level
405	No	6.3
421	No	10.5
13	No	8.6
273	No	9.3
203	No	7.7
681	No	5.6
683	No	3.1
231	No	9.8
545	No	11.2
399	No	12.1
541	No	9.9
69	No	8.8
126	No	5.2
643	No	5.3
347	No	5.2
719	No	6.4
613	No	7.5
601	No	5.2
207	No	5.0
55	No	13.1
455	No	7.0
118	No	7.9
471	No	12.3
549	No	5.7
		7.9





Lot 07RBSTT

- Injected Females
  - 97.1% 21 Day Survival
- Un-injected Females
  - 92.2% 21 Day Survival

Lot Years 2011 to 2014

- Brood Years 2010 to Present
  - All Injected at 18.4 mg / 10 lbs BW
- 2011
  - 97.8% with Flow-Thru
- 2012
  - 97.8% with Flow-Thru
  - 97.3% No F/T

- 2013
  - 97.3% No F/T
- 2014
  - 95.7% No F/T

Egg Thiamine Analysis

- Brood Lot 12INSTT-BR
  - Produced 13RBSTT Lot
  - 42 Females
  - 18.8 nmol/gm egg wt
    - 8.9 – 29.8
    - SD 5.0

Broodstock Survival

- Brood Lots 2000 – 2013
  - No Injections
    - 8 Years
    - 20.9% Mortality
      - 3.2 – 35.1% Range
  - Injections
    - 6 Years
    - 7.9% Mortality
      - 1.9 – 21.1% Range