GREAT LAKES FISH HEALTH COMMITTEE

2015 Summer Meeting
Ithaca, New York
July 28-29, 2015

Minutes
(with attachments)

Submitted By:

Andrew D. Noyes

New York State Department of Environmental Conservation

The data, results, and discussion herein are considered provisional; permission to cite the contents of this report must be requested from the authors or their agency.

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

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List of Attendees

John Coll	U.S. Fish and Wildlife Service- Pennsylvania
John Dettmers	Great Lakes Fishery Commission
Kevin Kayle	Ohio Department of Natural Resources
Sunita Khatkar	Fisheries and Oceans Canada
Kevin Loftus	Ontario Ministry of Natural Resources and Forestry
Dave Meuninck	Indiana Department of Natural Resources
Brian Niewinski	Pennsylvania Fish and Boat Commission
Andy Noyes	New York State Department of Environmental Conservation
Paula Phelps	Minnesota Department of Natural Resources
Ling Shen	Minnesota Department of Natural Resources
Gary Whelan	Michigan Department of Natural Resources
Coja Yamashita	Pennsylvania Fish and Boat Commission

Other Attendees included:

Vicki Blazer	U.S. Geological Survey
Paul Bowser	Cornell University
Gavin Glenney	U.S. Fish and Wildlife Service- Pennsylvania
Jenny Johnson	U.S. Fish and Wildlife Service- Pennsylvania
Bill Keleher	Kennebec River Biosciences
Michael Penn	U.S. Fish and Wildlife Service- Pennsylvania
Nick Phelps	University of Minnesota
Jacques Rinchard	The College at Brockport – State University of New York

Great Lakes Fish Health Committee Meeting

Hotel Ithaca

222 South Cayuga Street, Ithaca, NY 14850
July 28-29, 2015

Tuesday, July 28th 2015

8:30 am – 8:40 am	Welcome & Introductions (C. Yamashita)
8:40 am – 8:50 am	CLC update/Pathogen description update (J. Dettmers)
8:50 am – 9:30 am	Northeast Fish Health Committee and Guidelines for Fish Health Management in Northeastern States (C. O'Bara)
9:30 am – 10:00 am	MIDNR net pen aquaculture updates (G. Whelan)
10:00 am-10:15 am	Recommended actions for detection of significant pathogens in Great Lakes net pens (J. Dettmers)
10:15 am – 10:30 am	Break
10:30 am-10:45 am	Handling foreign import requests and African Longfin Eel risk assessment update (D. Meuninck)
10:45 am – 11:00 am	Discussion on how each state/province handles requests for aquaculture licensees to non-indigenous and exotic species in the context of managing the potential for import of pathogens (All)
11:00am – 11:10am	Recommendations regarding public pressure to change state VHSv regulations limiting movement of baitfish within Great Lakes basin (PA/All)
11:10 am - 11:30 am	MSU update (M. Faisal)
11:30 am – 12:00 pm	Salmon Herpes Virus update (G. Glenny)
12:00 pm – 1:30 pm	Lunch
1:30 pm – 2:00 pm	NYSDEC update on dead/sick steelhead in Salmon River (A. Noyes)
2:00 pm - 2:30 pm	Cornell thiamine presentation (P. Bowser)
2:30 pm – 3:00 pm	Thiamine research update/preview (J. Rinchard)
3:00 pm – 5:30 pm	Tour of Cornell Lab (P. Bowser)

<u>Wednesday, July 29th 2015</u>

8:30 am – 9:30 am	USGS update (V. Blazer)
9:30 am – 10:30 am	Agency updates / Weird and Unusual Cases (All)
10:30 am – 11:30 am	Department of Homeland Security - Perox-aid regulation (D. McKinney
11:30 am – 1:00 pm	Lunch
1:00 pm – 1:30pm	Kennebec River Biosciences update (B. Kelleher)
1:30 pm – 2:30 pm	Fish Kill Investigation Database and protocol (N. Phelps)
2:30 pm – 3:30 pm	Agency updates (All)
3:30 pm – 3:40 pm	Tech Advisors (C. Yamashita / C. Haska)
3:40 pm – 4:00 pm	Next Meeting (All)

1. Welcome & Introductions (C. Yamashita)

Coja welcomed committee members and guests to the meeting.

2. CLC update/Pathogen description update (J. Dettmers)

The pathogen descriptions are complete and ready to be posted. In his presentation to the CLC in April, Gary Whelan highlighted the Michigan aquaculture industry's plans to expand cage culture operations in Lake Michigan. The GLFC passed a resolution encouraging states to develop cage culture policy before additional culture operations begin and may seek further guidance from the GLFHC.

3. Northeast Fish Health Committee and Guidelines for Fish Health Management in Northeastern States (C. O'Bara)

Chris presented a history of the NEFHC committee and he suggested having a co-hosted meeting between GLFHC and NEFHC in in the future. The NEFHC coordinates fish health management among all agencies in NEFWA. The main focus has been the development of fish health guidelines. Their mission is to address fish health issues related to importation and transfer within member states, encourage communication between member agencies, and develop management to improve existing fish health strategies among member agencies. Fish health guidelines were initially developed based on trout and salmon disease concerns, then new sections were added dealing with facility classifications, bait transfers due to VHS, and other issues unrelated to trout and salmon. They initially addressed the development of guidelines and the problems they encountered along the way, then developed a new approach in 2012. By 2013, they reported their findings back to NEFAA and NEAFWA. Still needed were an additional risk assessment, biosecurity plan, and egg disinfection program. Once these were added, the cold and warm water sections were combined to make a single fish health management chapter. The document was approved by NEFC and NEFWAA and will be approved by NEAFWA by fall. The committee is currently drafting by-laws. A brief overview of the guidelines was presented. The guidelines apply to inter-basin and interstate transfer of wild and cultured fish and intrastate transfer of fish and transport water. They do not apply to fish not released from original shipping containers, fish headed to an approved quarantine, food fish, or tropical fish. Agencies will not knowingly extend the range of fish pathogens and will not transfer wild-acquired fish. The risk assessment will be used for wild-acquired transfer scenarios. Each agency's management plan should include transfer of wild-caught or cultured fish. A disease contingency plan should be developed to eliminate harmful pathogens from a facility and early testing is a key element and critical needs were highlighted. The risk assessment is based on three fish transfer scenarios and completed in an excel spreadsheet format. Pathogens were classified into different categories in the assessment. Testing criteria and sampling methods were discussed. Use of surrogate species for testing was addressed. Disease classification for wild populations was also addressed.

4. MIDNR net pen aquaculture updates (G. Whelan)

An overview of how the farm bureau views the great lakes was provided. Their goal is for the aquaculture industry in Michigan to grow from $5 million to $1 billion by 2025. Details of this plan are sparse, but they can be found at michiganaquaculture.org. This mission to operate such an expansive pen culture in Lake Michigan conflicts with DNR concerns. Escapement, biosecurity, and effluent management are all problematic. The plan calls for 10% of the industry to use recirculation culture systems, 10-20% flow-through systems, and 70-80% open water cage culture. This equates to 500 surface acres, 250 operations, and 1 million pounds per year combined in all operations. Many environmental concerns exist, including winter weather impact, wind, waves, etc. as well as interactions with charter boats and other vessels. Some areas include tribal areas, some of which do not have fishing rights. Very little public outreach has occurred so far. Fish species to be cultured include Rainbow Trout, Yellow Perch, and Whitefish. Important topics that have not been addressed include fish escape abatement, biosecurity plans disease mitigation, and effluent management. Effluent management concerns include phosphorus, suspended solids and fish pathogens. The proposed feeding rate is 5-7% and if all 250 facilities are operational, the resulting waste that would be produced equates to that of 2.8 million people. There is no investment stream in place currently. Two proposals exist for cage culture, one in Bay De Noc and Northern Lake Huron. Policy development is underway.

5. Recommended actions for detection of significant pathogens in Great Lakes net pens (J. Dettmers)

Any actions will stem from agency policy. The committee is concerned that fish grown in intensive cage culture operations will be vulnerable to an array of harmful fish diseases, and may be a serious risk to wild fish in the vicinity. Cage operations need to be considered as an extension of the hatchery system with similar disease policies.

6. Handling foreign import requests and African Longfin Eel risk assessment update (D. Meuninck)

There is a proposal to harvest glass eels from Madagascar, then transport to Wabash, IN, then rear for two years and sell to fish markets. Many risks were identified, including escapement, disease concerns, and wastewater treatment. If eels escape, the potential impact on American Eels was raised. The Indiana DNR originally intended to deny the proposal, then the Indiana Department of Agriculture intervened due to potential economic impact. The proposal is currently under review. Since the program is recirculation-system based, the risks during culture are likely minimal. The concern is what happens when the eels go to market.

7. Discussion on how each state/province handles requests for aquaculture licensees to non-indigenous and exotic species in the context of managing the potential for import of pathogens (All)

The committee discussed how each agency deals with aquaculture licenses for non-indigenous species. Each agency explained their own policy.

PA- has an approved species list for aquaculture and breaks it down by watershed and licenses are issued by PA Dept of Ag.
MN- developed a risk assessment.
MI- has an approved list for aquaculture species. Permits not on list may be approved after further review.
OH- has the right to review and can do risk assessment to evaluate the impact.
IN- has approved list for fish imports. DNR can review new species. Cannot simply deny without sound justification. Do not have power to approve all fish stocking.
OMNR-List has 45 species authorized for culture. Introductions and transfer committee exists to review others.

The committee may ask the GLFC to develop guidelines for having agencies develop regulatory packages.

8. Recommendations regarding public pressure to change state VHSv regulations limiting movement of baitfish within Great Lakes basin (PA/All)

Pennsylvania is being pressured by bait dealers to repeal testing requirements. APHIS made it clear that the federal rule revocation hinged on strong state regulation and any weakening or revocation of state agency rules may result in reestablished the federal rule.

9. MSU update (M. Faisal)

This presentation was pre-recorded digitally and the committee had technical difficulties viewing it. To save time, we all decided to view it on our own time later.

10. Salmon Herpes Virus update (G. Glenney)

The predominant salmonid herpesvirus in the Great Lakes basin is Epizootic Epitheliotropic Disease Virus (EEDv), member of the family Alloherpesviridae. The clinical signs and disease history were presented. Diagnosis was previously difficult until a PCR method was developed. The EPA currently has a grant to develop new methodology. The terminase sequence is highly conserved and useful for comparing to a wide variety of known herpes viruses to characterize specificity. The terminase sequence shares much homology with Salmonid Herpesvirus 4 (SalHv4). Both EEDv and SalH4 were isolated from Lake Trout in several New York locations and in Lake Champlain. As the isolate was further sequenced, a difference appeared between these isolates, suggesting the discovery of SalHV5. There is some diversity is in the terminase, glycoprotein and polymerase sequences. After this distinction was made, archived isolates previously thought to be SalHv4 were then re-tested to see if they were actually SalHv5. Both SalHv3 and SalHv5 have been isolated from both inland and Great Lakes locations. Species susceptibility was evaluated and only Lake Trout and Ciscoes are susceptible. Future research direction was addressed.

11. NYSDEC update on dead/sick Steelhead in Salmon River (A. Noyes)

Anglers fishing in Salmon River reported seeing lethargic Steelhead floating down the river. No infectious agents were isolated, however tissues sent to USGS-Wellsboro revealed thiamine deficiency. Some visibly lethargic fish were injected with thiamine and they recovered in 48 hours. Large numbers of fish had arrived at the Salmon River Hatchery to spawn, so DEC staff injected 1100 with thiamine hoping to replenish the thiamine. Unfortunately, 73% of the fish died, so the injection campaign did not work. The good news is late-arriving fish (not injected) had much higher egg survival than the early-arriving injected fish. In June, the thiamine researchers from many agencies met at the USGS-Tunison lab to discuss the future of thiamine research.

12. Cornell thiamine presentation (P. Bowser)

Did initial evaluation of Salmon River Steelhead. Found no significant findings, but recovered tissues for histology. Liver had greatly reduced glycogen in Salmon River SHD. In nerve tissue, advanced degeneration of brain.

13. Thiamine research update/preview (J. Rinchard)

Jacques discussed his research, and gave an overview of thiamine metabolism, chemistry, and detection methodology. Thiamine is important in glucose and lipid metabolism and nerve development. All fish life stages can effectively be treated with thiamine in cases of thiamine deficiency. Thiamine deficiency was first reported in 1974, although it was not truly identified as cause until 1990's. *Paenibacillus thiaminolyticus* is one potential source of thiaminase activity, but not the entire cause. The thiamine concentration in Lake Trout eggs from Lake Ontario was much higher than Cayuga Lake. From Lake Michigan, early mortality syndrome (EMS) is more prevalent in the southern end of lake. Thiamine has a key role in fatty acid metabolism. High condition factor in prey tends to lead to thiamine deficiency in predators. Lipid content in fish varies greatly by season. Steelhead eggs produced during the thiamine epizootic that were not treated with thiamine after fertilization mostly died, whereas thiamine-treated eggs survived. His plan is to study the lipid content in the major Great Lakes predators and the relationship between fatty acid content and thiamine deficiency in Lake Trout and Atlantic Salmon.

14. USGS update (V. Blazer)

An overview of Leetown activities was presented, including GLRI funded projects. The focus was abating toxics, like PAH's and PCB's, and better understanding issues like tumor development. The effects of contaminant exposure at various life stages were explained. The Susquehanna River Smallmouth Bass YOY had an array of several bacterial infections and parasitic infestations, suggesting some other underlying cause. Contaminants tend to be distributed in different tissues at very different concentrations. But ovaries tend to have high concentration of toxicants regardless. The skin had elevated arsenic concentration in May, but not in March or April. Sources of these emerging contaminants include wastewater treatment plants and agriculture. Recent news items suggest the role of endocrine disrupters. In the Great Lakes, an early warning program was developed to look at effects-based monitoring, using bio-indicators, like sentinel fish. In these wild fish assessments, brown bullhead, white sucker largemouth and smallmouth bass were used. The suite of bio-indicators was described, looking at blood, histology, morphology and molecular testing methods. For molecular work, a short list of approximately 50 genes of interest were identified for useful markers. Intersex expression is evident only in Smallmouth Bass from the Susquehanna and Chesapeake. Concentrations of compounds such as estrone, atrazine, and metolachlor were elevated in many locations around Great Lakes, but even higher in locations tested in Pennsylvania. Melanosis in Smallmouth Bass was discussed and the cause is unknown. In catfish, squamous cell carcinomas are common and were described. The mucoid lesions (hyperplasia) seen in White Suckers were similar to lesions reported in Smallmouth Bass, except they also can have liver or skin neoplasia. This was especially evident in Sheboygan. Risk factors for tumor formation were described. Hepadnaviruses (hepatitis) in fish were described in White Suckers and found throughout the Great Lakes. Whether this is related to tumor formation is unknown.

15. Agency updates / Weird and Unusual Cases (All)

<u>Rare Tumors in Smallmouth Bass (Mike Penn-USFWS-Lamar)</u> - A rare tumor observed in a single Susquehanna River Smallmouth Bass received an extensive media response. The fish was caught in November 2014 and samples of the tumor were sent to many labs for identification. It was described as a locally invasive, metastic carcinoma. Toxicology was also performed but results were inconclusive. The tumor was initially identified as an olfactory neuroblastoma; very rare and poorly understood in fish. The thought now is that the tumor is probably an esthesioneuroepithelioma and the cause is unknown.

<u>Rome Syndrome (Andy Noyes-NYSDEC)</u> - Brown Trout fry raised at the Rome State Fish Hatchery annually exhibit motor impairment soon after being moved to outside raceways in February. *Flavobacterium psychrophilum* was isolated from the brain of these fish, although no sign of cutaneous Bacterial Coldwater Disease is evident (BCWD) initially. Cutaneous BCWD does eventually appear in the weeks or months that follow, suggesting that the disease is not transmitted by water, but rather germinally via vertical transmission. The bacterium was isolated from eggs in the fall of 2014.

<u>Two Disease Cases in Michigan (Mohamed Faisal-Michigan State)</u> – Hatchery-raised Barramundi had elevated mortalities and clinical signs were described. Swim bladder hyperinflation was the predominant sign observed. *Edwardsiella tarda* was isolated, although Terramycin treatment was not effective. In another case, Tilapia suffering from a heavy *Gyrodactylus* infestation were presented. The fish were anorexic and had mottled gills.

16. Department of Homeland Security - Perox-aid regulation (D.McKinney)

Dave McKinney and Don Keen from the Department of Homeland Security (DHS) discussed nationwide measures to protect hydrogen peroxide storage at hatcheries from terrorism. DHS hired 125 inspectors nationwide to work with public and private entities that handle 325 known chemicals of interest to terrorists. Because hydrogen peroxide can be used in bomb-making, facilities having more than 400 pounds of hydrogen peroxide (≥35%) require abatement. A 200-liter (55 gallon) barrel weighs 513 pounds. The program is detailed on a DHS website. The process begins with a facility representative conducting a 'top screen' to evaluate if the chemical inventory is in excess of threshold standards. If so, then DHS will send an inspector to evaluate, then devise a site security plan (SSP) specific to that location. The goal is to have assurance that chemical inventories are secure and require locked storage with an alarm system. To date, 3100 SSP's have been conducted nationwide, 66 administrative orders have been done with compliance, and 3000 sites found ways to reduce their chemical inventory below threshold standards. There is no outside funding available for this.

17. Kennebec River Biosciences update (B. Kelleher)

Bill introduced himself to the committee and gave a brief overview of his lab. He went through 3 scenarios of the types of cases they see and discussed ongoing pathogen control plans. They strive to develop an integrated animal health approach using surveillance, inspections and other proactive measures to minimize disease impact and spread. He then discussed the difference between OIE and the AFS blue book testing approaches. International testing requirements can require OIE screening, but not always. Biosecurity measures are instrumental in plans he provides to fish producers. KRB develops USDA-approved, autogenous vaccines for use in hatcheries using local bacterial isolates.

18. Fish Kill Investigation Database and protocol (N. Phelps)

Nick defined a 'fish kill' as a localized event resulting in five or more fish dying from the same cause, although he suggested that definitions can vary among biologists. He pointed out that fish kills generally don't have a major impact on fish populations. Fish kills are good indicator of pathogen spread or for environmental problems.   Fish kill investigations can be hampered by poor response time, lack methods standardization, funding, and man power. Many agencies have developed fish kill databases and Minnesota has four. There is currently a project to advance the fish kill effort in Minnesota. From 2003 to 2013, 298 kills were reported, 236 were investigated, and 105 went to the pathology lab. Biologists feels the actual number of kills is ~500 per year and most occur in June. The cause of the kills are 33.8% environmental, 22% unknown, 22% infectious disease, 10% no information, and 10% chemical. Geographically, reporting bias occurs because of population base; more populated areas report more fish kills. Fish kill correlation with risk factors such as trophic state index, lake size, and tissue contamination was discussed. Kill data concerns have existed due to data reliability, under-reporting and missing data. The new approach includes more public involvement, methods standardization, improved database, and more timely response and reporting.

19. Agency updates (All)

New York:

- In May, there was a massive Atlantic Menhaden kill around Long Island and into the Hudson River. Disease appears to be 'Atlantic Menhaden Spinning Disease', a viral disease that first appeared in the Chesapeake in the 1950's. Virus identification is still underway.
- Sturgeon fin tissue was sent to Sharon Clouthier (FOC) in Winnipeg for Namaovirus testing. To date, only inland waters in Canada have been tested and this sample is the first from the Great Lakes. Results are pending.
- A study was conducted in 2014 to evaluate Chloramine T efficacy against *F. columnare* in Tiger Muskellunge at the South Otselic Fish Hatchery. Chloramine T was very effective and the FDA has agreed to approve the drug for all fish species.

Minnesota:

- Some hatcheries had mysterious disease Rainbow Trout and the cause is still unknown.
- At the Crystal Springs Hatchery, Lake Trout suffered from persistent furunculosis. Terramycin and Aquaflor treatments were ineffective, so they're considering ending the Lake Trout program since wild fish stocks are thriving.
- VHS surveillance continues and testing is required every two years.
- Minnow dealers no longer require transport permit, but disease testing is still required.

Michigan:

- Reported a novel detection of Heterosporis in Yellow Perch in Chicagan Lake.
- Brown Trout at the Oden Hatchery are suffering from IPN, although there may be other contributing causes.
- Yersinia ruckeri was isolated from Detroit River Muskellunge.
- No VHS was detected during the year.
- Crappie kill in Swan Lake likely caused by herbicide application.
- Using new approach to BKD-suspect adults by treating with erythromycin 28-days prior to spawn.

Ontario:

- Hired a new Fish Health Coordinator named Carrie Hobden.
- Broad fish pathogen survey of inland lakes was conducted looking for VHS and other pathogens. Results are pending.
- In the hatchery system, a few cases of furunculosis, BGD, and columnaris were reported. No serious problems have been reported.
- At-risk mussels and fish are becoming higher priority. Many of these species have been difficult to culture because little is known about them.
- OMNRF is working with the University of Guelph in launching a Lake Ontario Animal Health Network.
- Bloater reintroduction program is moving along.

Ohio

- Disease testing of wild and hatchery fish continues. Golden Shiner Virus was isolated from Fathead Minnows. Bluegill tested positive for Bluegill Picornovirus in Clearfork Reservoir.
- New bait dealer legislation is being developed that addresses inspections and certificate validity. Law enforcement now involved in checking certificates on the highways.

- Microcystis-related events have not been seen this year.
- *Bothriocephalus cuspidatus* infestion in Walleye is more prominent now.
- A new method to cryopreserve Sauger milt is being explored.

USFWS-Lamar

- No VHS was found in wild fish pathogen surveys this year.
- At Allegheny NFH, Lake Trout eye-up was 17%. Warm temperatures were to blame.
- There is interest in culturing coregonids at Lamar. Due to local IPN concerns, a UV system will be installed.

Indiana

- Wild fish from four locations were tested and no VHS or LMBV was found
- Aeromonas salmonicida has been a serious problem at the Mixsawbah Hatchery, BKD and ERM have not.
- For thiamine injection therapy in Steelhead, thiamine mononitrate is now used instead of HCL. Fish are injected at 40 mg/kg. EMS is not evident.
- A Purdue student surveyed green and bullfrog tadpoles for ranavirus and all tested positive for ranavirus 3.

Fisheries and Oceans Canada

- The CFIA launched a new VHS surveillance program using qPCR and virus isolation.
- Testing validation for an array of program pathogens is underway. The lab was audited and approved.
- Fish kill reports are less frequent now, so new fish kill hotline was developed.

Pennsylvania

- IPN is still problematic in culture operations. Looked for IPN-free Brown Trout sources for Lake Erie stocking program, so they are using Rome strain Brown trout from NYSDEC.
- Persistent furunculosis in several locations. Isolates are currently sensitive to both Rome and Terramycin.
- Cutthroat Trout Virus has been isolate in fish from three different hatcheries and not sure how to proceed. No mortalities have been attributed to it.
- Susquehanna Smallmouth Bass issues are lingering and time-consuming.
- Lake Erie research vessel has been funded, so wild fish will be collected for pathogen surveillance and sent to Lamar.

20. Technical Advisors (C. Yamashita)

Since George Ketola retired and Dale Honeyfield retiring soon, there is an urgent need to replace them with one or two other fish nutritionists. The committee suggested Ann Gannam (USFWS-Abernathy), Wendy Sealey (USFWS-Bozeman), or Jesse Trushenski (Southern Illinois University)

21. Next Meeting (All)

- The next meeting is in East Lansing on February 2nd and 3rd, 2016
- Summer meeting will be in Wisconsin.

Salmonid Herpesvirus Testing and Detection Update

Gavin Glenney, Patricia Barbash, Rick Cordes, Christina Cappelli, Michael Penn, Jennifer Johnson, and John Coll.

USFWS, Lamar Fish Health Center,
Lamar, PA 16848

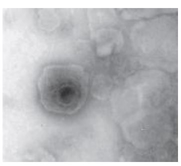
Epizootic epitheliotropic disease virus (EEDV/ SalHV3).

- A serious disease of yearling lake trout, *Salvelinus namaycush*, in the Great Lakes region of USA

Sue Marcquenski/Wisconsin DNR

Epizootic epitheliotropic disease virus (SalHV 3/EEDV).

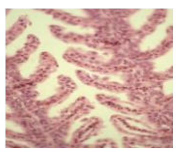
- *Alloherpesviridae*- fish and amphibians
- Enveloped icosahedral capsid
- Double stranded DNA genome



Ron Hedrick/University of California--Davis

Epizootic epitheliotropic disease virus (SalHV 3/EEDV).

- Clinical signs
  - -proliferative hyperplastic epithelial lesions
  - -rapid increase in mortalities, ataxia, spiral swimming
  - -hemorrhaging of the eyes
  - -lethargy with periods of hyperexcitability.(Bradely et al. 1988, Bradely et al. 1989, McAllister and Herman 1989)



Sue Marcquenski/Wisconsin DNR

Problems with EEDV diagnosis.

- 1. Can not culture EEDV on current cell lines.
- 2. Diagnosis by PCR. How do you confirm positives?
  - Terminase gene, polymerase, and glycoprotein genes (Waltzek et al. 2009)
  - sequence?
- 3. Histology- Screening wild pops.- costs $
- 4. At least in our hands, the current published PCR appears inconsistent with carrier or latent infections. (Kurobe et al. 2009)

What we decided to do:

- To increase sensitivity, we decided to develop real-time PCR assay.
- Selected terminase gene
- EPA Grant, Great Lakes Restoration Initiative-Project- Screen fish for emerging pathogens in Great Lakes.
- A tool to quantify viral loads in EEDV research.

- Real-time assay-Primer Express 3.0

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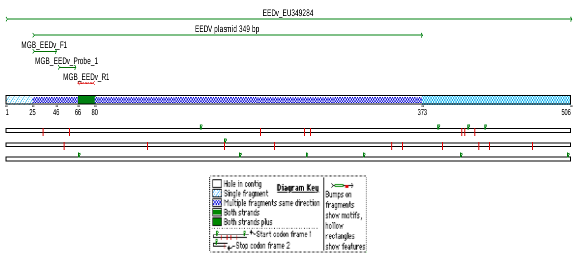
          A   F   V   N   L   T   S   I   T   S   L   I   P   L   M   L   V   A   G
SalHV3   GCC TTT GTG AAC CTC ACC TCC ATC ACT AGT CTG ATC CCC CTC ATG CTG GTC GCC GGG
SalHV1   GCC TTT GTC AAC CTC ACA TCC ATC ACT AGC CTC ATT CCG CTG ATG CTC GTC GCT GGC
SalHV2   GCC TTT GTC AAT CTC ACC TCG ATT ACC AGT CTC ATC CCT TTG ATG CTC GTC GCT GGT
SalHV4    CC TTT GTC AAC CTC ACC TCC ATC ACC AGT CTG ATT CCC CTC ATG CTG GTC GCC GGG
SalHV5                                 C ACA AGT CTG ATC CCC CTC ATG CTG GTC GCC GGG
```

Primers and TaqMan® MGB-probe locations are underlined within Epizootic epitheliotropic disease virus terminase gene (EU349284). 56 bp.

Herpesvirus salmonis- HPV/SalHV1 (EU349281)
Oncorhynchus masou virus- OMV/SalHV2 (EU349282)
Atlantic salmon papillomatosis virus- ASPV/SalHV4 (JX886026)
Namaycush herpesvirus- NamHV/SalHV5 (KP686092).

Positive Control- terminase gene

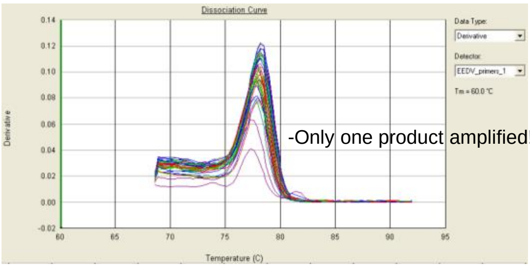
- Scott Weber's Lab, UC Davis, CA. –Kirsten Malm sent us positive control EEDV exposed fish skin.
- A plasmid was made containing 349bp EEDV insert.



Specificity?

Syber Green assay- primers only

Eight positive lake trout, (5) ten fold dilutions for a total of 40 samples (natural hatchery infection-samples from MSU)



-Only one product amplified!

Specificity?

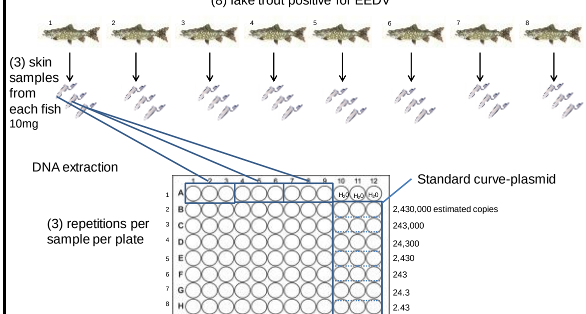
Pathogen	Source	Real-time PCR
SalHV1 (plasmid)	S. Weber, UCDavis	-
SalHV1	ATCC, cell culture	-
SalHV2 (plasmid)	S. Weber, UCDavis	-
CCV isolate 1	Lacrosse FHC, USFWS	-
CCV isolate 2	Lacrosse FHC, USFWS	-
ISAV cDNA	Lamar FHC, USFWS	-
IPNV (seg.A plasmid)	Lamar FHC, USFWS	-
LMBV	Lamar FHC, USFWS	-
R. salmoninarum	Lamar FHC, USFWS	-
F. psychrophilum	Lamar FHC, USFWS	-
M. cerebralis	Lamar FHC, USFWS	-
N. salmonis	Lamar FHC, USFWS	-

Specificity?

Pathogen	Source	Real-time PCR
SalHV1 (plasmid)	S. Weber, UCDavis	-
SalHV1	ATCC, cell culture	-
SalHV2 (plasmid)	S. Weber, UCDavis	-
SalHV4 (DNA, plasmid)	A. Doszpoly, Hungarian Acad. of Sciences	+
SalHV5 (DNA, plasmid)	Lamar FHC, USFWS	+
CCV isolate 1	Lacrosse FHC, USFWS	-
CCV isolate 2	Lacrosse FHC, USFWS	-
ISAV cDNA	Lamar FHC, USFWS	-
IPNV (seg.A plasmid)	Lamar FHC, USFWS	-
LMBV	Lamar FHC, USFWS	-
R. salmoninarum	Lamar FHC, USFWS	-
F. psychrophilum	Lamar FHC, USFWS	-
M. cerebralis	Lamar FHC, USFWS	-
N. salmonis	Lamar FHC, USFWS	-

Test for variance when using EEDV real-time assay-

(8) lake trout positive for EEDV



Total experiment was (5) assays on (5) separate days, multiple operators

This assay was able to detect a linear standard curve over nine logs of plasmid dilution (eight logs of naturally infected), and sensitive enough to detect single digit copies of EEDV.

-Consistent detection at the estimated 24.3 copy number dilution.

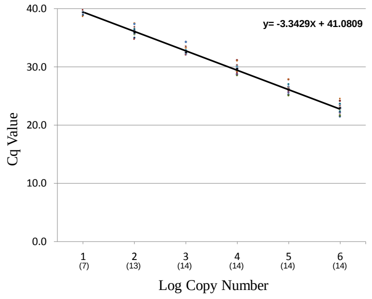
-Sporadic detection at the estimated 2.43 copy number dilution.

Both short and long-term precision of the EEDV real-time assay presented mean coefficient of variations below 10%.

These results are comparable to what has been found in a review of 33 published fish pathogen qPCR assays which found a majority of the assays had coefficients of variation under 15% for intra-assay variation (short-term precision/repeatability) (Getchell and Bowser, 2011).

Plasmid dilutions and PCR efficiencies.

A total of fourteen experiments.

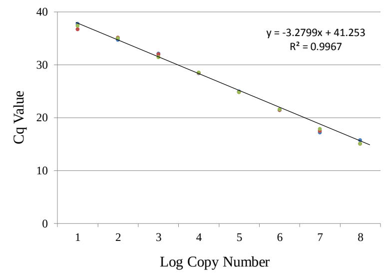


The mean PCR efficiency of the assay was 99.4 ±0.06% (SD), with a 95% confidence limit of 0.0296 (R^2= 0.994).

Efficiency = $-1 + 10^{(-1/slope)}$

Natural infection skin sample dilution#2

One standard curve- samples in triplicate

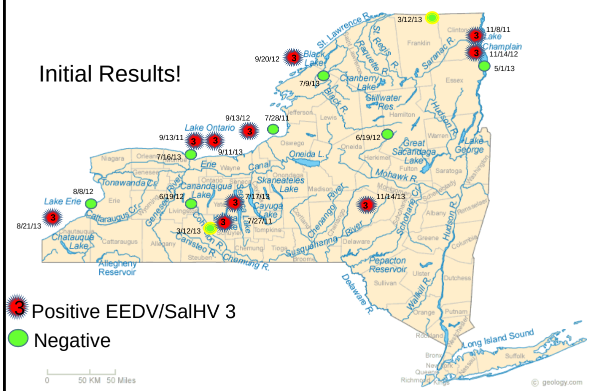


The assay's efficiency varied only slightly to 101.8% when we tested diluted naturally infected EEDV tissues (n=1).

Efficiency = $-1 + 10^{(-1/slope)}$

Lake trout, NY, VT- 2011-2013

Initial Results!

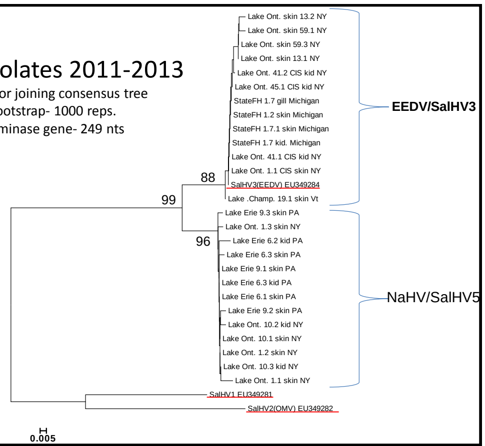


In wild lake trout showing no clinical signs of disease, SalHV detection tended to be on the lower end of viral detection of the real time assay, ranging between 4.0 to 827.8 copies/mg of tissue.

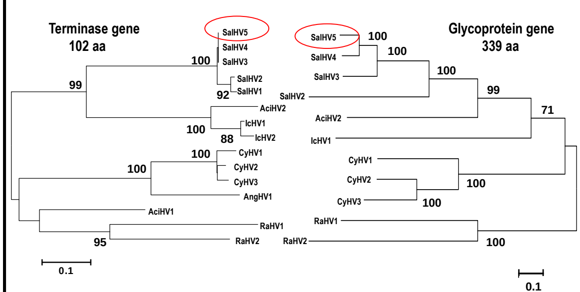
Needed nested PCR to get band for sequencing confirmations.

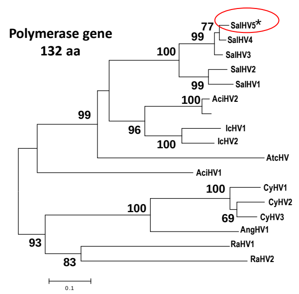
EEDV Isolates 2011-2013

Neighbor joining consensus tree
Bootstrap- 1000 reps.
terminase gene- 249 nts



Namaycush Herpesvirus/SalHV5

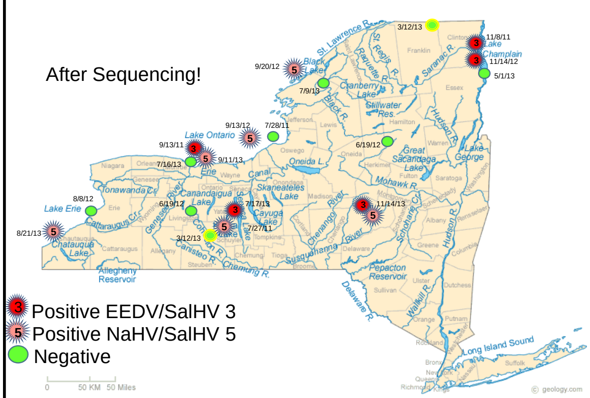




- According to Virus taxonomy, 9th report of the International Committee on Taxonomy of Viruses (Pellett et al. 2011), a herpesvirus may be classified as a species “if it has distinct epidemiological or biological characteristics and a distinct genome that represents an independent replicating lineage”.

- Distinct genetic difference (nts) between NaHV and EEDV.
  - Terminase >5%, glycoprotein >20%, polymerase- >10%
- NaHV and ASPV residing in different host species from separate geographic locations adds weight to NaHV being a separate species within the *Salmonivirus* genus.

Lake trout, NY, VT- 2011-2013



- How do we differentiate the various salmonid herpesvirus positives?

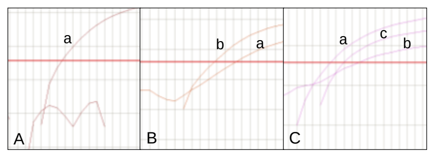
SalHV Multi-probe Assay Design

```
EEDV  SalHV3_EU349284   CCTTTGTGAACCTCACCTCCATCACTAGTCTGATCCCCCTCATGCTGGTCGCCGGG
ASPV  SalHV4_JX886026   CCTTTGTCAACCTCACCTCCATCACCAGTCTGATTCCCCTCATGCTGGTCGCCGGG
NHV   SalHV5                                     CACAAGTCTGATCCCCCTCATGCTGGTCGCCGGG
      SalHV3_F1         CCTTTGTGAACCTCACCTCCAT
      SalHV3_Probe                                ACTAGTCTGATCCCCC
      SalHV4_Probe                                  CAGTCTGATTCCCC
      SalHV5_Probe                               CACAAGTCTGATCCC
      SalHV3_R1                                                 ATGCTGGTCGCCGGG
```

a- SalHV3_Probe-FAM
b- SalHV4_Probe-VIC
c- SalHV5_Probe-NED

SalHV Multi-probe Results

a- SalHV3_Probe
b- SalHV4_Probe
c- SalHV5_Probe



Plasmids A. EEDV/SalHV3 B. ASPV/SalHV4 C. NaHV/SalHV5

SYBR® Green real-time PCR assay

```
SalHV3  430-TGGGAGTCCGTCGTCGAAAGTCCACGGAAGACCGAGGTGTTCGTGAGCTCCTGTGTGGAT-489
SalHV4      TGGAGCGCAGTGACCGAAAGCACCAAGAAGACCGAGGCCTTCGTCGACTCCTGTGTGGAG
SalHV5      TGGAGCGCAGTGACCGAAAGTGCCAAGAAGACCGAGGCGTTCGTCGGCTCCTGTGTGGGG

SalHV3  556-GGGGGTCCATCACCGTCGTTCACCAAGATAGCGGAGGCGTTCAGTAAAGTAATGGGGAGA-616
SalHV4      GGGGGACCCTCATTGGACTTTACCAAGATAGCCGAGGCCTTCAAGCGGGCAATTGGGGGGG
SalHV5      GGAGGACCATCATCGGACTTTACTAAGATAGCCGAGGCCTTCAAGCAGGCGATAGATGGGG

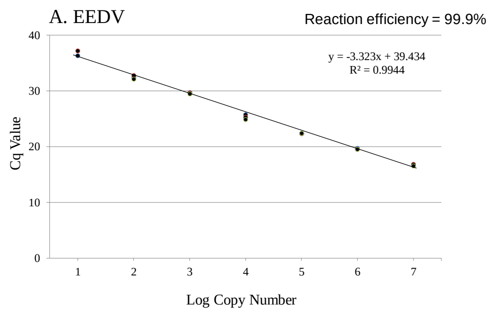
SalHV3 1310-GGACGGGACCGTTTACAGTATGTCCCGGGATTATAGCCACGATGGGCAAACTGGTCTTCA-1369
SalHV4      GCACGGGCGCGTTTGCAGTGTGTCCAAATATCATTGCCACAATGGGACGGTTGATCTTCA
SalHV5      GCACGGGTGCGTTTACAGTGTGTCCAAATATCATTGCCACAATGGGACGCTTGATCTTCA
```

Nucleotide numbering is based on EEDV glycoprotein sequence (JX886027).

Differentiation primer locations are underlined along respective aligned glycoprotein genes of salmonid herpesvirus members.
(EEDV/SalHV3- JX886027, NaHV/SalHV5- KP686091, and ASPV/SalHV4- JX886028).

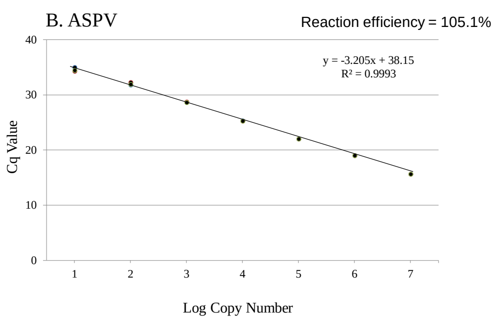
Nucleotide differences between genes are shaded.

EEDV SYBR® Green assay



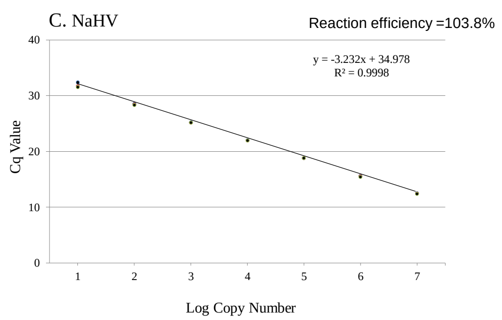
Plasmid was serially diluted tenfold between: (A. EEDV- 4.0 X 10^6 to 4.0 copies per reaction) (n=1).

ASPV SYBR® Green assay



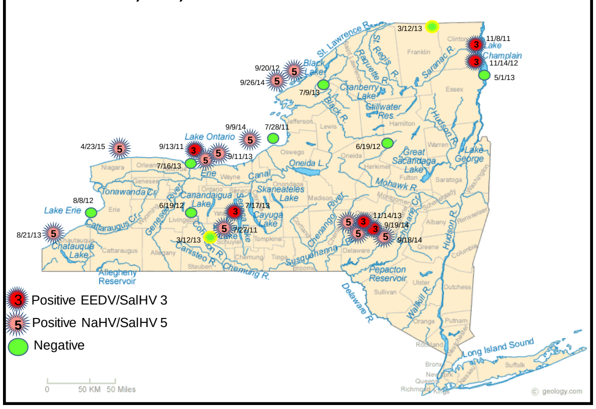
Plasmid was serially diluted tenfold between: (B. ASPV- 3.82 X 10^6 to 3.82 copies per reaction) (n=1).

NaHV SYBR® Green assay



Plasmid was serially diluted tenfold between: (C. NaHV- 3.19 X 10^6 to 3.19 copies per reaction) (n=1).

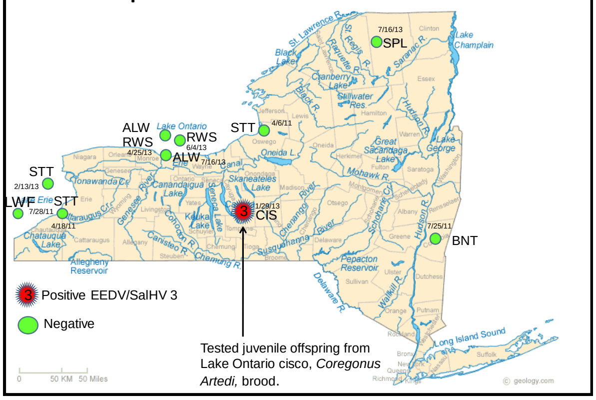
Lake trout, NY, VT- 2011-2015



New Hampshire- 2015

2012-13 Pennsylvania

Other species- 2011-2013



Hatcheries

Date	Water Body	Specie	State	Tissue sampled	n	Real-time PCR positives	Consistent real-time PCR positives[a]	Estimated range of virus copies detected per reaction <40 Ct	Ct range of real-time positives	Positive via semi-nested PCR assay	PCR amplicons cloned and confirmed by sequencing	Syber-Green glycoprotein PCR
11/8/12	Marquette State Fish Hatchery	LAT	MI	kidney	16	16	16	10.4 - 53,764.8	22-41	16[d]	1-SalHV3	N/A
				skin	16	16	16	272.0 - 952,923.0	18-29	16[d]	2-SalHV3	2-SalHV3
				gill	16	16	16	22.6 - 92,899.2	21-39	14[d]	1-SalHV3	N/A
3/12/13	Bath State Fish Hatchery	LAT	NY	kidney	64	0	N/A	N/A	N/A	N/A	N/A	N/A
3/12/13	Chateaugay State Fish Hatchery	LAT	NY	kidney	60	0	N/A	N/A	N/A	N/A	N/A	N/A
3/4/14	Les Voight State Fish hatchery	LAT	WI	kidney	20	0	N/A	N/A	N/A	N/A	N/A	N/A
1/29/13	Tunison Lab. of Aquatic Science, USGS	CIS	NY	skin	60	3	2	4.6 - 125.4	38-41	1	1	N/A
				kidney	60	5	3	5.5 - 37.2	37-42	2	2	N/A

Conclusions/Questions:

- EEDV seems more prevalent in wild fish than we first thought.
- Kidney, ventral skin, cranial skin, gill, and ovarian fluid.
- Skin samples appear to me more sensitive for detecting SalHV3 and 5 (head vs ventral body).
- Is the EEDV virus (low copy numbers) we are detecting latent? What is required for recrudescence?

Conclusions/Questions:

- Discovered new *Alloherpesviridae* member- Namaycush Herpesvirus/SalHV5. More prevalent than EEDV. Is this virus benign or pathogenic? Does it afford protection against EEDV?
- Found EEDV in domesticated *Coregonus artedi* (a.k.a. ciscos, or lake herring). Vertical transmission?
- EEDV TaqMan® assay is a sensitive and precise assay.
- It does cross-react with SalHV4 and SalHV5. When used with the SalHV SYBR® Green real-time PCR assay- it detects and differentiates between SalHV3, SalHV4, and SalHV5.

Questions?

Many thanks,

Scott Weber's Lab, UC Davis- SalHV1 and SalHV2 plasmid
Tom Waltzek's Lab, Univ. of Florida- SalHV4 sample
Mohamed Faisel's lab, MSU- SalHV3 infected tissues
USFWS- Lacrosse Fish Health Center- CCV DNA

Photos from: Fish Get Herpes, Too
Battling EED virus in lake trout.
Ken Phillips, USFWS
Eddies, Winter 2010/2011.

- To get a better understanding of EEDV prevalence in wild fish we initially screen with real-time assay. For confirmation- nested PCR, then sequencing, multi-probe assay, and now with SYBR® Green real-time PCR assay..

Namayacush herpesvirus percent identity with known members of *Alloherpeviridae*

Terminase gene

A	SalHV1	SalHV2	SalHV3	SalHV4	SalHV5	AciHV1	AciHV2	IcHV1	IcHV2	AngHV1	CyHV1	CyHV2	CyHV3
SalHV1		85.3	83.4	83.4	82.4	44.1	54.2	52.1	51.5	46.4	45.8	48.7	46.9
SalHV2	98.0		80.8	82.1	81.8	44.8	58.5	57.8	57.0	46.4	48.6	50.9	47.0
SalHV3	96.1	96.1		93.8	94.5	44.3	56.3	56.3	55.1	46.8	52.9	49.9	51.6
SalHV4	96.1	96.1	100.0		97.1	46.9	56.0	50.7	53.4	50.3	52.6	49.7	53.8
SalHV5	96.1	96.1	100.0	100.0		45.6	57.3	53.2	54.0	46.4	54.1	52.8	55.3
AciHV1	38.8	39.8	40.8	40.8	40.8		48.2	51.1	47.8	47.1	46.3	43.5	47.4
AciHV2	51.9	52.9	53.8	53.8	53.8	37.9		68.7	70.5	49.2	45.4	46.4	44.8
IcHV1	53.8	54.8	55.8	55.8	55.8	40.8	82.5		65.2	47.8	48.7	49.5	50.8
IcHV2	46.1	47.1	48.0	48.0	48.0	47.3	76.5	85.3		47.3	50.0	46.3	47.4
AngHV1	38.0	38.0	38.9	38.9	38.9	39.3	38.3	37.4	31.8		69.3	66.4	66.4
CyHV1	41.0	41.0	42.9	42.9	42.9	39.8	38.1	37.9	31.4	68.2		83.5	84.2
CyHV2	41.9	41.9	43.8	43.8	43.9	40.8	39.0	38.8	33.3	68.2	94.2		89.0
CyHV3	41.9	41.9	43.8	43.8	43.8	39.8	38.1	39.8	33.3	68.2	94.2	96.1	
RaHV1	38.9	38.9	39.8	39.8	39.8	44.2	36.5	37.5	32.7	36.1	40.4	40.4	38.3
RaHV2	41.0	41.0	41.9	41.9	41.9	44.2	39.6	42.1	38.5	41.7	42.1	43.0	43.3

Namayacush herpesvirus percent identity with known members of *Alloherpeviridae*

Glycoprotein gene

B	SalHV2	SalHV3	SalHV4	SalHV5	AciHV2	CyHV1	CyHV2	CyHV3	IchHV1
SalHV2		55.8	51.3	52.1	48.3	44.8	40.6	39.4	40.1
SalHV3	44.3		75.0	78.5	46.5	43.8	42.7	41.2	42.0
SalHV4	43.3	75.4		88.5	45.8	39.1	38.6	39.5	41.3
SalHV5	40.3	74.3	86.1		47.9	41.2	44.4	40.5	39.7
AciHV2	29.2	33.3	33.1	32.2		41.6	40.3	37.7	40.2
CyHV1	17.2	19.5	19.6	19.0	19.4		55.9	59.2	42.2
CyHV2	17.8	20.0	19.6	20.0	20.2	49.1		70.3	39.0
CyHV3	17.5	17.9	19.0	19.1	19.7	49.4	67.1		39.2
IchHV1	19.8	19.8	20.2	18.4	17.1	17.6	16.6	17.6	

Namayacush herpesvirus percent identity with known members of *Alloherpeviridae*

Polymerase gene

C	SalHV1	SalHV2	SalHV3	SalHV4	SalHV5	AciHV1	AciHV2	SbsHV	IcHV1	IcHV2	AtcHV	AngHV1	CyHV1	CyHV2	CyHV3	RaHV1	RaHV2
SalHV1		75.1	69.2	71.9	69.0	48.7	59.3	58.4	56.0	55.3	55.9	48.1	44.0	47.5	46.3	47.7	46.1
SalHV2	90.2		71.0	69.2	71.1	50.9	55.7	58.3	52.9	59.6	52.8	45.1	44.9	48.3	46.8	45.5	46.9
SalHV3	80.3	81.1		91.7	89.4	45.1	53.7	54.1	54.5	59.9	56.1	49.6	45.8	44.7	50.2	46.6	49.8
SalHV4	78.8	79.5	95.5		94.2	47.1	54.5	54.2	55.0	59.3	53.6	48.6	42.9	48.2	50.0	48.5	46.8
SalHV5	77.3	78.0	94.7	96.2		42.5	55.9	56.4	55.7	61.4	55.7	48.5	46.6	45.2	50.1	44.8	44.6
AciHV1	41.1	43.3	39.7	41.1	41.1		48.0	53.2	48.1	48.3	45.1	42.5	48.9	44.5	46.4	48.9	46.4
AciHV2	56.1	59.1	60.6	58.3	58.3	42.4		88.2	62.8	67.6	52.7	45.7	43.1	44.8	44.8	46.5	41.7
SbsHV	56.8	59.1	59.8	57.6	57.6	45.3	96.1		59.9	65.4	51.1	45.4	42.6	47.1	43.0	45.5	46.0
IcHV1	59.1	56.8	56.8	58.3	58.3	40.7	70.5	70.5		72.8	54.1	50.7	42.9	43.6	44.5	47.9	46.7
IcHV2	56.8	55.2	56.8	58.2	58.2	43.2	72.9	72.9	84.4		56.8	48.3	47.7	49.6	48.5	47.3	49.3
AtcHV	47.4	50.4	48.1	48.9	48.9	36.9	50.0	49.2	51.5	51.5		51.1	50.5	45.6	48.6	44.4	48.2
AngHV1	34.0	36.1	29.8	31.1	31.1	35.3	34.3	34.3	33.8	33.6	31.0		63.6	63.8	68.5	44.9	50.0
CyHV1	30.4	29.4	29.9	29.9	29.9	37.9	32.6	33.1	27.8	28.6	31.5	61.4		79.1	84.2	49.3	47.4
CyHV2	29.7	30.4	28.6	29.9	29.9	35.9	33.3	33.1	29.6	28.5	28.8	62.1	85.7		78.1	46.6	51.6
CyHV3	29.7	30.4	29.7	30.4	30.4	31.2	32.6	32.4	29.9	29.0	28.8	62.1	87.1	89.3		45.0	51.5
RaHV1	36.0	33.8	34.8	36.1	36.1	38.1	35.4	35.4	36.1	33.8	27.6	36.6	36.4	34.3	35.5		57.4
RaHV2	35.6	36.4	33.3	34.1	34.1	36.0	31.8	31.8	31.0	34.1	32.3	35.3	39.4	36.4	36.4	47.5	

Deduced amino acid sequences from 249 bp terminase PCR products

```
Lake_Ont._skin_13.2_NY        --------------AFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEVTGGTGDLGNLVSDS
Lake_Ont._skin_13.3_NY        --------------AFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Ont._skin_59.3_NY        --------------AFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake Ont. skin 59.1 NY        --------------AFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSGS
Lake_Champ.19.1_skin_Vt       NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Champ.19.2_skin_Vt       NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Erie_6.2_kid_PA          NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALPSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Erie_6.3_kid_PA          NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Erie_6.1_skin_PA         NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Erie_6.3_skin_PA         NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Erie_9.1_skin_PA         NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Erie_9.2_skin_PA         NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMSPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Erie_9.3_skin_PA         NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
State_FH_1.7a_gill_MI         NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
State_FH_1.2a_skin_MI         NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
State_FH_1.7.1_skin_MI        NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
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Lake_Ont._10.2_kid_NY         NNVGDIIDETTGEPAFHVISQKFKCGAHVHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Ont._10.3_kid_NY         NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Ont._1.1_skin_NY         NNVGDIIDETTGEPAFHVISQKSKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVALGGEMEITGGTGDLGNLVSDS
Lake_Ont._1.2_skin_NY         NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Ont._1.3_skin_NY         NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Ont._10.1_skin_NY        NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Ont._41.1_CIS_kid_NY     NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Ont._41.2_CIS_kid_NY     NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Ont._45.1_CIS_kid_NY     NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
Lake_Ont._1.1_CIS_skin_NY     NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
EEDv_EU349284                 NNVGDIIDETTGEPAFHVISQKFKCGAHMHLPGLTCPCEAVYCPSHIDMNPATQALLSCVAPGGEMEITGGTGDLGNLVSDS
                                            ******** *****:*******************.****** **** *****:************ *
```

All but two of the AA changes appear to be conserved

2011 EEDV Testing-Lake Trout

Sample Date	Water Body	State	Tissue sampled	Fish sampled	Positive via real-time PCR assay	Consistently positive via real-time PCR assay[a]	Estimated range of virus copies detected per reaction <40 Ct	Ct range of real-time positives	Positive via semi-nested PCR assay	PCR amplicons cloned and confirmed by sequencing	Multi-probe PCR confirmed	Case #
7/27/2011	Keuka Lake	NY	kidney	28	8	3	N/A	36-40	2[b]	N/A	3-SalHV5	11-327
7/28/2011	Lake Ontario	NY	kidney	15	0	N/A	N/A	N/A	N/A	N/A		11-328
			skin	15	0	N/A	N/A	N/A	N/A	N/A		
9/13/2011	Lake Ontario	NY	kidney	20	8	0	1.0-14.0	34-40	0	N/A		11-394
			skin	60	19	9	0.7-10.5	35-40	2[c]	2-SalHV3		
11/8/2011	Lake Champlain	VT	ovarian fluid	4(5fp)[e]	2(5fp)	2(5fp)	43.0 - 6,791.0	30-37	2(5fp)[b]	2(5fp)-SalHV3		12-049

Notes:

a. Samples positive by two repetitions in a single assay, and positive in two or more separate real-time assays upon re-extraction.
b. Semi-nested PCR primers for first round (40_F and 249_R), and second (150_F and 249_R).
c. Semi-nested PCR primers for first round (223_F and 224_R), and second (40_F and 224_R).
d. Semi-nested PCR primers for first round (194_F and 249_R), and second (214_F and 249_R).
e. 4(5fp) = A total of twenty fish pooled into four pools of five fish each.

N/A= not attempted

2012 EEDV Testing- Lake Trout

Sample Date	Water Body	State	Tissue sampled	Fish sampled	Positive via real-time PCR assay	Consistently positive via real-time PCR assay[a]	Estimated range of virus copies detected per reaction <40 Ct	Ct range of real-time positives	Positive via semi-nested PCR assay	PCR amplicons cloned and confirmed by sequencing	Multi-probe PCR confirmed	Case #
6/19/2012	Fourth Lake, Fulton Chain	NY	kidney	5	0	N/A	N/A	N/A	N/A	N/A		12-286
			skin	5	0	N/A	N/A	N/A	N/A	N/A		
			gill	5	0	N/A	N/A	N/A	N/A	N/A		
6/19/2012	Hemlock Lake	NY	kidney	20	0	N/A	N/A	N/A	N/A	N/A		12-287
			skin	20	0	N/A	N/A	N/A	N/A	N/A		
8/7/2012	Lake Erie	PA	kidney	14	1	1	3.4	37-38	1[d]	1-SalHV5		12-364
			skin	14	2	2	.38 - 3.9	36-41	2[d]	2-SalHV5		
8/8/2012	Lake Erie	NY	kidney	30	0	N/A	N/A	N/A	N/A	N/A		12-365
			skin	30	0	N/A	N/A	N/A	N/A	N/A		
9/13/2012	Lake Ontario	NY	kidney	12	1	0	-	43	N/A	N/A		12-398
			skin	12	4	2	-	41-45	0	N/A	2-SalHV5	
9/20/2012	Lake Ontario	NY	kidney	20	3	2	.38 -3.1	35-42	1[d]	1-SalHV5		12-406
			skin	20	15	7	.21 - 4.6	35-44	2[d]	2-SalHV5	4-SalHV5	
11/8/2012	Marquette State Fish Hatchery	MI	kidney	16	16	16	10.4 - 53,764.8	22-41	16[d]	1-SalHV3		13-44
			skin	16	16	16	272.0 - 952,923.0	18-29	16[d]	2-SalHV3	6-SalHV3	
			gill	16	16	16	22.6 - 92,899.2	21-39	14[d]	1-SalHV3		
11/14/2012	Lake Champlain	VT	kidney	18	6	4	.9 - 7.2	35-40	2[d]	-		13-52
			skin	18	15	11	.96 - 413.8	31-38	8[d]	1-SalHV3	6-SalHV3	
			ovarian	4	1	0	0.5	39	0	N/A		

Notes:

a. Samples positive by two repetitions in a single assay, and positive in two or more separate real-time assays upon re-extraction.

b. Semi-nested PCR primers for first round (40_F and 249_R), and second (150_F and 249_R).

c. Semi-nested PCR primers for first round (223_F and 224_R), and second (40_F and 224_R).

d. Semi-nested PCR primers for first round (194_F and 249_R), and second (214_F and 249_R).

2013 EEDV Testing- Lake Trout

Sample Date	Water Body	State	Tissue sampled	Fish sampled	Positive via real-time PCR assay	Consistently positive via real-time PCR assay[a]	Estimated range of virus copies detected per reaction <40 Ct	Ct range of real-time positives	Positive via semi-nested PCR assay	PCR amplicons cloned and confirmed by sequencing	Multi-probe PCR confirmed	Case #
3/12/2013	Bath State Fish Hatchery	NY	kidney	64	0	N/A	N/A	N/A	N/A	N/A		13-122
3/12/2013	Chateaugay State Fish Hatchery	NY	kidney	60	0	N/A	N/A	N/A	N/A	N/A		13-123
7/9/2013	Sixberry Lake	NY	skin	10	0	N/A	N/A	N/A	N/A	N/A		13-279
7/16/2013	Lake Ontario	NY	skin	2	0	N/A	N/A	N/A	N/A	N/A		13-285
7/17/2013	Seneca Lake	NY	skin	42	4	3	N/A	N/A	N/A	N/A	3-SalHV3	13-294
8/1/2013	Lake Erie	PA	skin	41	3	3	N/A	N/A	N/A	N/A	3-SalHV5	13-316
8/21/2013	Lake Erie	NY	skin	30	18	9	N/A	N/A	N/A	N/A	9-SalHV5	13-328
9/11/2013	Lake Ontario	NY	skin	20	5	3	N/A	N/A	N/A	N/A	3-SalHV5	13-350
11/14/2013	Otsego Lake	NY	skin	10	3	3	N/A	N/A	N/A	N/A	2-SalHV3 1-SalHV5	14-28
5/1/2013	Lake Champlain	VT	skin	15-fry	0	N/A	N/A	N/A	N/A	N/A		14-33

Notes:

a. Samples positive by two repetitions in a single assay, and positive in two or more separate real-time assays upon re-extraction.

b. Semi-nested PCR primers for first round (40_F and 249_R), and second (150_F and 249_R).

c. Semi-nested PCR primers for first round (223_F and 224_R), and second (40_F and 224_R).

d. Semi-nested PCR primers for first round (194_F and 249_R), and second (214_F and 249_R).

e. 4(5fp) = A total of twenty fish pooled into four pools of five fish each.

N/A= not attempted

2011-13 EEDV Testing- other species

Sample Date	Water Body	State	Species	Tissue sampled	Fish sampled	Positive via real-time PCR assay	Consistently positive via real-time PCR assay[a]	Estimated range of virus copies detected per reaction <40 Ct	Ct range of real-time positives	Positive via semi-nested PCR assay	PCR amplicons cloned and confirmed by sequencing
4/6/2011	Salmon River	New York	steelhead trout	kidney	60	0	N/A	N/A	N/A	N/A	N/A
				gill	60	0	N/A	N/A	N/A	N/A	N/A
				ovarian fluid	30	0	N/A	N/A	N/A	N/A	N/A
4/18/2011	Cattaraugus Creek	New York	steelhead trout	kidney	30	0	N/A	N/A	N/A	N/A	N/A
7/25/2011	Roeliff Jansen Kill	New York	brown trout	kidney	3(5fp)[e]	0	N/A	N/A	N/A	N/A	N/A
7/28/2011	Lake Ontario	New York	lake white fish	kidney	25	0	N/A	N/A	N/A	N/A	N/A
1/29/2013	Tunison Laboratory of Aquatic Science, USGS	New York	cisco	skin	60	3	2	4.6 - 125.4	38-41	1	1
				kidney	60	5	3	5.5 - 37.2	37-42	2	2
2/13/2013	Lake Erie	Penn	steelhead trout	kidney	60	0	N/A	N/A	N/A	N/A	N/A
4/25/2013	Lake Ontario	New York	rainbow smelt	kidney/ spleen	4(5fp)	0	N/A	N/A	N/A	N/A	N/A
			alwife	kidney/ spleen	6(5fp)	0	N/A	N/A	N/A	N/A	N/A
6/4/2013	Lake Ontario	New York	rainbow smelt	skin	20	0	N/A	N/A	N/A	N/A	N/A
7/16/2013	Lake Ontario	New York	alwife	skin	15	0	N/A	N/A	N/A	N/A	N/A
7/16/2013	Lake Ozonia	New York	splake	skin	4	0	N/A	N/A	N/A	N/A	N/A

Plasmid dilutions and PCR efficiencies.

A total of fourteen experiments over 1 year period.

No experiments	Estimated template copies	Mean Cq	SD	Interrun CV (%)
14	241000	22.92	0.881	3.845
14	24100	26.36	0.817	3.098
14	2410	29.63	0.812	2.74
14	241	32.92	0.379	2.245
13	24.1	36.09	0.803	2.223
7	2.41	39.34	0.48	1.219
			Mean	2.561667

The slope of the PCR should be (-3.1 to -3.6) yielding an (Eff. from 110% to 90%)

For a total of 14 assays-		
	Mean slope-	-3.343
	Mean efficiency	0.99
	Efficiency SD	0.06
	Efficiency 95%CI	0.0296

Efficiency = $-1 + 10^{(-1/slope)}$

Real-Time Quantitative PCR
Assay Data Analysis,
Evaluation
and
Optimization
A Tutorial on
Data Analysis and Evaluationand
Trouble-Shooting Sub-Optimal Real-Time
QPCR Experimentsby
Rainer B. Lanz,
M.S., Ph.D.
February 14. 2008

- Implications
- – The calculations of precision given above have been questioned in some peer-reviewed publications.
- – Replicate standard curves may produce potentially large inter-curve variations.
- – In general, the intra-assay variation of 10-20% and a mean inter-assay variation of 15-30% on molecule basis is realistic over the wide dynamic range (of over a billion fold range).
- – Variability is highest at >10 7 and <10 2 template copy ranges
- • Cut-off value: cycle 35, i.e. disregard CT values for cycle numbers 36 and higher.
- – For the threshold methods, the precision is dependent on the proper setting of the threshold, which itself is dependent on proper base line settings.

Repeatability (intraassay variance)

Fish	Sample	Estimated mean copies/rxn	SD	CV (%)
1	1	32660.5	2535.8	7.7
1	2	17189.7	2247.9	13.2
1	3	20299.4	2053.2	10.2
2	1	5947.2	452.0	7.6
2	2	4342.2	249.7	5.4
2	3	22315.6	1312.3	6.1
3	1	110879.3	5342.1	4.8
3	2	11726.2	955.5	8.3
3	3	10309.9	909.1	8.8
4	1	10670.8	1005.6	9.4
4	2	9840.5	750.7	8.0
4	3	13090.4	467.2	3.7
5	1	28042.3	2064.1	7.7
5	2	37087.0	3446.3	9.3
5	3	57439.5	3707.1	7.0
6	1	55826.1	5181.1	9.2
6	2	24919.5	2269.1	9.2
6	3	46211.3	3453.7	7.8
7	1	77252.2	8761.5	11.3
7	2	18761.3	1642.9	9.0
7	3	30675.1	3930.4	9.6
8	1	21588.3	1894.4	8.7
8	2	29005.3	2051.9	7.0
8	3	26893.3	4546.3	16.9

Reproducibility (interassay variance)

Fish	Estimated mean copies/rxn	SD	CV (%)
1	23383.2	1804.6	7.7
2	10868.4	812.4	7.5
3	44305.1	2172.4	4.9
4	11000.6	725.7	6.6
5	40856.3	4545.2	11.1
6	42318.9	3122.7	7.4
7	42229.5	3954.4	9.4
8	25829.0	3552.5	13.8

CV- Shows the extent of variability in relation to mean of the population.
It is often expressed as a percentage, and is defined as the ratio of the standard deviation to the mean (or its absolute value,).
The CV or RSD is widely used in analytical chemistry to express the precision and repeatability of an assay.

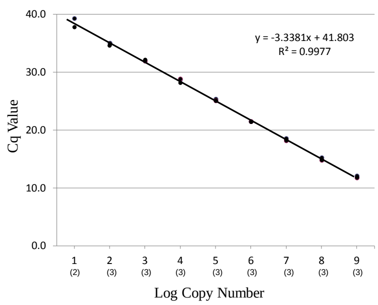
Samples (inter-tissue variance)

	Tissues within Fish		
Fish	Estimated mean copies/rxn	SD	CV (%)
1	23383.2	8198.2	35.1
2	10868.4	9949.9	91.5
3	44305.1	57659.8	130.1
4	11000.6	1407.3	12.8
5	40856.3	15389.0	37.7
6	42318.9	15836.9	37.4
7	42229.5	30923.1	73.2
8	25829.0	3901.2	15.1

Highest SD and CV % observed, could be due to error in tissue collection, and/or DNA extraction between tissues, or due to localization of virus in skin samples.

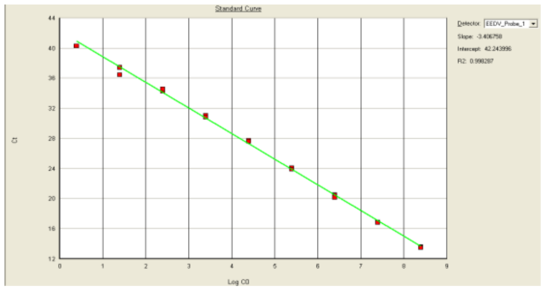
Plasmid dilutions and PCR efficiencies.

One standard curve- samples in triplicate



Efficiency = -1 + $10^{(-1/slope)}$

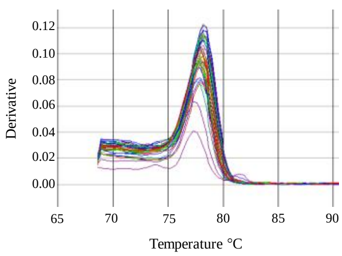
Analytical sensitivity- limit of detection (LOD)



-Plasmid standard curve is linear over 8 logs of plasmid dilution.

-Consistent detection at the estimated 24.3 copy number dilution.

-Sporadic detection at the estimated 2.43 copy number dilution.



Computer programs used:

- Real-time assay-Primer Express 3.0

- EEDV_EU349284_terminase_gene

ATTTCATCCTCGTCGACGAGGCCGCCTTTGTGAACCTCACCTCCATCACTAGTCTGATCC
CCCTCATGCTGGTCGCCGGGCGAAAGCAGATCCACATTTCTTCCCACGTGGCCAAATC
TTGGATTAACAACGTGGGCGACATTATCGACGAAACAACGGGGGAGCCGGCGTTTCA
TGTTATCTCTCAGAAGTTTAAATGCGGTGCGCACATGCACCTACCAGGTCTGACGTGTC
CCTGTGAAGCAGTCTACTGCCCCAGTCACATAGATATGAACCCCGCTACGCAGGCCCTG
CTCAGCTGTGTGGCCCCCGGGGGAGAAATGGAGATCACAGGTGGCACCGGTGACTT
GGGTAATCTGGTGTCGGACTCGACCTTCCCCTTCCCAGATGAGACGGTGCACAAGATA
ATGAACGATGTGATTGATATCAATGACCCGGGCGCCGAAGTTTCGGCTTTCTACATTGC
CATTGACCCCACCTATTCTTCCGGCAGCCAATCGTCAATG

Forward primer	5’-CCTTTGTGAACCTCACCTCCAT-3’	
Reverse primer	5’-CCCGGCGACCAGCAT-3’	56 bp
Hydrolysis probe	6FAMACTAGTCTGATCCCCCMGBNFQ	

NEW YORK STATE OF OPPORTUNITY | Department of Environmental Conservation

Thiamine Deficiency in Salmon River Steelhead

Andrew D. Noyes

November 2014 – Salmon River

- Anglers report distressed Steelhead in the river
- Swimming erratically
- Some mortality

NEW YORK STATE OF OPPORTUNITY | Department of Environmental Conservation

The Investigation Begins- Step 1

- 3 fish sent to Cornell for diagnosis

Cornell University College of Veterinary Medicine

NEW YORK STATE OF OPPORTUNITY | Department of Environmental Conservation

The Investigation Begins- Step 1

- 3 fish sent to Cornell for diagnosis
- **No Significant Findings**
- Tissues saved for further analysis……

Cornell University College of Veterinary Medicine

NEW YORK STATE OF OPPORTUNITY | Department of Environmental Conservation

The Investigation Begins- Step 2

- 6 fish sent to USGS in Wellsboro for tissue thiamine analysis

USGS
science for a changing world

The Investigation Begins- Step 3

- We injected fish with thiamine to see if they would recover

The Investigation Begins- Step 3

- We injected fish with thiamine to see if they would recover

  THEY DID

Total Thiamine (nmole/g)

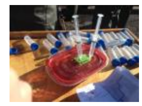
	Normal	SR SHD
Liver	20 - 25	
Muscle	2 - 4	

Total Thiamine (nmole/g)

	Normal	SR SHD
Liver	20 - 25	4.3 - 9.5
Muscle	2 - 4	0.4 - 0.6

Thiamine Therapy

- Injected 1100 fish returning to SRSFH
- 50 mg/kg of fish
- Goal = Revitalize for spawning.



Thiamine Therapy Outcome

- We thought we lost 30%.....

Thiamine Therapy Outcome

- We thought we lost 30%.....
- Only 300 fish left for egg take (73% loss)

Thiamine Therapy Outcome

- We thought we lost 30%.....
- Only 300 fish left for egg take (73% loss)
- Injectees and other early fish had poor egg quality
- Due to cold weather, gluco-reg collapse, unsuitable pond conditions….

Late Arrivals Saved the day

- Egg survival (eye-up)…..

Late Arrivals Saved the day

- Egg survival (eye-up)…..
  - From early arriving adults = 34.4% (213K)
  - From late arriving adults = 51.9% (947K)
  - NO EMS

Next Steps…

- Thiamine deficiency is still a mystery

Next Steps…

- Thiamine deficiency is still a mystery
- Thiamine Summit held at USGS Tunison

Next Steps…

- Thiamine deficiency is still a mystery
- Thiamine Summit held at USGS Tunison
  1. What have we learned?
  2. Where do we go from here?

Next Steps…

- Thiamine deficiency is still a mystery
- Thiamine Summit held at USGS Tunison
  1. What have we learned?
  2. Where do we go from here?
- NYSDEC trying to fund thiamine studies

Thank You

- **Andy Noyes**
- Pathologist 2 (Aquatic)
- Rome Field Station
- 8314 Fish Hatchery Rd
- Rome, NY 13440
- andrew.noyes@dec.ny.gov
- 315-337-0910

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Salmon River steelhead trout muscle thiamine values (nmol/g)

Fish Behavior	TPP	TP	T	Total T
Recently deceased female	0.32	0.06	0.08	0.46
Very lively male	0.55	0.07	0.0	0.62
Barely alive female	0.44	0.06	0.0	0.50
Barely alive male	0.38	0.08	0.04	0.50
Very lively male	0.56	0.07	0.0	0.63
Very lively female	0.34	0.06	0.0	0.40

Normal muscle thiamine (total) should be 2-4 nmol/g

Salmon River steelhead trout liver thiamine values (nmol/g)

Fish Behavior	TPP	TP	T	Total T
Recently deceased female	4.5	0.6	0.1	5.2
Very lively male	3.4	0.7	0.3	4.4
Barely alive female	3.8	0.9	0.2	4.9
Barely alive male	3.2	0.8	0.3	4.3
Very lively male	4.1	0.7	0.2	5.0
Very lively female	7.8	1.5	0.2	9.5

Normal liver thiamine (total) should be 20-25 nmol/g

NEW YORK STATE OF OPPORTUNITY | Department of Environmental Conservation

If You See Wigglers, Blame It On Their Diet

Rodman G. Getchell, Geofrey E. Eckerlin, Andrew D. Noyes, Steven R. LaPan, Dale C. Honeyfield, Kelly L. Sams, Hélène Marquis, and Paul R. Bowser

- "In October, there were a ton of dead steelhead at the bottom of the Douglaston Salmon Run. You had spin and bait guys blaming fly fishermen, and fly fishermen blaming gear fishermen. It got ugly," said one steelhead angler.

VS

Background

- "The bottom line is there were (fewer) salmon to catch this year and the steelhead took a beating. They need to do some testing, which will take a little time. Until then, everyone has their own theory, …and being caught multiple times isn't helping it."

Background

- Some of the early reports described fish as swimming in circles and appearing to be in distress, but dead fish also started turning up. Some fishing blogs used the term "wigglers" to describe these moribund steelhead.

Background

◆ **Scientists puzzled by dead steelhead in the Salmon River and other Lake Ontario tributaries** -- David Figura | dfigura@syracuse.com
Follow on Twitter on December 12, 2014 at 3:29 PM

◆ Dead steelhead have been turning up on the banks of the Salmon River in Oswego County in recent weeks. There have been anecdotal reports of the same thing happening in other Lake Ontario tributaries.

◆ NYSDEC received the first reports of steelhead swimming erratically during the third week of November. On Nov. 21, DEC staff submitted several dying fish for analysis to the Cornell Aquatic Animal Health Lab.

◆ **Cornell scientist: 'Nutritional disease' may have killed steelhead on Salmon River**

◆ Recent reports of steelhead exhibiting strange behaviors and dying along the Salmon River may be result of a nutritional issue.

◆ DEC fisheries biologists have speculated that a vitamin B_1 deficiency (thiamine) is the cause, and that in addition to Cornell, DEC has sent steelhead tissue samples to a USGS lab in Pennsylvania for testing.

◆ The DEC also injected several 'sick' fish with 25 mg/kg vitamin B_1, and another small group with saline solution.

◆ Great Lakes fish predators (including salmon and steelhead) that feed primarily on alewife are prone to thiamine deficiency. Little can be done to alleviate the mortality of adult steelhead that are unable to ascend the river and reach the hatchery's holding facilities.

◆ A thiamine deficiency can impact the survival of eggs and newly hatched fish, and, in severe cases, can cause the death of adult fish.

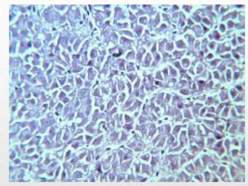
◆ Although moderate thiamine deficiencies are not uncommon in top predator fish in Lake Ontario, this year's acute deficiency is atypical in its severity.

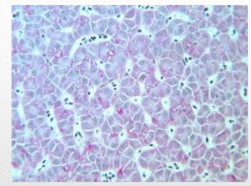
Thiamine Deficiency

◆ Three affected steelhead trout were injected with 25 mg/kg thiamine.
◆ Another three affected steelhead trout were injected with 25 mg/kg saline.
◆ Steelhead that received thiamine were active and alert after 48 hours, and those that received saline remained listless and unresponsive.

Salmon River steelhead trout
liver and muscle total thiamine values (nmol/g)

Fish Behavior	Liver	Muscle
Recently deceased female	5.2	0.46
Very lively male	4.4	0.62
Barely alive female	4.9	0.50
Barely alive male	4.3	0.50
Very lively male	5.0	0.63
Very lively female	9.5	0.40
Normal range	20-25	2-4



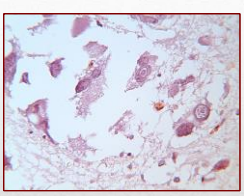


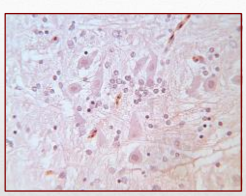
Salmon River steelhead   Chautauqua Creek steelhead

- Apparent decrease in glycogen granules (pink staining) in liver from moribund steelhead.

PAS Staining of steelhead liver

Salmon River steelhead   Chautauqua Creek steelhead

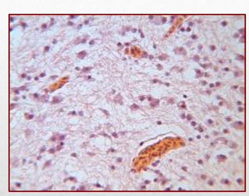


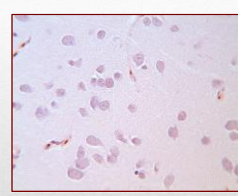


- Possible degenerative changes including neuropil vacuolation noted in the Salmon River steelhead samples.

H&E Staining of steelhead brain (40X)

Salmon River steelhead   Chautauqua Creek steelhead





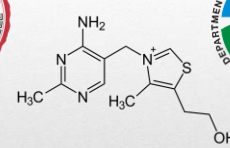
- Neuropil is the complex net of axonal, dendritic, and glial branchings that forms the bulk of the central nervous system.

H&E Staining of steelhead brain (40X)

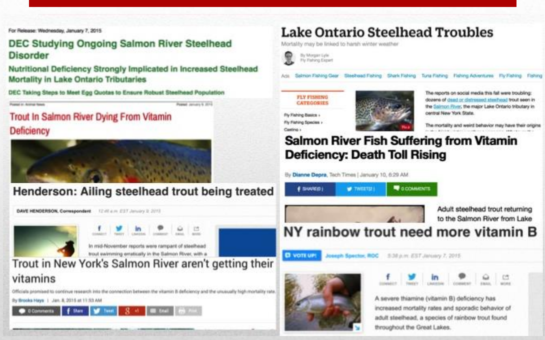
SUMMARY

- The NYSDEC sent dead fish samples to the Cornell Aquatic Animal Health Lab. Results were "inconclusive" though histological comparisons with reference steelhead may show glycogen differences in the liver and possible brain lesions. Further quantification of these glycogen differences is needed.
- Affected steelhead that received vitamin B_1 were active and alert after 48 hours, and those that received saline remained listless and unresponsive.
- Liver thiamine (total) should be 20-25 nmol/g and the Salmon River steelhead ranged from 4.3 to 9.5 nmol/g. Healthy muscle should have 2-4 nmol/g and these fish ranged from 0.32 to 0.56 nmol/g.
- Staff at the Salmon River Fish Hatchery in Altmar have been injecting steelhead captured on the river with 50 mg/kg of vitamin B_1, and then holding them in ponds and feeding a diet fortified with vitamin B_1 to improve the likelihood of successful steelhead egg collections in 2015.

Questions...



More Headlines



Salmon River steelhead trout muscle thiamine values (nmol/g)

Fish Behavior	TPP	TP	T	Total T
Recently deceased female	0.32	0.06	0.08	0.46
Very lively male	0.55	0.07	0.0	0.62
Barely alive female	0.44	0.06	0.0	0.50
Barely alive male	0.38	0.08	0.04	0.50
Very lively male	0.56	0.07	0.0	0.63
Very lively female	0.34	0.06	0.0	0.40

Normal muscle thiamine (total) should be 2-4 nmol/g

Salmon River steelhead trout
liver thiamine values (nmol/g)

Fish Behavior	TPP	TP	T	Total T
Recently deceased female	4.5	0.6	0.1	5.2
Very lively male	3.4	0.7	0.3	4.4
Barely alive female	3.8	0.9	0.2	4.9
Barely alive male	3.2	0.8	0.3	4.3
Very lively male	4.1	0.7	0.2	5.0
Very lively female	7.8	1.5	0.2	9.5

Normal liver thiamine (total) should be 20-25 nmol/g

Thiamine Research

Great Lakes Fish Health Committee Meeting

July 28, 2015

Thiamine

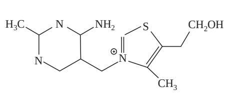
Vitamin B_1

Water soluble

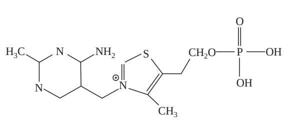
Required in diet

Major roles in growth, physiology, and metabolism

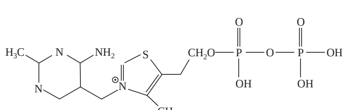
Thiamine



Thiamine (free base)
TH



Thiamine monophosphate
TMP



Thiamine pyrophosphate
(cocarboxylase)
TPP

Thiamine Measurement

HPLC Method

Brown et al. 1998

TH
TMP
TPP

Rapid solid-phase extraction fluorometric method

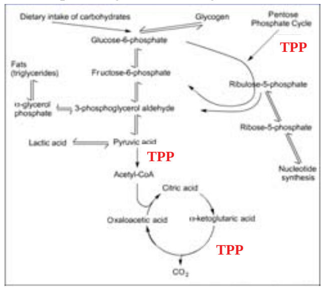
Zajicek et al. 2005

Phosphorylated thiamine
Unphosphorylated thiamine

Thiamine Roles

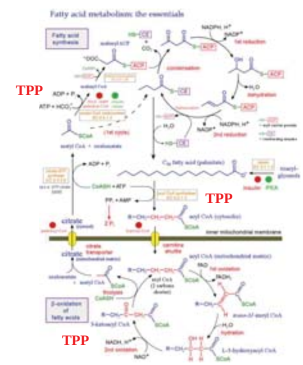
TPP is a coenzyme

Thiamine requirements in the metabolic pathways of carbohydrates



Thiamine Roles

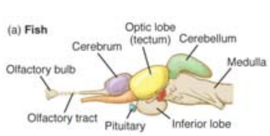
Thiamine requirements in the metabolic pathways of fatty acids



Thiamine Roles

Thiamine is uniformly distributed throughout the nervous system and appears to be highly localized in membrane structures

The most important function of thiamine in the nervous system (aside from providing energy for normal processes) is the production of acetylcholine, a neurotransmitter that transmits electrical signals between nerve endings



Thiamine Transfer

John D. Fitzsimons, Scott B. Brown, Dale C. Honeyfield and John G. Hnath

A Review of Early Mortality Syndrome (EMS) in Great Lakes Salmonids: Relationship with Thiamine Deficiency

Ambio 28: 9-15 (1999)

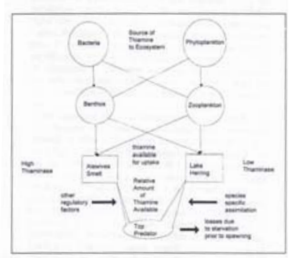
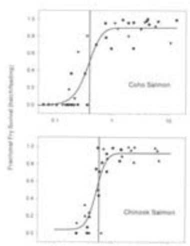


Figure 2. Production and flux of thiamine in a Great Lakes aquatic food chain and hypothesized linkage between diet and development of a thiamine deficiency in top predators.

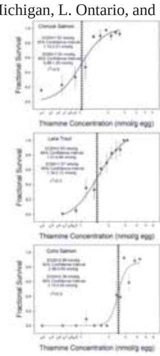
Thiamine Requirement

Coho and Chinook salmon
(L. Michigan and L. Huron)



Hornung et al. 1998

Chinook salmon, Lake Trout and Coho Salmon
(L. Michigan, L. Ontario, and L. Huron)



Fitzsimons et al. 1998

Thiamine Repletion

Thiamine treatment

- Adults
- Eggs
- Yolk-sac stage embryos
- Swim-up stage embryos

Methods

- Injection
- Immersion

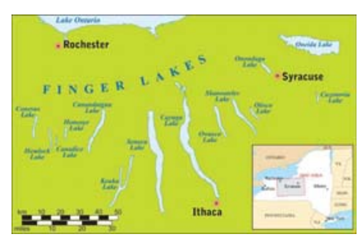
Thiamine Deficiency

• Early mortality syndrome – EMS (late 1960s – early 1970s) observed by hatchery personnel responsible for rearing progeny from feral broodstocks that mature in L. Ontario and L. Michigan and to a lesser extent L. Huron and L. Erie

Lake trout
Coho salmon
Chinook salmon
Brown trout
Steelhead trout

Thiamine Deficiency

• Cayuga syndrome (1974) observed in Cayuga Lake, Keuka Lake and Seneca Lake



Landlocked
Atlantic salmon

Thiamine Deficiency

• M74 (“miljobetingad” – environmentally related, 1974) observed in the Baltic Sea

Atlantic salmon
Sea trout

Thiamine Deficiency

• EMS was variable from 1968 through 1992 and tended not to exceed 20 to 30% for any species

• Hatcheries compensated by simply increasing the number of eggs collected during spawning

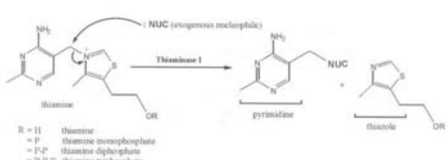
• In 1993, coho mortality dramatically increased to 60-90% in Wisconsin, Illinois, Indiana and Michigan hatcheries. Mortality of other Lake Michigan salmonids also increased

• Eggs from the Pacific coast could not be imported into the Great Lakes Basin because of potential pathogens (IHNV, VHS)

• GLFC sponsored several workshops and thiamine deficiency was proposed to be implicated as a possible cause for EMS

Thiamine Deficiency

Thiaminase I



Thiamine Deficiency

• Thiaminase is produced by several species of bacteria, and can be found in certain marine and freshwater fish species and shellfish, zooplankton, insects, and plants

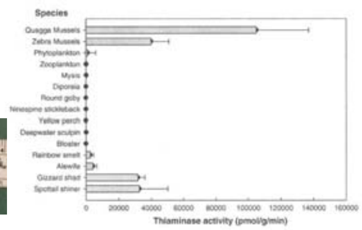
- *Bacillus thiaminolyticus*
- Bracken, Nardoo
- Cockle
- Shrimp *Penaeus*
- Silk worm
- Carp, goldfish, fathead minnow

Thiamine Deficiency

Journal of Great Lakes Research

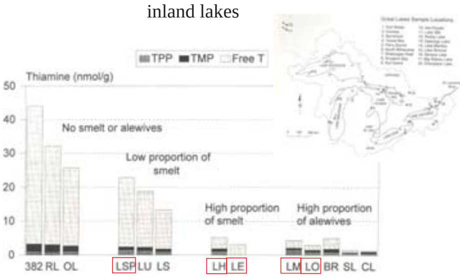
IAGLR

Dreissenid mussels from the Great Lakes contain elevated thiaminase activity



Thiamine Deficiency

Thiamine concentration in lake trout eggs from the Great Lakes and inland lakes



Fitzsimmons and Brown 1998

Thiaminase

1056

***Paenibacillus thiaminolyticus* is not the cause of thiamine deficiency impeding lake trout (*Salvelinus namaycush*) recruitment in the Great Lakes**

Catherine A. Richter, Allison N. Evans, Maureen K. Wright-Osment, James L. Zajicek, Scott A. Heppell, Stephen C. Riley, Charles C. Krueger, and Donald E. Tillitt

Abstract: Thiamine (vitamin B_1) deficiency is a global concern affecting wildlife, livestock, and humans. In Great Lakes salmonines, thiamine deficiency causes embryo mortality and is an impediment to restoration of native lake trout (*Salvelinus namaycush*) stocks. Thiamine deficiency in fish may result from a diet of prey with high levels of thiaminase I. The discoveries that the bacterial species *Paenibacillus thiaminolyticus* produces thiaminase I, is found in viscera of thiaminase-containing prey fish, and causes mortality when fed to lake trout in the laboratory provided circumstantial evidence implicating *P. thiaminolyticus*. This study quantified the contribution of *P. thiaminolyticus* to the total thiaminase I activity in multiple trophic levels of Great Lakes food webs. Unexpectedly, no relationship between thiaminase activity and either the amount of *P. thiaminolyticus* thiaminase I protein or the abundance of *P. thiaminolyticus* cells was found. These results demonstrate that *P. thiaminolyticus* is not the primary source of thiaminase activity affecting Great Lakes salmonines and calls into question the long-standing assumption that *P. thiaminolyticus* is the source of thiaminase in other wild and domestic animals.

Can. J. Fish. Aquat. Sci. 69: 1056-1064 (2012)

Lake Michigan
Lake Huron
Lake Trout

North American Journal of Fisheries Management 31:1052–1064, 2011

ISSN: 0275-5947 print / 1548-8675 online
DOI: 10.1080/02755947.2011.641066

ARTICLE

Increasing Thiamine Concentrations in Lake Trout Eggs from Lakes Huron and Michigan Coincide with Low Alewife Abundance

Stephen C. Riley* and Jacques Rinchard[1]
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U.S. Geological Survey, Northern Appalachian Research Laboratory, 176 Straight Run Road, Wellsboro, Pennsylvania 16901, USA

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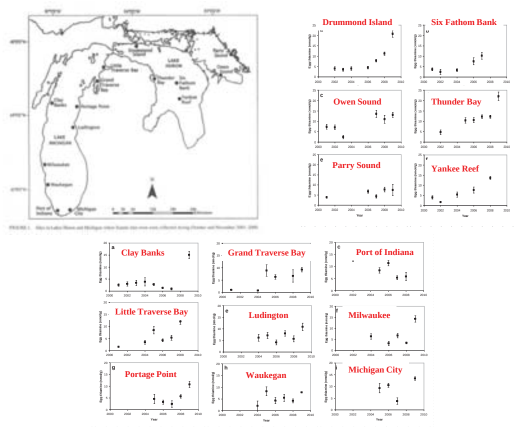
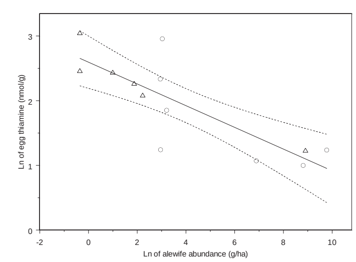
U.S. Geological Survey, Great Lakes Science Center, 1451 Green Road, Ann Arbor, Michigan 48105, USA

Lake Michigan
Lake Huron
Lake Trout

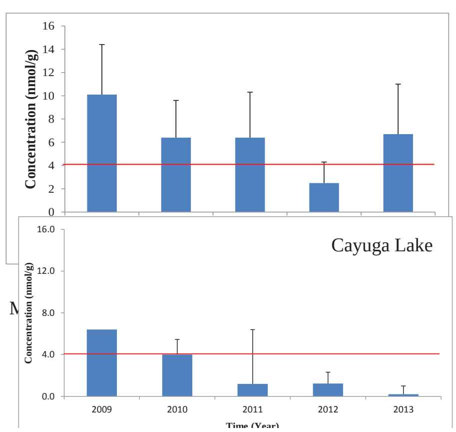


Lake Michigan
Lake Huron
Lake Trout

Lake trout egg thiamine concentration at Drummond Island (circles) and Thunder Bay (triangles) as a function of the mean yearling and older alewife abundance



Lake Ontario
Lake Trout



Thiamine Fatty Acids Embryo Mortality in Lake Trout

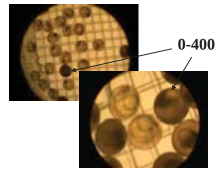
Thiamine Fatty Acids Embryo Mortality in Lake Trout

North n=19 **South** n=21

Samples of eggs frozen for thiamine and FA analyses;
Eggs fertilized
During water hardening, eggs were treated or not with allithiamine

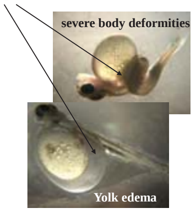
Fertilized eggs incubated, hatched and reared for 1000 DD post fertilization;
Mortality recorded daily

Thiamine Fatty Acids Embryo Mortality in Lake Trout



0-400 CDD – pre hatch mortality

400-600 CDD – post hatch mortality



600-1000 CDD – Swim-up stage mortality - EMS

Loss of equilibrium

Lying on the sides

Hyperexcitability

Spiral swimming

Thiamine Fatty Acids Embryo Mortality in Lake Trout

Pre- and post-hatch mortality in lake trout embryos from two sampling sites (North and South) and two experimental groups (allithiamine treated – T and non-treated – NT)

	North (n = 19)		South (n = 21)	
Mortality	NT	T	NT	T
Pre-hatch (%)	17.01 ± 15.64[a]	21.88 ± 19.50[a]	11.08 ± 9.76[a]	19.65 ± 16.64[a]
Yolk edema (%)	3.54 ± 3.58[a]	5.92 ± 4.81[a]	42.19 ± 25.15[b]	49.92 ± 24.49[b]
EMS (%)	11.46 ± 16.96[a]	0.31 ± 0.51[b]	0.98 ± 3.05[b]	0.06 ± 0.19[b]

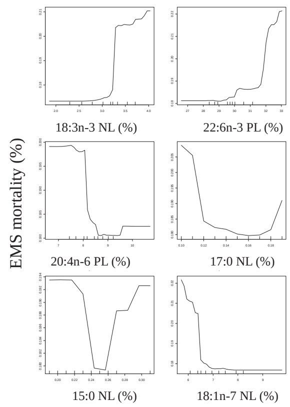
Thiamine Fatty Acids Embryo Mortality in Lake Trout

Radom Forest Regression models with ranking of predictor variables of lake trout mortalities

Non-treated			**Treated**		
Mortality	% mean standard error increase	Variation explained	Mortality	% mean standard error increase	Variation explained
Yolk edema		**32.59%**	**Yolk edema**		**34.79%**
Location	28.42%		Location	34.05%	
20:3n3 PL	16.20%		20:5n3 PL	20.68%	
20:3n3 NL	16.00%		22:6n3 PL	14.67%	
TPP	14.37%		20:3n3 NL	12.86%	
22:6n3 PL	11.39%		20:3n3 PL	11.80%	
20:2n6 NL	10.75%		22:5n3 NL	11.70%	
20:5n3 NL	10.17%		22:5n3 PL	11.25%	
16:0 NL	9.00%		20:2n6 NL	10.11%	
EMS		**26.1%**	**EMS**		**14.05%**
TT	11.69%		18:3n3 NL	10.72%	
TMP	10.79%		22:6n3 PL	9.03%	
TH	10.34%		20:4n6 PL	8.64%	
20:1n9 NL	10.37%		17:0 NL	8.23%	
18:2n6 NL	8.54%		15:0 NL	7.97%	
20:2n6 NL	7.43%		18:1n7 NL	7.78%	
22:6n3 NL	7.33%		TT	7.36%	

Thiamine Fatty Acids Embryo Mortality in Lake Trout

Partial dependence plots for six most important predictor variables for random forest predictions of mean EMS mortality among families treated with allithiamine



Thiamine Deficiency

ICES Journal of Marine Science Advance Access published September 29, 2011

Relationships between fish stock changes in the Baltic Sea and the M74 syndrome, a reproductive disorder of Atlantic salmon (*Salmo salar*)

Jaakko Mikkonen[1], Marja Keinänen[1*], Michèle Casini[2], Jukka Pönni[3], and Pekka J. Vuorinen[1]

The thiamine deficiency syndrome M74, a reproductive disorder of Atlantic salmon (*Salmo salar*) feeding in the Baltic Sea, is related to the fat and thiamine content of prey fish

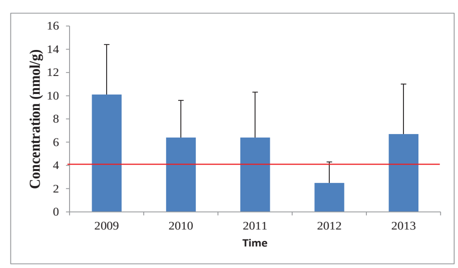
Marja Keinänen[1*], Annika Uddström[1†], Jaakko Mikkonen[1], Michele Casini[2], Jukka Pönni[3], Timo Myllylä[4], Eero Aro[1], and Pekka J. Vuorinen[1]

Thiamine Deficiency

This study clarifies how the diet of Baltic salmon leads to thiamine deficiency in eggs, and consequently to M74 mortality of yolk-sac fry. The main prey species, sprat (*Sprattus sprattus*) and herring (*Clupea harengus membras*), and their biomass in the Baltic Proper (BPr) and the Bothnian Sea, the two feeding grounds of salmon originating from the northern Gulf of Bothnia rivers, are compared. The thiamine concentration of both prey species is lowest in the youngest age groups. Because average fat content and energy density are greater in sprat than in herring, and greatest in youngest sprat, the supply of thiamine per unit energy is least in a diet containing many young sprat. Also, the greater is the supply of thiamine and fat from sprat in the southern BPr in the preceding year, the lower the concentration of thiamine in salmon eggs. Thiamine deficiency in eggs results from an unbalanced diet abundant in fatty prey fish, such as young sprat, from which the supply of thiamine is insufficient in proportion to the supply of energy and unsaturated fatty acids for salmon, which must undergo a long prespawning fasting period.

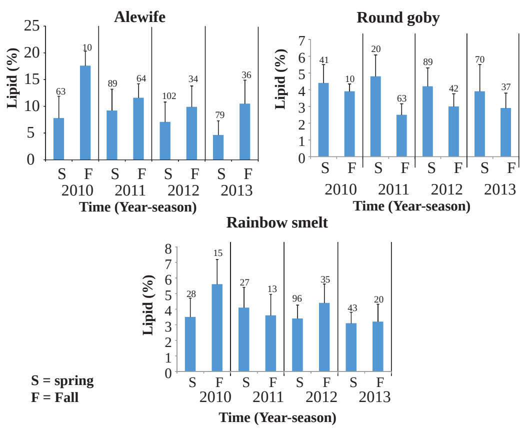
Keywords: Atlantic salmon, energy density, fat, herring, lipid, reproduction, sprat, thiamine.

Lake Ontario Lake Trout



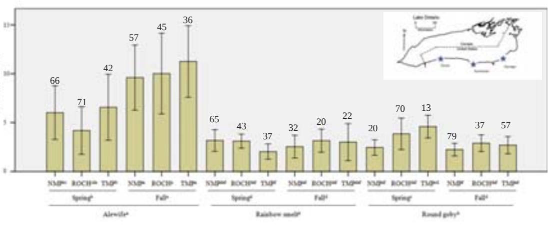
Mean annual total thiamine concentration in eggs of lake trout collected in Hamlin Beach (Lake Ontario)
The red line indicates the threshold (4 nmol/g) recommended by Great Lakes fishery managers for successful lake trout reproduction (Bronte et al. 2008)

Lipids (Rochester)



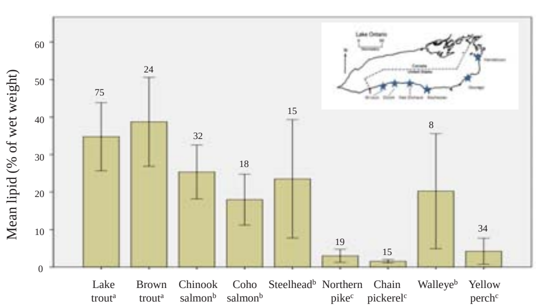
Lipids

Lake Ontario prey fish mean lipid content in 2013 separated by location, season, and species respectively. Each separation was analyzed separately using Kruskal-Wallis test. Means with different superscript letters indicate statistical difference ($p < 0.05$). Error bars indicate standard deviation.

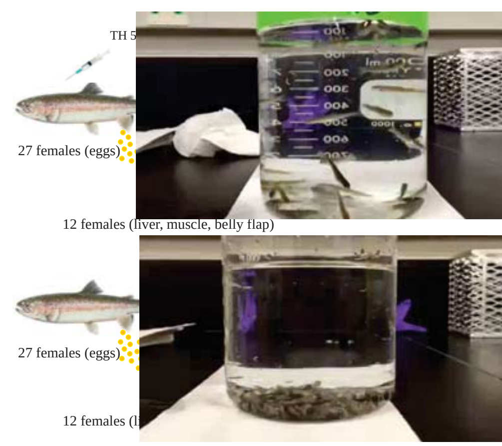


Lipids

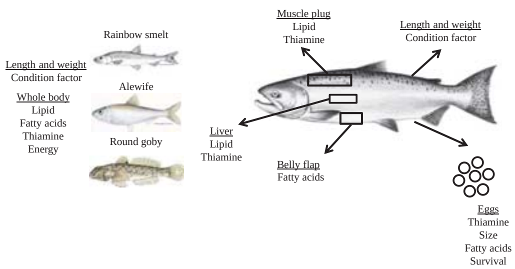
Lake Ontario predator fish mean lipid content in belly flap collected in 2013. Differences in lipid content analyzed using Kruskal-Wallis test. Means with different superscript letters indicate statistical difference ($p < 0.05$). Error bars indicate standard deviation.



Steelhead trout



Coho Salmon
Chinook Salmon
Lake Trout
Brown Trout



Other Ongoing Projects

- Great Lakes Fishery Commission: Can early feeding in lake trout fry ameliorate thiamine deficiency? PI: E. Marsden (University of Vermont), A. Evans (Oregon State University), and J. Rinchard (Department of Environmental Science and Biology, The College at Brockport - SUNY).

- US Fish and Wildlife Service: Lake trout thiamine and fatty acid study (2013 monitoring both US and Canadian waters). PI: Dr. Jacques Rinchard (Department of Environmental Science and Biology, The College at Brockport - SUNY) in collaboration with USGS, NYSDEC, Ontario Ministry of Natural Resources.

- US Fish and Wildlife Service: Thiamine status of Lake Champlain Landlocked Atlantic Salmon. PI: Dr. Jacques Rinchard (Department of Environmental Science and Biology, The College at Brockport - SUNY) in collaboration with Bill Ardren.

Questions?

Great Lakes Fish Health Research

National Fish Health Research Laboratory
Leetown Science Center

Vicki Blazer
Kearneysville, WV

USGS
science for a changing world

Health Assessments of Wild Fishes Indicators of Ecosystem Health

- Determining if there are indicators of exposure to chemicals of emerging concern
  - FWS, EPA, USGS MN Water Center
- Addressing the fish tumor Beneficial Use Impairment (BUI)
  - Primarily working with state agencies – Ohio EPA, PA DEP, WI DNR, MN Water Pollution Board

Great Lakes Restoration Initiative

- One priority:
  Cleaning up toxics and Areas of Concern
  – delisting Areas of Concern

Toxic concerns at AOCs have been focused on legacy contaminants, particularly PAHs and PCBs

IJC has recognized that contaminants of emerging concern may also be having significant effects on the health of fish and wildlife

Effects of Contaminant Exposures

- Thousands on chemicals in complex mixtures
- Timing of sensitive exposure periods versus water/sediment sampling
- Effects of early life stage exposure that may not be evident until adult
- Effects on disease resistance that require understanding the fish immune response and the pathogens involved

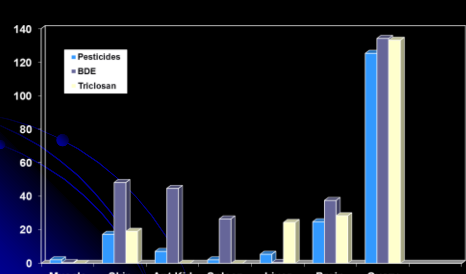
Organisms Observed in YOY Susquehanna

- *Aeromonas hydrophila* and other motile Aeromonads
- *Flavobacterium columnare*
- Largemouth Bass Virus
- Trematodes
- Myxozoan parasites

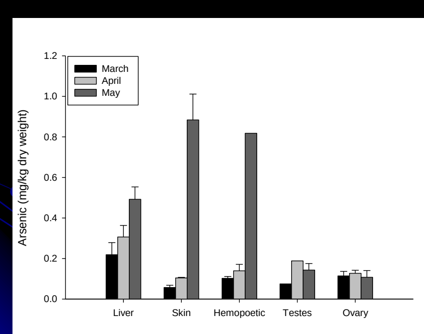
Issues with Addressing "Toxic" Chemicals

- Monitoring concentrations in water/sediment provide only a snapshot in time
- Most monitoring of fish tissue chemical contaminants is on whole body or fillet
  - Evidence for differential tissue accumulation
- See serious biological effects when no one chemical is above "threshold benchmarks"
  - Complex mixtures – additive, synergistic, antagonistic

Comparison of Tissue Contaminant Concentrations Potomac Bass



Arsenic Tissue Contaminants Smallmouth Bass



Chemicals of Emerging Concern

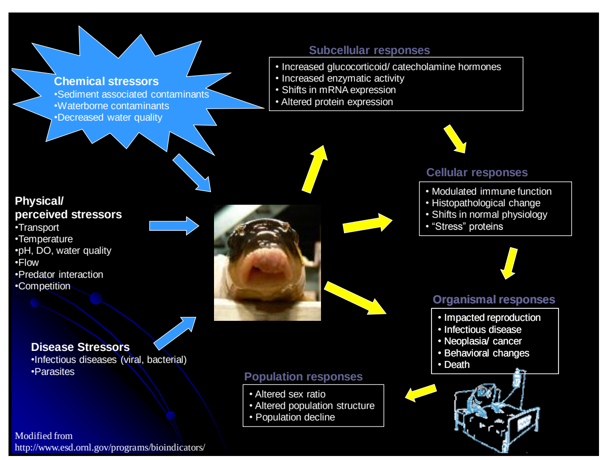
- WWTP
  - Pharmaceuticals – human and animal
  - Hormones – natural and synthetic
  - Personal care products – triclosan, fragrances
  - Flame retardants - polybrominated
- Agricultural
  - Current use pesticides
  - Hormones

Effects
Chemical of Emerging Concern

- Endocrine disruption
- Immune system/disease resistance
- Cancer/Neoplasia - promoters
- Numerous physiological and pathological effects
- Behavior

Great Lakes
Fish Health Assessments

- "Early Warning Project"
- FWS Contaminants program, USGS, WVU
  - Effects-based monitoring at Areas of Concern (AOC) and other sites
  - Bioindicators of exposure to legacy and chemicals of emerging concern
  - Suite of chemicals in discrete water and sediment samples – USGS MN Water Center and Denver NWQL
  - Caged fathead minnow studies by investigators from Duluth and Athens EPA labs and collaborators



Wild Fish Assessments

- Target species
  - Brown bullhead or white sucker
  - Largemouth or smallmouth bass
- Seasonal comparison
  - spring and fall
- Site comparisons

Model Versus Non-model Species

- Comparison of results with the short-term fathead minnow exposures
- Much information on gene expression and adverse outcome pathways for model species such as fathead minnow, zebrafish
- Also known that fish differ greatly in sensitivity and response

Suite of Biological Indicators

- Morphometric and necropsy-based
  - Comparisons based on sex, age,
  - identifies visible abnormalities,
  - condition factor/relative weight, hepatosomatic/gonadosomatic indices
- Blood/Plasma
  - Hormones – estrogen, testosterone, thyroid
  - Vitellogenin
  - Micronuclei and other RBC abnormalities
- Histopathological
  - Diagnose causes of gross observations, identify emerging pathogens, identify specific effects of contaminants, with image analyses quantify parasites, macrophage aggregates
- Molecular
  - mRNA for reproductively related genes (vitellogenin, estrogen receptors), immune system indicators (TGF-β, hepcidin), contaminant-related (CYP1A, oxidative stress), stress (glucocorticoid receptors)

Gene Expression Analysis

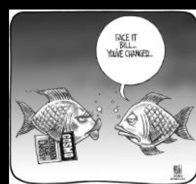
- Next Generation sequencing project
  - Transcriptome analyses

- Hepatic gene expression (NanoString Technologies)
  - Barcode-based approach using the nCounter Analysis system
  - Direct detection of mRNAs with molecular barcodes
  - Quantitative data on the modulation of each gene of interest
  - Targeting 50 genes (including 5-6 housekeepers)
- Hope to corroborate expression results with water quality and histological data
- Comparisons with EPA fathead minnow cage studies

USGS

Short List of Genes of Interest

- Estrogen Receptor (α, β1, β2)
- Androgen Receptor
- Thyroid Hormone Receptor (α, β)
- Glucocorticoid Receptor
- Steroidogenic Acute Regulatory Protein (STAR)
- CYP17, CYP19A1A, 17β-HSD
- Aryl Hydrocarbon Receptor
- CYP1A, CYP3A
- PPARs
- Glutathione Peroxidase
- Glutathione-S-Transferase
- Heat Shock Proteins

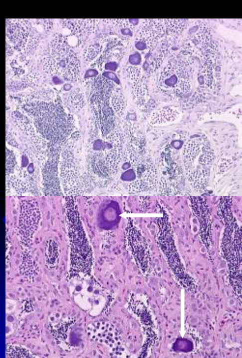


USGS

Multiple Endpoints

- Intersex – testicular oocytes
  - Most likely induced early in life, may increase in severity with age
- Plasma vitellogenin in male fish
  - Days to months
- Expression of the vitellogenin gene
  - Hours to days

Intersex in Normally Gonochorist Fishes



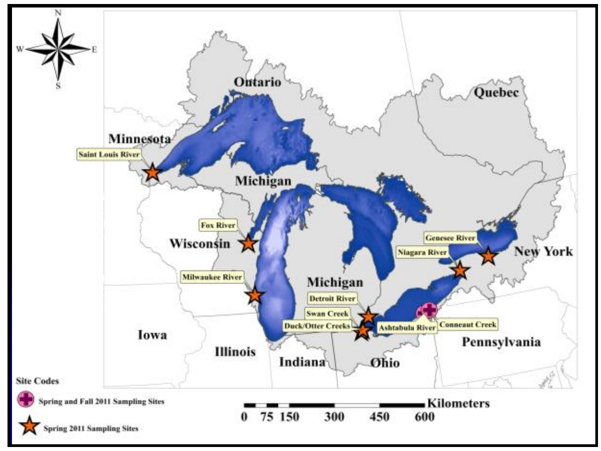
- Immature oocytes within testes
- Suggested as a marker of endocrine disruption
- Used as an indicator of exposure to estrogenic compounds

Testicular Oocytes

- Correlations with:
  - % agriculture in watershed above sample site
  - # animals in animal feeding operations
  - total estrogenicity
  - water estrone concentrations
  - concentrations of herbicides – atrazine, simazine, metolachlor

Reference of “Least Impacted Sites”

- Presque Isle Bay
  - Atrazine 25ng/L; Metolachlor 3.0 ng/L; estrone 1.6 ng/L
- Long Point
  - Atrazine 413 ng/L; Metolachlor 210 ng/L; estrone 1.5 ng/L



Summary - Species Comparisons

- Bass were the only species that demonstrated intersex (testicular oocytes)
  - Generally higher prevalence and severity in SMB
- Males of all species demonstrated plasma vitellogenin
- White sucker had testicular germ cell tumors at a number of sites
  - Milwaukee, Sheboygan
- White sucker and brown bullheads demonstrate liver and skin tumors
- All species had some red blood cell micronuclei/nuclear abnormalities
  - Bass had higher rate than white sucker and bullhead

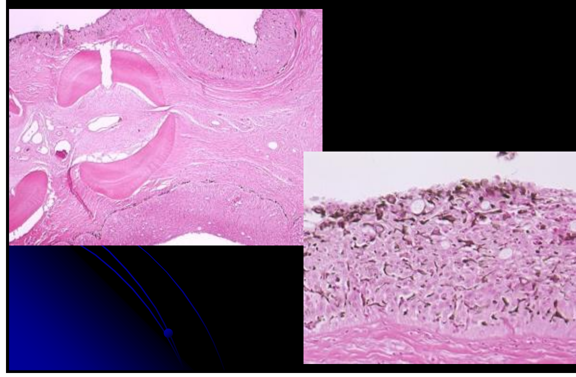
Species Comparisons Bass

- In general smallmouth bass have a higher prevalence and severity of testicular oocytes
- Smallmouth also demonstrate a higher prevalence of males with vitellogenin
  - Almost all smallmouth males had some measurable vitellogenin
  - Many sites/seasons had no male largemouth bass with measurable vitellogenin and in the fall only a few females

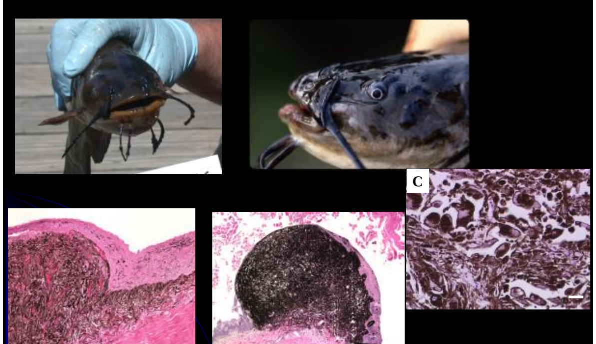
Molecular analyses is providing some explanations – differences in estrogen receptors

Skin Lesions
Melanistic Areas

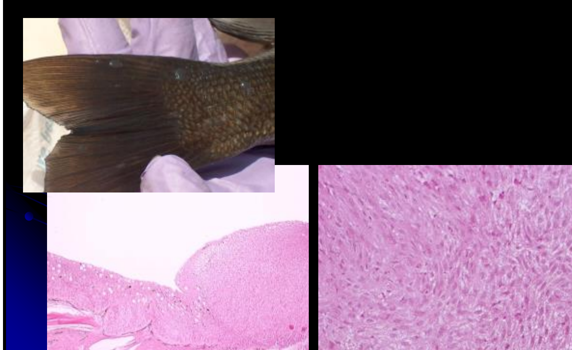
Melanistic Areas



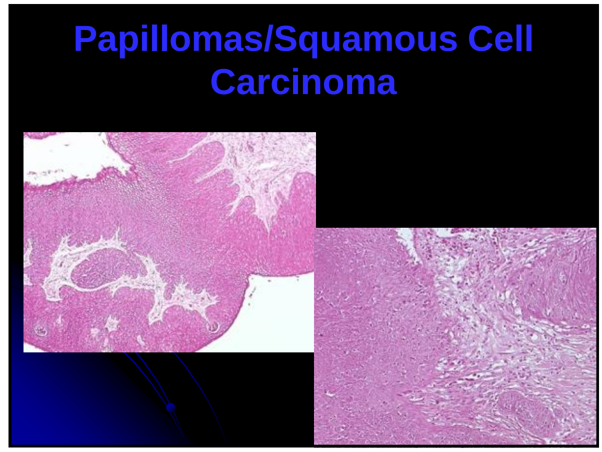
Actual Melanomas in Bullhead



Bass Mucoid Lesions




Papilloma
Squamous Cell Carcinoma


Papillomas/Squamous Cell Carcinoma


Observed External Lesions in White Sucker


White Sucker Mucoid Lesions

Site Comparisons
Skin and Liver Lesions in Suckers

	St. Louis AOC	Sheboygan AOC
Sample size	200	193
Raised skin lesions	31.0%	38.3%
Skin Neoplasia	4.5%	32.6%
Altered foci	4.5%	5.2%
Liver Neoplasia	4.5%	8.3%

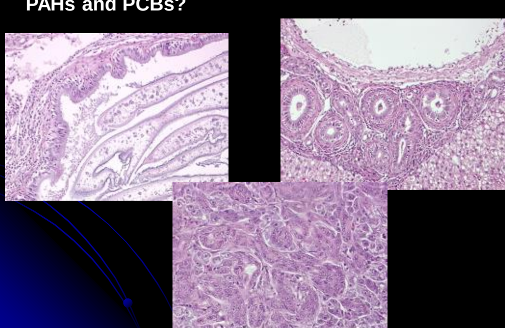
St. Louis – only papillomas and bile duct tumors
Sheboygan – papillomas, squamous cell carcinoma, hepatic cell, bile

Skin and Liver Neoplastic and Preneoplastic Lesions

- Are the slightly raised skin mucoid lesions that microscopically are hyperplasia preneoplastic lesions?
- Are there different risk factors/causes for lip versus body surface/fin neoplasms and for hepatic cell versus bile duct neoplasms?
- Are there viruses inducing the hyperplastic responses and subsequent chemical exposure is necessary for carcinogenesis?
- Is the bile duct myxozoan a risk factor for bile duct carcinogenesis?

Risk Factors for Liver Tumors?

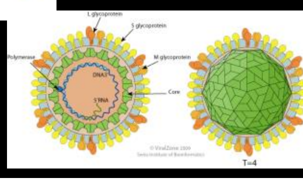
Should we be considering factors other than PAHs and PCBs?



Risk Factors for Tumors

- PAHs and PCBs
  - Do we need to move beyond PAHs as the only factor?
- Mammalian species
  - Estrogens as promoters
  - Arsenic, other contaminants
  - Viruses, parasites
- Chemical analyses of individual tissues along with histopathology and gene expression

The First Report of a Hepadnavirus from Fishes: Molecular Evidence for a Novel Genus of Hepatitis B Virus in White Sucker (*Catostomus commersonii*) that inhabit the Great Lakes Region.



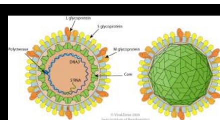
Virus discovery via NGS

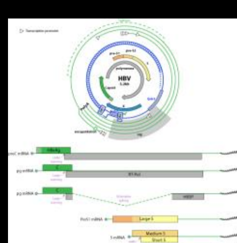
- Total RNA was extracted (ribosomal RNA depleted) and samples were prepared for sequencing on a HiSeq2000 (2 x 100bp PE)
- Read pairs were quality trimmed and *de novo assembly was* conducted using CLC Genomics Workbench *v.* 7
- Resulting contigs were included in a local blastx query against the virus database (NCBI)
- A linear 3519 bp contig (135052 reads) was identified
  - Similarity to Duck Hepatitis Virus (35% ID; 2e-065)

USGS

Hepadnaviruses
(Hepatitis B)

- Enveloped, spherical (~42 nm; Dane particle)
- Partially dsDNA, circular genome
- Genome (~3200 bp)
- 3 or 4 partially overlapping reading frames (RF+1, RF+2 & RF+3)
- Reverse transcribing (DNA virus)
  - Replicate via reverse transcription of pgRNA
  - pgRNA contains ~300bp of untemplated sequence
- Integrating virus
- Oncovirus

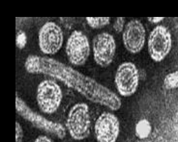




http://viralzone.expasy.org/all_by_species/605.html

Hepadnaviruses
(Hepatitis B)

- Two recognized genera
  - Orthohepadnavirus (mammal)
  - Avihepadnavirus (birds)
- Not yet identified in fishes
- Variable liver pathology dependent on species
  - Inflammation
  - Cirrhosis
  - Neoplasia
- Annually accounts for 1 million deaths in humans (cirrhosis, liver failure and HCC)
- Hepatocellular carcinoma associated with Orthohepadnavirus infections but not in Avihepadnaviruses

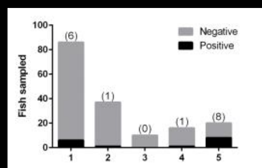


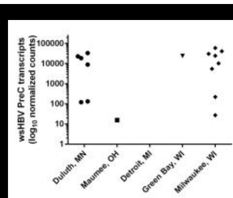
http://intranet.tdmu.edu.ua/data/kafedra/internal/infect_desease/classes_stud/en/med/lik/ptn/Infectious%20diseases/5/07.%20General%20description%20of%20viral%20hepatitis.htm

WSHBV Prevalence

(transcription)

- Livers from 169 fish evaluated for core protein expression
  - 9.4% were positive
  - 40% of fish from Milwaukee River postitve
- Fish were also collected from the Root River.
  - 20% (n=20) were positive for WSHBV DNA in liver and plasma





USGS

Is This Virus Associated with Disease?

- The association of the WSHBV with neoplasia or liver disease is currently unclear

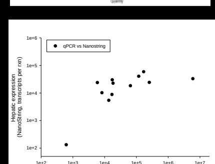
Prevalence of virus = 9.5%
Prevalence hepatic tumors = 4.9%
Positive for both = 2.4%

Site	Sample Size	Virus Only	Tumor Only	Virus & Tumor
St. Louis River	86	5	2	1
Maumee	37	1	0	0
Detroit River	10	0	0	0
Fox River	16	0	0	1
Milwaukee River	20	6	3	2

USGS

qPCR for Virus Screening

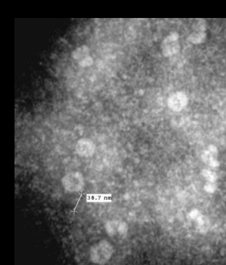
- SYBR green qPCR assay developed to quantify viral DNA in plasma samples (extracted DNA)
  - 16 fish positive in liver
  - 17 positive by plasma qPCR
- Whole blood collected on FTA worked for presence/ absence
- Approach for non-lethal sampling/ epidemiological surveillance



USGS

Viral DNA in the plasma, what about virus?

- Shipped PCR positive plasma to Jim Winton, USGS Seattle lab for EM
- Crude preparation for first run
- Evidence of Dane particles



USGS

"Rome Syndrome"

Andrew D. Noyes

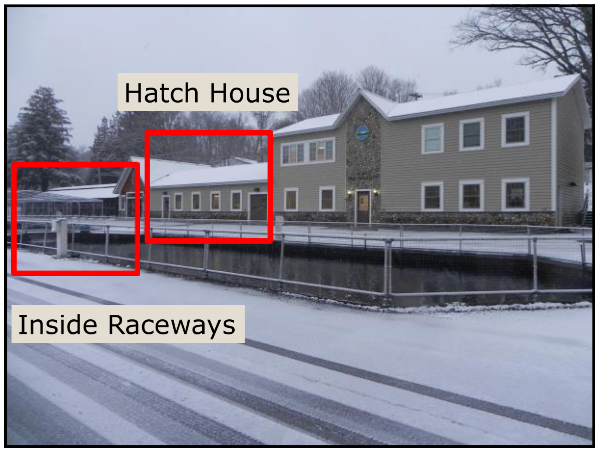
Rome Field Station
New York State Department
of Environmental Conservation

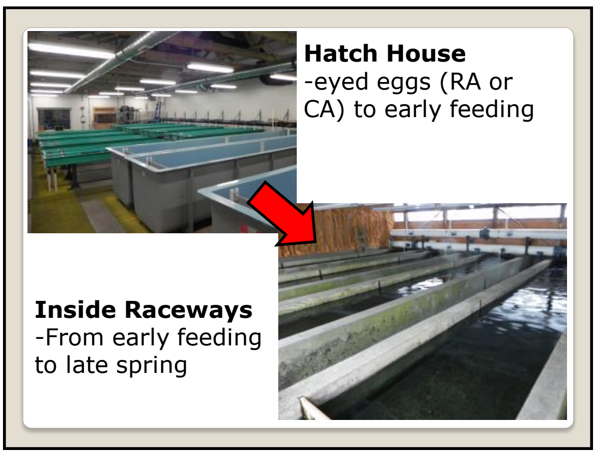
12 DEC Hatcheries

12 DEC Hatcheries

12 DEC Hatcheries


Rome State Fish Hatchery


Hatch House
Inside Raceways


Hatch House
-eyed eggs (RA or CA) to early feeding
Inside Raceways
-From early feeding to late spring


By late January……
• Lay on side

By late January……

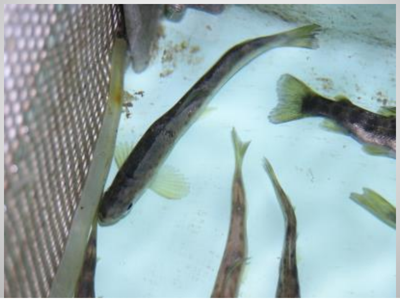
- Lay on side
- Morts rarely high

By late January……

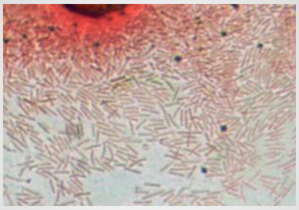
- Lay on side
- Morts rarely high

- Affected fish permanently impaired

Unilateral Discoloration

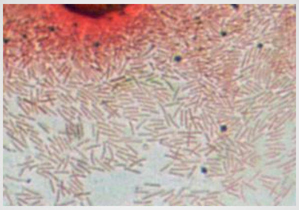


Brain inoculated onto media



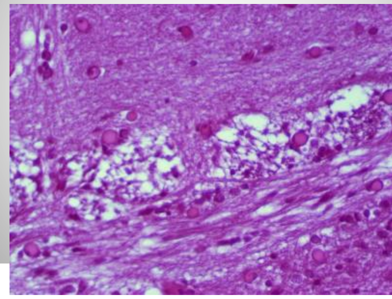
- *F. psychrophilum*

Brain inoculated onto media

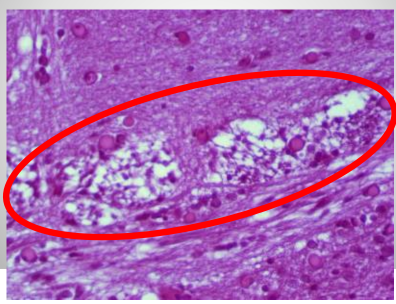


- *F. psychrophilum*
- **Scarce on skin**

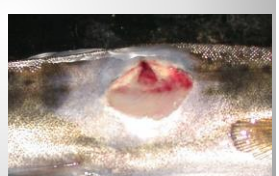
Brain from Brown Trout (7 cm)
H&E



Brain from Brown Trout (7 cm)
H&E



- By March or April
  - Brain form disappears
  - Cutaneous form predominates



"Rome Syndrome" Summary

- *F. psychrophilum-*
  - Appears in brain first, skin second

"Rome Syndrome" Summary

- *F. psychrophilum-*
  - Appears in brain first, skin second
  - Suggests initial disease onset from vert. transmission?

"Rome Syndrome" Summary

- *F. psychrophilum-*
  - Appears in brain first, skin second
  - Suggests initial disease onset from vert. transmission?
- *F. psych* confirmed in BT eggs
  - Randolph SFH (10/14)

FISH HEALTH MANAGEMENT
"A Proactive Approach"

Great Lakes Fish Health Committee
July 28, 2015
Ithaca, NY

Bill Keleher

KENNEBEC RIVER BIOSCIENCES

Overview

- Three case studies:
  - Bait/Game fish: pond operation
  - Atlantic salmon: sea cage operation
  - Marine finfish spp.: recirculating aquaculture system (RAS)
- Pathogens of concern
- Health management approaches



Case Study 1
Bait & Game Fish

- **Species**: multiple (minnows, sucker, pike)
- **Water**: spring (protected)
- **Operation Type**: pond
- **Fish Source**: Internal
- **Biosecurity**: High

Case Study 1
Pathogens of Concern

- **Regulatory**
  – VHSV
- **Production**
  – None

Case Study 1
Health Program

- **Biosecurity**
  - Movement Controls
  - Disinfection
  - Documentation
- **Surveillance**
  - Regulatory Testing (VHSV)
  - "Bluebook" – Annual 60 fish per lot
  - Mortality event investigations
- **Vaccination**
  - **None**

Case Study 2
Atlantic salmon

- **Species**: single (Atlantic salmon)
- **Water**: fresh (protected) and salt (open)
- **Operation Type**: vertically integrated
- **Fish Source**: Internal (several facilities)
- **Biosecurity**: High

Case Study 2
Pathogens of Concern

- **Regulatory**
  - ISAV, IHNV, IPNV, OMV, SVCV, VHSV, SAV
  - Aeromonas salmonicida, Yersinia ruckeri, Renibacterium salmoninarum
  - Myxobolus cerebralis, Ceratomyxa shasta, Gyrodactylus salaris
- **Production**
  - ISAV
  - Aeromonas salmonicida, Yersinia ruckeri, Listonella anguillarum, Vibrio ordalii, Flavobacterium spp.
  - Moritella viscosa, Tenacibaculum maritimum

Case Study 2
Health Program

- **Biosecurity**
  - Movement Controls
  - Disinfection
  - Documentation
- **Surveillance**
  - Regulatory Testng (Salmonid inspection incl. ISAV, SAV, G. salaris)
  - OIE, FHPR, "Bluebook", State/Province/Country (175/60)
  - Mortality event investigations
- **Vaccination**
  - Autogenous immersion (2 x bivalent)
  - Licensed injectable (tetravalent + ISAV)

Case Study 3
Marine Species

- **Species**: multiple (bream, sea bass, yellowtail)
- **Water**: brackish (protected)
- **Operation Type**: vertically integrated
- **Fish Source**: Internal & Third party
- **Biosecurity**: Low

Case Study 3
Pathogens of Concern

- **Regulatory**
  - IPNV, SVCV, VHSV
  - Aeromonas salmonicida, Yersinia ruckeri
- **Production**
  - VNNV
  - Vibrio harveyi, Photobacterium damesla piscicida, Edwardsiella tarda
  - Oodinium spp.

Case Study 3
Health Program

- **Biosecurity**
  - Semi-quarantine
  - Limited Movement Controls
  - Disinfection
- **Surveillance**
  - Regulatory Testing (IPNV, SVCV, VHSV, As, Yr)
  - "Bluebook" & State
  - Mortality event investigations & Routine screening
- **Vaccination**
  - Autogenous immersion (2 x bivalent)
  - Vaccination protocols tailored to RAS

Integrated Animal Health Approach

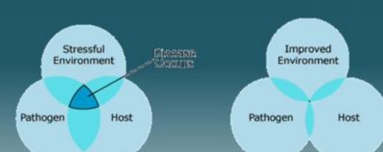


Figure 1 Figure 2

- Surveillance
- Biosecurity Measures
- Proactive measures - vaccines

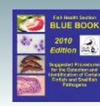
Surveillance

- Diagnostic
  - Flexibility on testing
  - Reported only to farmers (unless OIE reportable)
  - Production related pathogens
- Regulatory
  - Testing protocols set by “Bluebook” or OIE
  - May or may not be pathogen of concern to farmer
  - Sampling/Pathogen regime can be complicated

Inspection & Testing Standards

- AFS-FHS “Bluebook”
- USDA APHIS - OIE
- Fisheries & Oceans Canada FHPR
- US Fisheries & Wildlife Service Title 50

Country/State/Provincial Requirements



“Bluebook” vs OIE

- Lot vs facility level
- 60 per lot vs 150-175 per facility
- Salmonid focused vs amphibian, crustaceans, fish, & molluscs
- Annual vs biannual inspections
- Domestic* vs international movements

* “Bluebook” referenced by many states in statute or regulations. OIE starting to be included by some states and required for international movements.

Domestic Movements

State Requirements….a continuum

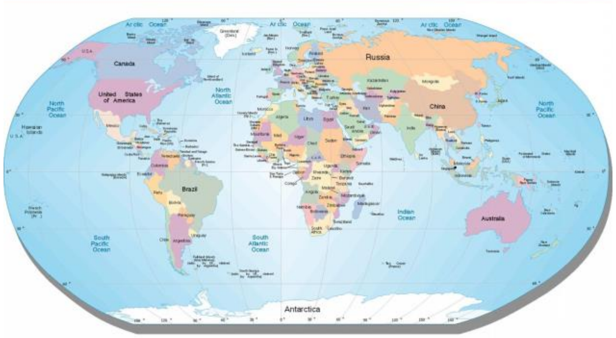
- Rigorous requirements for import - many/all species and numerous pathogens
- Restricted pathogen testing – VHSV only
- No requirements

Often times a lack of process for new species being cultured with regard to testing requirements. Disconnect between regulatory pathogens and pathogens farmers are concerned with.

Natural Resource vs Ag View of World

- Different inherent missions e.g. resource protection vs agriculture health
- Expansive vs restrictive pathogen lists
- Different authority depending on state
- Little harmonization between states

International Exports



Progress

- USDA APHIS - Competent Authority
- USDA Export Testing Laboratories
- Facilities Registration
- Veterinary Accreditation Aquatics
- USDA Health Certificates & Export Signing

Country Requirements

- Country specific
- Can include OIE and non-OIE pathogens
- Work w/ USDA to meet import requirements
- Facility registration
- Not always an easy process

Biosecurity

- Is the water protected vs. unprotected?
- Does the fish/egg source(s) have relevant health history?
- Is the farm controlling access and have any biosecurity measures in place?
- Plan development (living document)

Autogenous Vaccine

Custom Vaccine Model

Diagnostic/Surveillance

Vaccine

Monitoring/Refinement

‘Full Cycle’ Aquatic Health Approach: Hatchery-Production-Broodstock-Gametes

- **Management goals reviewed**
  - *Production and movement plan*
  - *Susceptibility factors analyzed*
  - *Past disease patterns*
- **Diagnostic work-ups**
- **Pathogens isolated, identified and purified**
- **Vaccine manufacture**
- **Vaccination regimens developed**
- **Pathogen surveillance & efficacy data**

Summary

- Aquaculture industry very diverse
- No one size fits all approach
- Every operation has different goals
- Need integrated health approach
  - Surveillance
  - Biosecurity Plan
  - Proactive Measures

Fish kills: Common annoyance or valuable indicator?

Dr. Nick Phelps

Great Lakes Fish Health Committee
July 29, 2015

University of Minnesota College of Veterinary Medicine

What is a "fish kill"

"Localized die off of more than 5 fish of the same species with similar clinical signs of disease"

Why look at fish kills?

Advance fish health and fisheries management

Why look at fish kills?

- Public concern

small town in northwest Arkansas, and observers spotted the massive drum fish kill near the town of Ozark. There were numerous reports of UFOs hovering over the fish kill.

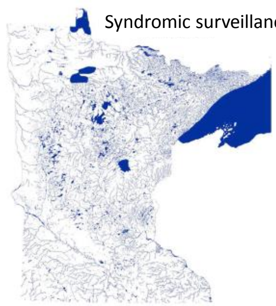
There is a massive government cover-up to keep all this information from the public. But, Frank Lake was down in Arkansas today with Dr. Anna Pfeffer, the world's foremost ichthyologist and Olympic badminton champion, examining the dead drum fish and the Mark of the Alien found on each of the fish.

Why look at fish kills?

- Cost effec@ve

Expensive cleanup

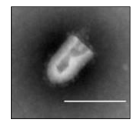
Syndromic surveillance



Why look at fish kills?

- Prepare, iden@fy, and respond to new threats

1990s?



Why look at fish kills?

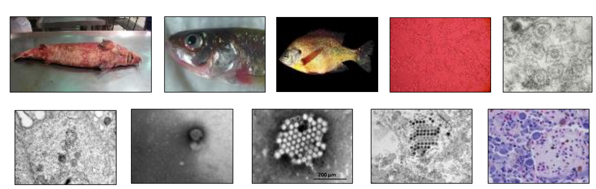
- Prepare, iden@fy, and respond to new threats

2005

Why look at fish kills?

- Prepare, iden@fy, and respond to new threats

2015



Why look at fish kills?

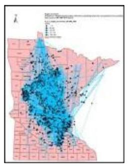
- Prepare, iden@fy, and respond to new threats

Fish kills don't happen in isola@on…

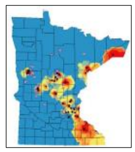
Water connec@vity

BaiLish movement

Boater movement



Linear distance



Why look at fish kills?

- Iden@fy trends over@me

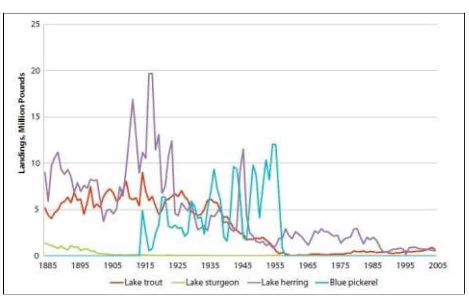
As state warms, a few spots keep their cool

Dan Kraker · Brainerd, Minn. · Feb 3, 2015 Environment

Ten Mile Lake, north of Brainerd, is one of 176 "refuge lakes" the DNR has identified where cisco, also known as lake herring, can survive even in a significantly warmer climate. Dan Kraker / MPR News

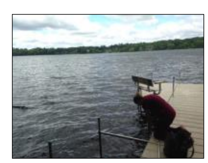
Why look at fish kills?

- Ecosystem sustainability



Challenges to fish kill inves@ga@on

- Rapid response is difficult
- LiUle standardiza@on
- Many stakeholders, with varying levels of experience
- Assump@ons made in the field
- "Natural events"
- LiUle money for inves@ga@on
- Not priority



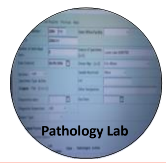
Project to advance fish kill efforts in Minnesota

1. Compile, organize and analyze historical data related to fish kill events in Minnesota
2. Create an online user--friendly database to report fish kill events in Minnesota

Exis@ng FK databases in Minnesota

MN Dept of Natural Resources Databases

Winterkill Database



Area and Regional Offices

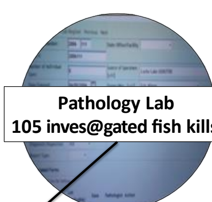
Other Databases

Minnesota Veterinary Diagnos@c Laboratory

Minnesota Pollu@on Control Agency

Retrospec@ve FK Analysis: 2003--2013

Spills and Kills
236 reported fish kills



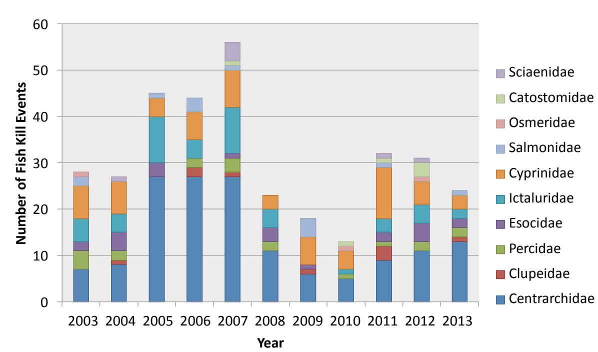
Pathology Lab
105 inves@gated fish kills

298 reported fish kill events from 2003–2013

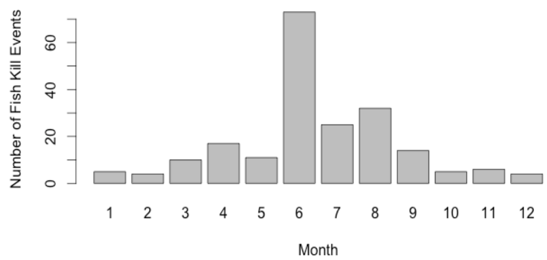
≠

Consensus poll of DNR biologists = ~500 fish kills per year

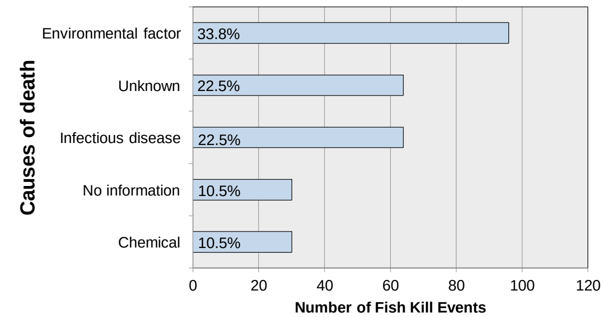
Retrospec@ve FK Analysis: 2003--2013

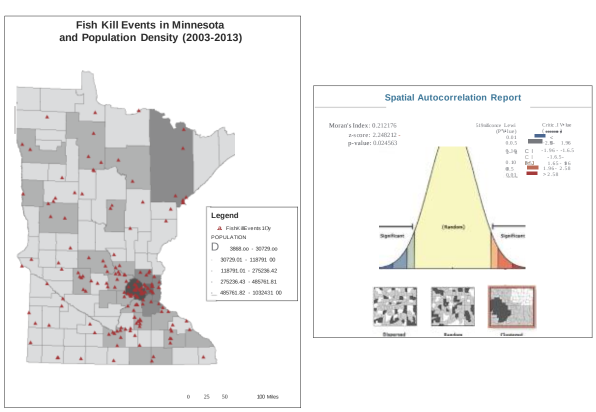


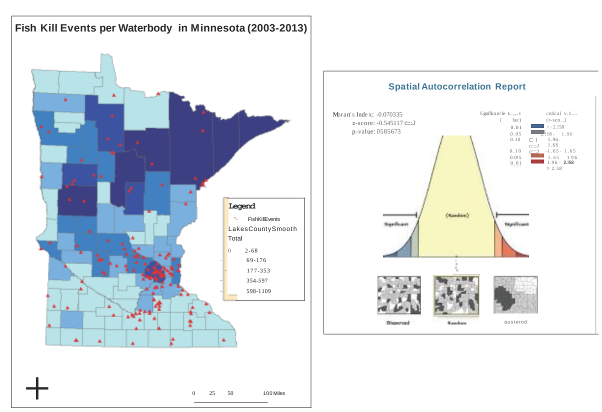
Retrospec@ve FK Analysis: 2003–2013



Retrospec@ve FK Analysis: 2003–2013







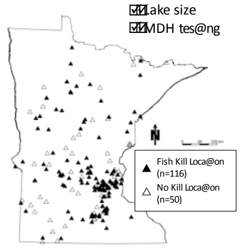
Correla@on with poten@al risk factors

TSI	Mean	Std Dev	P--value
Kill	60.0	11.5	0.069
No Kill	63.8	14.9	

Lake Size (acres)	Mean	Std Dev	P--value
Kill	5,372	30,962	0.259
No Kill	353	672	

Tissue contamina@on	Posi@ve	Nega@ve	Percent
Kill	80	36	69%
No Kill	10	40	20%

☑TSI
☑Lake size
☑MDH tes@ng



Limitations

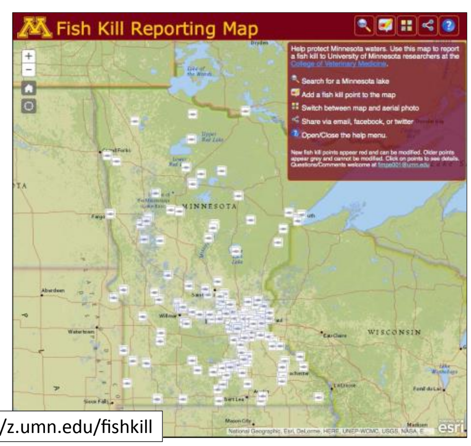
- Type and reliability of data
  - Underrepor@ng and bias
  - 17% of the entries were missing essen@al informa@on for the analysis (Loca@on, Species)
- Limited diagnos@c inves@ga@on
  - Assump@ons made in the field
- Must improve repor@ng…

Advancing fish kill inves@ga@on in MN: A way forward

1. Communica@on with public and DNR
2. Standardized protocol for fish kill response
3. Fish kill differen@al list for field and lab
4. Online, user--friendly database

h\p://z.umn.edu/fishkill

Fish Kill Reporting Map



hUp://z.umn.edu/fishkill

hUp://z.umn.edu/fishkill

hUp://z.umn.edu/fishkill

Conclusions

- Fish kill events occur frequently
- Repor@ng and inves@ga@on is limited
- Online, user friendly database now available
- Repor@ng fish kill events is important:
  - Emerging threats
  - Long--term trends

Recommenda@ons

- Communicate value of fish kill repor@ng and inves@ga@on with field biologists and public
- Document all fish kill events in searchable database
- Pursue fish kill inves@ga@on if appropriate
- Communicate findings with submiUer and other fish health professionals

Acknowledgements

- U of MN
  - Dr. Irene Bueno–Padilla (PhD student)
  - Sarah Knowles (DVM student)
  - Sarah Massarani (DVM student)
- MN DNR
  - Ling Shen (Pathology lab supervisor)
  - Marilynn Danks (Program coordinator)
  - Paula Phelps (Aquaculture/Fish Health consultant)
- Everyone who reports fish kills!!

Request

Send me your dead carp!!

- Currently funded to inves@gate pathogens of common and Asian carps in the Upper Midwest
  - No cost to you!
  - Will send all results asap from full workup