GREAT LAKES FISH HEALTH COMMITTEE

2012 Winter Meeting
Sandusky, Ohio
February 8-10, 2012

Minutes
(with attachments)

Submitted By:

Christina Haska
Great Lakes Fishery Commission

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

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List of Attendees

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

Great Lakes Fish Health Committee Meeting
February 8-10, 2012
Kalahari Resort and Convention Center
7000 Kalahari Drive
Sandusky, OH 44870

Agenda

Wednesday, February 8

8:00 am-8:10 am	Welcome (Ken Phillips/Christina Haska)
8:10 am-8:20 am	Approval of Meeting Minutes (Ken Phillips)
8:20 am-8:30 am	Communications (Ken Phillips)
8:30 am-9:00 am	Infectious Salmon Anemia Update and Discussion (John Coll)
9:00 am- 10:00 am	Michigan State University Research Update (Mohamed Faisal)
10:00 am-10:15 am	Break
10:15 am-10:30 am	Overview of the Purdue University ADDL (Tsang Long Lin)
10:30 am-Noon	Agency Updates (All)
Noon-1:00 pm	Lunch (on your own)
1:00 pm-1:30 pm	*Anguillicoloides crassus* Update (Ken Phillips)
1:30 pm-2:30 pm	Subcommittee on Aquatic Animal Health/APHIS VHSv Rules Update (Janet Whaley)
2:30 pm-3:00 pm	GLFHC Relationship with State & Federal Ag Departments (Ken Phillips)
3:00 pm-3:15 pm	Break
3:15 pm-3:30 pm	CLC Update (John Dettmers)
3:30 pm-5:30 pm	Model Program Review and Discussion (Ken Phillips/John Dettmers)
5:30 pm	Adjourn for the day

Thursday, February 9

8:00 am- 10:00 am	Model Program Review & Discussion (Ken Phillips/John Dettmers)
10:00 am- 10:15 am	Break
10:15 am-12:15 pm	Model Program Review & Discussion (Ken Phillips/John Dettmers)
12:15 pm-1:15 pm	Lunch (on own)
1:15 pm-3:15 pm	Model Program Review & Discussion (Ken Phillips/John Dettmers)
3:15 pm-3:30 pm	Break
3:30 pm-5:30 pm	Tour of Castalia SFH (Tim Parrett)
5:30 pm	Adjourn for the day

Friday, February 10

8:00 am-9:00am	Agency Updates (All)
9:00 am- 9:30 am	Research Priorities (Ken Phillips)
9:30 am- 10:00 am	Review and Discussion of Research Preproposals (Ken Phillips)
10:00 am-10:15 am	Break
10:15 am-11:30 am	Model Program Wrap-up/Parking Lot/Action Items (Ken Phillips/John Dettmers)
11:30 am-11:45 am	Summer 2012 meeting Discussion (Phillips)
11:45 am-Noon	Selection of Dates & Location for Winter 2013 meeting (Phillips)
Noon	Adjourn Meeting

Day 1: 8 February 2012

1. Welcome and Introductions (K. Phillips)

2. Approval of Meeting Minutes

The minutes from the August 2011 meeting were approved.

3. Communications (K. Phillips)

John Coll had contacted the committee about a detection of *Didymo* at a Vermont hatchery which supplies fish to Pennsylvania. It was decided that those lots would be destroyed and not imported to any state hatchery.

4. Infectious Salmon Anemia (ISA) virus update (J. Coll)

See Appendix 1 for the presentation.

5. Michigan State University Research Update (M. Faisal)

See Appendix 2 for the presentation.

6. Overview of the Purdue University ADDL (T. Lin)

See Appendix 3 for the presentation.

7. Agency Updates I (All)

USFWS- Pennsylvania (J. Coll): The Allegheny hatchery has been renovated, disinfected, and held brook trout as a test to see if IPN was detectable. All tests indicated it was negative for all pathogens. The future broodstock is currently there and it has eggs from Region 3. The White River hatchery picked up the productions while Allegheny was down. It flooded last year, and the fish were sacrificed because of a Didymo infiltration. GLRI has funded 2 projects. Surveillance has found VHS, *Nucleaspora,* and EEDv. In Lake Ontario, BKD and LMBv have been detected.

Minnesota DNR (L. Shen): Renibacterium detections (through ELISA and culture) have downgraded a hatchery. A broodstock source lake which provides eggs to an A1 hatchery was also tested, and Renibacterium was found there as well. The hatchery did not import eggs from there this year. A small fish kill (200-300 carp) occurred last July from SVC in a confluence of the Mississippi River. VHS surveillance continues this year, and thus far all tests have been negative.

New York State DEC (A. Noyes): Examined 23 sites for wild pathogen detections and found EEDv outside Rochester in Lake Ontario. There were 51 total hatchery inspections, and A. sal was higher than last year. The two main concerns are that, 1) This was the worst coho egg take

year on record, and 2) they are in the third year of a contract with a diet company and they aren't happy with the multi-generational effects with the brook trout.

Wisconsin DNR (S. Marcquenski): There have been two isolations of VHS: the first was in a fish kill of YOY gizzard shad in the Milwaukee Harbor, and the second was in spawning yellow perch populations last June that showed minimal milt production. Due to personnel issues, APHIS surveillance did not survey the quota of lakes. A vaccination program for furunculosis has been very successful this year. Hatcheries are trying to manage Renibacterium in coho, and one new facility has increased the velocity of the intake water which forces the fish to continually move. This means they don't have diet restrictions, and so far this seems to be reducing pathogen presence. Lake trout and splake were tested for EEDv and came out negative, but wild herring was tested from an inland lake and had positive PCR hits but sequencing did not confirm its presence. Tables are attached to the end of her annual report, and the committee is encouraged to review them.

Ohio DNR (T. Parrett): No new pathogens have been found since the last report.

8. *Anguillicoloides crassus* Update (K. Phillips)

Information on *A. crassus* has been sent to committee members via email (see Appendix 4). It has been detected in the upper St. Lawrence River. Copepods are the intermediate host, while the final host is the North American eel.

When the Model Program was sent to the CLC for advice and comments, the question was raised why *A. crassus* is not included in the document. This can be discussed later during the Model Program sessions, if need be.

Also, Andy Todd and John Casselman can be contacted to enquire on the status of their publication regarding *A. crassus.*

9. Subcommittee on Aquatic Animal health/APHIS VHSv Rules Update (J. Whaley)

APHIS would appreciate hearing from members of the GLFHC regarding the following issues about VHS:

- When you enacted your rules, were they effective? Are they now? Should they be changed? Did you overreact and do too much?
- What was the dollar value of our actions? Eg., Private producers and bait collectors; How many people may have gone out of business? Provide a table with number of aquaculture and bait licenses from 2005 and now (expecting this number to decrease substantially); compare this with number of fishing licenses as well
- List in lay terms what the rules are for importation. What species are susceptible?
- Would your rules still be effective without APHIS's list of susceptible species?
- If the federal rule were revoked, would your state regulations be revoked at the same time? Are you dependent on the federal rule?

- Do your rules apply to outside the basin or just the Great Lakes? Consider the southern states.
- If the APHIS order left, would your state develop its own rules?

GLFHC VHS recommendations from a previous meeting are included in Appendix 5.

10. GLFHC Relationship with State and Federal Ag Departments (K. Phillips)

The following are suggestions to enhance this relationship:

- Send Ag Depts the agenda for the meeting beforehand; if they have input, we could arrange a conference call for that session
- Send minutes to key officials after the meeting
- Consider including them in joint meetings every ~4yrs.
- Committee members should compile a list of potential respondees to send the agenda/minutes --- send to Ken
- Hold a summit meeting with ALL Great Lakes fish health officials- NOAA, GLERL, EPA, CFIA, etc

11. CLC/GLFC Update (J. Dettmers)

The CLC and Coast Guard have an agreement to notify each other of fish kills in the Great Lakes basin (not inland lakes). Upon receiving information from the CLC, the Coast Guard would notify mariners to follow best management practices. Over the last few years, states did not notify the GLFC until the pathogen was confirmed. It is encouraged that agencies do not wait this time period and notify at the beginning stage at whatever extent is possible. Contact your CLC representative (cc John) and they will notify the Coast Guard.

Ken spoke with CLC at the October meeting regarding the Model Program. They returned with comments (editorial and substantive) for the committee to consider.

Day 2: 9 February 2012

1. Model Program Discussion (All)

The GLFC will formally edit the document prior to posting it online.

The timeline should be as follows:

- Final version needs to be submitted to the GLFC by **March 31, 2012**
- Conference call with the committee March 26
- Send to the committee by March 9
- Conference call with writing subcommittee by February 23-24
  - Scope
  - Edits to document, including "Wordsmithing"
  - Risk Assessment values
  - Pathogen descriptions- send to committee as they are completed
  - Comment Disposition Statement for CLC

Ideas for the Scope section include addressing the role hatcheries play in the Great Lakes--- conservation, etc.--- and including a purpose (get Gary's wording).

2. Agency Updates II (All)

Ontario Ministry of Natural Resources (B. Locke): VHS was found in Lake Simcoe in gobies, perch, and bullheads. A broad scale monitoring effort detected this, and it has very important implications for the recreational fishery to the Toronto area. Restrictions are in place for bait movement, and the zones will be re-mapped to reflect this detection.

Indiana DNR (D. Meuninck): A reference regarding Brookfield Lake in the latest report needs to be corrected as it was NOT on the list. Inspections have detected A. sal and Renibacterium throughout the last few years, but there were never any clinical signs of disease. There was a furunculosis outbreak at the Curtiss Creek trout station. Approximately 3000 brown trout from Wolf Creek were affected, but there was low mortality due to a treatment of medicated feed. There were only a few fish kills overall (excluding carp), and APHIS money funded the inspection of 6 waterbodies. There were 4 carp kills throughout the state due to the koi herpesvirus. This was the 2nd year of injecting broodstock with thiamine using a procedure from Dr. Honeyfield. Overall there was good survival throughout the past year, and control trials will begin in 2012. Coho had an increase in mortality because of work with another hatchery, resulting in the death of approximately half of the fry during transport. EMS mortality in approximately 20% of coho fry was discussed with the Wolfe and Platte hatcheries. During mass marking event, Chinook salmon came down with gill disease after marking.

Day 3: 10 February 2012

1. Agency Updates III (All)

Pennsylvania FBC (C. Yamashita): IPN was detected in steelhead and brown trout destined for Lake Erie. They are euthanizing ~80,000fish and disinfecting hatcheries. Biosecurity protocols are being written for nurseries throughout the state, especially Lake Erie drainage nurseries. Nucleospora was found in broodfish in the wild and 3 lots were found positive in the hatcheries in approximately 300,000 steelhead. Smallmouth bass are undergoing a full genome sequencing of LMBv to see if it has mutated.

Michigan DNR (G. Whelan): Whirling disease is in the system but not in hatcheries. It's been found in low titres and only with PCR. VHS policy and surveillance did not found the pathogen, and people are beginning to question if this is all necessary (non-DNR). A publication is being released soon about the history of VHS in Michigan. There have been high TDS levels this year and it is necessary to treat with thiamine. There were concerns about the muskie broodstock in Lake St. Clair, but all VHS tests came back negative. There were many kills throughout the state last year from koi herpesvirus.

USFWS- Wisconsin (K. Phillips): There is a new annual vaccination program for IPN for coastal brook trout and lake trout broodfish. Furunculosis injections are being used where appropriate. A bait fish study is being wrapped up regarding the Lacey Act importation rules. Some bait dealers were not following the guidelines. In 45 cases of virology, 24 were found to contain viruses. Thirteen were novel. Of 82 lots, 34 were positive, including the golden shiner virus and fathead minnow virus. Construction projects are proceeding at Jordan River with new buildings over the raceways. Todd Turner is the new assistant regional director.

2. FHC Research Priorities (All)

The Research Priorities are due to be updated. See Appendix 6 for the current priorities. Below are suggested revisions, to be approved by the committee during its summer meeting.

Research Priorities

- What non-lethal field sampling techniques and tissue/fluid samples are equivalent to lethal field sampling methods to determine fish disease status?
- What is the ecology of important fish pathogens and diseases?
- Examples of GLFHC important disease and pathogens include VHSv Genotype IVb, Heterosporis sp., and other emerging diseases.
- What is the effectiveness of the GLFHC disinfection protocols in eliminating key pathogens of interest from fish eggs? There is a need for a reliable disinfection methodology to prevent pathogen transmission via eggs and sperm.

Additional Research Interests

1. Development and validation of new methods for detecting emerging fish pathogens or pathogens of concern in the Great Lakes Basin.
    a. What factors affect sample integrity during collection, shipment, and storage?

2. Disease Ecology and Epidemiology
    a. What is the susceptibility of Great Lakes fish species to emerging fish pathogens in the Great Lakes?
    b. Identification of reservoirs and vectors (including ballast water) for fish pathogens in the Great Lakes Basin
    c. What factors affect the virulence of fish pathogens?
    d. What is the effect of population size on disease expression?
    e. What are the effects of multiple pathogens or combination of pathogens and nutritional deficiency and/or contaminant exposure on disease expression?

3. Nutritional Aspects of Fish Health in the Great Lakes
    a. What is the role of lipids or other nutrients in determining and predicting health status?
    b. What is the role of thiaminase-producing organisms in Great Lakes ecosystems?
    c. What affect do invasive species have on nutrient stores in the Great Lakes and what are the associated affects on fish health?
    d. What is the effect of nutrition on reproductive success?
    e. Does protein substitution in hatchery feeding formulations or extrusion manufacturing methods have a negative impact on survivorship, migratory behavior or reproductive success of hatchery-reared salmonids?

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

3. Research Pre-Proposals (All)

The committee was asked to review four research proposals concerning fish health topics.

4. Summer 2012 meeting (K. Phillips)

It will be held in LaCrosse, WI from Monday, July 30th to potentially Tuesday, July 31st. Other meetings are being held that week at the hotel, including the AFS Fish Health Section meeting (Wednesday-Thursday) and a Continuing Education Workshop (Friday).

Homework for the next meeting is to think of pathogens in the GL basin without a chemical or drug that can be used for treatment (eg., improved antibiotic feed for smaller diets).

5. Date and Location of Winter 2013 meeting (All)

The winter meeting will be held Tuesday, February 5 - Thursday, February 7 in Indiana (near South Bend). The committee should consider inviting the Department of Agriculture.

Minnesota will be the host for the summer 2013 meeting.

APPENDIX 1

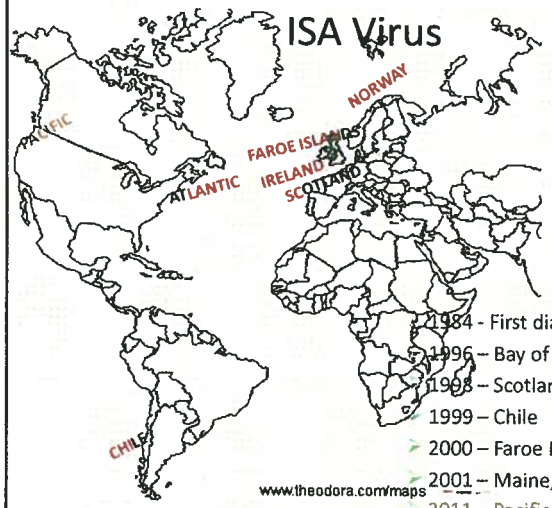
ISA Virus

OIE reportable disease of farmed Atlantic salmon

Other salmonids and several non-salmonids can function as carriers

Though to a lesser degree than Atlantic salmon, the virus can infect and replicate in brown trout and rainbow trout

ISA Virus



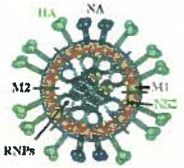
- 1984 - First diagnosed in Norway
- 1996 – Bay of Fundy, Canada
- 1998 – Scotland
- 1999 – Chile
- 2000 – Faroe Islands
- 2001 – Maine, USA
- 2011 – Pacific BC

ISA Virus

- Orthomyxovirus = influenza viruses
- North American and European genotypes
  - Both possess pathogenic types
  - Atlantic cases mostly NA, some EU
  - Mostly intermediate to highly pathogenic
  - A non-pathogenic ISAV strain –HPR 0
    - Is a EU strain, but found in Atlantic

ISA Virus- HPR 0 strain

- Non-pathogenic
- Non-cultivable in cell culture
- Detected solely by PCR
- HPR = Highly Polymorphic Region of segment 6 of the genome that codes for protein responsible for receptor binding and cleavage for virus entry to host cell



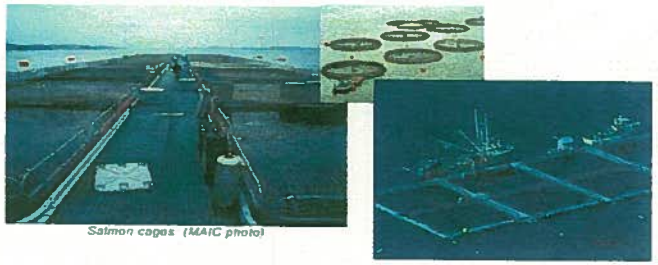
=pathogenicity

HPR0s ubiquitous in regions w/ previous disease

APPENDIX 1

ISA Virus

- Transmission
  - Horizontal - through water column
  - Vectors – sea lice
  - Vertical - true vertical or surface of gametes unknown



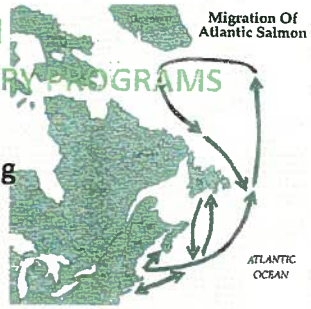
Salmon cages (MAIC photo)

ISA Virus

- Control
  - Surveillance
  - Depopulation/ indemnification
  - Zone Fallowing
  - Vaccination

ATLANTIC SALMON RESTORATION/RECOVERY PROGRAMS

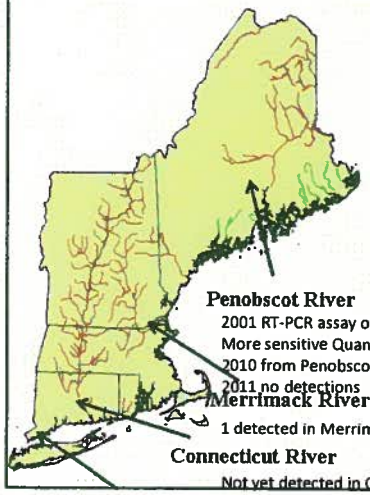
- Wild ATS adults captured during freshwater spawning migration for use as broodstock



Salmon Pen Sites in Maine

- Risk of amplification and distribution of pathogens to culture facilities and watersheds through gametes

ISA virus in **Wild** Atlantic salmon



Penobscot River
2001 RT-PCR assay of blood
More sensitive Quantitative RT-PCR detected a handful in 2009 and 2010 from Penobscot sea runs – HPR 0
2011 no detections

Merrimack River
1 detected in Merrimack sea run in 2010

Connecticut River
Not yet detected in Connecticut River - serum antibodies

APPENDIX 1

ISAV detection and Management

- **DNA Sequence analysis identified non-pathogenic (HPRO) genotype found to be carried by wild ATS**
- **Possible mutation of HPRO to pathogenic strain in captivity causes risk to ATS programs**
- **Individual fish identified as positive either released back into rivers, or culled from population before spawning.**

ISA Virus- NEW FINDING

2009 collapse of the Fraser River sockeye salmon fishery,

Leukemia/parvovirus as possible decline for wild salmon

The virus was found in two of 48 sockeye smolts collected as part of a long-term study led by Simon Fraser University professor Rick Routledge on the collapse of Rivers Inlet sockeye salmon populations.

Dr. Fred Kibenge of the reference laboratory for infectious salmon anemia at the Atlantic Veterinary College at the University of Prince Edward Island made the diagnosis and notified the Canadian Food Inspection Agency of the positive results for the European strain of ISA virus.

Politics – "DNA fragments"- exotic species -reports of archived

ISA Virus Surveillance Plan

- OIE basis/schemed for determination of ISAV status in sectors/zones/farms
- Very intensive; Funding presently uncertain
- Many collaborators

Alaska Dept Fish & Game	NW Indian Fisheries Commission
USDA APHIS ISA Program	USDA APHIS Natl Animal Health Policy
USDA Natl Vet Services Lab	USDA APHIS Washington Area Office
USDA APHIS West. Reg Off	US Dept Comm – NMFS
US Dept Int – USFWS	US Dept Int – USGS

Washington Dept Fish & Wildlife

Washington Animal Disease Diagnostic Lab

ISA Virus Surveillance Plan goals & strategies

- Regional ISAV status: zonation - movement decisions
- ISA disease freedom status for facility exports
- Investigated response to stakeholder concerns
- Prevention & early detection of new introductions
  - Natural and Enhancement Salmonids and Pacific Herring
  - Atlantic Salmon Populations
  - Salmonid Hatcheries Producing Fish/Eggs for Export
  - Future Surveillance

APPENDIX 1

ISA Virus Surveillance Plan

- Real-time RT-PCR segment 8 of genome – if suspect
- Cell culture of -80 archived on CHSE and ASK or SHK

If suspect, then follow up:

- 2 RT-PCR assays –segments 6 & 8 of genome
- Any PCR product(s) will be sequenced
- Suspects to NVSL

ISA Virus Surveillance Plan

- Presumptive Positive Sample
  - Positive by 2 molecular tests (segment 6 & 8)
  - Agreement between originating lab & NVSL
    - Including sequence of at least 100 base pairs of each segment
- Confirmed Positive Sample
  - Presumptive requirements, plus
  - Successful ISA virus isolation
- Confirmed Positive Population
  - Two or more confirmed positive samples as defined above. Or
  - One confirmed, a second presumptive, and mortality, pathology, clinical signs

Natural and Enhancement Salmonids + Pacific Herring

- 2 year surveillance to evaluate ISAV status
- Steelhead ideal
  - Broad ranging
  - Known susceptible to ISAV
  - Readily accessed during spawning
- Other species with potential susceptibility
  - Pacific herring
  - Five Pacific salmonids (chum, Chinook, pink, Coho, sockeye)

Natural and Enhancement Salmonids + Pacific Herring

95 % confidence if at 1% incidence

presuming test sensitivity 85% and specificity 100%

= 350 fish sampled per species survey

10 -11 surveys / year = 3500 – 3850 fish sampled

1% incidence lower than OIE 2 % because susceptibilities uncertain

To enhance, focus on high population susceptibility (e.g. stress); marine will target sea-run adults, moribund Tribal/State net pen fish, freshwater exposure in anadromous zones

APPENDIX 1

Natural and Enhancement Salmonids + Pacific Herring

- Total sample volume of 3,500 – 3,850 fish/yr
  - 1,400 steelhead (700 returning adults, 700 juveniles)
  - 700 sockeye (350 returning adults, up to 350 juvenile sockeye and/or Kokanee adults)
  - 350 coho (returning adults / net pen moribunds)
  - 350 pink (returning adults in odd years)
  - 350 Chinook (returning adults)
  - 350 chum (returning adults)
  - 350 Pacific herring (marine net pens)

Atlantic Salmon Populations

- 2 year surveillance to establish regional freedom from ISAV
- Not native so commercial populations are focus
  - Feral (escapees) collected during other surveillance will be included.
  - Focus on moribund to enhance detection
  - Eggs will be imported so broodstock are tested under Washington State Import Regs

Atlantic Salmon Populations

95 % confidence if at 2% prevalence

presuming test sensitivity 85% and specificity 100%

4 times per year in net pens

2 times per year for freshwater juveniles

Focus on moribund fish, using Maine/New Brunswick Model 5-fold detection enhancement = 15 moribunds 4 x /yr, per farming area

8 facilities, 4- 5 geographic areas= 60 samples x 5 areas = 300 samples per year

(freshwater non-clinical 175 fish, 2 x a year)

Atlantic Salmon Populations

- Total sample volume of 650-1000 fish/year
  - 300 moribunds from marine net pens
  - 350 juveniles from the single facility
  - 0-350 broodstock (if not all eggs are

APPENDIX 1

Salmonid Hatcheries Producing Live Fish/Eggs for Export

- Again at 1% level of assumed prevalence, susceptibility unknown
- Due to biosecurity, can aggregate,
  - 95% / 1% = 350 annually
- Total sample volume of 1400 fish/year
  - 350 juveniles (1 juvenile facility , 350 fish per facility)
  - 1050 spawners (3 broodstock facilities, 350 fish per facility)

Future Surveillance All Populations

- Early Detection
  - Enhance passive surveillance
  - Continue active surveillance on moribunds
  - SHK & ASK cells use routinely
  - Tribal marine passive surveillance
- Basic Biosecurity
  - Review and update regulations
  - Conduct audits
- Certification
  - Voluntary USDA Cert Program
  - Standardizations, training, reporting

APPENDIX 2

MICHIGAN STATE UNIVERSITY

Mohamed Faisal, DVM, PhD

College of VETERINARY MEDICINE
Michigan State University

Department of Fisheries and Wildlife, Michigan State University

MICHIGAN STATE UNIVERSITY

Order Mononegavirales

- Family: *Bornaviridae* (1 Genus)
- Family: *Filoviridae* (2 Genera)
- Family: *Paramyxoviridae* (2 Subfamilies)
- Family: *Rhabdovirida*
  - Genus: *Cytorhabdovirus*
  - Genus: *Ephemerovirus*
  - Genus: *Lyssavirus*
    - *Rabies Virus*
  - Genus: *Novirhabdovirus*
  - Genus: *Nucleorhabdovirus*
  - Genus: *Vesiculovirus*
    - *Infectious Hematopoietic Necrosis Virus, Viral Hemorrhagic Septicemia Virus*

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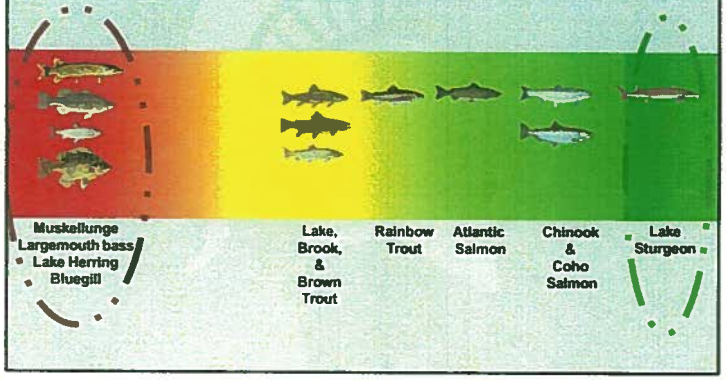
MSU Differential Susceptibility Study



APPENDIX 2

MICHIGAN STATE UNIVERSITY

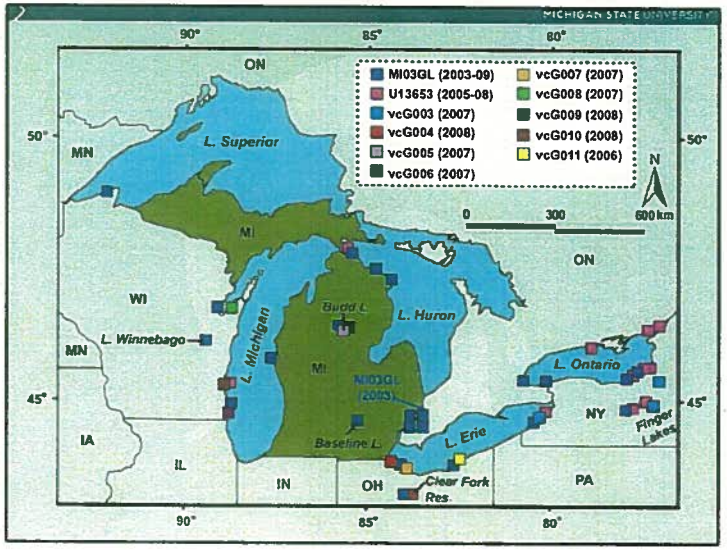
Genetic Typing of 108 Isolates through 2009 shows extremely low genetic diversity

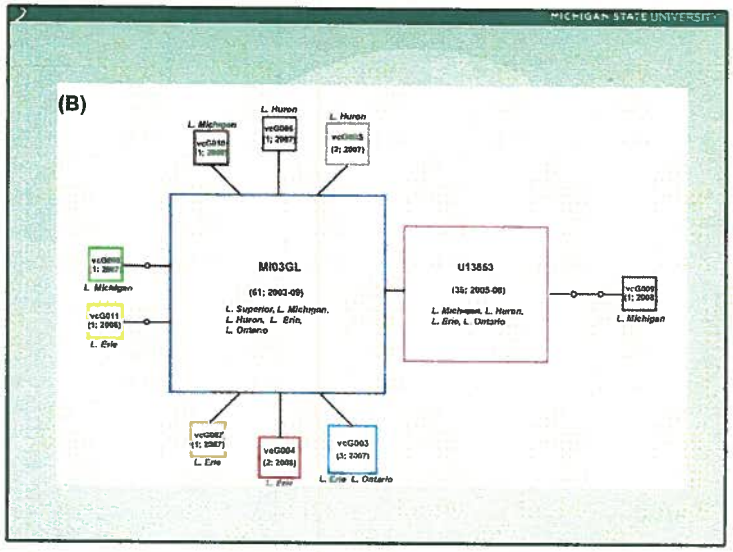
-There are 11 sequence types, cvG001-vcG011

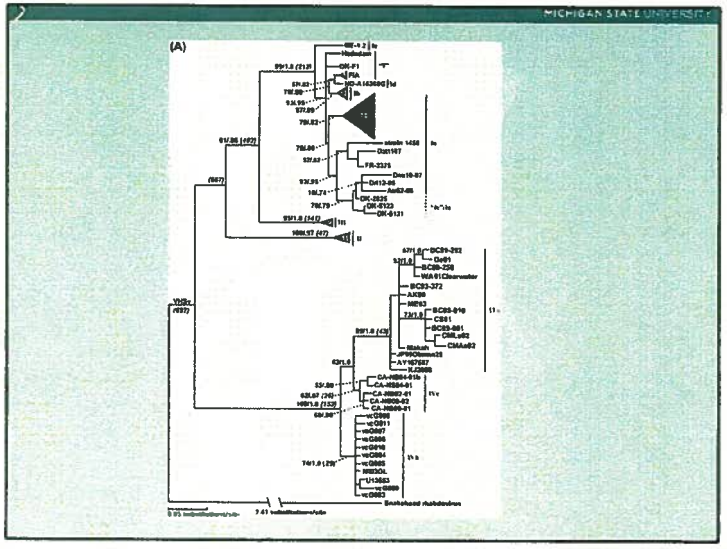
- Sequence type vcG001 is most common and contains the original 2003 Lake St. Clair muskellunge isolate

Types vcG001 and vcG002 are dominant, accounting for 97/108 isolates

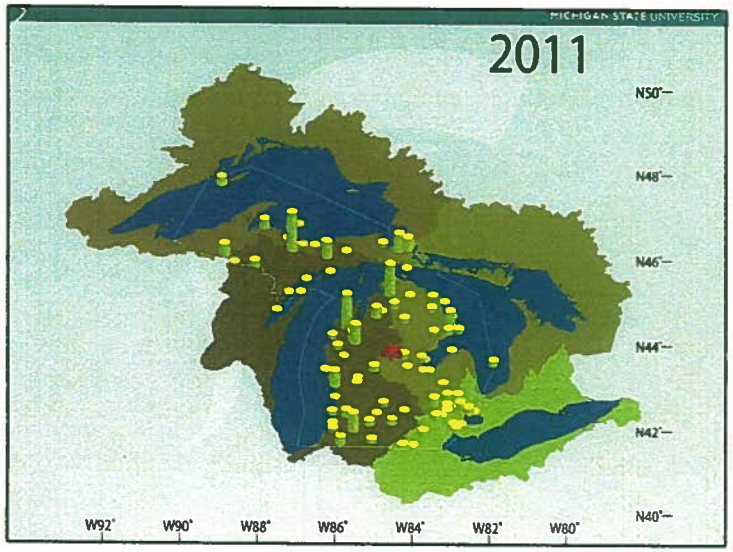
Sequence Type	*# of isolates*
vcG001	61
vcG002	36
vcG003	3
vcG004	2
vcG005	2
vcG006	1
vcG007	1
vcG008	1
vcG009	1
vcG010	1
vcG011	1

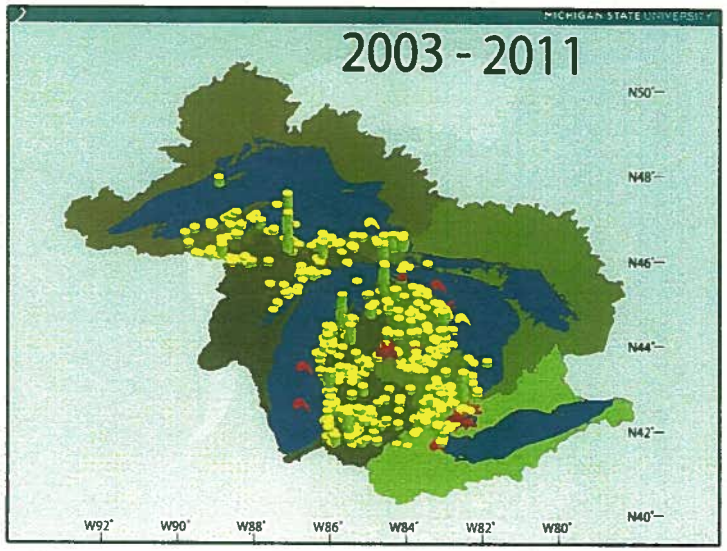




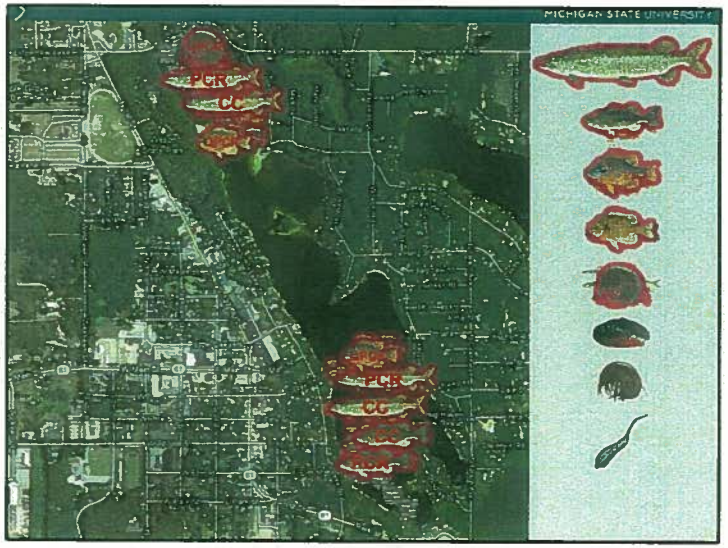


APPENDIX 2

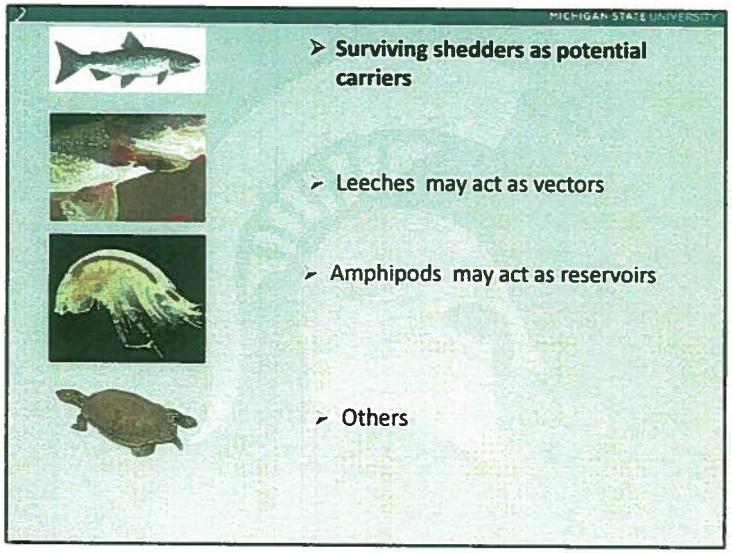

MICHIGAN STATE UNIVERSITY
2011
N50°
N48°
N46°
N44°
N42°
N40°
W92°
W90°
W88°
W86°
W84°
W82°
W80°

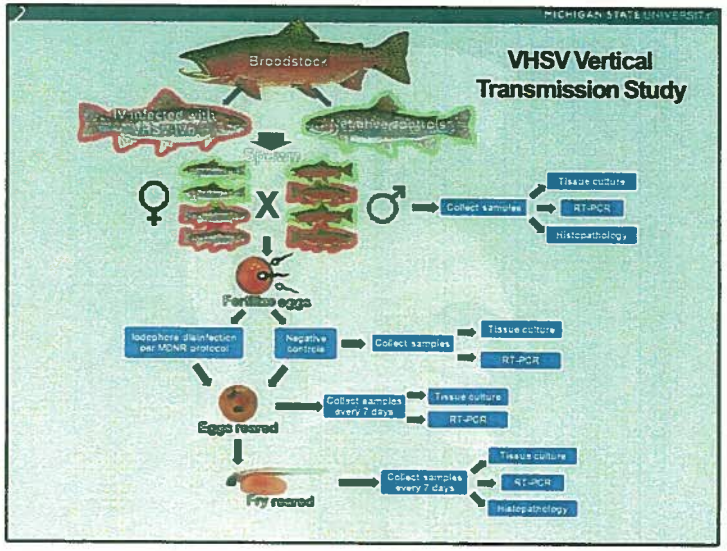

MICHIGAN STATE UNIVERSITY
2003 - 2011
N50°
N48°
N46°
N44°
N42°
N40°
W92°
W90°
W88°
W86°
W84°
W82°
W80°

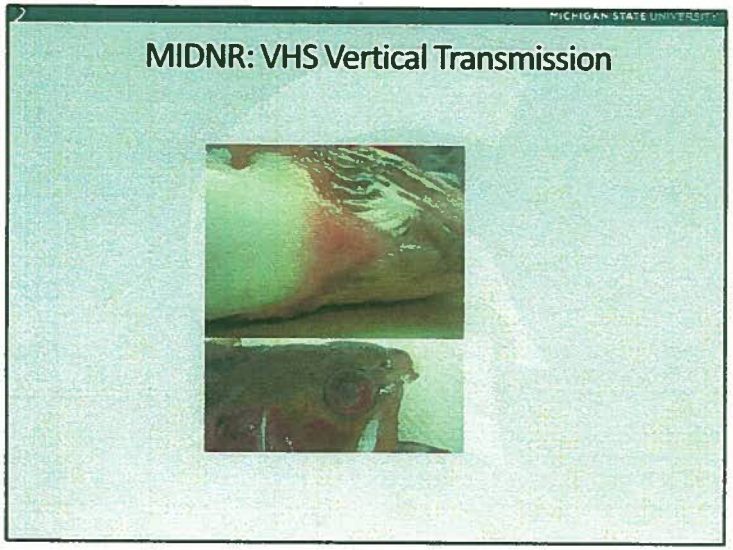

MICHIGAN STATE UNIVERSITY

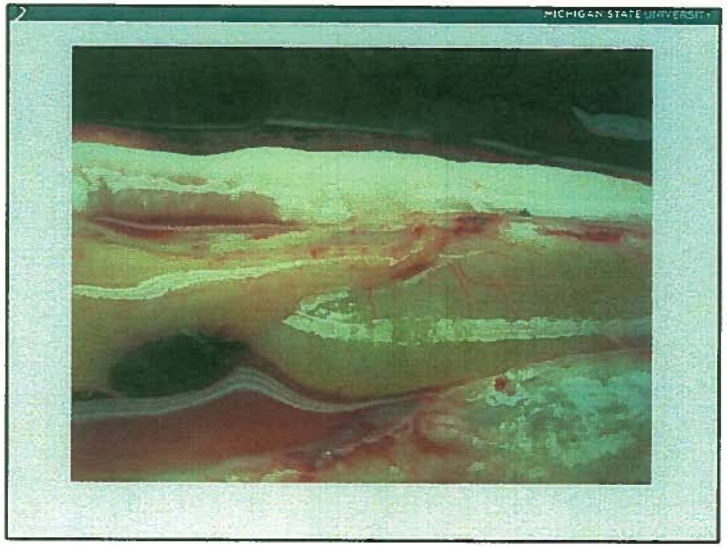

MICHIGAN STATE UNIVERSITY

APPENDIX 2

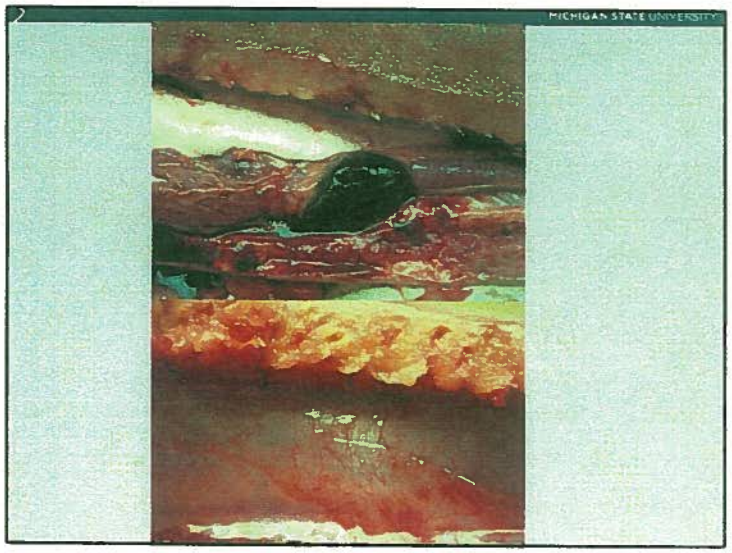

MICHIGAN STATE UNIVERSITY
Surviving shedders as potential carriers
Leeches may act as vectors
Amphipods may act as reservoirs
Others

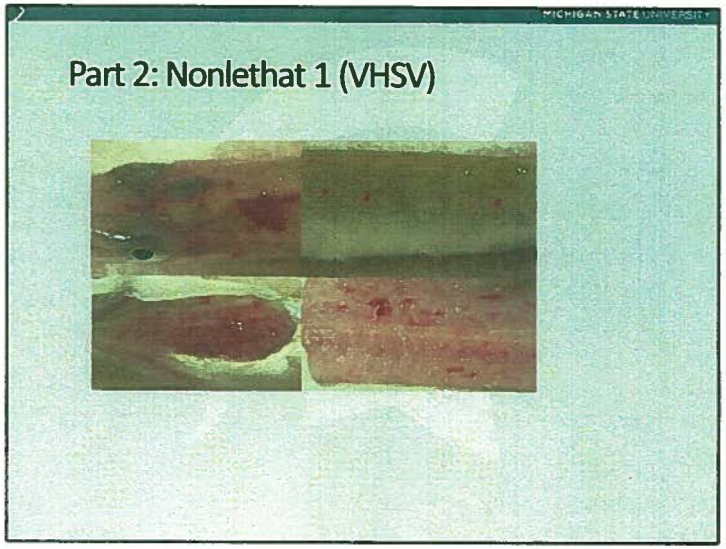

MICHIGAN STATE UNIVERSITY
VHSV Vertical Transmission Study
Broodstock
Collect samples
Tissue culture
RT-PCR
Histopathology
Negative controls
Collect samples every 7 days
Eggs reared
Fry reared

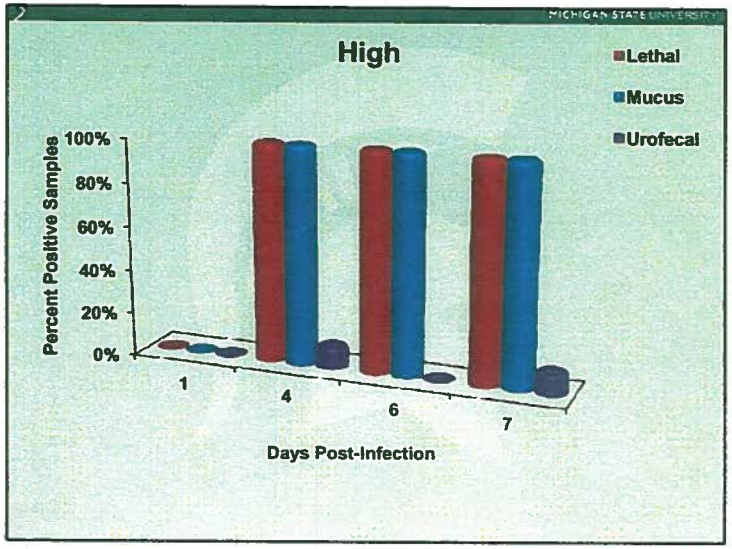

MICHIGAN STATE UNIVERSITY
MIDNR: VHS Vertical Transmission

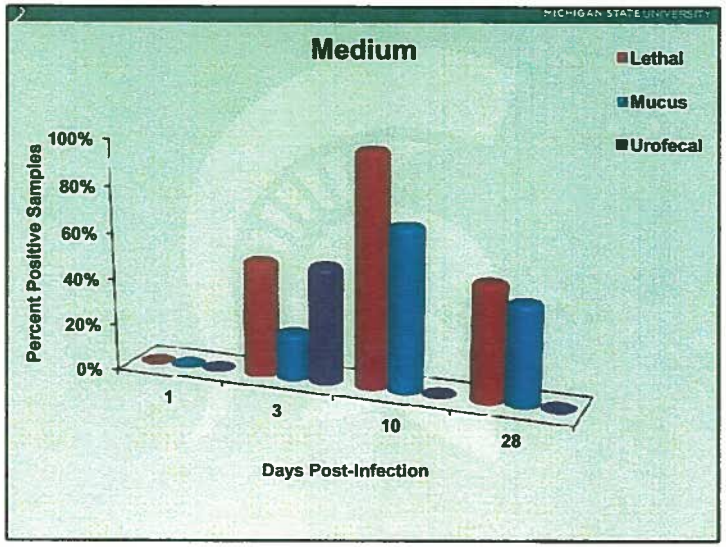

MICHIGAN STATE UNIVERSITY

APPENDIX 2

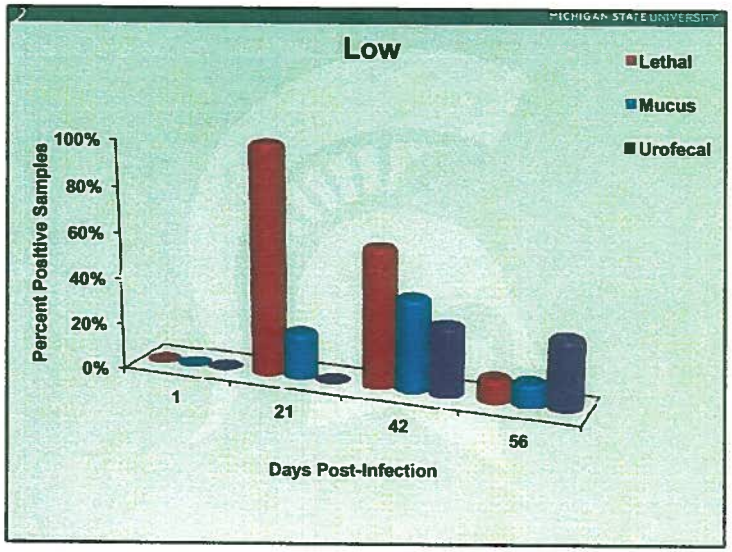


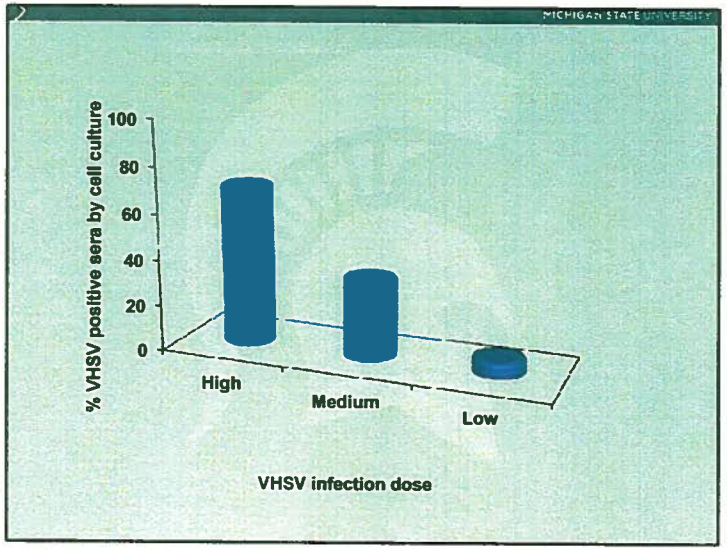

Part 2: Nonlethat 1 (VHSV)

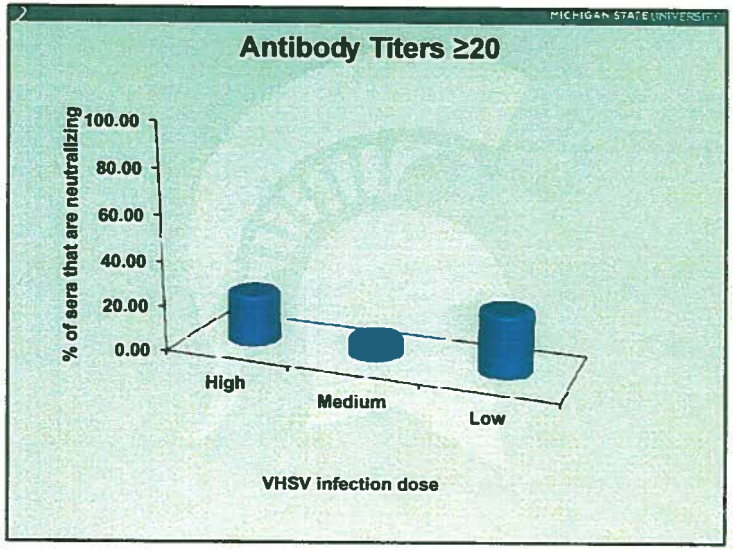

High
Lethal
Mucus
Urofecal
Percent Positive Samples
100%
80%
60%
40%
20%
0%
1
4
6
7
Days Post-Infection


Medium
Lethal
Mucus
Urofecal
Percent Positive Samples
100%
80%
60%
40%
20%
0%
1
3
10
28
Days Post-Infection

APPENDIX 2


MICHIGAN STATE UNIVERSITY
Low
Lethal
Mucus
Urofecal
Percent Positive Samples
100%
80%
60%
40%
20%
0%
1
21
42
56
Days Post-Infection


MICHIGAN STATE UNIVERSITY
% VHSV positive sera by cell culture
100
80
60
40
20
0
High
Medium
Low
VHSV infection dose


MICHIGAN STATE UNIVERSITY
Antibody Titers ≥20
% of sera that are neutralizing
100.00
80.00
60.00
40.00
20.00
0.00
High
Medium
Low
VHSV infection dose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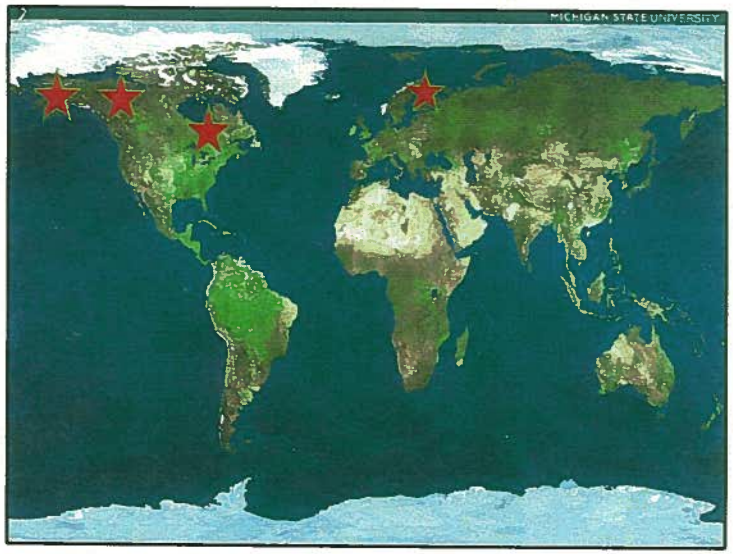
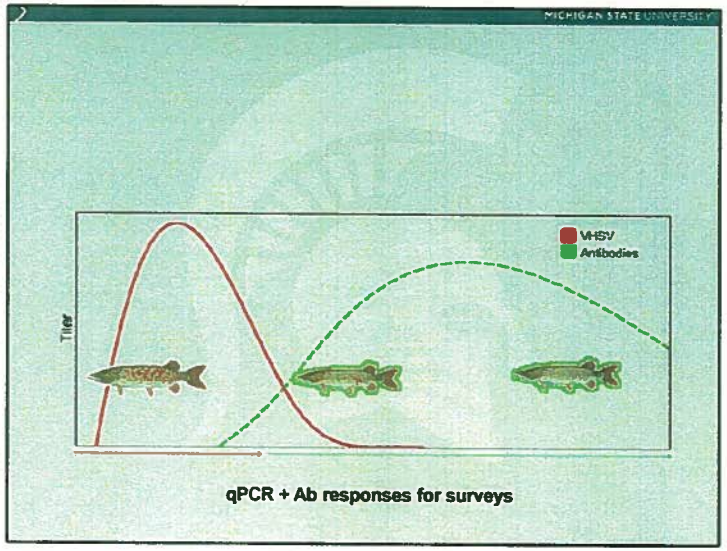
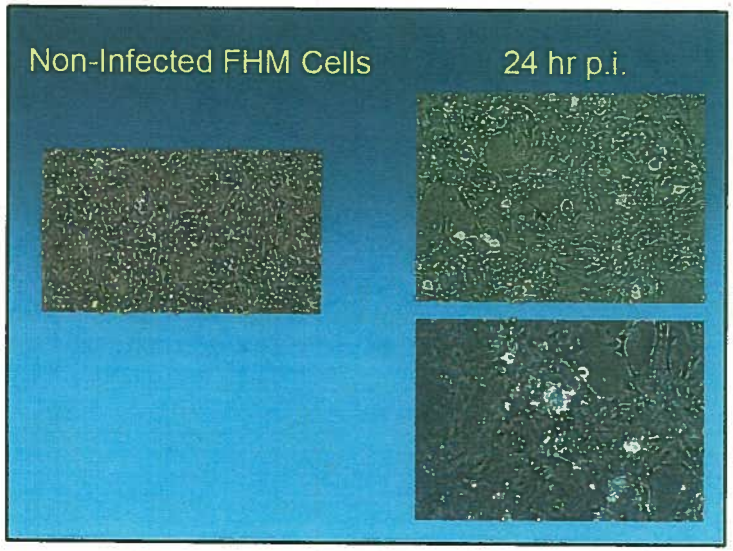
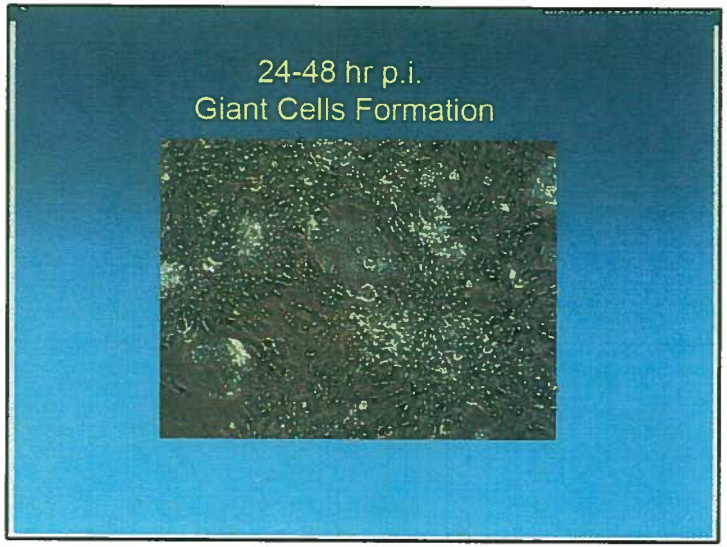
APPENDIX 2


MICHIGAN STATE UNIVERSITY

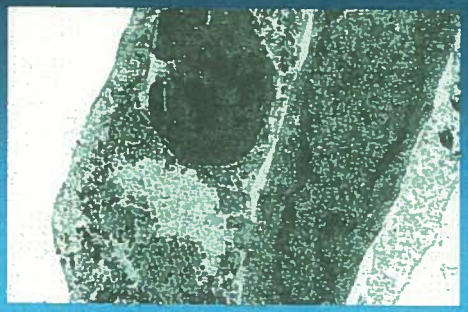

MICHIGAN STATE UNIVERSITY
VHSV
Antibodies
Titer
qPCR + Ab responses for surveys


Non-Infected FHM Cells
24 hr p.i.


24-48 hr p.i.
Giant Cells Formation

APPENDIX 2

40,500 X



Intracytoplasmic icosahedral Virus

Morphology of Purified Virus Particles

Spotted Musky Virus

- RNA virus
- Forms giant cells in FHM
- Double capsid
- Ether resistant
- Resists pH 3.0
- Survives 56 °C for 30 min

suspect reovirus !!

Family *Reoviridae*

- Orthoreovirus
- Orbivirus
- Rotavirus
- Coltivirus
- Aquareovirus
- Cypovirus
- Fijivirus
- Phytoreovirus
- Oryzavirus

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Genus Aquareovirus

- Created by ICTV in 1991
- Infects aquatic animals:
  - Mollusks: Oysters
  - Finfish: cyprinids, CCF, salmonids
  - Crustaceans

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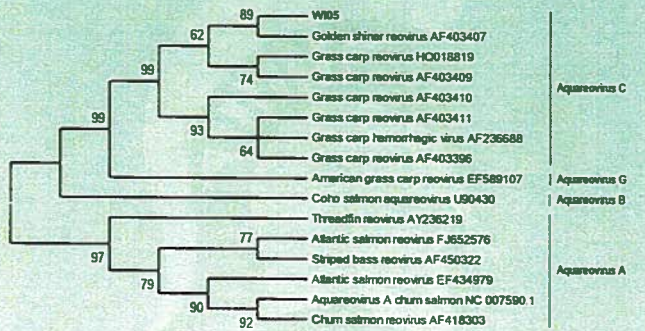

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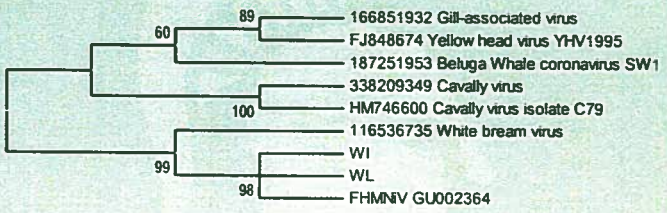
Muskellunge Nidovirus



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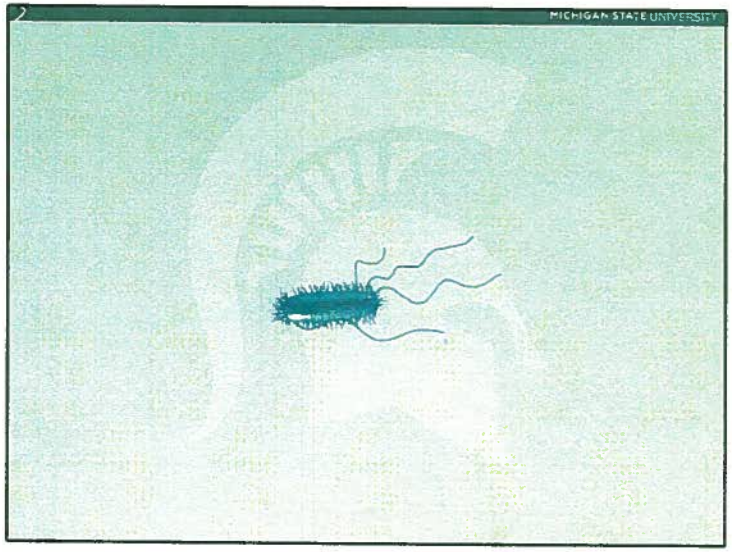
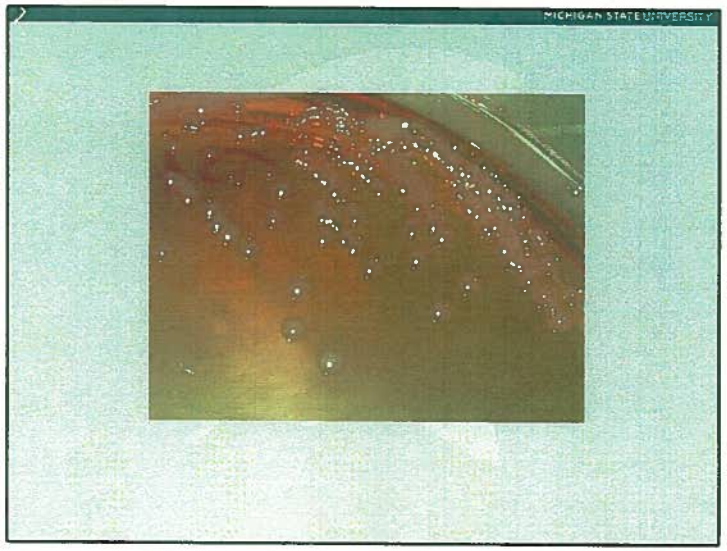
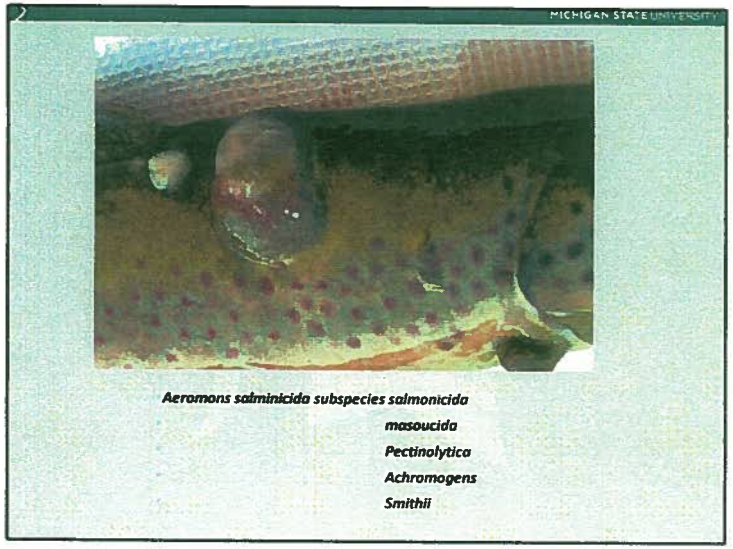
Koi Herpesvirus

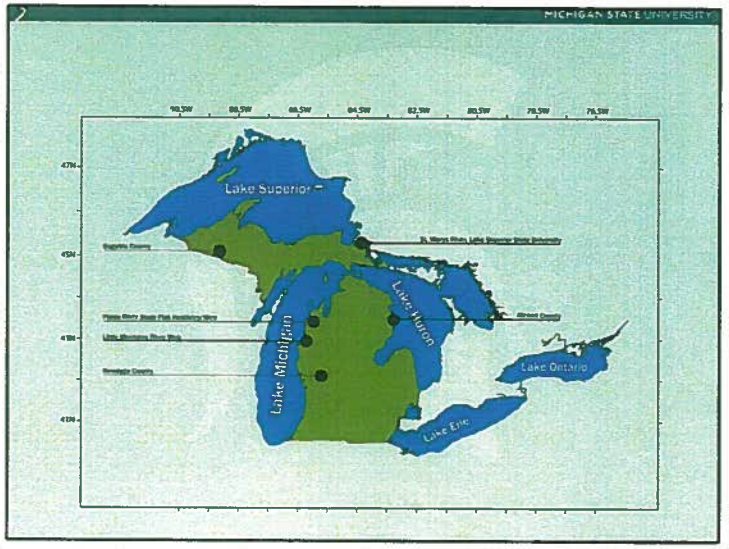
- **CC: Carp die-off event in June in Kent Lake (Oakland County),**
- **CC: Carp die-off event in August in Silver Lake (Oceana County)**
- **qPCR: October, Wolverine Lake and Kent Lake (both Oakland County)**

APPENDIX 2

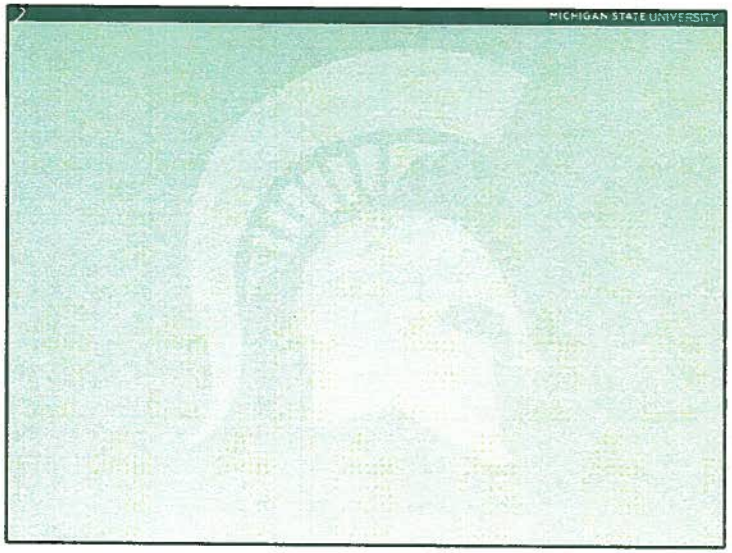

MICHIGAN STATE UNIVERSITY

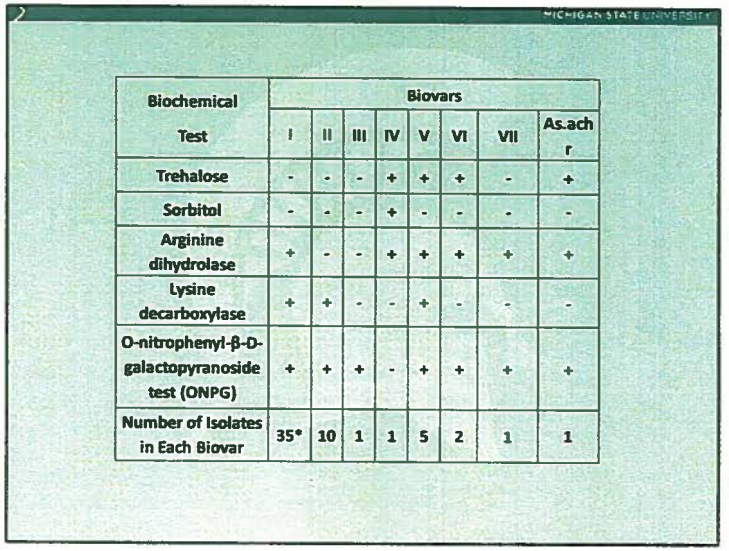

MICHIGAN STATE UNIVERSITY


MICHIGAN STATE UNIVERSITY
Aeromons salminicida subspecies salmonicida
masoucida
Pectinolytica
Achromogens
Smithii

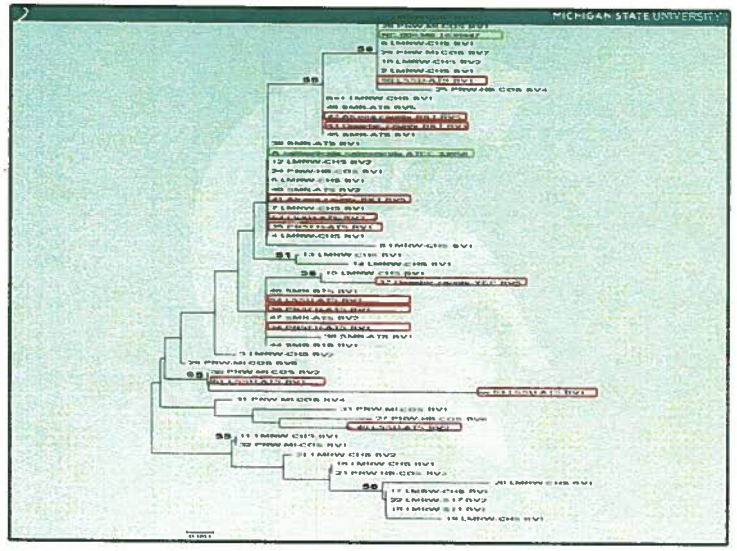

MICHIGAN STATE UNIVERSITY
Lake Superior
Lake Michigan
Lake Huron
Lake Erie
Lake Ontario

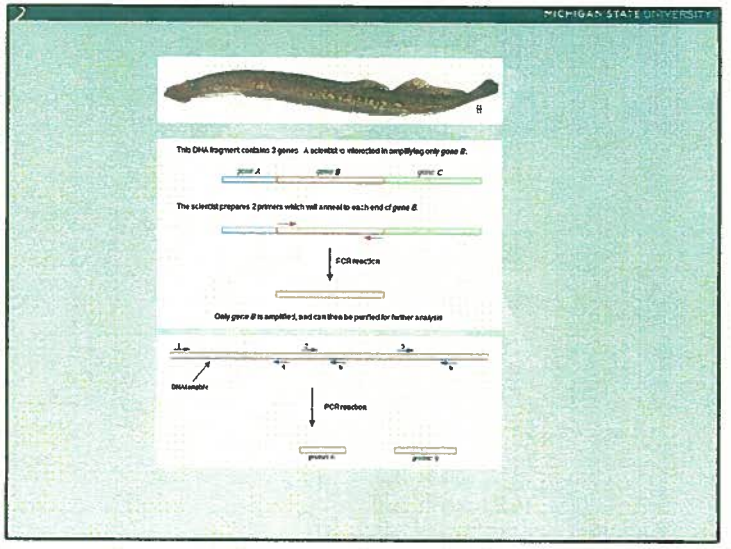
APPENDIX 2





Biochemical Test	Biovars							
	I	II	III	IV	V	VI	VII	As.achr
Trehalose	-	-	-	+	+	+	-	+
Sorbitol	-	-	-	+	-	-	-	-
Arginine dihydrolase	+	-	-	+	+	+	+	+
Lysine decarboxylase	+	+	-	-	+	-	-	-
O-nitrophenyl-β-D-galactopyranoside test (ONPG)	+	+	+	-	+	+	+	+
Number of Isolates in Each Biovar	35*	10	1	1	5	2	1	1





APPENDIX 2

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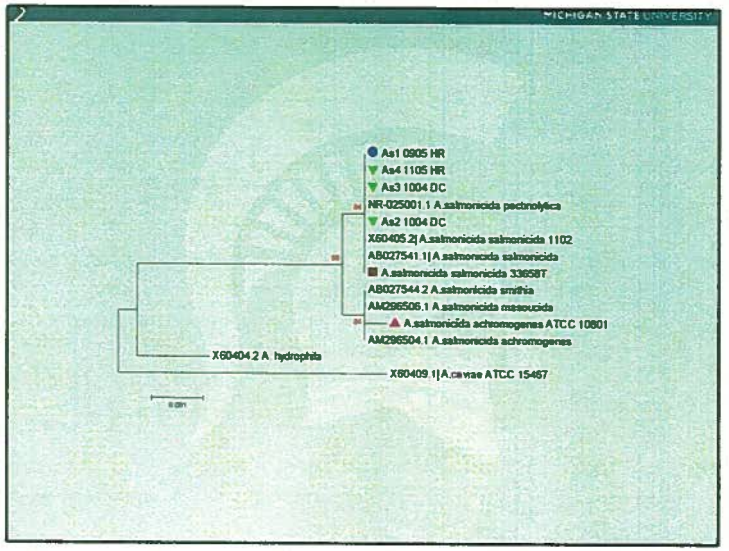
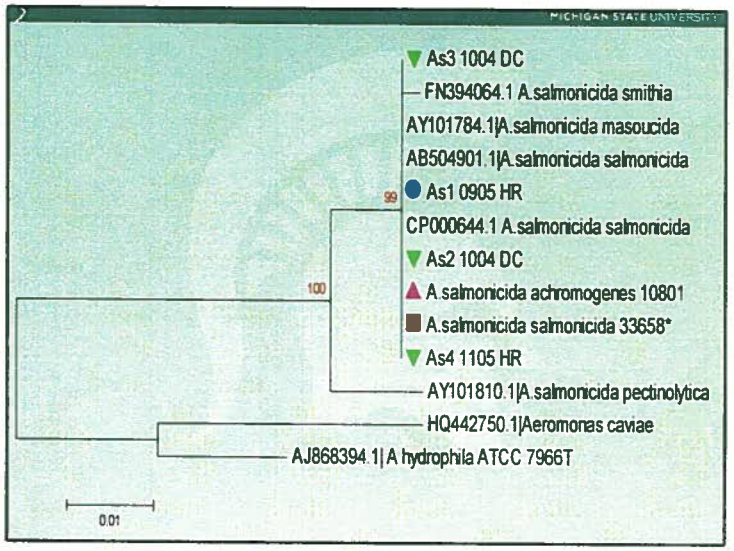
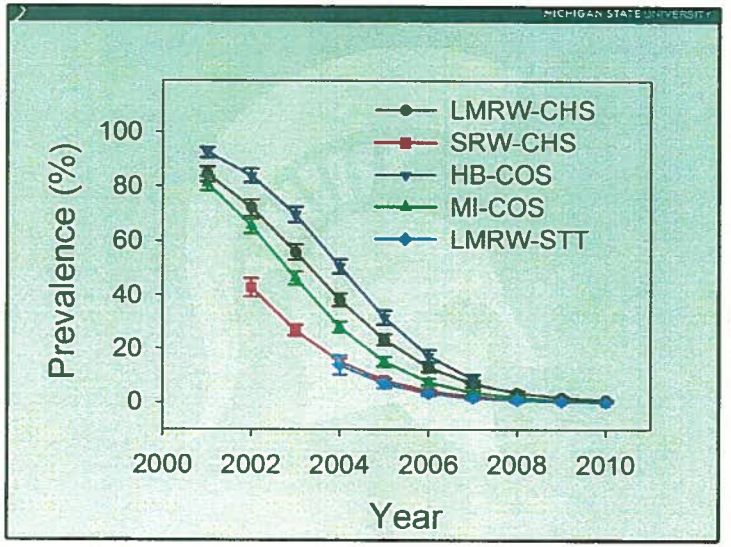
Miyata Primers (Miyata et al. 1996)

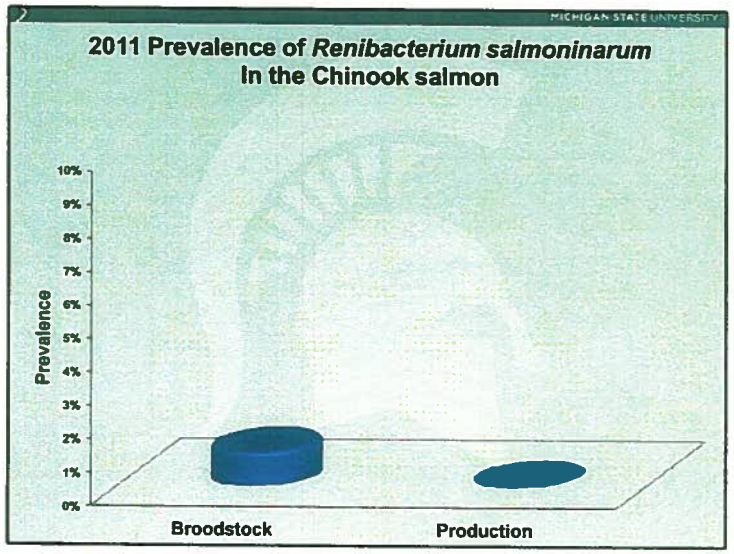
- RAPD profile from *A. salmonicida* subsp. *salmonicida*
- Products cloned
- Specificity assessed with DNA-DNA hybridization
- 680 bp sequence was determined
- Primer set designed to amplify fragment (512 bp)
- 100% specific for *A. salmonicida* subsp. *salmonicida*

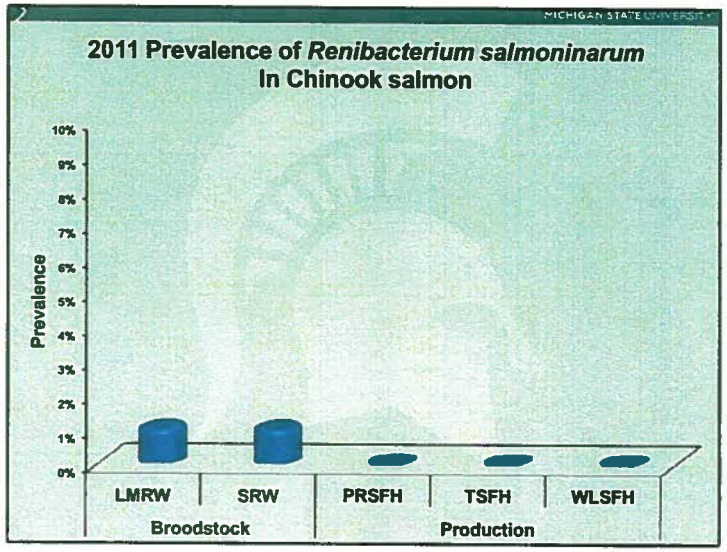


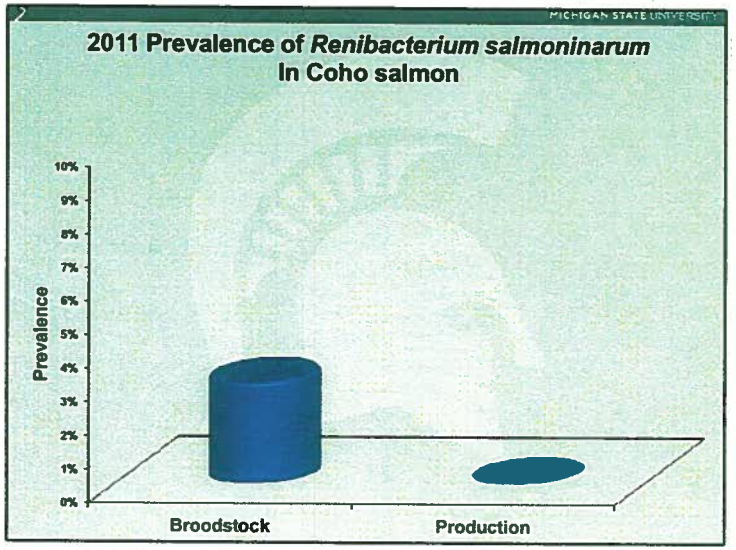


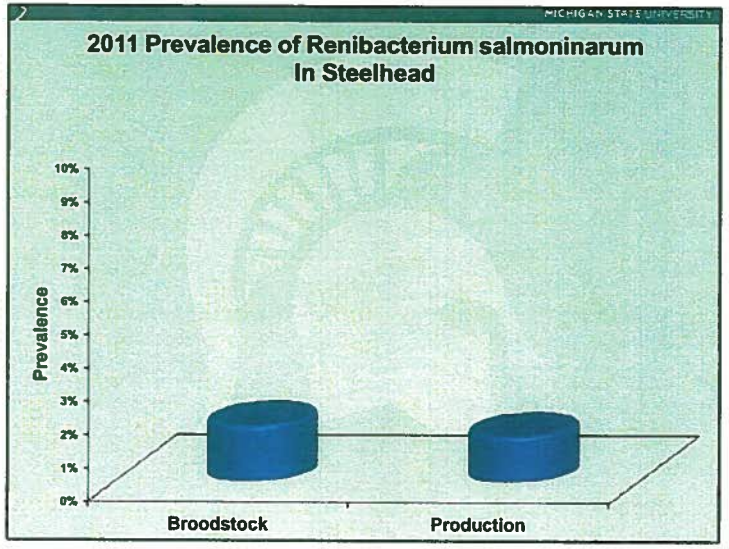


APPENDIX 2

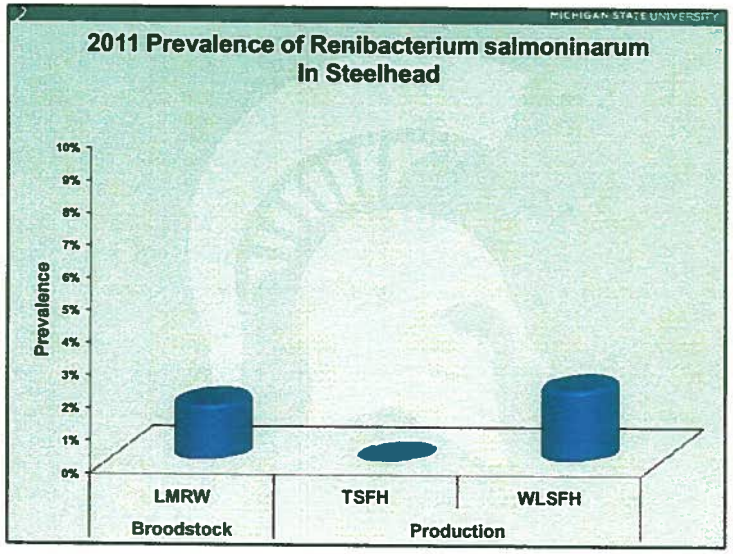

MICHIGAN STATE UNIVERSITY
2011 Prevalence of *Renibacterium salmoninarum*
In the Chinook salmon
Prevalence
10%
9%
8%
7%
6%
5%
4%
3%
2%
1%
0%
Broodstock
Production

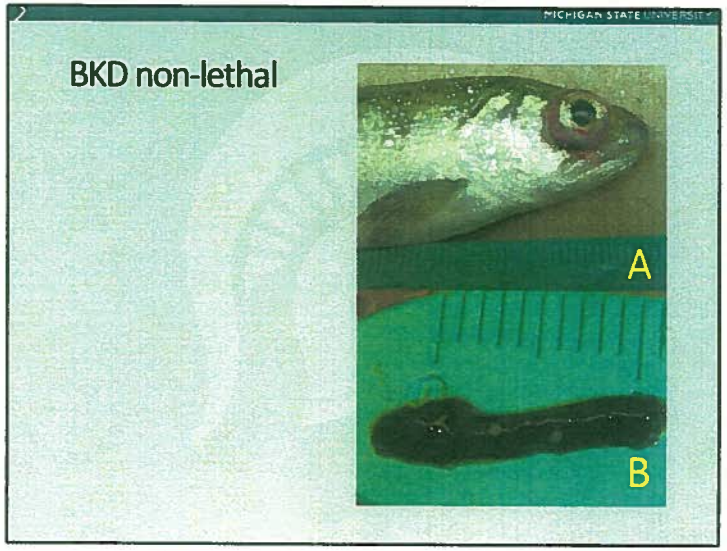

MICHIGAN STATE UNIVERSITY
2011 Prevalence of *Renibacterium salmoninarum*
In Chinook salmon
Prevalence
10%
9%
8%
7%
6%
5%
4%
3%
2%
1%
0%
LMRW
SRW
PRSFH
TSFH
WLSFH
Broodstock
Production

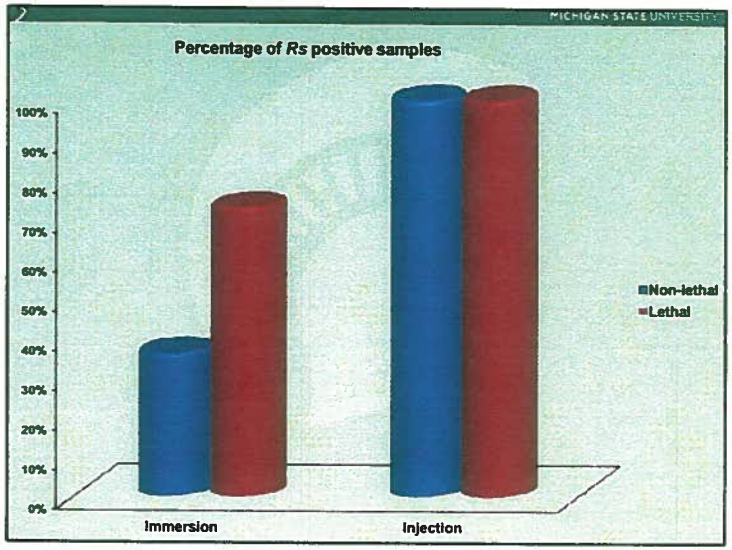

MICHIGAN STATE UNIVERSITY
2011 Prevalence of *Renibacterium salmoninarum*
In Coho salmon
Prevalence
10%
9%
8%
7%
6%
5%
4%
3%
2%
1%
0%
Broodstock
Production

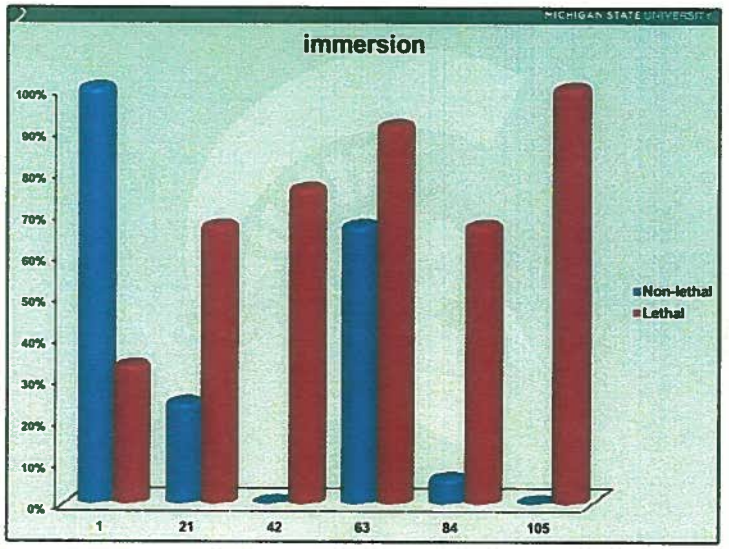

MICHIGAN STATE UNIVERSITY
2011 Prevalence of Renibacterium salmoninarum
In Steelhead
Prevalence
10%
9%
8%
7%
6%
5%
4%
3%
2%
1%
0%
Broodstock
Production

APPENDIX 2

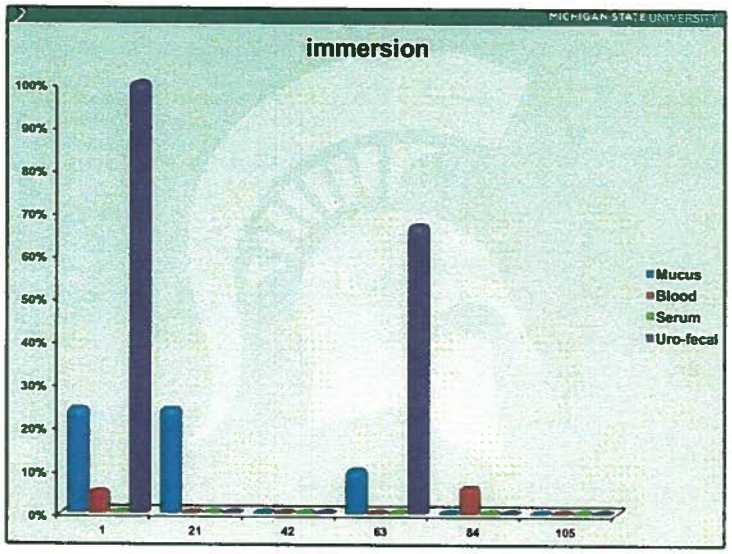

MICHIGAN STATE UNIVERSITY
2011 Prevalence of Renibacterium salmoninarum In Steelhead
Prevalence
10%
9%
8%
7%
6%
5%
4%
3%
2%
1%
0%
LMRW
TSFH
WLSFH
Broodstock
Production

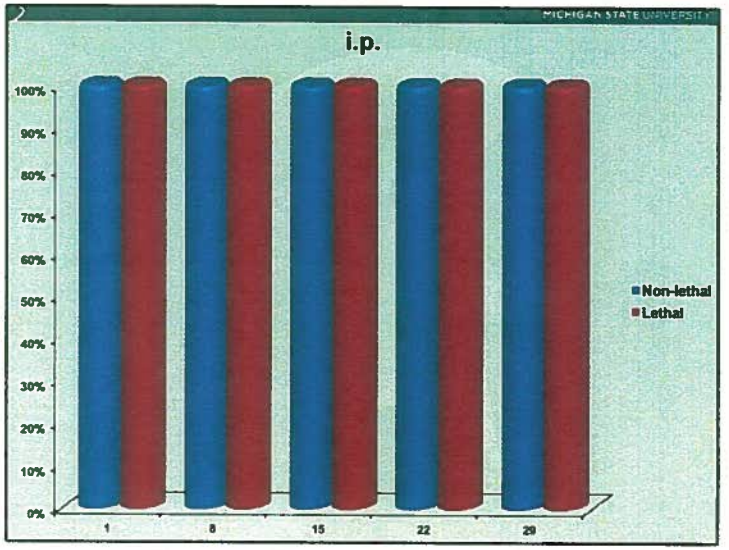

MICHIGAN STATE UNIVERSITY
BKD non-lethal
A
B

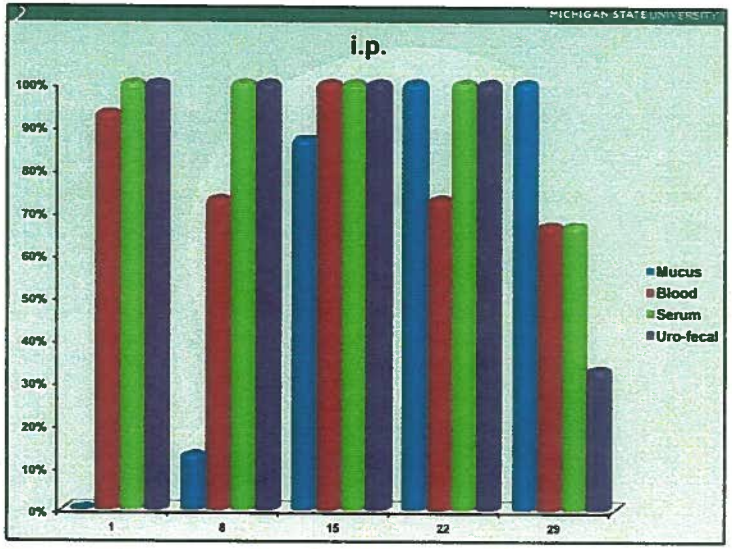

MICHIGAN STATE UNIVERSITY
Percentage of Rs positive samples
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Non-lethal
Lethal
Immersion
Injection

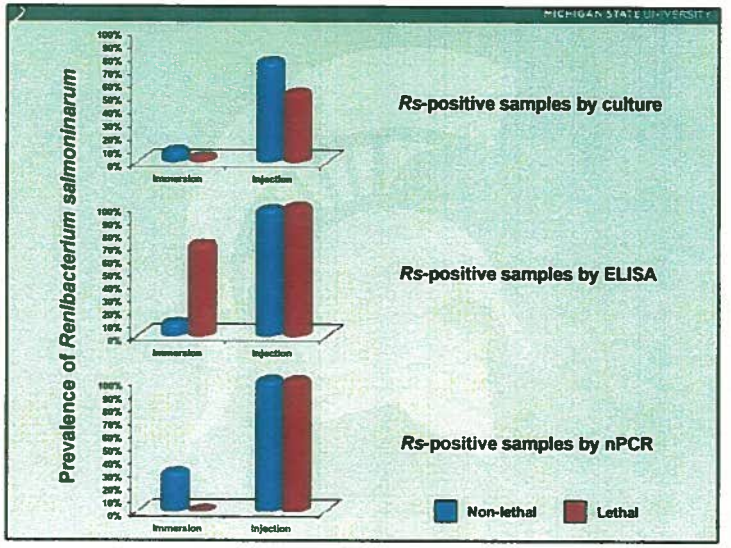

MICHIGAN STATE UNIVERSITY
immersion
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Non-lethal
Lethal
1
21
42
63
84
105

APPENDIX 2

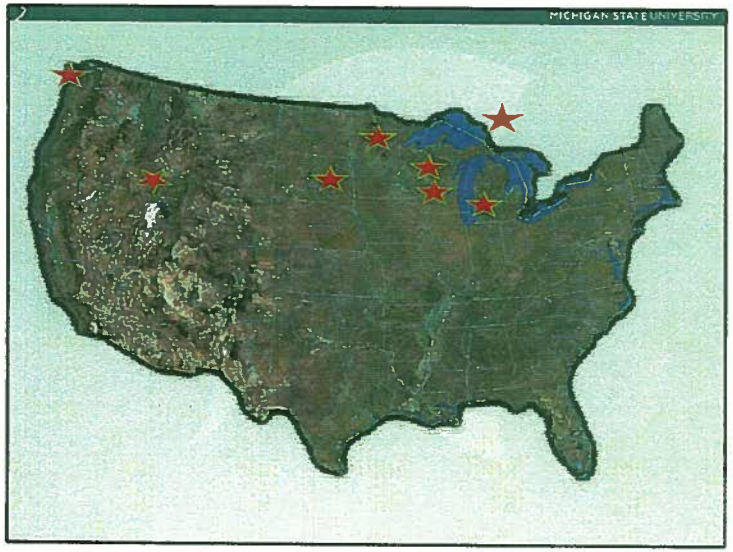

MICHIGAN STATE UNIVERSITY
immersion
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
21
42
63
84
105
Mucus
Blood
Serum
Uro-fecal

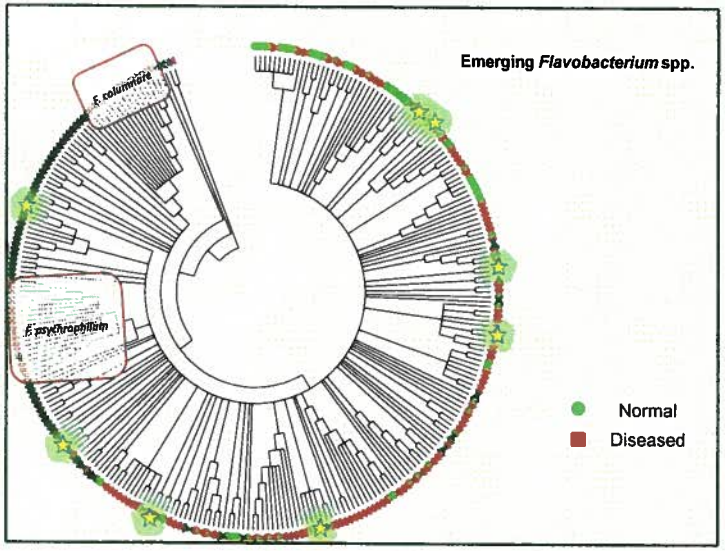

MICHIGAN STATE UNIVERSITY
i.p.
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
8
15
22
29
Non-lethal
Lethal

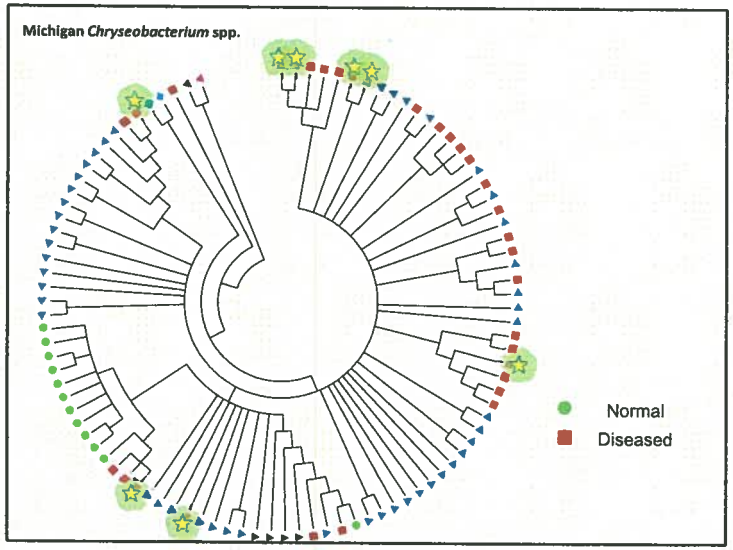

MICHIGAN STATE UNIVERSITY
i.p.
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
8
15
22
29
Mucus
Blood
Serum
Uro-fecal


MICHIGAN STATE UNIVERSITY
Prevalence of Renibacterium salmoninarum
Immersion
Injection
Rs-positive samples by culture
Rs-positive samples by ELISA
Rs-positive samples by nPCR
Non-lethal
Lethal

APPENDIX 2







16 Flavobacteria for Polyphasic Characterization

	Isolate	Host Fish Species	Assoc. w/ Mortalities	Assoc. w/ Disease Signs	Organ of Recovery
Chryseobacterium spp.	T86	Muskellunge	Yes	Yes	Kidney
	T28	Coho salmon	Yes	Yes	Kidney
	T68	Lake Trout	No	Yes	Kidney
	T72	Lake Herring	No	Yes	Kidney
	T83	Lake Herring	No	Yes	Kidney
	T31	Steelhead Trout	No	No	Kidney
	T24	Steelhead Trout	Yes	No	Gills
	T115	Chinook Salmon	No	Yes	Kidney
Flavobacterium spp.	T91	Channel Catfish	No	Yes	Kidney
	T75	Brown Trout	Yes	Yes	Kidney
	T18	Brown Trout	Yes	Yes	Kidney
	T16	Chinook Salmon	No	Yes	Kidney
	S87	Largemouth Bass	No	Yes	Kidney
	S12	Chinook Salmon	Yes	Yes	Kidney
	S21	Rainbow Trout	Yes	Yes	Ulcer
	T76	Rainbow Trout	Yes	Yes	Kidney

APPENDIX 2

Fatty Acid	T68	1	2	3	4	5	6	7	8	9
iso-C13: 0	Tr	1.3	Tr	-	-	1.2	3.0	Tr	1.3	2.0
iso-C15: 0	30.9	35.1	41.8	50.3	35.6	36.8	56.3	39.0	43.9	46.4
anteiso-C15: 0	2.6	9.1	1.9	3.8	-	1.1	2.5	1.2	1.1	1.0
16:1 w6c/ [illegible]	26.6	-	•	-	-	-	-	7.7	-	-
16:1 w5c	1.5	-	-	-	-	-	-	-	-	-
C16:0	3.8	Tr	3.4	-	1.3	1.4	1.3	1.3	Tr	3.2
iso-C15:3-OH	2.5	2.6	2.7	5.2	2.5	2.7	2.6	3.3	2.6	3.0
[illegible]	Tr	Tr	•	-	-	-	-	Tr	-	-
iso-C15 : 0 2-OH	-	10.6	-	-	-	-	-	-	-	-
iso-C17: 1 w9c	1.4	16.9	14.6	9.3	20.2	27.5	4.8	22.0	7.8	6.6
iso-17: 0	Tr	Tr	Tr	[illegible]	1.3	1.6	2.1	1.6	Tr	Tr
16:0 3OH	5.1	1.2	-	-	1.2	1.2	Tr	1.4	2.6	3.0
C18 : 1w5c	•	1.1	Tr	•	•	•	•	•	Tr	Tr
iso-C17: 0 3-OH	16.1	10.0	17.7	21.9	20.8	16.3	15.9	19.4	14.6	15.3
C17:0 2-OH	1.9	Tr	Tr							
12:0 aldehyde	Tr	-	-	-	-	-	-	-	-	-
[illegible]	•		9.7	9.5	14.0	8.4	9.4	•	19.6	17.0

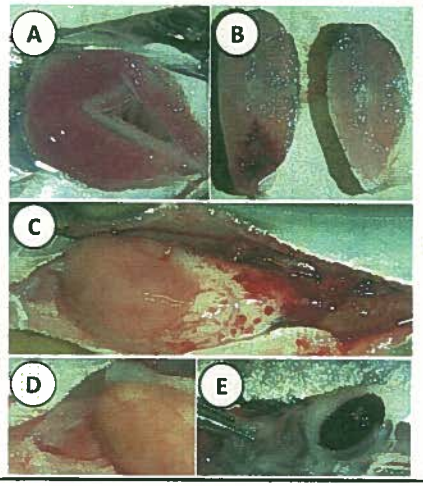
Antibiotic susceptibility results for the 16 flavobacterial isolates selected for polyphasic characterization. NZ, no zone of inhibition.

	Isolate	SXT	PB	N	P	O129	FFC	AMP	E	T
Chryseobacterium spp.	T86	25	NZ	13	NZ	23.5	NZ	NZ	NZ	NZ
	T28	22	NZ	11	NZ	21.5	NZ	NZ	13	11.5
	T68	26	NZ	13	NZ	25	NZ	NZ	18.5	17
	T72	16	9.5	15	NZ	25	NZ	NZ	NZ	NZ
	T83	27	9.5	14.5	NZ	29.5	NZ	9	13.5	14
	T31	27	8.5	13.5	NZ	30.5	NZ	9	13	13.5
	T24	24.5	8.5	13	NZ	34	NZ	NZ	12	12.5
	T115	NZ	12	17.5	13	NZ	NZ	NZ	NZ	NZ
Flavobacterium spp.	T91	12	9.5	13	NZ	NZ	32	11.5	16	23
	T75	11	10.5	15.5	30	NZ	33	29	31	14.5
	T18	14	9.5	14.5	NZ	NZ	32	13	24.5	30
	T16	11.5	10.5	14.5	NZ	NZ	16	15	18	17
	S87	14	8	15	13	15	25	15.5	17.5	30
	S12	14	11	14.5	NZ	NZ	17	NZ	18.5	16.5
	S21	NZ	11.5	14.5	NZ	NZ	27.5	NZ	18	27
	T76	18	13	16	NZ	NZ	24	13	24.5	17

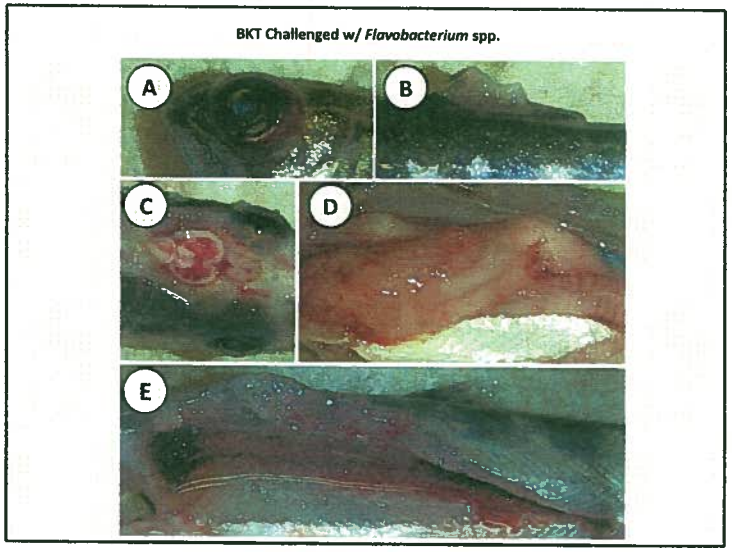
Percent mortality following ip experimental infection with *Flavobacterium* spp.

Isolate	CHS	BKT	BNT
	% Mortality	% Mortality	% Mortality
T91	0	0	20
T75	0	0	0
T18	0	0	0
T16	80	40	80
S87	0	0	0
S12	20	20	60
S21	20	40	20
T76	0	0	40
Neg	0	0	0

CHS Challenged w/ *Flavobacterium* spp.

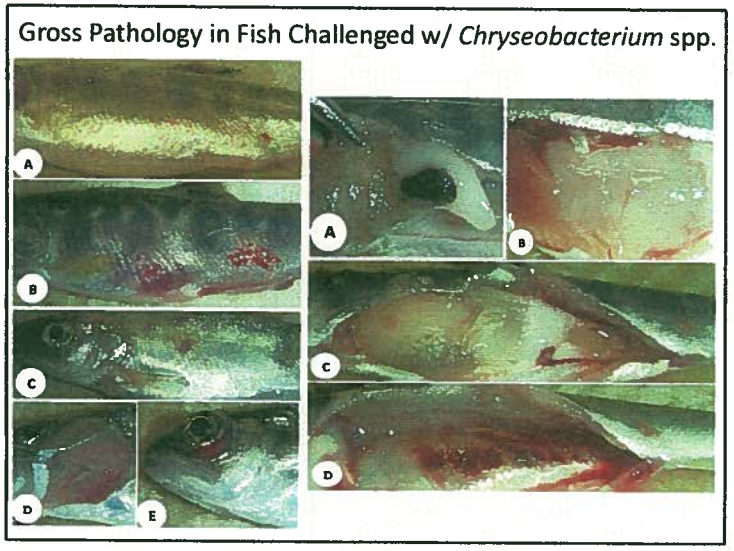


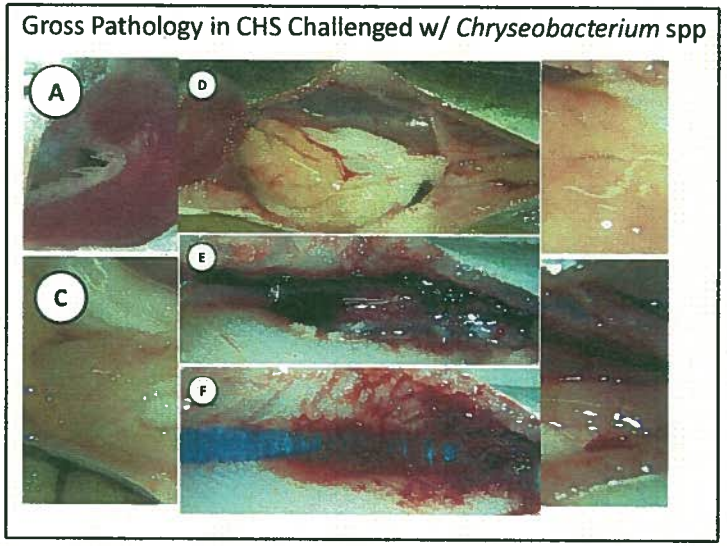
APPENDIX 2



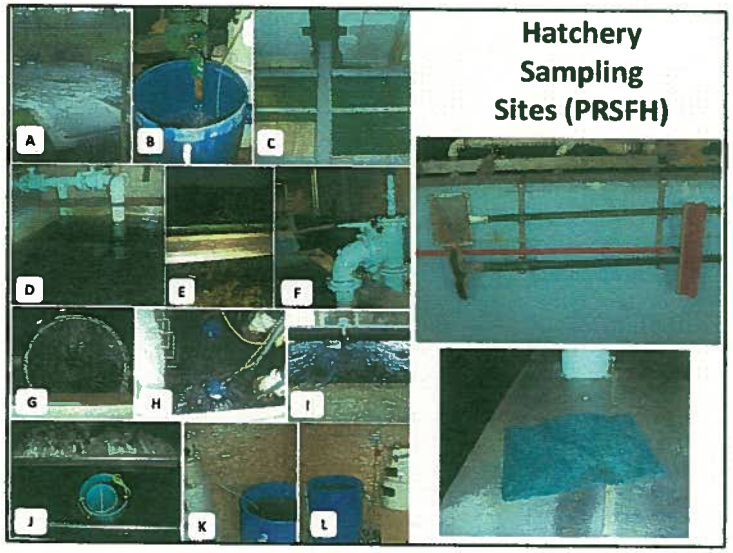
Percent mortality in fish ip infected with *Chryseobacterium* spp.

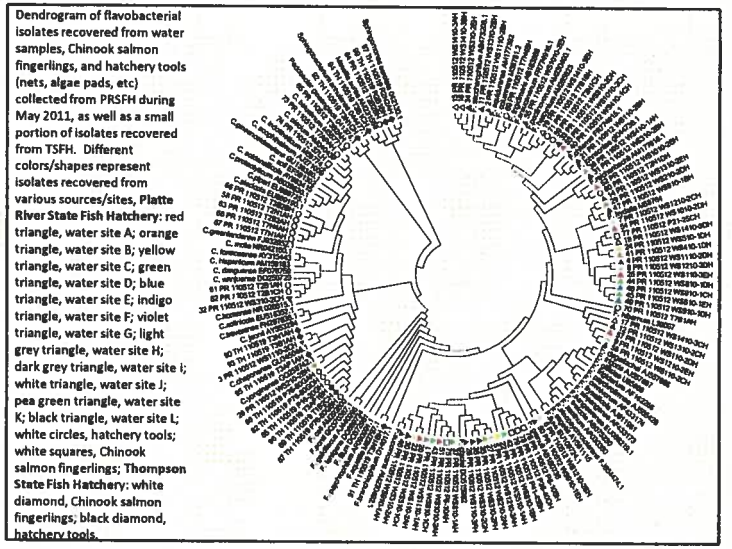
Isolate	CHS	BKT	COS & MUE	BNT
T86	80	40	20	20
T28 ★	60	60	40	60
T68 ★	20	20		20
T72	80	60		20
T83	0	0		40
T31	50	20		0
T24	60	20		20
T115	0	0		0
Neg	0	0		0



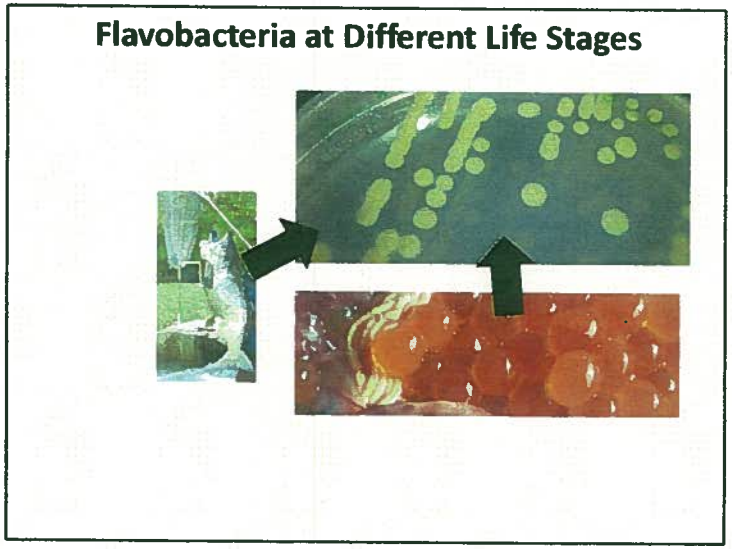


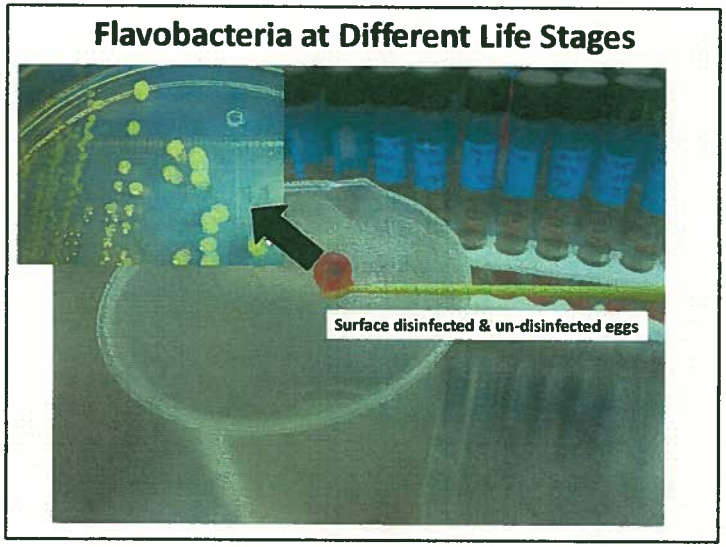
APPENDIX 2



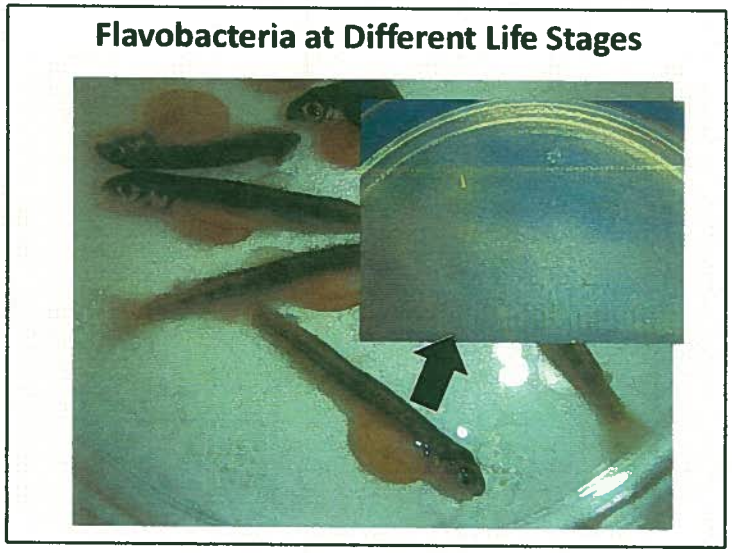


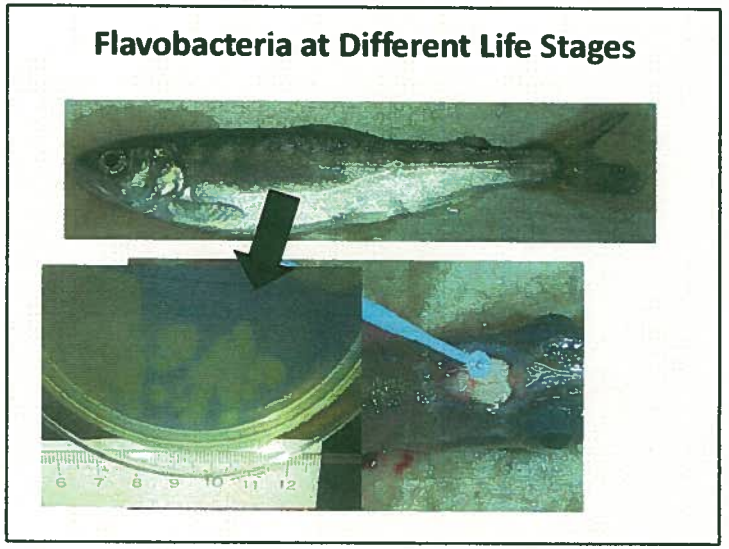
Dendrogram of flavobacterial isolates recovered from water samples, Chinook salmon fingerlings, and hatchery tools (nets, algae pads, etc) collected from PRSFH during May 2011, as well as a small portion of isolates recovered from TSFH. Different colors/shapes represent isolates recovered from various sources/sites, **Platte River State Fish Hatchery:** red triangle, water site A; orange triangle, water site B; yellow triangle, water site C; green triangle, water site D; blue triangle, water site E; indigo triangle, water site F; violet triangle, water site G; light grey triangle, water site H; dark grey triangle, water site i; white triangle, water site J; pea green triangle, water site K; black triangle, water site L; white circles, hatchery tools; white squares, Chinook salmon fingerlings; **Thompson State Fish Hatchery:** white diamond, Chinook salmon fingerlings; black diamond, hatchery tools.

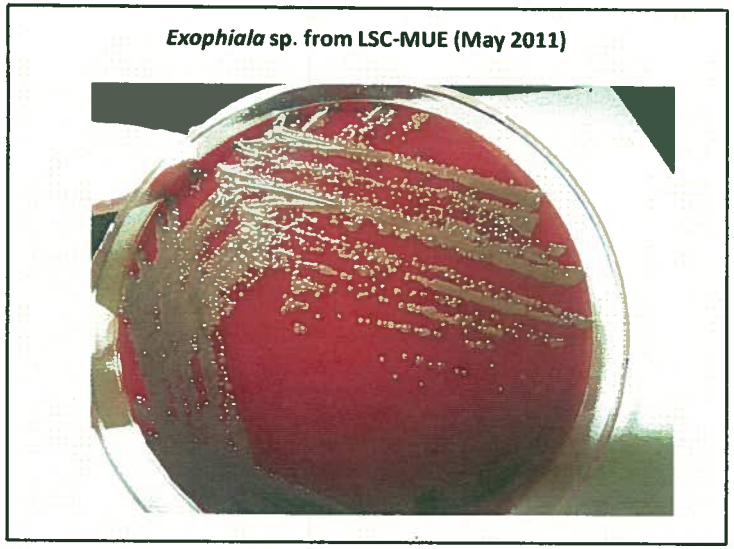


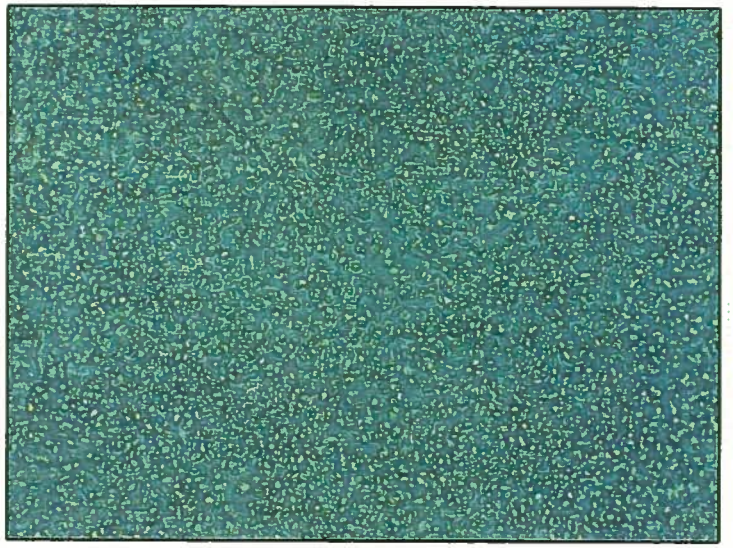


APPENDIX 2

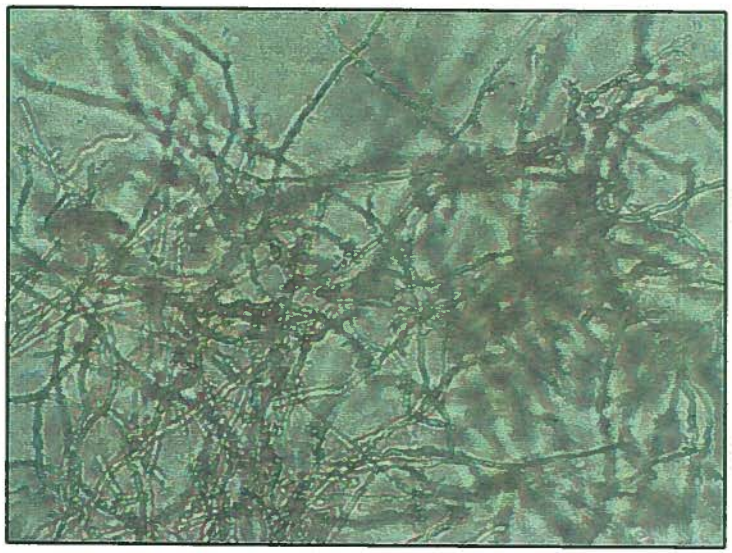

Flavobacteria at Different Life Stages

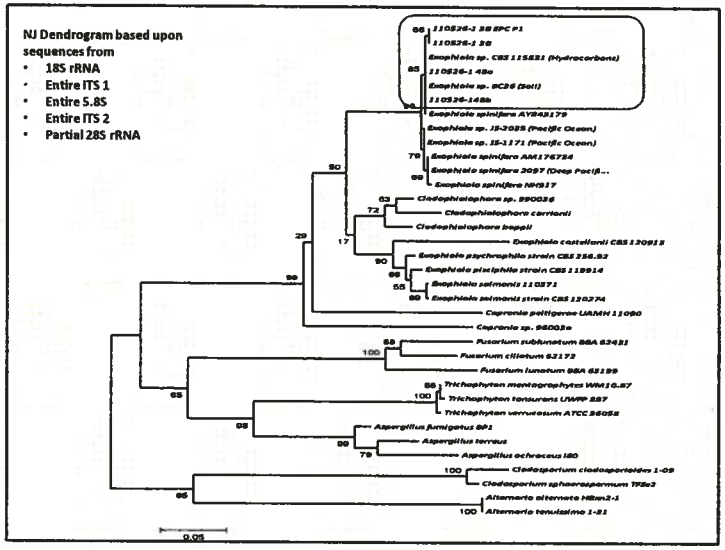

Flavobacteria at Different Life Stages

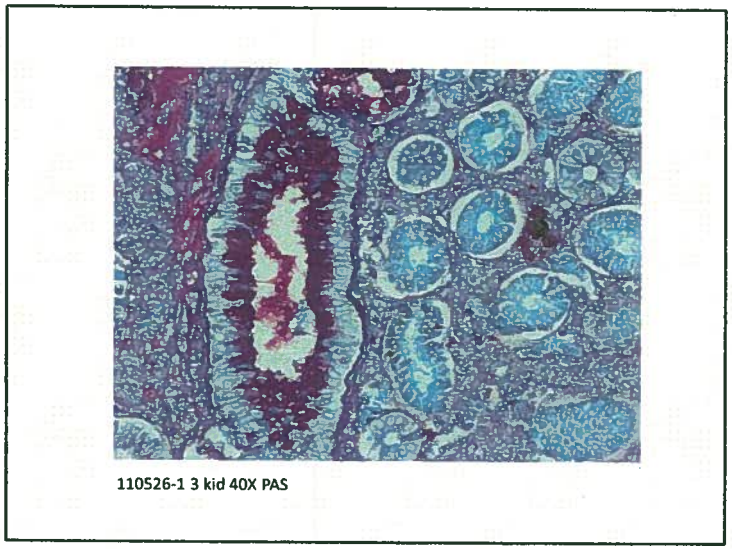

Exophiala sp. from LSC-MUE (May 2011)

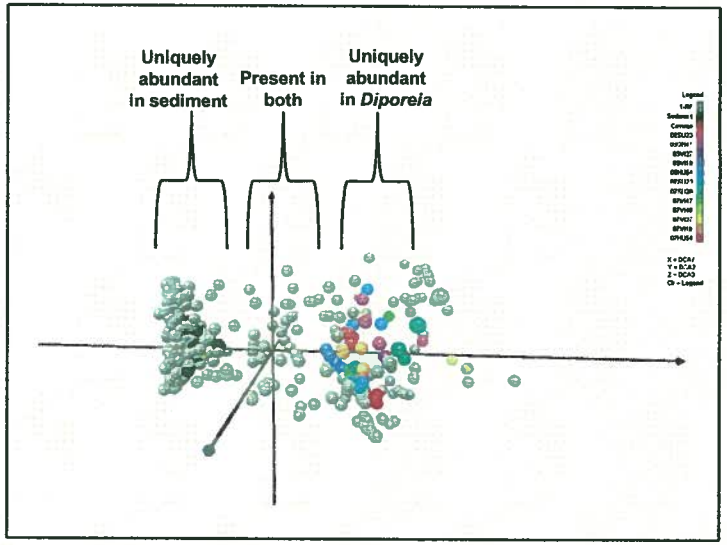


APPENDIX 2

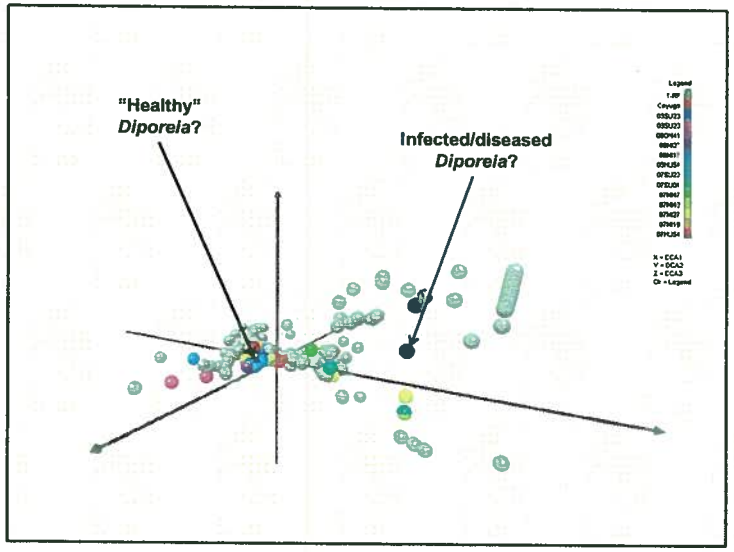


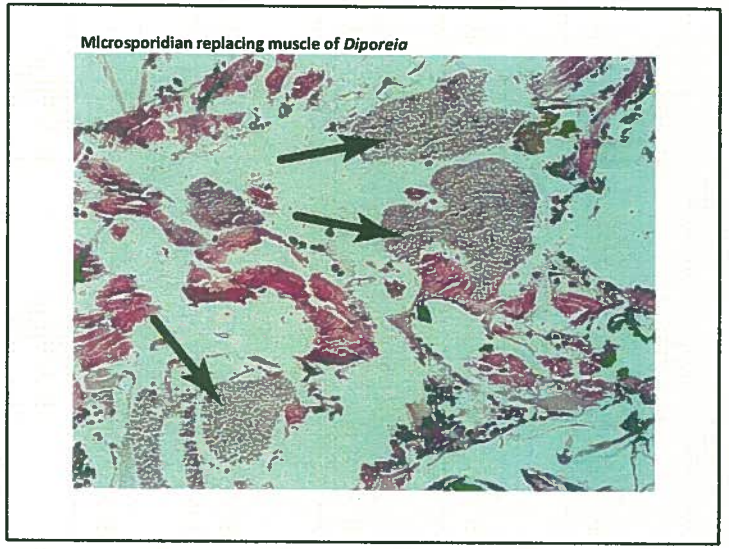

NJ Dendrogram based upon sequences from
• 18S rRNA
• Entire ITS 1
• Entire 5.8S
• Entire ITS 2
• Partial 28S rRNA
0.05

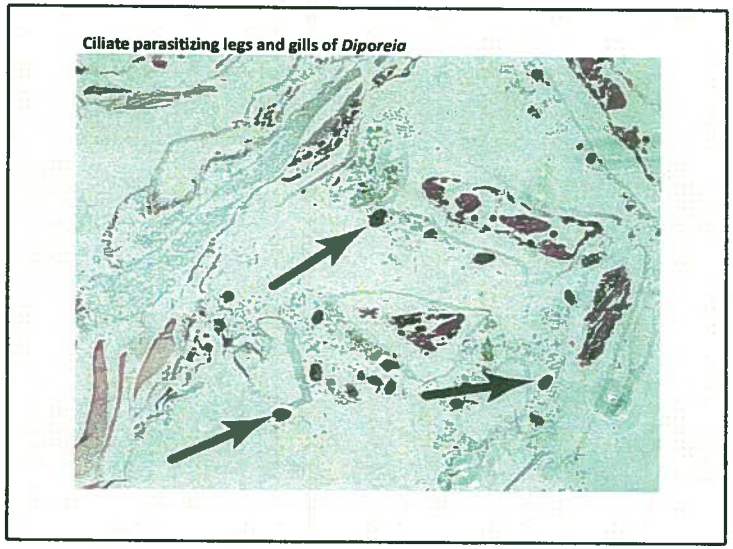

110526-1 3 kid 40X PAS

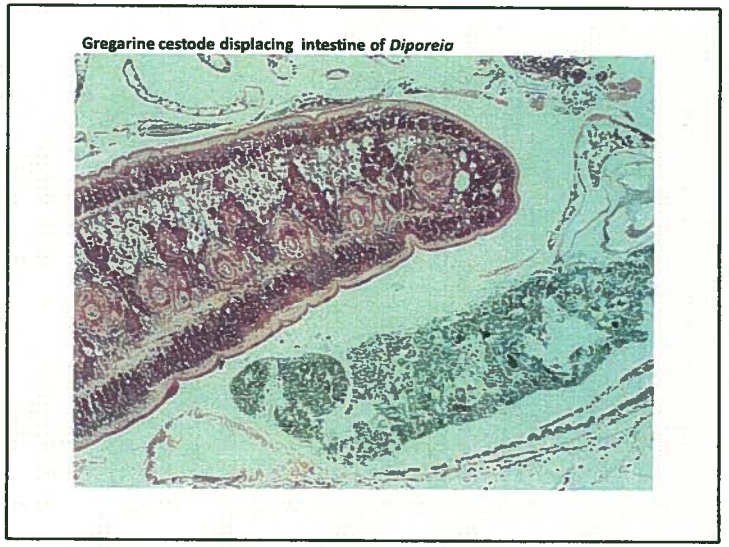

Uniquely abundant in sediment
Present in both
Uniquely abundant in Diporeia

APPENDIX 2


"Healthy"
Diporeia?
Infected/diseased
Diporeia?

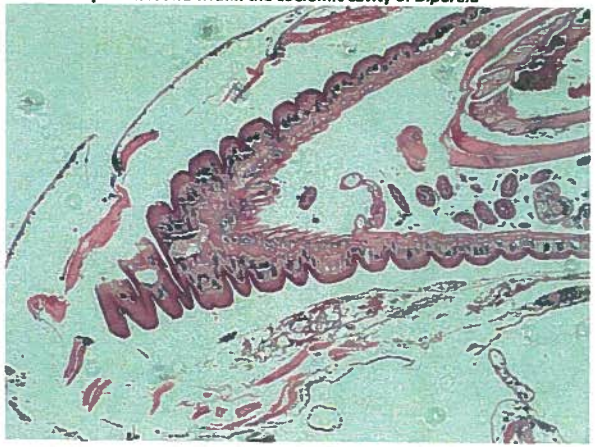

Microsporidian replacing muscle of *Diporeia*


Ciliate parasitizing legs and gills of *Diporeia*

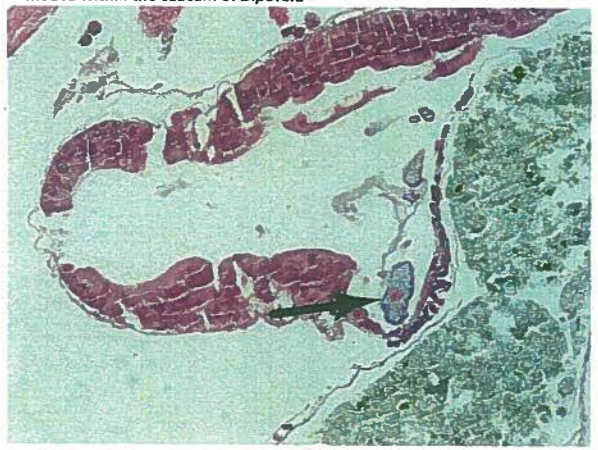

Gregarine cestode displacing intestine of *Diporeia*

APPENDIX 2

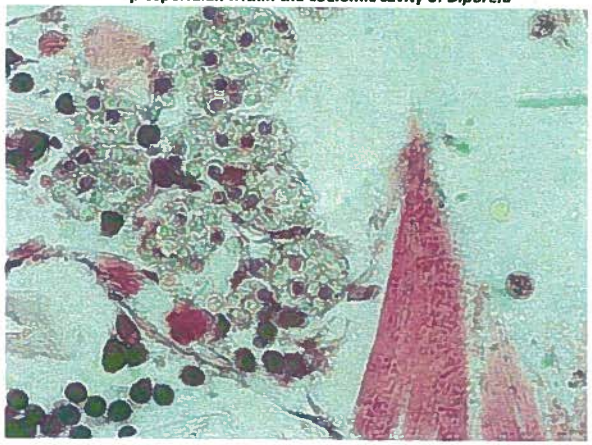
Acanthocephalan found within the coelomic cavity of *Diporeia*

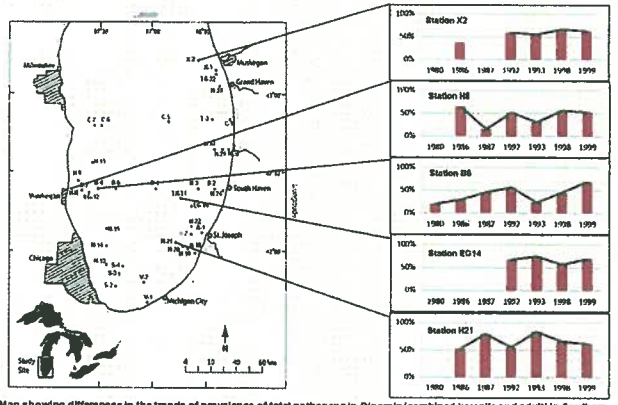


Amoeba within the ceacum of *Diporeia*



Unidentified haplosporidian within the coelomic cavity of *Diporeia*





Map showing differences in the trends of prevalence of total pathogens in *Diporeia* (combined juvenile and adult) in Southern Lake Michigan over a period of two decades.

APPENDIX 2

Online Training Course

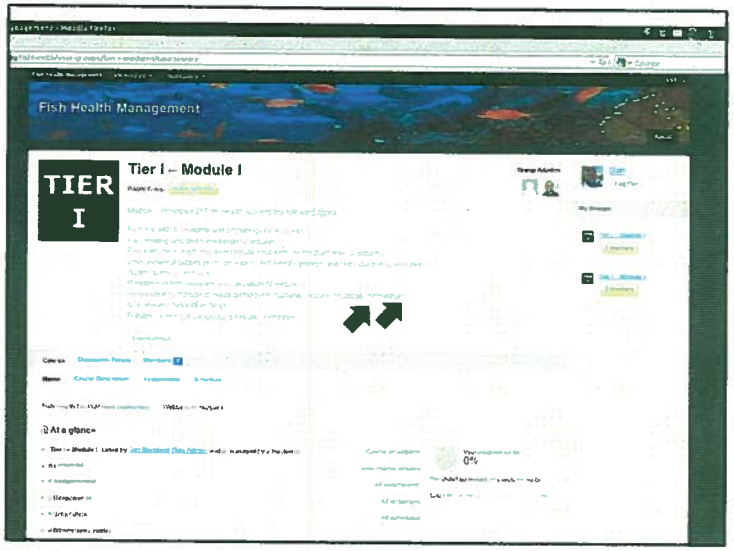
- Consists of 3 Tiers
- Online Lectures
- Interactive Video Conferences
- Network of Fish health

TIER I

- **Module I:** Principles of Fish Health
- **Module II:** Fish and Shellfish Diseases and their Investigation
- **Module III:** Principles of Fish and Shellfish infections
- **Module IV:** Differential diagnosis
- **Module IIV:** Development of managerial decisions and strategies to control diseases

TIER II

TIER III



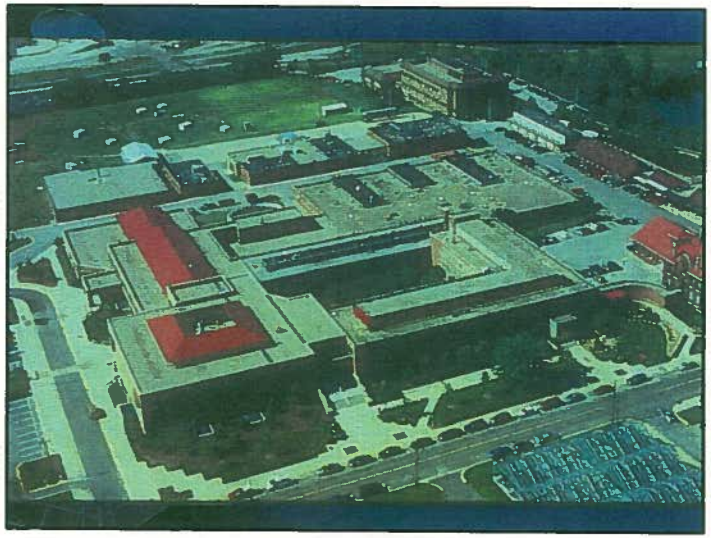
APPENDIX 3

An Introduction to ADDL

Tsang Long Lin, DVM, PhD
Professor of Pathology

Department of Comparative Pathobiology
And
Animal Disease Diagnostic Laboratory

Purdue University
West Lafayette. Indiana. USA



APPENDIX 3

Mission Statement

"Provide accurate and prompt diagnostic services to veterinary practitioners, animal producers, companion animal owners, wildlife conservationists, animal researchers, and state/federal regulatory officials."

Accredited Laboratory

- Providing a full range of diagnostic services
  - Necropsy
  - Pathology/Histopathology
  - Clinical pathology
  - Bacteriology/Mycology
  - Virology
  - Serology
  - Chemistry/Toxicology
  - Molecular diagnostics

- Avian Diagnostics
  - Includes poultry, pet birds, exotic and wildlife avian species
  - Available tests include:
    - Serology
    - Chick embryo inoculation
    - Chlamydia detection
    - Mycoplasma detection
    - ELISA for IBDV, AE, etc.
- Aquaculture Diagnostics
  - Fish Health Inspections
  - Necropsy/Histopath.
  - BKD testing
  - WD testing
  - Virus isolation

Pathology

- Services include necropsy (insurance/legal, cosmetic)
- Histopathology
- Electron microscopy
- Farm consultations
- Lesion photography

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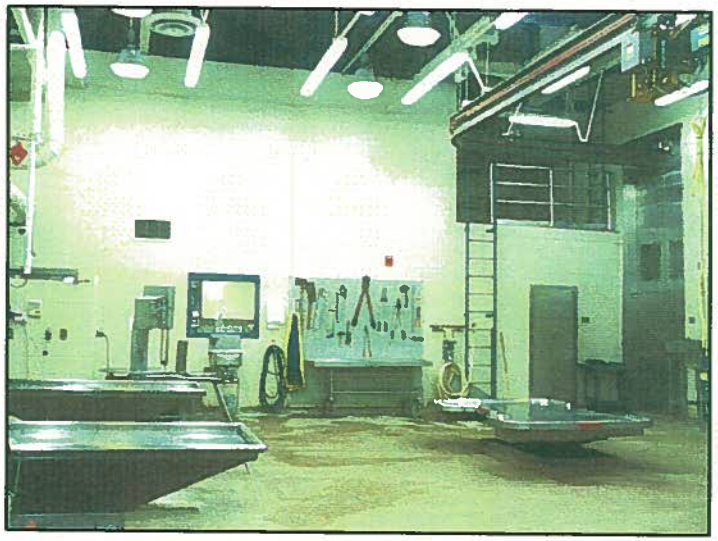

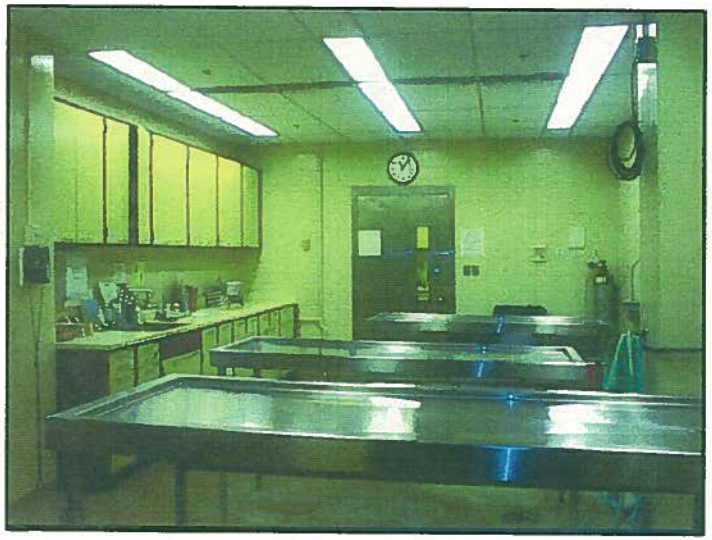

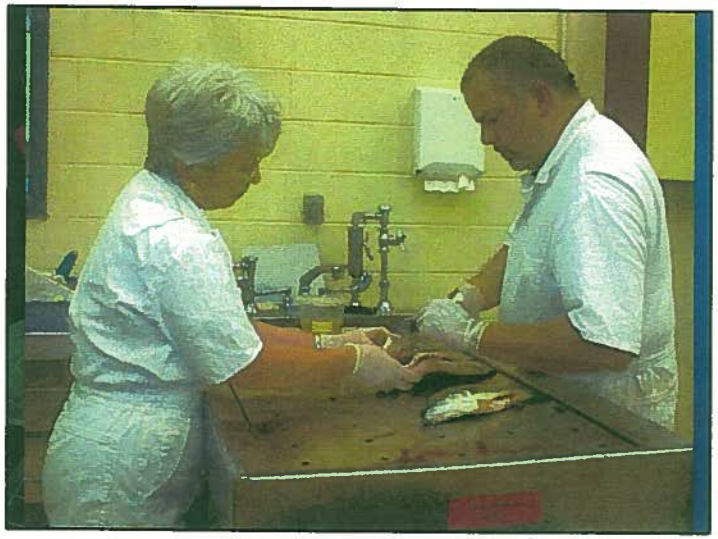

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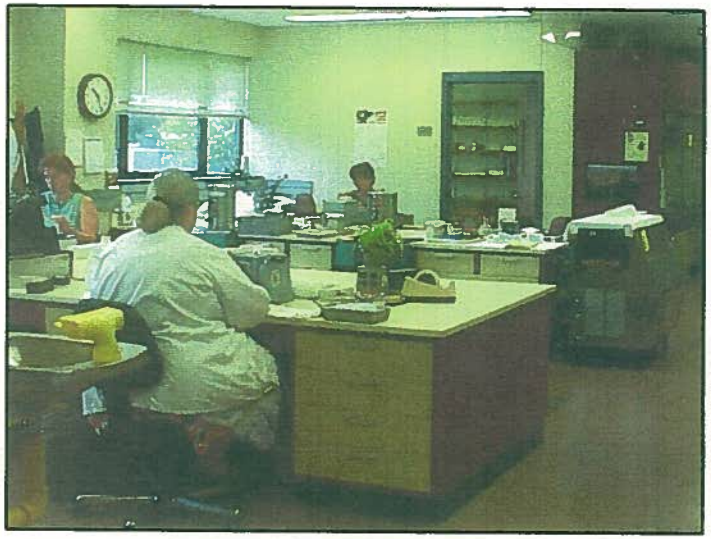
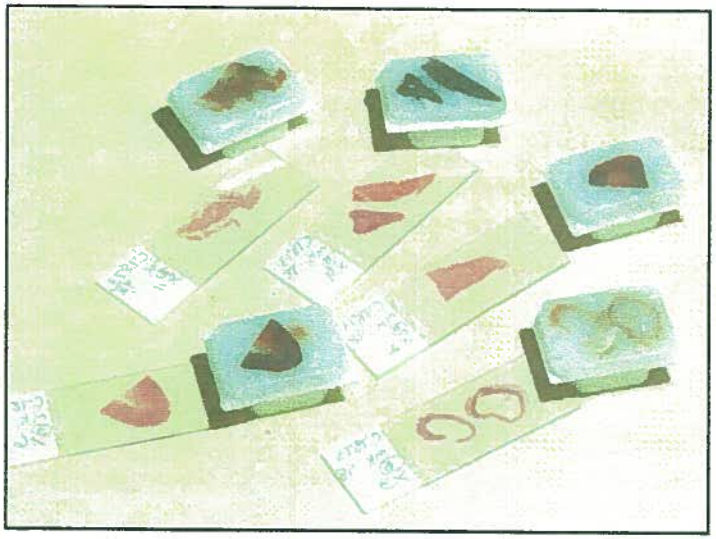




Bacteriology

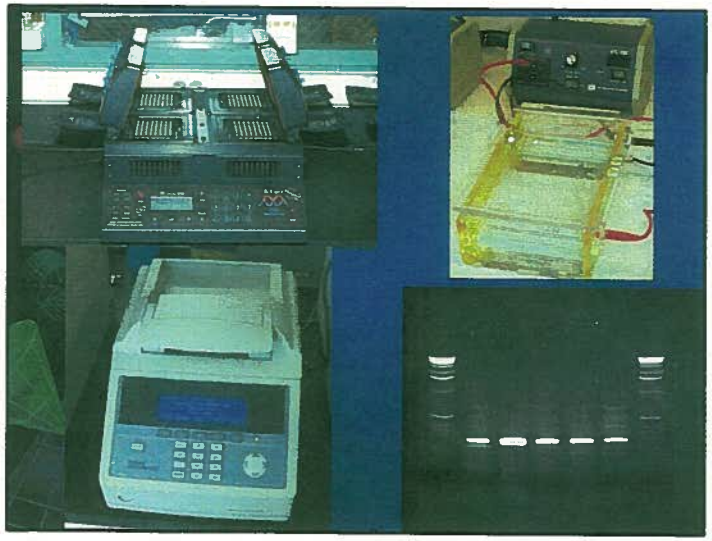
- Services provided include:
  - Aerobic/Anaerobic culture
  - Sensitivity
  - PCR tests for bacterial toxins
  - FA for bacterial identification
  - Automated identification systems (Vitek® and Sensititre®)

APPENDIX 3

Molecular Diagnostics

- PCR (polymerase chain reaction; RT-PCR (reverse-transcriptase polymerase chain reaction)
- Detects DNA/RNA for agents such as Group A influenza viruses, BVD, PRRS, TGE, *Leptospira*, *Chlamydia*, *Neospora caninum*, West Nile virus, Turkey coronavirus, *Lawsonia intracellularis*, *Brachyspira* spp., *Salmonella*, *Mycobacterium paratuberculosis*, *Listeria monocytogenes*, *Renibacterium salmonarium*
- *E. coli* typing (F18, 987P and F41 pili, eae attachment gene, LT, StaP, Stb, and Stx2e toxins)
- *Clostridium perfringens* typing (a,ß,ε,ı and ß2 toxins, enterotoxin)
- *Clostridium difficile* toxins A and B
- Additional PCR tests are constantly being developed at ADDL

APPENDIX 3



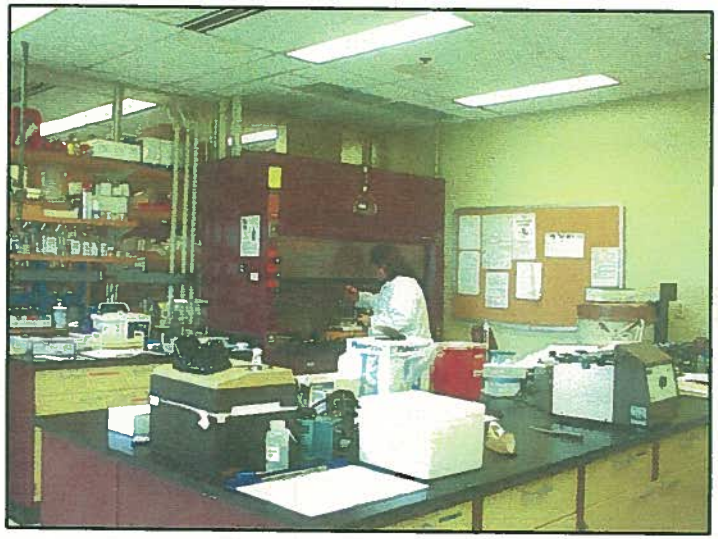
Serology

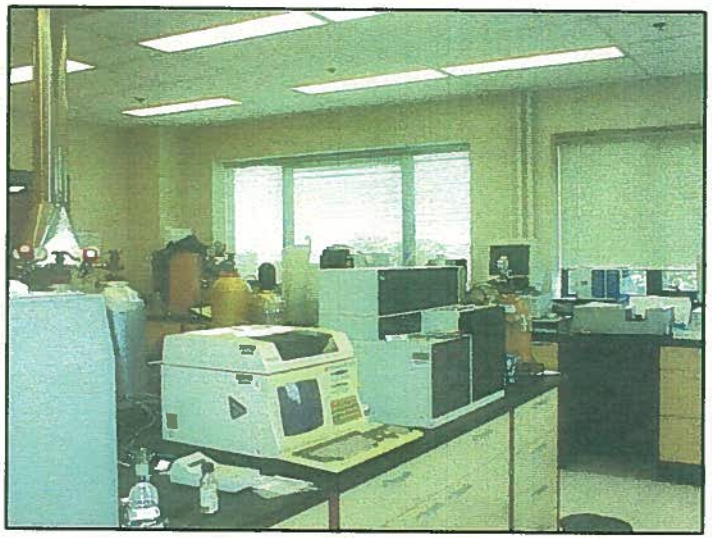
- Regulatory: Provides all regulatory serologic testing for the state of Indiana in accordance with the State Board of Animal Health (ex: Pseudorabies of swine)
- Diagnostics: Provides diagnostic testing for necropsy cases and practitioner samples (ex: Lepto abortions)

APPENDIX 3

Toxicology

- Testing of animal tissues, feed or environmental substances for the detection of naturally-occurring and man-made toxicants
- Performs assays for toxic or trace minerals, elements and vitamins
- Techniques include HPLC, GC, GC-Mass spectrometry

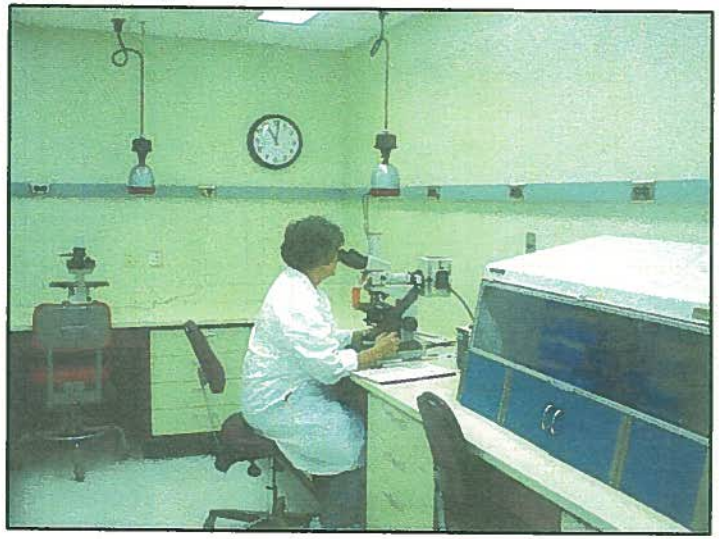




APPENDIX 3

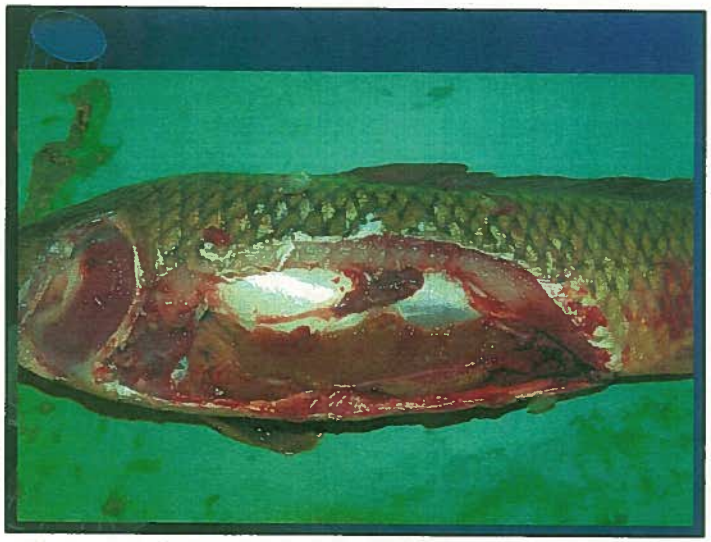
Virology

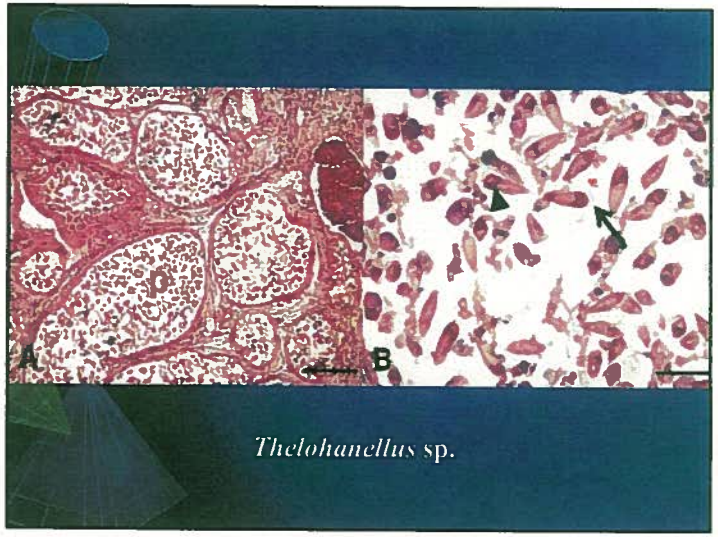
- Isolation and identification of pathogenic viruses from animal tissues or feces
- Techniques include:
  - Virus Isolation
  - Electron microscopy
  - Fluorescent Antibody
  - PCR
- Samples must be chilled during shipment

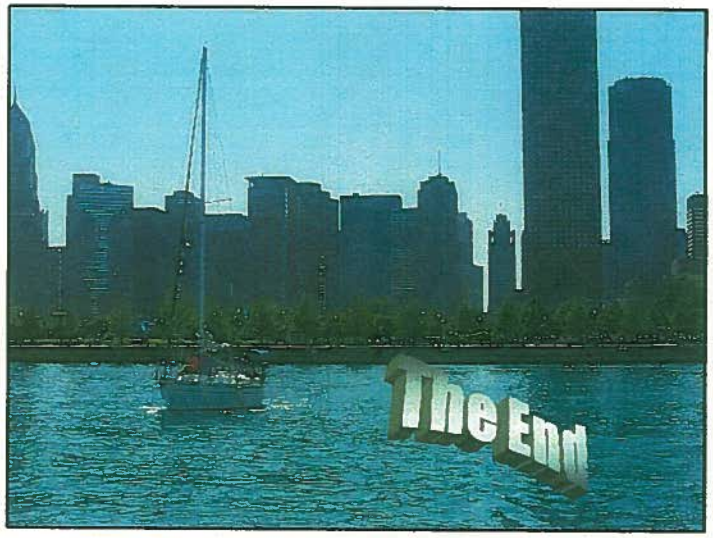


Koi herpesvirus infection in carp

APPENDIX 3







GLFHC members,

Please see email and the attached memo below from Andy Todd regarding the recent confirmation of the nematode *Anguillicoloides crassus* in American eel collected from the Upper St. Lawrence River. Andy has asked that people not contact Dr. Casselman at this time (Dr. Casselman is working on the final report and he will not be releasing additional information until his report is submitted to OMNR). Please conatact Alastair Mathers Alastair.mathers@ontario.ca. at OMNR with any questions.

Happy Thanksgiving,

Ken

Ken Phillips
Microbiologist
U.S. Fish and Wildlife Service
La Crosse Fish Health Center
555 Lester Avenue
Onalaska, WI 54650
(608) 783-8447
(608) 783-8450 Fax

----- Forwarded by Kenneth Phillips/R3/FWS/DOI on 11/22/2011 01:47 PM -----

"Todd, Andy (MNR)" <andy.todd@ontario.ca>

11/22/2011 01:21 PM

To"John Dettmers" <jdettmers@glfc.org>, <Kenneth_Phillips@fws.gov>

cc"Marc Gaden" <marc@glfc.org>

SubjectConfirmation of A. crassus in the Upper St. Lawrence River

Hi Kenneth

Please see the attached memo about the recent confirmation of *A. crassus* in the Upper St. Lawrence River.

The purpose of this memo is to keep the GL Fish Health Committee in the loop as this situation develops. While it is still early in the process, we wanted our partner agencies to know about the recent finding, prior to the science meeting next week.

DFO, OPG, New York State DEC and Quebec have been notified.

We will keep you informed as more information becomes available.

Thank you.

Andy Todd
Manager, Lake Ontario Management Unit,
Fish and Wildlife Services Branch
Ontario Ministry of Natural Resources
Glenora Office: 613 476 3147
Email: Andy.Todd@ontario.ca

(See attached file: A-Crassus-_Nov-22-GLFC.doc)

November 22, 2011

To: American eel Restoration Partners; GLFC and Great Lakes Fish Health Committee

From: Andy Todd, Manager Lake Ontario Management Unit.
Andy.todd@ontario.ca
613-476-3147

ISSUE: First confirmed occurrence of *Anguillicoloides crassus (A. crassus)* in American eels in the upper St. Lawrence River

Other Agencies that have been notified: New York State DEC, Canada DFO, Ontario Power Generation, Quebec Natural Resources.

The purpose of this memo is to provide early notification to our American eel Restoration Partners about a recently confirmed occurrence of *Anguillicoloides crassus (A. crassus)* in American eels in the upper St. Lawrence River.

The information below provides a factual account as of November 22, 2011.

This information is not confidential but the recent finding is not being broadly communicated at this time since the final report from Dr. Casselman has not been published. This recent finding will be discussed at the Canadian Eel Science Working Group meeting, which will be held in Montreal on November 28 and 29, 2011. Once OMNR receives the final report from Dr. Casselman, broader notification may be considered.

Please respect that Dr. Casselman is working on the final report and he will not be releasing additional information until his report is submitted to OMNR. Please direct questions to Alastair Mathers Alastair.mathers@ontario.ca.

Recent Developments - Positive identification of *A. crassus* in Ontario

- During the summer of 2011, Dr. J. Casselman from Queens University, conducted an adult eel survey in the upper St. Lawrence River and Main Duck Island under contract with the Lake Ontario Management Unit. This survey has been conducted annually since 1984.
- As part of the survey, 100 eels were collected for biological examination.
- Three of the eels each had one swim bladder parasite – at the time Dr. Casselman felt these were likely *A. crassus*.
- The three eels with the suspected *A. crassus* parasite were stocked eels.
- Pictures of the parasites were sent to Dr. Ken Oliveira, University of Massachusetts for identification. Dr. Oliveira reported to Dr. Casselman that two of the three were *A. crassus*. The third parasite was likely *A. crassus*, but a less mature stage.

- The parasites (actual specimens) were also sent to Dr. Frantisek Moravec (Ph.D., D.Sc.; Biology Centre of the Academy of Sciences of the Czech Republic Institute of Parasitology) for confirmation of their identity on October 7, 2011.
- **On November 4, 2011, Dr. Moravec confirmed via email that the parasites provided by Dr. Casselman were *A. crassus*.**

BACKGROUND:

- American eel is listed as *Endangered* under Ontario's *Endangered Species Act* (ESA) and a variety of actions have been taken to help restore eel populations including:
  - Ontario is developing a Recovery Strategy under the ESA.
  - Lake Ontario / St. Lawrence River - since 2006, MNR, in partnership with DFO and Ontario Power Generation (OPG), has been implementing OPG's Action Plan ($2.5 million spent by OPG over 5 years) as part of the ESA water power agreement for the operation of R.H. Saunders Generating Station.
  - The OPG Action Plan includes:
    - Stocking 4 million 'glass' eels (early life stage) from 2006 to 2010 that were collected from the wild in Nova Scotia / New Brunswick by commercial fishers.
    - Trapping adult eels in Lake Ontario and the upper St. Lawrence River and transporting them downstream below the turbines.
    - Eel population monitoring at the eel ladder at the Saunders Generating Station, in the estuary of the St. Lawrence River, the upper St. Lawrence River and Lake Ontario and tributaries.

- Fish Health Risks:
  - One of the risks identified with the eel stocking program was the potential transmission of disease or parasites and specifically the nematode *Anguillicoloides crassus (A. crassus)*.
  - *A. crassus* is considered to be the most aggressive fish parasite to have been introduced anywhere in the world and is thought to be one of the threats to the recovery of eel populations.
  - <u>This parasite does not pose a threat to human health.</u>
  - *A. crassus* infections cause damage to the swim bladder of eels and create a drain on the eel's energy reserves. It is widely assumed that infestation is very likely to impair an eel's ability to migrate normally and reproduce successfully.
  - This parasite was introduced to Europe in the 1980s when Japanese eels were imported to Europe for aquaculture. It was first found in the American eel in 1995 both in Texas aquaculture facilities and in a single wild American eel from South Carolina.
  - Since American eels migrate from the Atlantic Ocean into fresh water to mature, it is expected that in time, this parasite would eventually become established throughout the entire range, including the Lake Ontario, Upper St. Lawrence and Ottawa River systems.
  - *A. crassus* has been detected in American eel populations along the eastern seaboard of North America to the south of Nova Scotia, but until 2011, it had not been positively identified in the St. Lawrence River / Lake Ontario population.

- Risk Management
  - Agencies (DFO, MNR, OPG) have taken steps to reduce the risk of spreading this parasite via OPG's eel stocking program.
  - Prior to support for stocking wild glass eels, extensive health testing is required for a variety of diseases and *A. crassus*.
  - Health assessment protocols were based on advice received from:
    - Great Lakes Fishery Commission (GLFC) Fish Health Committee
    - Ontario Introductions and Transfers Committee
    - Workshop on stocking eels in Canadian Waters held in Montreal in 2007
  - The decision to stock eels was supported by: MNR, DFO, Quebec Ministry of Natural Resources and Wildlife, New York State Department of Environmental Conservation (NYSDEC), GLFC.
  - *Note that A. Crassus* was detected in one batch of fish during 2007 – this batch of fish was not stocked.

Management Implications

- As prescribed in the OPG Action Plan – eels were not stocked in 2011 and plans to stock them in the future are under review.
- It is not known at this time how the presence of *A. crassus* in Lake Ontario will impact the broader restoration efforts of American eel in Ontario or Quebec.
- Some key questions that need to be answered:
  - Is *A. Crassus* only found in stocked eels and only in specific year classes or is the parasite more widespread including naturally migrating eels?
  - What are the management implications if the parasite is isolated to one year class of stocked eels?
  - Could the parasite be cleared from the system when the eel migrate back to the sea?
  - If the parasite is determined to be established in Lake Ontario, would there be support for continued stocking?
  - Should stocking as a management tool be restricted in other areas such as the Ottawa River?

Communications:

- No broad public announcement is planned since there are no human health risks.
- The recent finding will be reported by Dr. John Casselman in a formal report to MNR and possibly a science journal in the future.
- The observation of *A. crassus* is an important event that may shape future approaches to management and restoration of this species. The observation of *A. crassus* will be reported to the scientific community at the Canadian Eel Science Working Group meeting, which will be held in Montreal on November 28 and 29, 2011.
- The OMNR, (Lake Ontario Management Unit) will keep agencies informed as new information becomes available.

In anticipation of CLC member agency needs for guidance in managing this new pathogen, the Great Lakes Fish Health Committee (GLFHC) has developed a set of management recommendations that we request be considered for adoption by all member agencies. The adoption of these recommendations will slow the spread of this pathogen, providing additional time for new options to be developed to more effectively contain this pathogen.

Most of the recommendations had full consensus by all GLFHC and these are requested to be immediately adopted by all CLC member agencies. GLFHC could not reach consensus on a few recommendations because of: differences in management approaches; likely inability of the member agency to be able to effectively implement the recommendations; or other large scale priorities that must be considered such as sea lamprey control. With respect to these recommendations where consensus could not be reached, we recommend that the adoption of the recommendation is up to the discretion of the individual management agencies as they are more protective actions that enhance the recommended actions with consensus.

There are a large number of information needs on this virus and the GLFHC has developed a list of the most important needs for consideration by the Great Lakes Fishery Commission in their research programs along with those of member agencies.

GLFHC Consensus Recommendations

Fish Health Testing

1. GLFHC member agencies should use the most sensitive cell lines in all samples processed for VHS virus.
2. GLFHC member agencies should request that all laboratories used by the agencies conducting VHS virus sample analysis undertake cell line susceptibility analysis, determine best performing cells, clone them, and distribute among all Great Lakes laboratories for sample analysis.
3. GLFHC member agencies should require that periodically all laboratories testing for VHS virus will share cell lines with at least one other laboratory to allow for quality control and assurance analysis to be conducted on the cell lines.

Hatchery Operations – Coolwater Culture

1. GLFHC members should refrain from taking non-salmonid eggs and sperm from any waters that are positive for the VHS virus until more is known about the success of disinfection methods with these species and the VHS virus.
    a. If there are no feasible alternatives to using wild broodstock from waters that are positive for VHS virus and the fish are absolutely necessary for fish management purposes, production fish beginning with egg and sperm from waters positive for VHS virus can be stocked back into those waters already determined to be positive for VHS virus.

2. All non-salmonid eggs from Great Lakes wild fish sources would be surface treated with iodophor during water hardening in using a known effective concentration and duration.
3. All non-salmonid broodstock lots should be tested annually using standard fish health inspection protocols for VHS virus prior to the stocking of their production lots, where possible, and production fish from positive broodstock lots should be tested for VHS virus.
4. All production lots should be at minimum annually tested for VHS virus using standard fish health sampling protocols and those production lots found to be positive for VHS virus should not be stocked at this time.
5. All fish in a given hatchery will carry the same hatchery fish health designation to ensure full disclosure of potential fish health concerns.
6. GLFHC member agencies should strongly consider the development of protected Great Lakes non-salmonid broodstock lines using isolation or quarantine facilities and holding them in either captive situations or in isolated inland lakes.

Hatchery Operations - Salmonid Culture

1. All GLFHC members should disinfect all salmonid eggs during water hardening from Great Lakes waters using iodophor compounds using a known effective concentration and duration.
2. All adult salmonid broodstock lots should be sampled annually using standard fish health inspection protocols during egg take operations and tested for VHS virus.
3. All production lots with fish larger than fry size should be tested for VHS virus prior to stocking. Those production lots found to be positive for VHS virus should not be stocked.
4. All fish in a given hatchery will carry the same hatchery fish health designation to ensure full disclosure of potential fish health concerns.
5. All GLFHC member agencies consider the development of protected Great Lakes salmonid broodstock lines using isolation or quarantine facilities and holding them in either captive situations or in isolated inland lakes.

General Hatchery Guidance

1. GLFHC member agencies should destroy all fish at hatchery facilities that are found to be infected with VHS virus based on a management plan developed after consultation with GLFHC member agencies.
2. All eggs moved between GLFHC member agency facilities must be surface disinfected using an iodophor compound prior to transfer.
3. GLFHC member agency hatchery equipment and trucks should be fully disinfected after each use and between uses between hatcheries.
4. GLFHC member agencies should not allow the use of untreated water for moving fish from Great Lakes Basin waters testing positive for VHS virus.

Fish Management Activities – Fish Transfers

1. GLFHC members should test all species targeted for transfer from all potential donor waters before fish transfers occur.

Fish Management Activities – Others

1. All GLFHC member agencies should clean and disinfect all sampling gear, personal protective clothing and boots, boats and vehicles after sampling VHS virus positive waters.
2. All investigators under GLFHC member agency control that are sampling VHS positive waters under some type of sampling or collectors (investigators or harvesters) permit should be required to clean and disinfect all sampling gear, personal protective clothing and boots, boats and vehicles after sampling as a condition of any such permit.

Commercial Fishing Activities

1. GLFHC member agencies should periodically test all species of fish used in the live commercial fish trade for the presence of VHS virus.
2. GLFHC member agencies should prohibit the transfer of live fish species that are known to be from fish populations infected with VHS virus and are moving from Great Lakes commercial fishing operations to either live markets or fee-fishing lakes.
    a. Alternatively, GLFHC members should appropriately test individual shipments from waters positive for VHS virus and prohibit the transfer of shipments that test positive for VHS virus from commercial fishing operations.
3. GLFHC member agencies should require that all live fish shipments from commercial fishing operations being imported for use in public waters be tested for and be certified free of VHS virus.
4. GLFHC members should ensure that all waste products from processed fish collected by commercial fisheries from waters positive for VHS be properly disposed of in either sanitary sewer systems or in licensed landfills.

Bait Industry

1. GLFHC member agencies should at minimum annually test, using standard fish health inspection protocols and proper timing, all wild Great Lakes Basin baitfish sources for VHS virus to determine which locations are positive for the pathogen.
2. GLFHC member agencies should test, or require testing of all imported baitfish sources in the Great Lakes Basin, prior to importation, for VHS virus to determine which vendors' facilities and sources are infected using standard fish health inspection protocols and proper timing to best detect the pathogen.
3. GLFHC member agencies should prohibit the importation of bait that is found to be infected by VHS virus.

4. Any baitfish source testing positive for VHS virus should not be allowed to be sold in any GLFHC member agency jurisdiction.

Non-member Agency Aquaculture Operations

1. All fish tested for VHS virus by non-member agency aquaculture operations for stocking in Great Lakes Basin public waters should use the most sensitive cell line for VHS virus.
2. GLFHC member agencies should recommend or ensure the destruction of all fish at non-member hatchery facilities that are found to be infected with VHS virus based on a management plan developed after consultation with GLFHC member agencies.
3. GLFHC member agencies should ensure or recommend that non-member hatchery equipment and trucks should be fully disinfected after each use and between uses between hatcheries.

Public Information

1. All GLFHC member agencies should jointly develop information sheets, boat launch information, and a website on VHS virus and other pathogens to highlight how the public can prevent their spread.
2. GLFHC member agencies should take every opportunity to inform the public about VHS virus and its potential affects in press interviews, press releases and popular articles.
3. GLFHC member agencies or the GLFC should sponsor a 1-800 number and a website on fish pathogens, their potential affects, and current distribution.
4. The GLFC should assist the GLFHC in using the existing internal website for the posting of information to allow for the rapid dissemination of public information materials among member agencies.
5. The GLFHC strongly encourages the development of a North American website for the posting of current and emerging fish pathogen information that is jointly managed by state, provincial, tribal and federal fisheries agencies.

Other Preventive Measures

1. GLFHC member agencies should undertake all possible measures to prevent the discharge of untreated ballast water within Great Lakes waters.
2. GLFHC member agencies should use the U.S. Coast Guard abilities to prohibit ballast water exchange in areas of high pathogen density and in areas of active mortality events.
3. GLFHC member agencies strongly encourage all possible measures to prevent the use of untreated water for any purpose from Great Lakes Basin waters positive for VHS virus that maybe possibly discharged into waters not yet exposed to VHS virus.

GLFHC Recommendations – Without Full Consensus

Fish Management Activities – Fish Transfers

1. GLFHC members should not move fish from waters positive for VHS virus.
2. GLFHC members can move fish that test negative for VHS virus from waters with other positive VHS virus detections in fish to other waters that have tested positive for VHS virus in fish.
3. GLFHC members can move fish lots that test negatively for VHS virus from waters with positive VHS virus detections in other fish to any water.

Bait Industry

1. GLFHC member agencies can allow the use of bait collected from waters testing positive for VHS virus in fish in other waters testing positive for VHS virus in fish.

Key Information and Management Needs

1. Systematic wild fish surveys to determine the location of VHS virus in the Great Lakes Basin.
2. Improved understanding of host-pathogen-disease relationship for key management species with a high priority on sea lampreys.
3. The length of time VHS virus is viable in the environment.
4. Geographic distribution of VHS virus for all affected fishes in the Great Lakes.
5. The effectiveness of iodophor disinfection of non-salmonid eggs.
    a. Appropriate safe levels of disinfection need to be determined for each non-salmonid species and that physical manipulation at this stage will not kill the newly fertilized non-salmonid embryos.
6. Determination of which non-salmonid species are susceptible, infectious and carrier species.
7. Improvements in the detection tests for VHS virus
    a. Evaluation of all available cell lines to determine the most sensitive.
        i. Most sensitive should be cloned and provided to all fish health labs in the Great Lakes region
    b. Full development of rapid field and laboratory virus detection tools to include rPCR tests.
    c. General methodology improvements are needed to include which is the best tissue to test and what is the best way to ship and store samples.
8. Develop a full understanding of the extent of the live fish market for commercially caught fish along with distribution network for these fish to greatly improve trace-back options.
9. Develop a full set of options to use Aquatic Nuisance Species and Department of Homeland Security funds to combat fish health problems that could affect commercially important species.

10. Understand how the baitfish industry operates, the effects of the above recommendations on bait availability for anglers, and extent of bait importation into and movement around the Great Lakes.
11. Understand the seasonal variability of infection in key baitfish species, in particular lake emerald shiners and golden shiners.
12. A systematic survey of VHSv and other pathogens carried in Great Lakes ballast water.
13. An analysis of the movement of fish, pathogens and ballast materials through the Great Lakes should be conducted to examine if any relationships exist among these factors that could inform management decisions.

FISHERY RESEARCH PRIORITIES: GREAT LAKES FISH HEALTH COMMITTEE

Great Lakes Fishery Commission

Version October 31, 2009

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

Research Priorities

- *What non-lethal field sampling techniques and tissue/fluid samples are equivalent to lethal field sampling methods to determine fish disease status?*
- *What is the ecology of important fish pathogens and diseases?*
- *Examples of GLFHC important disease and pathogens include VHSv Genotype IVb, Heterosporis sp. among other emerging diseases.*
- *What is the effectiveness of the GLFHC disinfection protocols in eliminating VHSv and Nucleospora from fish eggs? There is a need for a reliable disinfection methodology to prevent VHSv and Nucleospora transmission via eggs.*

Additional Research Interests

i) Development and validation of new methods for detecting emerging fish pathogens or pathogens of concern in the Great Lakes Basin.
   (a) What factors that affect sample integrity during shipment and storage

ii) Disease Ecology and Epidemiology
   (a) What is the susceptibility to of Great Lakes fish species to emerging fish pathogens in the Great Lakes?
   (b) Identification of reservoirs and vectors for fish pathogens in the Great Lakes Basin
   (c) What factors affect the virulence of fish pathogens?
   (d) What are the effects of synthetic estrogen or other hormones on gonad development in Great Lakes fish?
   (e) What is the effect of population size on disease expression?
   (f) What are the effects of multiple pathogens or combination of pathogens and nutritional deficiency and/or contaminant exposure on disease expression?

iii) Nutritional Aspects of Fish Health in the Great Lakes
   (a) What is the role of lipids or other nutrients in determining and predicting health status?
   (b) What is the role of thiaminase-producing organisms in Great Lakes ecosystems?

(c) What affect do invasive species have on nutrient stores in the Great Lakes and what are the associated affects on fish health.
(d) What is the effect of nutrition on reproductive success?
(e) What is the relationship between genetics and thiamine deficiency complex?
(f) Does protein substitution in hatchery feeding formulations or extrusion manufacturing methods have a negative impact on survivorship, migratory behavior and reproductive success of hatchery-reared salmonids?

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