GREAT LAKES FISH HEALTH COMMITTEE

2012 Summer Meeting
La Crosse, Wisconsin
July 30, 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

Table of Contents

List of Attendees

John Dettmers	Great Lakes Fishery Commission
Mohamed Faisal	Michigan State University
Christina Haska	Great Lakes Fishery Commission
Sunita Khatkar	Fisheries and Oceans Canada
Randy Lang	Indiana Department of Natural Resources
Sue Marcquenski	Wisconsin Department of Natural Resources
Dave Meuninck	Indiana Department of Natural Resources
Brian Niewinski	Pennsylvania Fish and Boat Commission
Paula Phelps	Minnesota Department of Natural Resources
Ken Phillips	U.S. Fish and Wildlife Service- Wisconsin
Ling Shen	Minnesota Department of Natural Resources
Gary Whelan	Michigan Department of Natural Resources
Coja Yamashita	Pennsylvania Fish and Boat Commission

Other attendees included:

Kerry Collins	Kennebec River Biosciences
Hui-Min Hsu	Wisconsin Veterinary Diagnostic Laboratory
Becky Lasee	USFWS- La Crosse Fish Health Center
Beka McCann	USFWS- La Crosse Fish Health Center

Great Lakes Fish Health Committee Meeting

July 30, 2012
Radisson Hotel
La Crosse, WI

Agenda

Monday, July 30

8:00 am-8:15 am	Welcome & Introductions (Ken Phillips)
8:15 am-8:30 am	Approval of Meeting Minutes (Ken Phillips)
8:30 am-8:45 am	GLFC Update (John Dettmers)
8:45 am-9:15 am	Model Program Update (Ken Phillips)
9:15 am-10:00 am	WFRC Update (Diane Elliot)
10:00 am-10:15 am	Break
10:15 am-10:30 am	Leetown Science Center Update (Vicki Blazer)
10:30 am-11:30 pm	Agency Updates (All, 10-15 min each)
11:30 pm-12:45 pm	Lunch (on own)
12:45 pm-1:45 pm	Agency Updates Continued
1:45 pm-2:45 pm	Pathogens of Baitfish (Gary Jagodzinski/Corey Puzach)
2:45 pm-3:00 pm	Break
3:00 pm-4:00 pm	Subcommittee on Aquatic Animal Health/APHIS VHSv Rules Update Infectious Salmon Anemia Update (Janet Whaley)
4:00 pm-4:20 pm	Infectious Salmon Anemia Surveillance and Control (Kerry Collins)
4:20 pm-4:30 pm	Dates/Location for summer 2013 meeting (Ken Phillips/Paula Phelps)
4:30 pm-4:45 pm	GLANSIS (Ken Phillips)
4:45 pm-5:00 pm	Meeting Wrap-up
5:00 pm	Adjourn

1. Welcome and Introductions (Phillips)

2. Approval of Meeting Minutes (All)

The minutes from Winter 2012 were approved.

3. GLFC Update (Dettmers)

For those going to the national AFS meeting in Minneapolis, there will be a workshop on aquatic connectivity. One aspect is the transmission of pathogens. If attending, you should consider participating in this workshop on Wednesday/Thursday.

4. Model Program Update (Phillips)

The Model Program was approved at the CLC meeting in April. The CLC members were pleased with the document and offered the GLFHC praise. Now the document is undergoing the copyediting process. Upon completion, Ken and Ling will ensure that the intent and meaning of the document has remained intact. The pathogen descriptions are not yet completed, but Gary and Andy Noyes hope to have them written by the end of September.

Recent emails have been sent regarding movement of fish that haven't been tested (steelhead from Castalia, lake sturgeon from Genoa; Appendix 1). Perhaps this should be included in the Model Program when the document is revised at the Winter 2013 meeting. If this problem occurs often, then it should become a part of the Model Program; if not, then the process currently in place is adequate. An eye can be kept on this to make a determination of how to proceed. This fall, the committee will be sent assignments to review certain sections, and this could be one of them if committee members feel it is necessary.

The CLC recommended the GLFHC consider writing a wild fish disease model program, creating flow charts for reassigning pathogens to different lists, and adding *A. crassus* and *A. invadans* to the lists of pathogens. Another consideration is rhinoviruses---they infect frogs but can be transferred to fish. There will be a talk at the AFS-Fish Health Section meeting. That presenter could be invited to talk to a GLFHC meeting if the members think it is necessary.

Once the copyediting is completed, it will be printed for distribution. The living document will reside on the GLFHC's webpage.

5. Western Fisheries Research Center Update (Elliott)

See Appendix 2 for the presentation.

6. Leetown Science Center Update (Blazer)

See Appendix 3 for the presentation.

7. Agency Updates (All)

Ohio DNR (T. Parrett through email): He gave special thanks to everyone who provided comments about the surplus steelhead trout that were at the Castalia State Fish Hatchery. These fish will be tested as soon as possible and stocked once the results are obtained. David Insley is no longer on ODNR's staff, and Andy Jarrett is the acting Fish Hatchery Superintendent until the position is filled permanently. With Dave Insley's departure, ODNR does not have an AFS Certified Fish Health Inspector on staff. This year, a veterinarian has overseen sample collection, but while coordination went well, it's quite expensive. So far, all hatchery and brood fish have tested negative for all pathogens of concern. There are no significant fish health concerns at any facility.

Wisconsin USFWS (K. Phillips): There was a change in status at Genoa, due to detection of *Y. ruckerii* in smallmouth bass. The fish were destroyed. As a result of the finding, the facility was split into cool and warmwater sections and was subsequently disinfected. The isolation facility had already been split from the rest. The hatchery will undergo an inspection next week. In January, there will be an upgrading of the isolation facility to full quarantine facility (UV on effluent waters). Recent completed projects include: covered raceways at Pendle Creek and Jordan River hatcheries; replaced water intake at Sulvan Lake; construction of an eDNA lab for Asian carp in La Crosse; vaccinated Iron River broodfish for A. Sal in brook trout and lake trout; and vaccinated Jordan River brook trout fry for furunculosis.

Michigan DNR (G. Whelan): There have been substantial budget cuts, larger than previous years. It's unclear how that will affect work. Large cuts in Chinook stocking for Lake Michigan have implications for BKD control and herd immunity. There have been lots of fish kills so far this year--- most were in streams exceeding thermal tolerances, and northern pike were especially hit. This resulted in lots of press interest. Nucleospora was detected in 6 of 7 hatcheries for salmonids, but not in high numbers. Coldwater disease has been an ongoing problem in hatcheries. A. sal was found in some fish (steelhead, Atlantic salmon), but not to a high degree. BKD is suppressed. Whirling disease is potentially at Platte, and the investigation is ongoing. Other detections include: vireo virus in muskies, one koi herpesvirus detected, and VHS is prevalent in some smaller lakes. Asian carp work is starting and MIDNR is trying to extirpate grass carp (diploid), which are proving very difficult to remove.

Michigan State University (M. Faisal): Nucleospora detections were found via PCR in various salmonids. Levels are low in the fish.

Fisheries and Oceans Canada (S. Khatkar): DFO is encouraging people to use their VHS surveillance website which is updated every month. There is also an email subscribing list to find out what's happening. Currently doing diagnostics surveys for finfish and testing the specificities of different detection methods. Results will be published in upcoming years. The CFIA is taking over domestic movement of fish, and there will be minor amendments to compliance. Get website links from her or Ken.

Indiana DNR (D. Meuninck): VHS surveillance was negative in hatcheries. There were no fish kills in northern IN, but there were some in ponds in the southern part of the state. Bodine SFH had gill bacteria this year with a typical mortality curve for steelhead, but after treatment there were no significant

changes. Samples were sent to Purdue to look for coldwater disease and Nucleospora. Some fish had lesions on their back, but results came back negative for everything. Dr. Lin could see bacteria but nothing would grow on media. Hopefully mortality will subside on its own. If not, the lab may look into using a different type of agar. Dr. Faisal found Nucleospora when samples were sent to him.

Minnesota DNR (L. Shen): The fishing license fee increased this year, which will hopefully help budget issues. Renibacterium was detected at 5 state hatcheries at low prevalence, with no mortalities. One hatchery will vaccinate the fish to try to get rid of the bacteria. A few fish kills, one involved northern pike, were likely due to higher water temperatures. VHS surveillance is ongoing. So far, there have been no detections. One mortality was recorded at a warmwater hatchery among the muskie fingerlings. Samples were collected and an Aeromonas species was found. Mortality improved after treatment. The hatchery manager wondered if the feed they are using was the culprit---- and it was completely covered in bacteria! They tried to isolate and identify the bacteria, but were unsuccessful. They contacted the company, who said there is an allowable amount of bacteria in the feed. So, likely this was how the fish contracted the pathogen. The morphology was different between food and fish though. There were questions regarding whether or not lake sturgeon should be stocked (see Appendix 3), but the sturgeon were released within the Red River basin.

Pennsylvania Fish and Boat Commission (C. Yamashita): Hatcheries are at status quo. There have been fewer furunculosis cases due to vaccinations last year. Also, there's less IPN in the whole system and in fewer lots. Approximately 90k fish were euthanized where IPN was found and the hatcheries are being disinfected (see February 2012 update). The hatcheries will undergo testing this fall to ensure they are IPN free. They are still having smallmouth bass YOY problems, and samples are undergoing testing. Presque Isle Bay had its first VHS detection in bluegills. The Lake Erie R/V has a new captain so they will be able to go out in the field again. Brian Newinsky has replaced Andy Shiels.

Wisconsin DNR (S. Marcquenski): The last APHIS VHS surveillance investigated 27 locations, and no VHS found; however, there was one detection of largemouth bass virus. A current project is working with the La Crosse fish health lab, testing fathead minnows that feed walleye and muskie. WVDL isolated golden shiner virus, and at the end of June, found fathead minnow nidovirus. So essentially, these areas are free of VHS but have other viruses. Great Lakes spotted muskie which are fed fathead minnows can acquire this disease. It seems like is a dangerous potential pathogen. Lori Gustafson is requesting VHS surveillance data for wild fish or hatcheries. Finally, there have been a number of northern pike fish kills due to the heat.

8. Pathogens of Baitfish (Jagodzinski/Puzach)

See Appendix 4 for the presentation.

9. APHIS/ VHSv Rules Update and ISA Update (Whaley)

An Advisory Committee has been set up, with 18 members from industry, federal, and state agencies. Priority diseases are very important, especially in regards to the NAAHP. They are looking into a voluntary surveillance program for industry, using the poultry plan as a guideline. Reporting/

ramifications/ communications are still being looked into. State veterinarians would document reportable diseases. Questions remain about how to access to this information and how to manage VHS. $7 million has already been spent. Should federal regulations be created? Should states manage it? The bottom line is: at the end of fall, a recommendation on how to go forward should be in place.

Thoughts from the fish health committee: In general, the concern from here is what would replace federal regulation. What would changes look like? It's hard to support something you don't know. Complete elimination would likely be a mistake simply because some states have nothing else. Also, the feds pulling out would send the message that no regulation is needed anymore. The emergency rule as currently written has quirks but works overall. Some of the non-APHIS-supported surveillance has shown that it's persisting in the environment and hasn't gone away. To protect the U.S., there is a need for some federal oversight on the movement of these fish. Lots of money has been spent, but a lot has also been learned. Budget restraints are likely going to make a continued surveillance at this level difficult, but some surveillance needs to be ongoing. Surveillance has shown the distribution among larger fish, but hasn't done so for smaller fish or eggs. The story of VHS is not over, and it may be working at a level that is currently unknown. The repercussions of its existence are still unknown. The wild fish surveillance should continue especially at early life stages and to continue learning about the ecology of the disease. Anything that is in the wild will show up in aquaculture at some point due to surface water exchange.

See Appendix 5 for the ISA presentation.

10. ISA Surveillance and Control (Keleher)

See Appendix 6 for the presentation.

11. Dates/Location of the Summer 2013 Meeting (Phillips/Phelps)

The Winter 2013 meeting will be in northern Indiana (near Notre Dame most likely) from February 5-7th. The GLFHC should invite researchers from the university to present their projects.

The Summer 2013 will be in Duluth, Minnesota from August 7-8th.

12. GLANSIS (Phillips)

The NOAA Great Lakes Environmental Research Lab is creating a database on aquatic invasive species (Appendix 7). For this, pathogen fact sheets have been generated which would be placed on the website. NOAA-GLERL has asked the GLFHC to review these fact sheets and provide input. Each GLFHC member will receive 1-2 pathogens to review later in the month.

13. Meeting Wrap-up (All)

The GLFHC website needs updating. Please review and provide suggestions on any links that may be important to have on there.

A new vice-chair will be elected in February. Send nominations to Ken.

Lori Gustoffson is looking for VHS data. Mohamed had sent her an email with information to all Michigan surveillance data, but she may need additional information. If you have data you would like to share that has not been submitted to APHIS, please contact her. Please mirror Mohamed's Excel sheet.

14. Adjourn

From: Kenneth_Phillips@fws.gov
Sent: Tuesday, July 24, 2012 9:53 AM
To: adnoyes@gw.dec.state.ny.us; alfred.kaas@wisconsin.gov; Andy.dwilow@dfo-mpo.gc.ca; ashiels@state.pa.us; Christina Haska; cyamashita@state.pa.us; dave.insley@dnr.state.oh.us; dmeuninck@dnr.in.gov; faisal@cvm.msu.edu; gwright@sault.com; John Dettmers; jgdaley@gw.dec.state.ny.us; john_coll@fws.gov; kevin.loftus@ontario.ca; ling.shen@dnr.state.mn.us; bmattes@glifwc.org; rlang@dnr.in.gov; steve.krueger@illinois.gov; susan.marcquenski@wisconsin.gov; whelang@michigan.gov; Tim.Parrett@dnr.state.oh.us; paula.phelps@dnr.state.mn.us; Sunita.Khatkar@DFO-MPO.GC.CA
Cc: Doug_Aloisi@fws.gov; Angela_Baran@fws.gov; Becky_Lasee@fws.gov; Kurt_Schilling@fws.gov
Subject: Stocking LST from Genoa NFH into MN waters
Attachments: GEN LST RA.pdf

Hi everyone,

The Genoa NFH and MN DNR have tentative plans to stock Rainy River-strain lake sturgeon fingerlings reared at the Genoa NFH into several sites in MN, all within the Red River Basin. The stocking by the Genoa NFH will take place in tribal waters, including 11,000 LST into the Black Duck River and 8,000 into the Wild Rice River. The MN DNR will be stocking an additional 11,000 LST from the Genoa NFH into other waters within the Red River Basin. Although both of these rivers are in the Red River Basin, and not the Great Lakes Basin, Ling and I feel it is important to inform the GLFHC of this plan and ask for feedback from the GLFHC membership.

Background information. One lot of SMB at the Genoa NFH warm/cool water facility tested positive for *Yersinia ruckeri* during a routine fish health inspection this spring. The lot of SMB that tested positive were destroyed, the building where the LST are kept was completely disinfected prior to the arrival of the LST eggs, and the pond where the SMB were reared was drained, dried, and treated with lime. The eggs for the LST to be stocked were collected from the Rainy River (southern Ontario, Canada) in May, disinfected with iodophor, and reared at the Genoa NFH. The La Crosse Fish Health Center screened the fish for viruses in June (histology for iridovirus; cell culture on EPC, CHSE-214, WSS cell lines); no viruses were detected. Due to the small size of the fish in early June, the fish were not tested for bacterial pathogens. Thirty thousand fish from this lot will remain at Genoa for stocking this fall. The La Crosse Fish Health Center will screen the remaining 30,000 LST from this lot for bacterial and viral pathogens prior to release.

In the revised Model Program approved by the CLC in April, *Y. ruckeri* is considered to be a Level 1 Restricted pathogen, which would allow positive fish to be stocked under certain conditions. It should be noted that this lot of LST has not tested positive for Y. ruckeri, but because of the recent history at Genoa and the fact that they have not been screened for *Y. ruckeri*, they should be treated as if they are suspect positive until they are tested. One of the criteria to release fish with a Level 1 Restricted pathogen is to complete the Risk Assessment. Doug Aloisi (Manager, Genoa NFH) and I completed a risk assessment for the proposed stocking, which was also reviewed by Ling. The completed risk assessment indicated that the fish health risk for these fish was low, indicating that it would be acceptable to stock the LST as proposed. I've attached a PDF of the completed Risk Assessment.

(See attached file: GEN LST RA.pdf)

The initial was to release these fish Monday, July 23. However, the release date was delayed until Wednesday, July 25 (tomorrow) to allow for evaluation of the risk involved. The Genoa NFH does not have the capacity to

continue rearing all 60,000 Rainy River-strain LST beyond this week, and will need to destroy the 30,000 fish in question if they are not stocked. Any feedback provided by the COB today would be appreciated. I appologize for the short notice.

Thanks,

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

Form MP-3. Risk assessment for pathogen movements out of a facility. Complete this form when transferring fish to the wild during stocking events.

Prevalence of pathogen in the hatchery (10)		
High (67-100) – 5		10
Medium (33-66) – 3		
Low (1-32) – 1	1	
None – 0		
Unknown- 5		
Pathogen transmission through fish or their gametes (10)		
Vertical – 5		10
Horizontal – 1	1	
Unknown – 5		
Prevalence of the pathogen in the receiving water (5)		
High – 1		50
Medium – 3		
Low – 5		
None – 10		
Unknown- 10	10	
Current geographic distribution in the Great Lakes basin (10)		
Presence – 1	1	10
Absence – 3		
Indicate which vectors are accessible to the pathogen (10) E.g., Ballast, bait (preserved, frozen, live), fish stocking (state, federal, certified private hatchery, uncertified private hatchery), fish-eating birds and mammals, bilge and live wellwater in recreational boats, commercial fishing, weed harvesting, etc.		
0-1 vectors – 1		30
2-4 vectors – 3	3	
5-7 vectors – 5		
8+ vectors – 7		
Is the activity of introducing these fish likely to increase a pathogen's prevalence/intensity? (5)		
Yes – 5		15
Maybe – 3	3	
No – 0		
Is the activity of introducing these fish likely to increase a pathogen's geographic range? (10)		
Yes – 5		30
Maybe – 3	3	
No – 0		
Describe the potential for disease to other animals or species (10)		
No other species affected – 0	0	0
1-2 species affected – 1		
3-4 species affected – 3		
5+ species affected – 5		
Describe the potential for an epidemic in wild stocks (20)		
Known to cause epidemics elsewhere – 5		20
Does not cause epidemics – 1	1	
Unknown – 5		

Confidence in the pathogen test methods in the hatchery (5)		
Standard methods – 1	1	5
Non-traditional (non-representative) methods – 3		
No surveillance – 7		
Fish health history of lot (5)		
No history of significant pathogens – 1		
History of significant pathogens – 5		
No history – 5	5	25
Fish health history of broodstock (5)		
No history of significant pathogens – 1		
History of significant pathogens – 5		
No history – 5	5	25
Fish health history of facility (5)		
No history of significant pathogens – 1		
History of significant pathogens – 5	5	25
No history – 5		
Pathogen surveillance in the receiving hatchery or waterbody (10)		
High level of population pathogen history		
Significant pathogen(s) detected – 5		
No significant pathogen(s) detected – 1		70
Low level of population pathogen history		
Significant pathogen(s) detected		
No significant pathogen(s) detected		
No population pathogen history	7	
Total Risk Score		325

From: John_Coll@fws.gov
Sent: Tuesday, July 24, 2012 10:58 AM
To: Kenneth_Phillips@fws.gov
Cc: adnoyes@gw.dec.state.ny.us; alfred.kaas@wisconsin.gov; Andy.dwilow@dfo-mpo.gc.ca; Angela_Baran@fws.gov; ashiels@state.pa.us; Becky_Lasee@fws.gov; bmattes@glifwc.org; Christina Haska; cyamashita@state.pa.us; dave.insley@dnr.state.oh.us; dmeuninck@dnr.in.gov; Doug_Aloisi@fws.gov; faisal@cvm.msu.edu; gwright@sault.com; John Dettmers; jgdaley@gw.dec.state.ny.us; kevin.loftus@ontario.ca; Kurt_Schilling@fws.gov; ling.shen@dnr.state.mn.us; paula.phelps@dnr.state.mn.us; rlang@dnr.in.gov; steve.krueger@illinois.gov; Sunita.Khatkar@DFO-MPO.GC.CA; susan.marcquenski@wisconsin.gov; Tim.Parrett@dnr.state.oh.us; whelang@michigan.gov
Subject: Re: Stocking LST from Genoa NFH into MN waters
Attachments: GEN LST RA.pdf

Hello Ken & All,

I am no longer aware of when Genoa had the *Y.ruckeri* isolation. Has the facility been inspected since, with no occurrence? We do need two years freedom for it to be a mute issue, but information such as one year of freedom would be helpful. Were any other fish on station screened (for bacterial pathogens) in June?

The issue about what does disinfection (or in this case decontamination?) provide in regard to hatchery classification / disease risks has come up before. Although the newest Model Program doesn't factor disinfection into the classification scheme, logically, in our minds we must.

The other side of risk assessment, benefit analysis must also be factored. One may accept a moderate risk if the benefit is very high. How much benefit this stock provides must be weighed against the (low) disease risk. That would logically be something the tribes and the Minnesota DNR would have to assess. That said, as a rep of the third party in this issue (GLFHC), I recommend that if (and only if) the benefit of stocking these fish is substantial, the low disease risk should be accepted and stocking proceed.

One final note for discussion purposes If Genoa did not have a recent finding of *Y ruckeri*, would this issue of stocking fry from untested adults still be brought forward?

Thank you

John

John Coll
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From: Lang, Randy [RLANG@dnr.IN.gov]
Sent: Tuesday, July 24, 2012 2:02 PM
To: Kenneth_Phillips@fws.gov; adnoyes@gw.dec.state.ny.us; alfred.kaas@wisconsin.gov; Andy.dwilow@dfo-mpo.gc.ca; ashiels@state.pa.us; Christina Haska; cyamashita@state.pa.us; dave.insley@dnr.state.oh.us; Meuninck, Dave; faisal@cvm.msu.edu; gwright@sault.com; John Dettmers; jgdaley@gw.dec.state.ny.us; john_coll@fws.gov; kevin.loftus@ontario.ca; ling.shen@dnr.state.mn.us; bmattes@glifwc.org; steve.krueger@illinois.gov; susan.marcquenski@wisconsin.gov; whelang@michigan.gov; Tim.Parrett@dnr.state.oh.us; paula.phelps@dnr.state.mn.us; Sunita.Khatkar@DFO-MPO.GC.CA
Cc: Doug_Aloisi@fws.gov; Angela_Baran@fws.gov; Becky_Lasee@fws.gov; Kurt_Schilling@fws.gov
Subject: RE: Stocking LST from Genoa NFH into MN waters

Ken-

I consulted with Dave Meuninck and Indiana supports the stocking.

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Cikana State Fish Hatchery
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Cellphone: 765-346-3421
FAX: 765-349-1692

From: Marcquenski, Susan V - DNR [Susan.Marcquenski@Wisconsin.gov]
Sent: Thursday, July 26, 2012 5:28 PM
To: John_Coll@fws.gov; Kenneth_Phillips@fws.gov
Cc: adnoyes@gw.dec.state.ny.us; Kaas, Alfred - DNR; Andy.dwilow@dfo-mpo.gc.ca; Angela_Baran@fws.gov; ashiels@state.pa.us; Becky_Lasee@fws.gov; bmattes@glifwc.org; Christina Haska; cyamashita@state.pa.us; dave.insley@dnr.state.oh.us; dmeuninck@dnr.in.gov; Doug_Aloisi@fws.gov; faisal@cvm.msu.edu; gwright@sault.com; John Dettmers; jgdaley@gw.dec.state.ny.us; kevin.loftus@ontario.ca; Kurt_Schilling@fws.gov; ling.shen@dnr.state.mn.us; paula.phelps@dnr.state.mn.us; rlang@dnr.in.gov; steve.krueger@illinois.gov; Sunita.Khatkar@DFO-MPO.GC.CA; Tim.Parrett@dnr.state.oh.us; whelang@michigan.gov
Subject: RE: Stocking LST from Genoa NFH into MN waters

John brings up a good point which ties this case with the previous situation OH faced in spring regarding stocking surplus steelhead that had had no testing at all. It seems these situations are coming up more frequently and the current Model Program is silent on this combination of factors. Can we plan to discuss this broadly at the winter mtg when most of the member agencies can be present? With the intent of revising the MP to cover these situations.

I am sorry I missed the chance to respond before now, but I have one question: Were the lake sturgeon reared in the same pond that the SMB occupied earlier this spring when the Y r. was detected? Are there any hints regarding the source of the Y.r. ? (OK, two questions..)
Sue

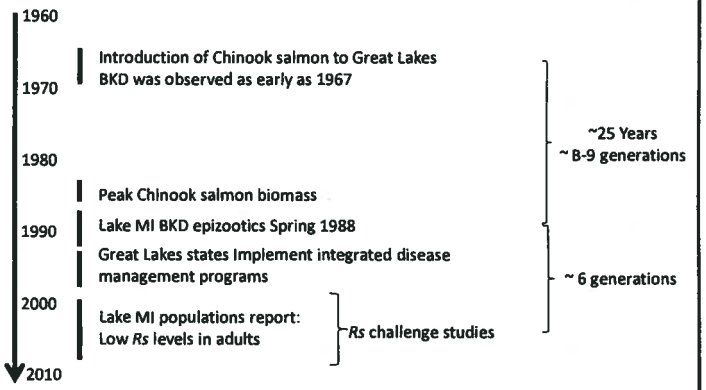
USGS

Characterization of the Adaptive Potential of Chinook Salmon to Resist Bacterial Kidney Disease

Maureen Purcell, Jeff J. Hard, Linda K. Park,
James R. Winton and Diane Elliott

Western Fisheries Research Center; USGS
Northwest Fisheries Science Center; NOAA
Seattle, WA

Timeline



USGS

R. Salmoninarum Genetics Cohort Years 2008 and 2009

Genetics

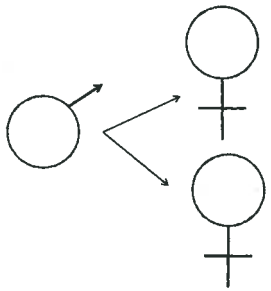
1. Is there a genetic basis to the phenotypic difference in *R. salmoni*narum resistance between WA and WI?
2. Is *R. salmoninarum* resistance a stable trait under different environmental conditions?

Biomarkers

3. Are there host biomarkers that are prognostic of BKD mortality or pathology?
4. Mechanisms of differential survival?

USGS

Nested Half-Sib Breeding Design



Cohort Year 2008 – 69 Families

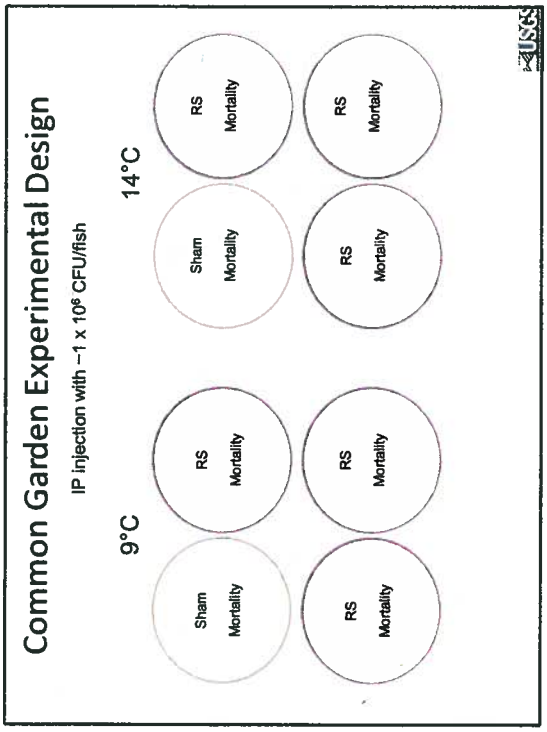
WA x WA
n=28 full-sibs / n= 14 half-sibs
WI x WI families
n=26 full-sibs / n= 13 half-sibs

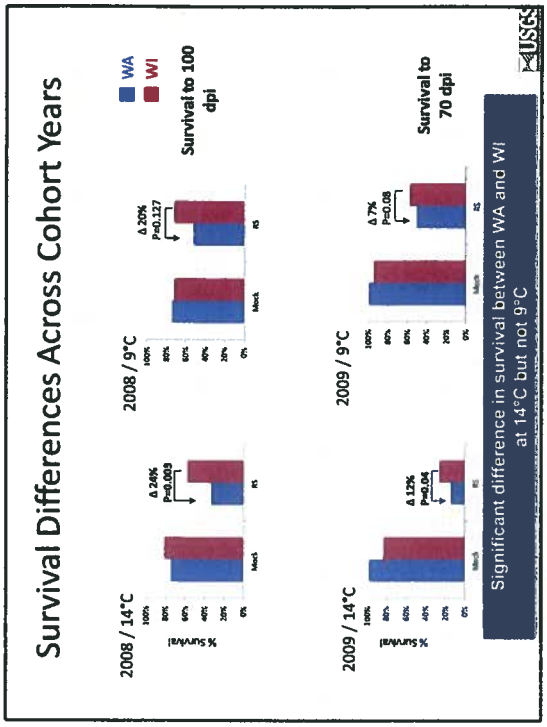
WI x WA Families n =7
WA x WI Families n=8
5300 tagged fish at challenge

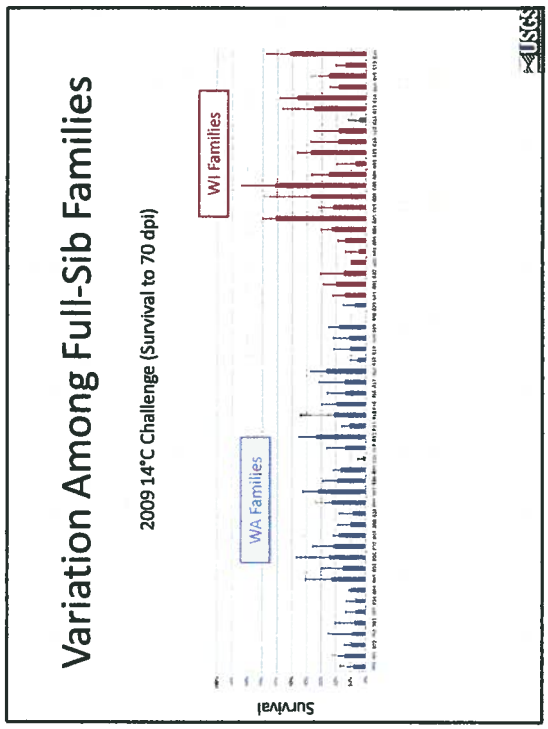
Cohort Year 2009 – 58 Families

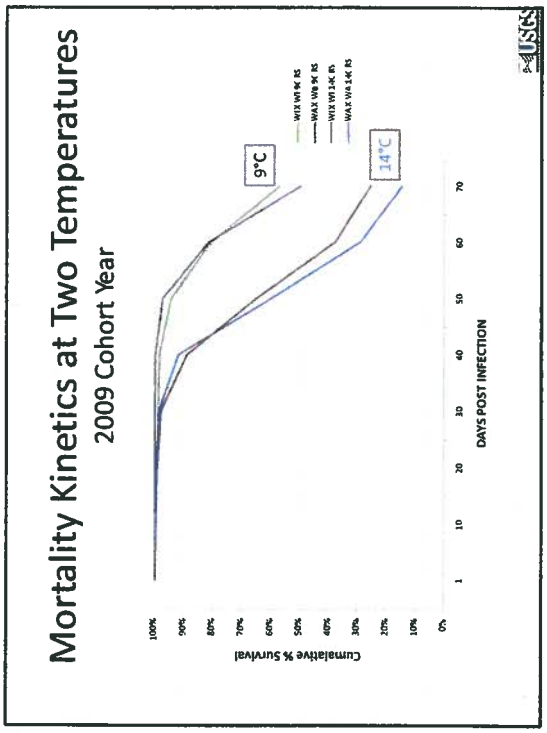
WA x WA
n=34 full-sibs / n= 16 half-sibs
WI x WI families
n=24 full-sibs / n= 10 half-sibs
6500 tagged fish at challenge

USGS


Common Garden Experimental Design
IP injection with ~1 x 10⁶ CFU/fish
9°C
14°C
Sham Mortality
RS Mortality
RS Mortality
RS Mortality
Sham Mortality
RS Mortality
RS Mortality
RS Mortality
USGS


Survival Differences Across Cohort Years
WA
WI
2008 / 14°C
2008 / 9°C
2009 / 14°C
2009 / 9°C
Survival to 100 dpi
Survival to 70 dpi
% Survival
Mock
RS
Δ 24%
P=0.069
Δ 20%
P=0.127
Δ 12%
P=0.04
Δ 7%
P=0.08
Significant difference in survival between WA and WI at 14°C but not 9°C
USGS


Variation Among Full-Sib Families
2009 14°C Challenge (Survival to 70 dpi)
WI Families
WA Families
Survival
USGS


Mortality Kinetics at Two Temperatures
2009 Cohort Year
9°C
14°C
Cumulative % Survival
DAYS POST INFECTION
USGS

Heritability Estimates

Cohort Year	Population	H_2 mortality (SE)*	H_2 days to death (95% HPD)**
2008	WA	0.04 (0.01)	0.001 (0.00 – 0.002)
	WI	0.04 (0.01)	0.09 (0.04 – 0.15)
2009	WA	0.04 (0.01)	0.15 (0.09 – 0.23)
	WI	0.07 (0.02)	0.31 (0.20 – 0.58)

*Corrections for H_2 based on the binary trait mortality using methods of Dempster and Lerner 1950
**Animal model estimate (Bayesian) of H_2 based on days to death conditioned on temperature as fixed and tank as random effect; HPD (highest posterior density)

Conclusion
H_2 estimates are low overall (higher for 2009)
No evidence of reduced additive genetic variation in the WI population

USGS

Most but not all studies have found that BKD survival is a heritable trait

Species	Population	H_2 (time to death)	Significant > 0	Source
Chinook	Robertson Ck.	0.00 – 0.20	No	Beacham & Evelyn 1992a
Chinook	Carson H.	0.35 - 0.89	Yes	Hard et al. 2006
Chinook	Katimat, Nitinat, Quinsam	0.00 – 0.50	Yes/No	Beacham & Evelyn 1992b
Atlantic salmon	AKVAFORSK Natl. Breed. Program	0.23 – 0.45	Yes	Gjedrem & Gjøen 1995

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R. Salmoninarum Genetics

1. Is there a genetic basis to the phenotypic difference in *R. salmoni*narum resistance between WA and WI?
   - Demonstrated significant heritability of trait with 2009 broodyear
   - No evidence of reduced genetic variation in WI population relative to WA population
   - Hybrid crosses performed in 2008 did not have sufficient power to determine if phenotypic difference between WA and WI was due to selection
2. Is *R. salmoninarum* resistance a stable trait under different environmental conditions?
   - -Statistical analyses of phenotypic plasticity are ongoing
   - -Preliminary results indicate trait is stable at both temperatures

USGS

R. Salmoninarum Genetics

Genetics
1. Is there a genetic basis to the phenotypic difference in *R. salmoni*narum resistance between WA and WI?
2. Is *R. salmoninarum* resistance a stable trait under different environmental conditions?

Biomarkers
3. Are there host biomarkers that are prognostic of BKD mortality or pathology?
4. What contributes to differential survival?

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Research Question

A number of diagnostic tests to measure *R. salmoninarum* prevalence and intensity but patterns among tests are not always concordant

Need for predictive tools to monitor fish health in natural population

Are there host biomarkers that are prognostic of BKD mortality or pathology?

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Mechanisms of Higher Survival?

- Pathogen pressure can lead to two evolutionary trajectories
- Resistance
  - Ability to limit the bacterial load such as by reducing bacterial replication
- Tolerance
  - Ability to limit disease severity by compensating or reducing pathological damage

USGS

Prognostic Biomarkers for BKD?

Renibacterium salmoninarum infection

Molecular & cellular response

Tissue/ organ response

Disease Outcome

High Survival Low Survival

Bacterial Load
qPCR to detect *R. salmoninarum* DNA
Immunohistochemistryin kidney

Gene expression biomarkers
Host immune response (Type II interferon / iNOS)
Inflammation (Interleukin 1 beta, TNF-alpha)

Pathology and Pathophysiological biomarkers
Granulomatous lesion/ membranous naphropathy
Clinical signs
Hematocrit / leukocrit
Serum nitrate
Lysozyme

Disease Outcome
Population (WI vs. WA)
Progeny of full-sibling families: cumulative survival

Bacterial Replication and/or Biomarkers Predict Survival?

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Biomarker Experimental Design

WA Full-Sibling Progeny Families
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10

WI Full-Sibling Progeny Families
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10

PIT-tagged progeny at 5g

Common Garden

pre-challenge → 160 Fish

Sham (PBS-peptone)

Day 30 → 160 Fish
Day 50 → 160 Fish

R. salmoninarum 5.0 × 10⁵ CFU/Fish

Day 30 → 160 Fish
Day 50 → 160 Fish

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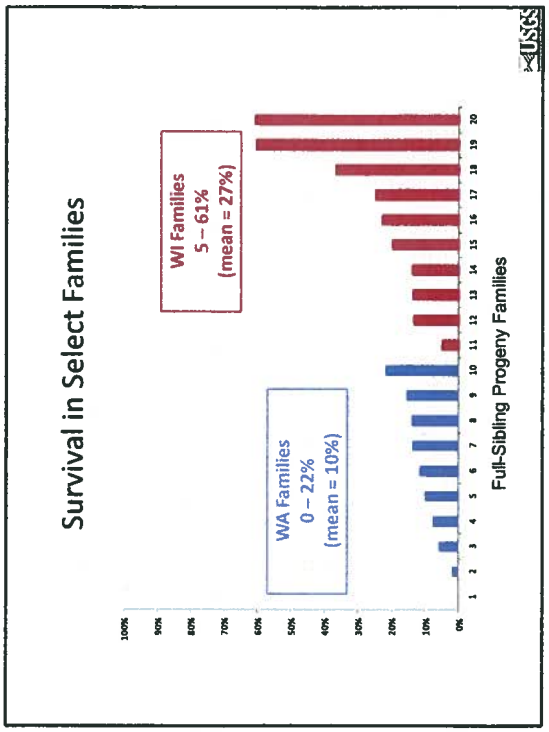
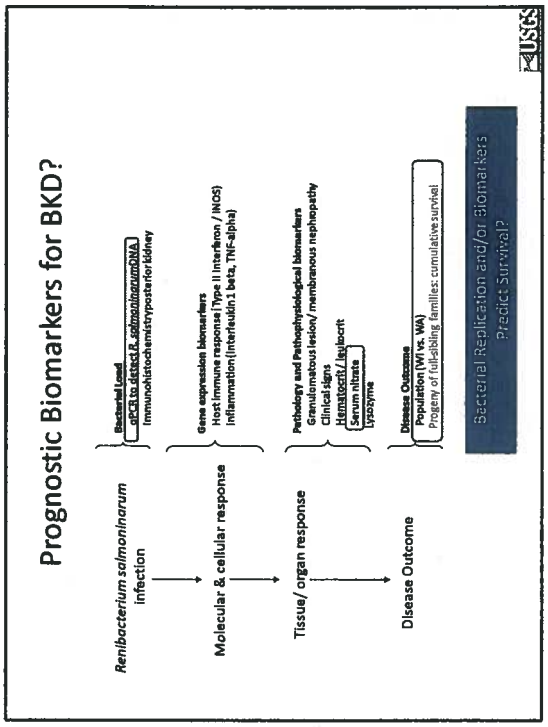
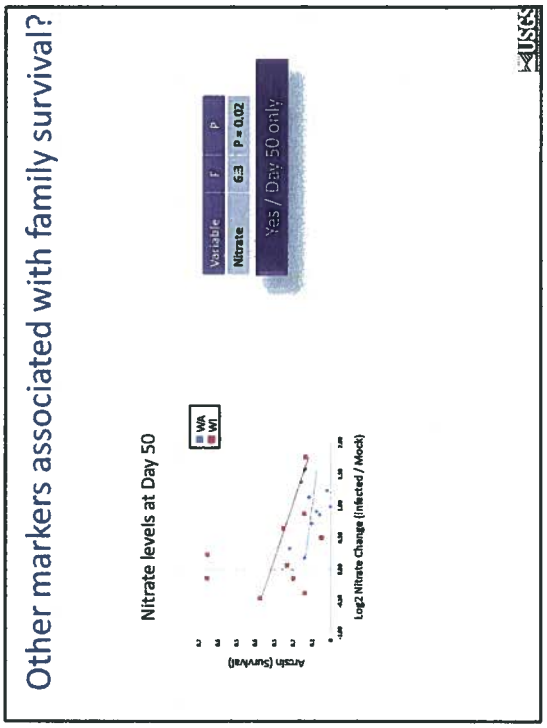
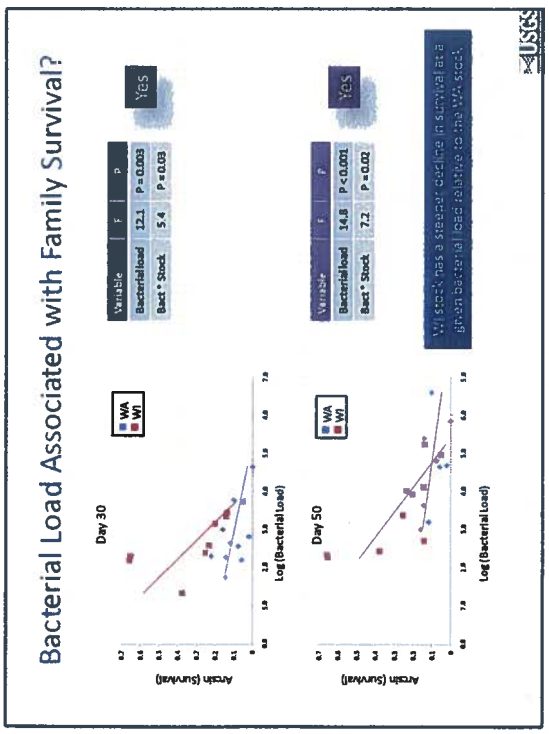

Survival in Select Families
WI Families
5 – 61%
(mean = 27%)
WA Families
0 – 22%
(mean = 10%)
Full-Sibling Progeny Families
USGS


Prognostic Biomarkers for BKD?
Renibacterium salmoninarum infection
Molecular & cellular response
Tissue/ organ response
Disease Outcome
Bacterial Load
qPCR to detect R. salmoninarum DNA
Immunohistochemistry posterior kidney
Gene expression biomarkers
Host immune response (Type II interferon, / iNOS)
Inflammation (interleukin 1 beta, TNF-alpha)
Pathology and Pathophysiological biomarkers
Granulomatous lesion/ membranous nephropathy
Clinical signs
Hematocrit / leukocrit
Serum nitrate
Lysozyme
Disease Outcome
Population (WI vs. WA)
Progeny of full-sibling families: cumulative survival
Bacterial Replication and/or Biomarkers Predict Survival?
USGS


Other markers associated with family survival?
Nitrate levels at Day 50
WA
WI
Arcsin (Survival)
Log2 Nitrate Change (Infected / Mock)
Variable
F
P
Nitrate
6.3
P = 0.02
Yes / Day 50 only
USGS


Bacterial Load Associated with Family Survival?
Day 30
Day 50
WA
WI
Arcsin (Survival)
Log (Bacterial Load)
Variable
F
P
Bacterial load
12.1
P = 0.003
Bact * Stock
5.4
P = 0.03
Yes
Bacterial load
14.8
P < 0.001
Bact * Stock
7.2
P = 0.02
Yes
WI stock has a steeper decline in survival as a given bacterial load relative to the WA stock
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Summary of Variables Predictive of Survival

	Pre-Challenge (Day 0)	Day 30	Day 50
Bacterial Replication			
Kidney Bacterial Load	-NA-	P = 0.003	P = 0.001
Post Kidney IHC	-NA-	P < 0.001	P=0.01
Molecular and Cellular Level			
TNF-α gene expression	No	No	No
IFN-γ gene expression	No	No	No
IL-1β gene expression	No	No	No
iNOS gene expression	No	No	No
Tissue and Organ Level			
Hematocrit	No	P = 0.04	P = 0.09
Leukocrit	-NA-	No	No
Serum Nitrate	No	No	P = 0.02
Fin Erosion	No	No	No
Ascites	-NA-	P=0.04	P=0.025
Histopathology	-NA-	P < 0.001	P=0.007

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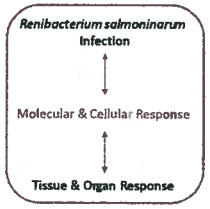
Mechanisms of Higher Survival?



What is the relationship among these levels?

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Biomarkers significantly associated with bacterial load?

	Day 30	Day 50
Hematocrit	P < 0.001 (negative)	P < 0.001 (negative)
Serum Nitrate	P < 0.001 (positive)	P < 0.001 (positive)
TNF-alpha gene expression	P = 0.04 (negative)	no
IFN-γ gene expression	no	P <0.001* (positive)
IL-1β gene expression	P=0.02 (positive)	P < 0.001* (positive)
iNOS gene expression	P = 0.03 (negative)	P = 0.004 (positive)
Kidney Histopathology	P<0.001 (positive)	P<0.001 (positive)

*Direct and/or interactive effect of population

All genes were up-regulated in response to *R. salmoninarum*
Up-regulated response greater at day 30
Association with bacterial load much stronger at day 50

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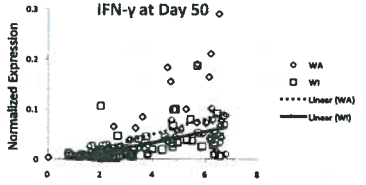
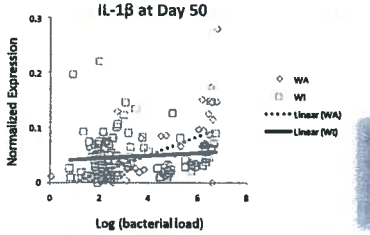
Biomarkers significantly associated with bacterial load?



Variable	F	P
Bacterial load	93.7	P < 0.001
Stock	7.8	P = 0.006



Variable	F	P
Bacterial load	19.2	P < 0.001
Stock	4.6	P = 0.03
Bact * Stock	6.2	P = 0.01

At a given bacterial load, we observe a disproportionately higher IFN-γ and IL-1β transcript level in the WA stock

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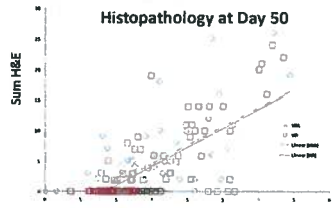
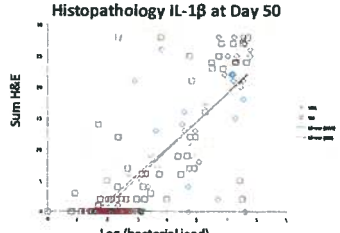
Biomarkers significantly associated with bacterial load?

Histopathology at Day 50



Variable	F	P
Bacterial load	146.8	P <0.001

Histopathology IL-1β at Day 50



Variable	F	P
Bacterial load	206.4	P <0.001

Posterior kidney histopathology scores increases proportionally with bacterial load

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Mechanisms of Higher Survival?

Renibacterium salmoninarum infection

↓

Molecular & cellular response

↓

Tissue/ organ response

↓

Disease Outcome

High Survival | Poor Survival

WI families trended towards lower bacterial load but steeper decline in survival as bacterial load increased

WA families trended towards proportionally higher IFN and inflammatory cytokine transcript levels for a given bacterial load

WA families trended greater marker change but marker response to a given bacterial load was proportional in both stocks

Overall higher survival is most strongly associated with the ability to limit bacterial replication 'Resistance'

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Prognostic Biomarkers for BKD?

Renibacterium salmaninarum infection

↓

Molecular & cellular response

↓

Tissue/ organ response

↓

Disease Outcome

High Survival | Poor Survival

Bacterial load is a good predictor of disease outcome

Gene expression markers change in response to infection but variable and lacked predictive power

Responses at the tissue or whole animal level have some predictive capability

Future Potential for Field Studies?

Laboratory evidence that blood indices may have utility when combined with quantitative measures of bacterial load

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Timeline

1960

1970 — Introduction of Chinook salmon to Great Lakes
BKD was observed as early as 1967

1980 — Peak Chinook salmon biomass

1990 — Lake MI BKD epizootics Spring 1988

Great Lakes states implement integrated disease management programs

2000 — Lake MI populations report: Low *Rs* levels in adults (*Rs* challenge studies)

2010

~25 Years
~ 8-9 generations

~ 6 generations

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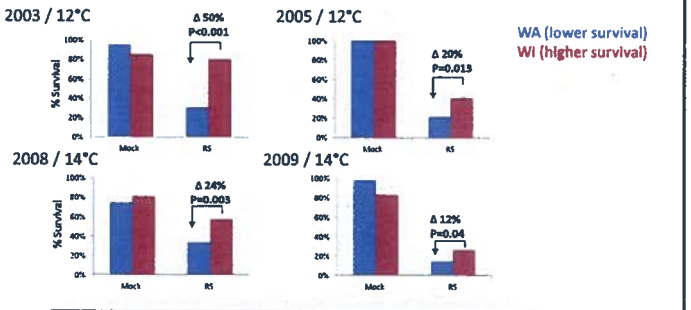
Cumulative % Survival
Four Cohort Years



WA (lower survival)
WI (higher survival)

Comparison of survival data across years is confounded by different challenge conditions and various host factors. However, there is a trend towards increasing susceptibility in the WI stock over time (relative to WA)

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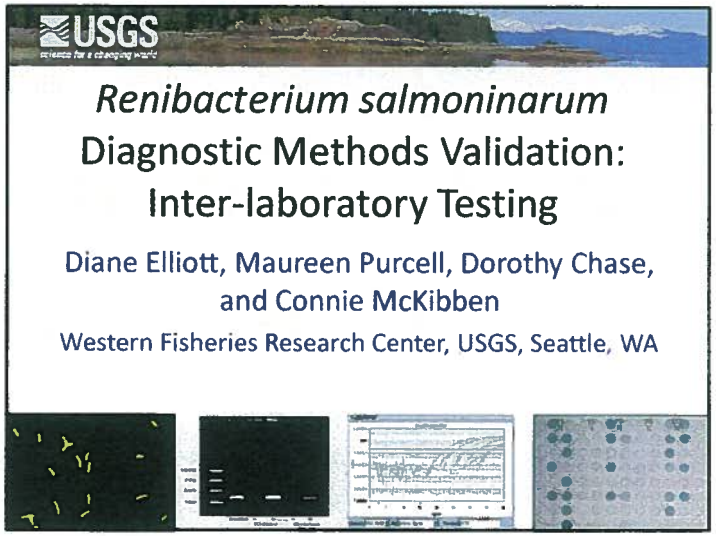
USGS
science for a changing world

Acknowledgements

- Fish
  - Sue Marcquenski and Wisconsin Dept. Nat. Resources
  - Mike Wilson and Washington Dept. Fish & Wildlife
- Technical assistance
  - Sue Marcquenski and her team (WI DNR)
  - Rachel Thompson (WFRC; USGS)
  - Taylor Alton (WFRC; USGS)
  - Samantha Badil (WFRC; USGS)
  - James Woodson (WFRC; USGS)
  - Connie McKibben (WFRC; USGS)
  - Carla Conway (WFRC; USGS)
  - Andrew Wargo (UW)
- Funding
  - Great Lakes Fishery Trust

GLFT Project 2009.966



Inter-laboratory assay comparisons

Can be performed for a variety of reasons

- Assessing the capability of an individual laboratory to conduct a diagnostic test
- Checking the performance of a particular operator
- Checking the performance or calibration of instrumentation
- Evaluating a new method
- Resolving inter-laboratory differences
- Harmonization / standardization of existing methods

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Test Methods

- **Bacteriological culture** (Jansson et al. 1996 DAO 27:197)
- **Polyclonal ELISA** (Pascho et al. 1991 DAO 12:25)
- **Direct FAT** (DFAT; Pascho et al. 1991 DAO 12:25)
- **Membrane filtration-FAT** (MF-FAT; Elliott and McKibben 1997 DAO 30:37)
- **Nested PCR** (nPCR; Chase and Pascho 1998 DAO 34:223)
- **Real-time quantitative PCR** (qPCR; Chase et al. 2006 J Vet Diagn Invest 18:375)

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Participating Laboratories

- **University of Guelph, Ontario Canada**
- **Wisconsin Veterinary Diagnostic Laboratory**
- **Minnesota DNR**
- **Michigan State University**
- **La Crosse WI Fish Health Center (USFWS)**
- **Dworshak Idaho Fish Health Center (USFWS)**
- **Eagle Idaho Fish Health Center (ID Dept. Fish and Game)**
- **Pfizer Animal Health (B.C., Canada)**
- **Western Fisheries Research Center (USGS)**

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1. *Renibacterium salmoninarum* workshop

2. Develop and test reference materials

3. Inter-laboratory testing

4. Analysis and Communication

USGS

1. *Renibacterium salmoninarum* workshop

Goals

- Dissemination of results of bench validation study (WFRC)
- Presentations on BKD status in the Great Lakes
- Round-table discussion of challenges in BKD diagnostics
- Coordination of inter-laboratory testing

USGS

1. *Renibacterium salmoninarum* workshop

2. Develop and test reference materials

3. Inter-laboratory testing

4. Communication

USGS

2. Develop and test reference materials

Goal

- Determine homogeneity and stability of reference materials (standards & controls)

Purpose

- Ensure that the properties of the reference materials do not change during shipment and storage
- Each reference aliquot gives similar results
- Develop a specific protocol for receipt and storage of each reference sample

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Standards & Controls

- Standard: a sample of a known pathogen level often used to construct a standard curve
- Control: various samples that ensure the validity of positive and negative results

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2. Develop and test reference materials

Goal
- Develop large pool of reference material
- Distribute reference materials to laboratories for testing

Purpose
- Each assay uses positive and negative controls to verify validity of results
- Standards are for quantitative assays (e.g. qPCR)
- Materials distributed to participating laboratories to verify that their assays are functioning optimally prior to inter-laboratory testing

USGS

1. *Renibacterium salmoninarum* workshop
2. Develop and test reference materials
3. Inter-laboratory testing
4. Analysis and communication

USGS

3. Inter-laboratory testing

Goal (Phase I, initial strategy)
- Inter-laboratory testing of seeded kidney tissue and/or ovarian fluid

Purpose
- Relatively unlimited amounts of this material can be produced in a uniform manner
- Phase I will provide an initial evaluation of inter-laboratory results
- Phase I will test both culture and non-culture methods

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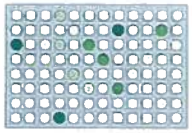
3. Inter-laboratory testing

Problems with initial Phase 1 plan

- Stability of culturable Rs in seeded kidney material was variable during transport and storage experiments
- Difficult to achieve high ELISA OD values with seeded samples
  - Most soluble antigen released into medium and lost during seeding process



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3. Inter-laboratory testing

Goal (Phase I, revised strategy)

- Inter-laboratory testing using infected kidney tissues and ovarian fluids

Purpose

- Kidney tissue from challenged fish and ovarian fluid from spawning Chinook salmon
- More realistic test (relative to seeding)
- Restricted to non-culture methods
- Identify and correct problems with protocols, reagents and test materials, testing procedures used by participants, etc. before Phase II

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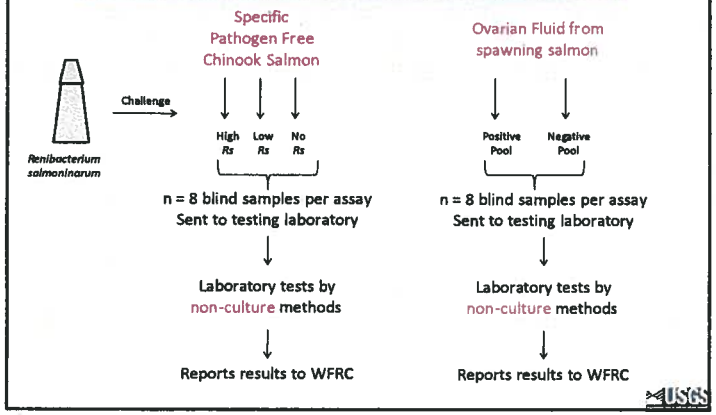
3. Inter-laboratory testing



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3. Inter-laboratory testing

Goal (Phase II)

- Inter-laboratory testing using infected kidney tissues and ovarian fluids (same as Phase I)
  - Materials, distribution and testing procedures will be similar to Phase I

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4. Analysis and communication

Analysis

- Agreement among laboratories
- Reproducibility of the different methods
- Ruggedness of the different methods

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Reproducibility /Ruggedness

- Reproducibility
  - 'The ability of a test method to provide consistent results, as determined by estimates of precision, when applied to aliquots of the same samples tested in different laboratories'
- Ruggedness
  - 'A measure of a test method's capacity to remain unaffected by substantial changes or substitutions in test conditions anticipated in multi-laboratory utilization'

*Definitions from OIE Manual of Diagnostic Tests for Aquatic Animals

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4. Analysis and communication

Communication

- Protocols posted on USGS Microbiology website and distributed to participants
- Laboratories will be notified as far as possible in advance about pending shipment (ideally 1+ month)
  - Including projected date and shipment method
  - We will be flexible to avoid peak field periods
  - Laboratories should request replacement if the samples arrive in questionable condition
  - Laboratories should acknowledge receipt of samples

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4. Analysis and communication

Communication

- A data recording sheet will be shipped with the samples and returned to the WFRC
  - We will acknowledge receipt of the results
- A summary of the results will be sent to all participating laboratories
  - Results will be coded to maintain confidentiality of individual laboratories
  - Laboratories will receive feedback on their individual performance (confidential written and telephone consultation)

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Preliminary Analysis: Phase I

	Number of Laboratories Testing Samples	
Assay	Kidney	Ovarian Fluid
DFAT	4	—
MF-FAT	—	3
ELISA	7	—
nPCR	5	3
qPCR	6	5

Need a minimum of 3 laboratories testing a given assay for valid ring test

Preliminary Analysis: Phase I Kidney DFAT

Sample No.	Expected result	No. of results matching expected (%)*
1	Neg	3 (75%)
4	Neg	4 (100%)
7	Neg	2 (50%)**
3	Pos (low)	3 (75%)
5	Pos (low)	4 (100%)
8	Pos (low)	4 (100%)
2	Pos (high)	4 (100%)
6	Pos (high)	4 (100%)

*% of 4 labs ** One lab reported detection of a single Rs cell on slide

Preliminary Analysis: Phase I Kidney ELISA

		No. of results matching expected (% of 7 labs)	
Sample No.	Expected result	Cutoff: 2 SD	Cutoff: OD 0.100
2	Neg	2 (29%)	6 (86%)
5	Neg	3 (43%)	7 (100%)
7	Neg	3 (43%)	7 (100%)
1	Pos (low)	7 (100%)	7 (100%)
3	Pos (low)	7 (100%)	7 (100%)
7	Pos (low)	7 (100%)	7 (100%)
4	Pos (high)	7 (100%)	7 (100%)
8	Pos (high)	7 (100%)	7 (100%)

Preliminary Analysis: Phase I Kidney nPCR

Sample No.	Expected result	No. of results matching expected (%)*
2	Neg	3 (60%)
3	Neg	3 (60%)
8	Neg	2 (40%)
4	Pos (low)	5 (100%)
5	Pos (low)	5 (100%)
6	Pos (low)	5 (100%)
1	Pos (high)	5 (100%)
7	Pos (high)	5 (100%)

*% of 5 labs

Preliminary Analysis: Phase I Kidney qPCR

		No. of results matching expected (% of 7 labs)	
Sample No.	Expected result	Cutoff: detectable in both replicates	Cutoff: mean C_T <38
2	Neg	4 (67%)	5 (83%)
3	Neg	3 (50%)	4 (67%)
8	Neg	2 (33%)	3 (50%)
4	Pos (low)	6 (100%)	6 (100%)
5	Pos (low)	6 (100%)	6 (100%)
6	Pos (low)	6 (100%)	6 (100%)
1	Pos (high)	6 (100%)	6 (100%)
7	Pos (high)	6 (100%)	6 (100%)

Preliminary Analysis: Phase I OF MF-FAT

Sample No.	Expected result	No. of results matching expected (%)*
3	Neg	2 (67%)
4	Neg	2 (67%)
6	Neg	3 (100%)
2	Pos (low)	3 (100%)
5	Pos (low)	3 (100%)
7	Pos (low)	3 (100%)
1	Pos (high)	3 (100%)
8	Pos (high)	3 (100%)

*% of 3 labs

Preliminary Analysis: Phase I OF nPCR

Sample No.	Expected result	No. of results matching expected (%)*
2	Neg	1 (33%)
6	Neg	2 (67%)**
7	Neg	2 (67%)**
3	Pos (low)	3 (100%)
4	Pos (low)	3 (100%)
8	Pos (low)	3 (100%)
1	Pos (high)	3 (100%)
5	Pos (high)	3 (100%)

*% of 3 labs **weak positive reported by 1 lab

Preliminary Analysis: Phase I OF qPCR

		No. of results matching expected (% of 5 labs)	
Sample No.	Expected result	Cutoff: detectable in both replicates	Cutoff: mean C_T <38
2	Neg	4 (80%)	5 (100%)
6	Neg	4 (80%)	4 (80%)
7	Neg	4 (80%)	4 (80%)
3	Pos (low)	5 (100%)	5 (100%)
4	Pos (low)	5 (100%)	5 (100%)
8	Pos (low)	5 (100%)	5 (100%)
1	Pos (high)	5 (100%)	5 (100%)
5	Pos (high)	5 (100%)	5 (100%)

Preliminary Analysis: Phase I

- False negative results were rare regardless of Rs level
  - Single false negative by kidney DFAT for low-Rs sample
- False positives were observed in all assays
  - Possible explanations for false positive results varied by assay

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False Positive Results: Phase I

- **Contamination**
  - —**FAT**
    - —Cross-contamination of slides during rinse steps
    - —Separate slides on rinse rack; rinse carefully
  - —**ELISA**
    - —Splashing of samples between wells
    - —Examine % CV of OD values in replicate wells for evidence of contamination (e.g. >10% CV of negative control wells)

USGS

False Positive Results: Phase I

- **Contamination**
  - —**PCR**
    - —DNA contamination by amplified PCR products
    - —Contamination can occur in both nPCR and qPCR during DNA extraction and PCR setup
    - —nPCR more susceptible to contamination than qPCR because of second round of PCR amplification and post-PCR manipulation of amplified products
    - —Results from negative extraction controls and and negative PCR controls (no template) very important for detection of contamination

USGS

False Positive Results: Phase I

- **Selection of Negative-Positive Cutoff**
  - —**ELISA**
    - —Lower ELISA OD values from some negative test samples than from negative control kidney tissue pool resulted in "positive" designation for some negative test samples when 2 SD cutoff used
    - —Raising cutoff to OD 0.100 eliminated all but one false positive
    - —Selection of cutoff will depend on relative importance of eliminating false positives vs eliminating false negatives (cutoffs may differ for inspection screening vs population monitoring)

USGS

False Positive Results: Phase I

- **Selection of Negative-Positive Cutoff**
  - **—qPCR**
    - —Reduction of qPCR cutoff from "detectable amplification" to mean <38 CT (with both replicates showing amplification) eliminated some false positives (but not those likely due to contamination)
      - —Cutoff mean <38 C_T represents theoretical lower limit for consistent qPCR detection (≥ 5 Rs/reaction)
    - —Similar to ELISA, selection of qPCR cutoff will depend on relative importance of eliminating false positives vs false negatives

USGS

False Positive Results: Phase I

- **Mis-interpretation of Results**
  - **—FAT (DFAT and MF-FAT)**
    - —Mis-identification of fluorescing particles as Rs
    - —Ridges in some MF-FAT filters after filtration can make observation/interpretation of results difficult
    - —Staining and morphological features on positive control slides and filters can help with ID
    - —Filtration of conjugated antibody (0.2 µm) before runs helps to remove aggregates

USGS

False Positive Results: Phase I

- **Mis-interpretation of Results**
  - **—nPCR**
    - —May be some nonspecific (spurious) bands in gels
      - —Sequencing can be used to confirm that bands are Rs
      - —However, sequencing will not distinguish between Rs originating from infection from that resulting from contamination

USGS

Other Possible Issues: Phase I

- **Unclear or incomplete protocols or instructions**
  - Mis-reading of instructions
- **Problems with shipment or storage of reagents or samples**
- **Mistakes in analysis or reporting of data**

USGS

Problems are Common in Ring Tests

Example: PCR of *Mycobacterium tuberculosis* (Nordhoek et al. J Clin Micro v. 34)

- 20 samples & 30 laboratories
- Only 5 laboratories obtained results that were 100% correct
- Variety of factors differed among the lab:
  - Lack of protocol standardization
  - Inconsistent use of positive-negative controls

Example: Koi Herpesvirus Ring Test (2008)

- 44 laboratories in 32 countries
- Used 2 OIE conventional PCR tests and one qPCR test
- 58% of labs reported incorrect results with 44% of the labs reporting false positives

USGS

Opportunities Provided by Ring Tests

- Assessing the capability of an individual laboratory to conduct a diagnostic test
- Checking the performance of a particular operator
- Checking the performance or calibration of instrumentation
- Evaluating a new method
- Resolving inter-laboratory differences
- Harmonization / standardization of existing methods; improving protocols and instructions

USGS

What's Next?

- Each participant will be sent confidential Phase I report showing how their lab compared with other labs performing the same assays
- Follow-up phone conversations will be used for more detailed discussions of assays and problem-solving where needed
- Phase II samples will be shipped to participating laboratories

USGS

Acknowledgements

- University of Guelph, Ontario Canada
- Wisconsin Veterinary Diagnostic Laboratory
- Minnesota DNR
- Michigan State University
- La Crosse WI Fish Health Center (USFWS)
- Dworshak Idaho Fish Health Center (USFWS)
- Eagle Idaho Fish Health Center (ID Dept. Fish and Game)
- Pfizer Animal Health (B.C., Canada)
- Western Fisheries Research Center, USGS (Taylor Alton)
- Wisconsin DNR (Sue Marcquenski)

Funding: Great Lakes Fishery Trust Project 2010.1158

Leetown Science Center Overview

Vicki Blazer
National Fish Health Research Laboratory
Kearneysville, WV

USGS
science for a changing world

Biosecurity Level-3 Aquaculture Laboratory

Frank Panek, Christine Densmore, Chris Ottinger

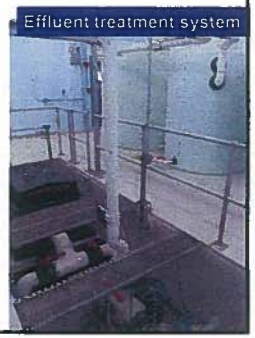
- **Wet Lab for holding fish or other aquatic animals under a high level of biocontainment**
- **Disease-related investigations or diagnostic procedures**
- **Added containment to prevent the release of pathogens or invasive species to the environment**

LSC BSL-3 Laboratory

- Planned to be operational early Fall 2012
- Consists of two separate wet laboratories
  - 1140 and 140 square feet

Key Features

- **UV irradiation of the influent water supply**
- **Redundant treatment systems for disinfection of effluent water**
  - **Microscreen filtration**
  - **High intensity UV arc tubes**
  - **Chlorination**
- **Uninterruptible power supply**



Features Shared with Biosafety Level-3 Facilities

- Primary hygiene room for entry and exit
- Negative pressure ventilation with HEPA filtration of exhaust air
- Room pressure monitoring
- Sealed and water resistant surfaces
- Implementation of standard Biosafety Level-3 operational policies for access and use

Anticipated Uses

- Research investigations involving new, emerging, or non-endemic pathogens of aquatic animals.
- Research involving pathogens of aquatic animals with particularly severe consequences, such as OIE-notifiable pathogens.
- Research investigations involving aquatic pathogens with zoonotic potential.
- Diagnostic procedures involving unknown aquatic animal pathogens that are potentially non-endemic, highly virulent, or zoonotic.
- Research investigations utilizing invasive aquatic species.

Fish Health Studies in the Great Lakes Watersheds

Vicki Blazer and Luke Iwanowicz

Great Lakes Fish Health Assessments

- Great Lakes Restoration Initiative
  - Collaborating on the FWS "Early Warning Project" for effects of exposure to chemicals of emerging concern
  - Working with state and other agencies to address the "Tumor and Other Deformities" Beneficial Use Impairment
    - St. Louis River
    - Sheboygan River
    - Niagara
    - Black, Cuyahoga, Ashtabula, Maumee
    - Reference sites

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

Chemicals of Emerging Concern

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

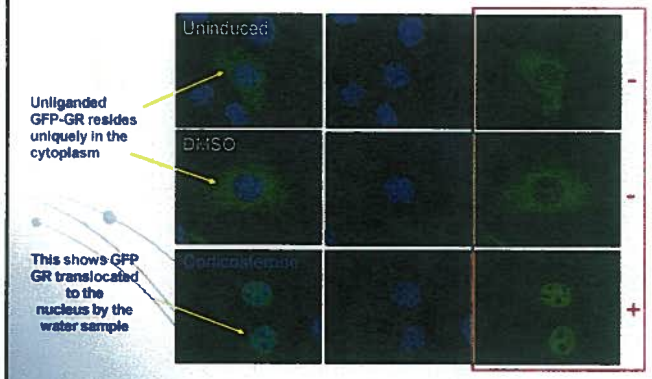
Effects Chemical of Emerging Concern

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

Biological Effects

- Effects Monitoring of wild fishes
- In vitro receptor-based assays
  - Discrete water samples, extracted and tested
  - Total estrogenicity – BLYES
  - Androgenicity
  - Glucocorticoid

Nuclear Translocation Assays



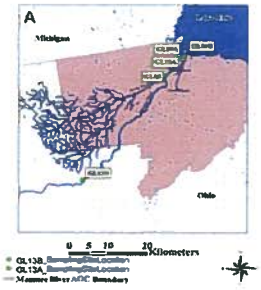
Wild Fish Assessments

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

Sites

Initial Five sites Fall 2010
Added Fox River in Spring 2011

Spring 2012



Fish Health Methods

- Anesthetize, weigh, measure
- Bleed – blood smears, drop on FTA card, blood sample on ice
- Visual assessment of external organs
- Visual assessment of internal organs
- Remove liver and gonads and weigh
- Pieces of liver, spleen, anterior kidney in RNAlater for molecular analyses; obvious skin lesions/tumors
- Pieces of all tissues and abnormalities in fixative for histopathological analyses
- Otoliths removed for aging
- Piece of liver and carcasses frozen

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

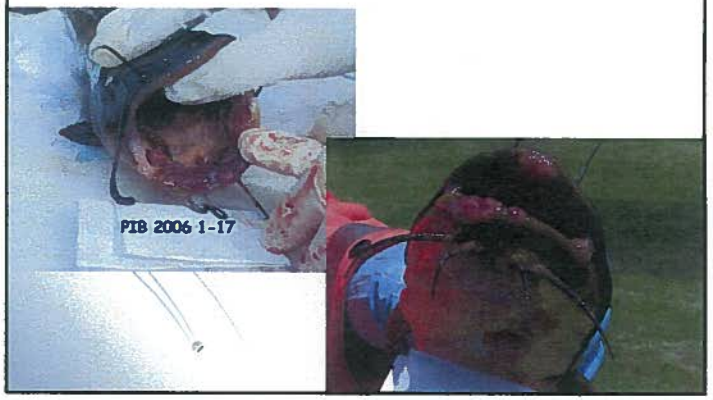
Histopathological

- Diagnose causes of gross observations, identify emerging pathogens, identify specific effects of contaminants, w ith image analyses quantify parasites, macrophage aggregates

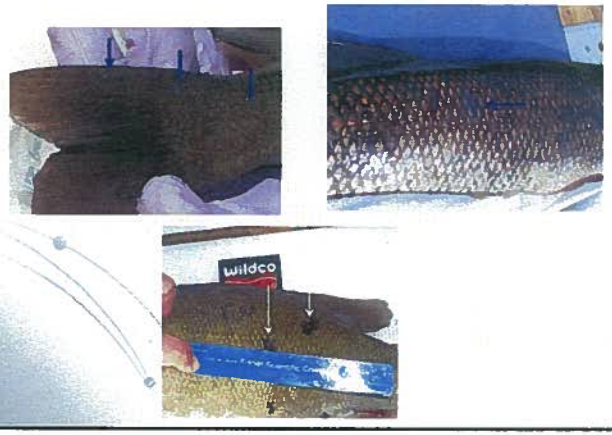
Molecular

- mRNA for reproductively related genes (vitellogenin, estrogen receptors), immune system indicators (TGF-β, hepcidin), contaminant-related (CYP1A, oxidative stress), stress (glucocorticoid receptors)
- Mechanisms

Raised Skin Lesions Papilloma



Skin Lesions



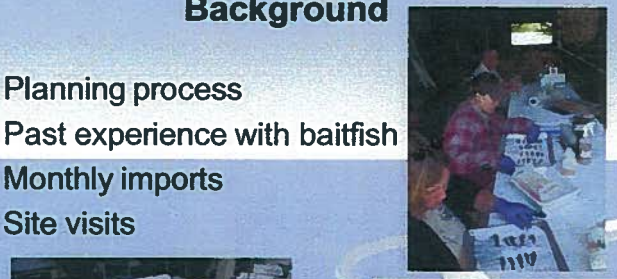
Movements of Fish Pathogens with Interstate Shipments of Baitfish

La Crosse Fish Health Center
Law Enforcement

U.S. Fish & Wildlife Service,
La Crosse Fish Health Center, 555 Lester Avenue, Onalaska, Wisconsin, 54650

Background

- Planning process
- Past experience with baitfish
- Monthly imports
- Site visits



Shipping

- Fish are shipped live overnight
- AM delivery
- Arrive in good condition
- Samples are taken same day
- Virology samples are processed same day


Disease Signs

- Lesions
- Scale loss
- Eroded or frayed fins
- Mucus production
- External parasites
- Hemorrhaging
- Behavioral- off feed/gathering at inlet/listless



Fish Health Sampling

USFWS and AFS-FHS (U.S. Fish and Wildlife Service and American Fisheries Society-Fish Health Section). 2010 (2007). Standard procedures for aquatic animal health inspections. *In* AFS-FHS. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2007 edition. AFS-FHS, Bethesda, Maryland.

Wild Fish Health Survey

Bacteriology Results

- Sampled 30 fish from each lot
- Screened for the certifiable pathogens *Aeromonas salmonicida*, *Yersinia ruckeri*, *Edwardsiella ictaluri*, and *Renibacterium salmoninarum*
- Many more pathogens out there

Methods- Bacteriology

- Aseptically cut open fish
- Sample kidney with 1 µL loop
- Streak sample on TSA slant
- Biochemical tests
- ELISIA
- DFAT
- PCR confirmation

Bacteriology Results

- Screened 2,381 fish for *A. salmonicida*, *Y. ruckeri*, *E. ictaluri*
- One isolation of *A. salmonicida* from creek chub
- Screened 14 cases for *R. salmoninarum*
- 2 positive cases from fathead minnows, and golden shiners
- Confirmed by PCR

Methods Parasitology

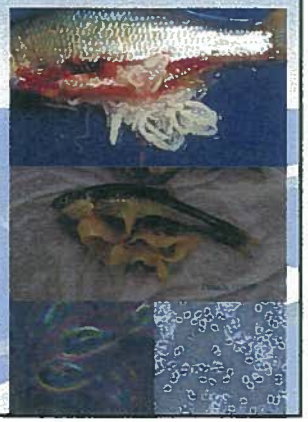
- Examine fresh or after flash freezing
- Perform parasite searches on all exterior surfaces, internal body cavity and internal organs.
- Preserve parasites and stain for identification

Parasitology

- Microsporidia (2)
- Myxosporidia (?)
- Monogenetic Trematodes (5)
- Digenetic Trematodes (10) (metacercarial)
- Cestoidea (6) (tapeworms)
- Nematoda (6) (round worms)
- Acanthocephala (1) (spiny headed worms)
- Copepoda (1)

Parasites of concern

- *Bothriocephalus acheilognathi* (Asian tapeworm)
- *Ligula intestinalis*
- *Myxobolus sp.*
- Microsporidea



Methods-Virology

- Samples can be pooled up to five fish (Kidney/spleen, whole visera)
- Diluted with transport medium (HBSS) and centrifuged
- Placed on CHSE (15 °C), BF-2 (25°C), EPC (15°C), and EPC (20°C)
- Blind pass (10 to 14 days)
- Observed for 28 days
- PCR Confirmation

Viruses

- Infectious Pancreatic Necrosis Virus (IPNV)
- Infectious Hematopoietic Necrosis Virus (IHNV)
- Viral Hemorrhagic Septicemia Virus (VHSV)
- Oncorhynchus masou Virus (OMV)
- Largemouth Bass Virus (LMBV)
- Spring Viremia of Carp Virus (SVCV)
- Fathead Minnow Virus (FHMV)
- Golden Shiner Virus (GSV)
- Unknown virus (replicating agent)

Virology Summary (2010-2011)

	# Positive	% positive
Total cases		
45	24	58%
Total lots		
82	34	44%

Virus Isolations

Virus	# positive lots	% Isolated
GSV	20	51%
FHMNV	4	10%
Novel viruses	13	39%

Species

Virus	Species
GSV	FHM, GOS, WHS, EMS, CKC
FHMNV	FHM, CKC
Novel viruses	FHM, GOS, WHS, CMS, CSR, CMM, LDC, NRD

Virology results from imported baitfish

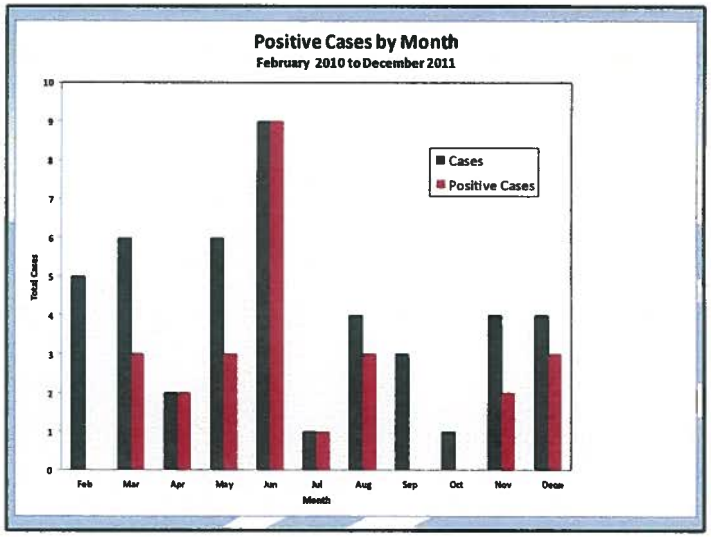
	# Positive	% positive
Total lots 66	31	47%

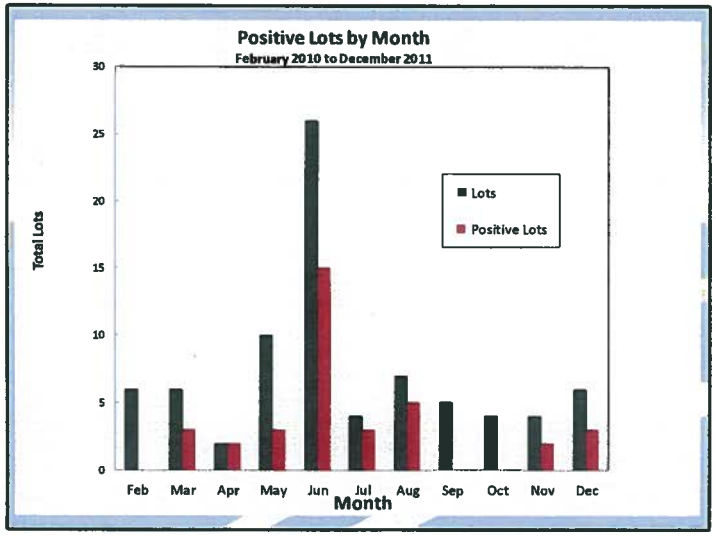
Virology results from wild harvest baitfish

	# Positive	% positive
Total lots 16	5	31%

Species Breakdown

Species Tested	# tested	% of total	# Positive Lots	% positive
FHM	2210	51%	23	62%
GOS	551	13%	5	42%
WHS	1204	28%	5	25%
other	121	7%	-	-





Viruses Isolated from each host species

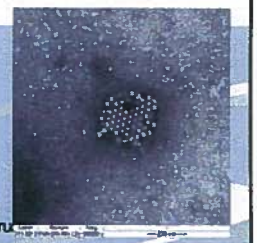
Species	Golden Shiner Virus	Fathead Minnow Nidovirus	Unknown Viruses	Total	Positive Lot %
Fathead Minnows	15	3	0	18	62%
Golden Shiners	5	0	0	5	42%
White Suckers	1	0	4	5	25%
Other species	2	1	2	5	39%
Total	23	4	6	33	

Virus prevalence among baitfish dealers and import states

Dealer & Sources	Number of Positive Lots	Total Lots	% Positive
Dealer #1	**5**	**19**	**26%**
Minnesota	5	19	26%
Dealer #2	**6**	**12**	**50%**
Minnesota	6	12	50%
Dealer #3	**10**	**23**	**44%**
Arkansas	7	12	58%
South Dakota	3	8	37%
Wild-Harvested (WI)	0	3	0
Dealer #4	**15**	**28**	**54%**
Minnesota	7	10	70%
Arkansas	3	5	60%
Wild-Harvested (WI)	5	13	38%

Unknown Viruses

- 8 Fathead Minnow Picornavirus (FHMPV)
  - via electron microscopy, sequencing, and PCR
  - Two distinct FHMPV identified
- 2 unknown *Picornaviridae-like*
  - via electron microscopy
  - negative for FHMPV via PCR
- 1 unknown *Bunya/Paramyxo/Corona*
  - via electron microscopy
- 4 unknown *Orthomyxoviridae*-like
  - via electron microscopy
- 7 unknown *Astroviridae*
  - via sequencing, and PCR
  - most were co-infections with the Picornaviru
- 7 unknown Astroviridae



Anibal Armien

Future work with novel viruses

- More EM
- High definition EM
- Histopathology
- Subtractive molecular method

Conclusion

- Isolations of certifiable bacteria
- Several parasites of concern
- Viruses in 58% of the cases
- Viruses in 44% of the lots
- At least 7 different viruses isolated
- 12.2% of all WI imports (n=491) were tested from the four companies

A NATIONAL APPROACH TO SURVEILLANCE FOR INFECTIOUS SALMON ANEMIA VIRUS IN THE PACIFIC NORTHWEST

USDA APHIS: Janet Whaley, Lynn Creekmore, Lori Gustafson, Teresa Robinson, Janet Warg, and Jill B. Rolland
NOAA NMFS: Kevin Amos
USFWS: Joel Bader

June 13, 2012

Unconfirmed ISA report in Pacific Northwest

- Reported by Canadian OIE reference lab and researchers at Simon Fraser Univ. in October 2011
- Part of ongoing investigation of fluctuating returns of sockeye salmon in Fraser River watershed in BC, Canada
- Canadian officials (Canadian Food Inspection Agency – lead aquatic animal health issues) investigated claims and found no ISAV. CFIA immediately notified and coordinated with U.S.
- U.S. Congress requested further information from *Task Force* – National Aquatic Animal Health
  - U.S. capacity to detect and respond to ISAV in Pacific Northwest and the potential impact on wild salmon

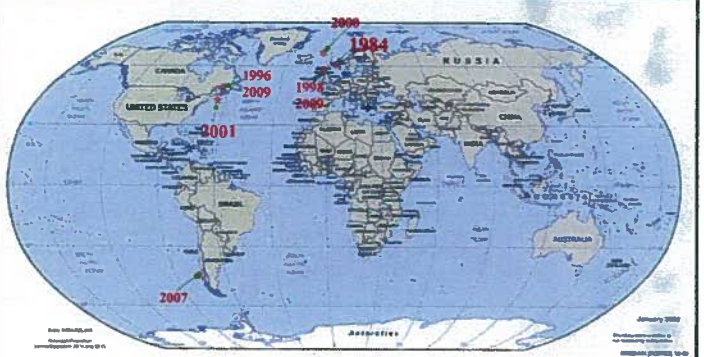
National Aquatic Animal Health *Task Force*

- Established in 2001 under the Joint Subcommittee on Aquaculture to develop the **National Aquatic Animal Health Plan**
- Three federal agencies with lead oversight for U.S. aquatic animal health
  - USDA Animal and Plant Health Inspection Service (Lead)
  - NOAA National Marine Fisheries Service
  - USFWS
- Provide a "Report" to Congress

Infectious Salmon Anemia

- First reported in 1984 in Norway.
- OIE recognized the disease in 1990 & named it ISA.
- ISA virus was characterized by Falk *et al.*, in 1997 (now classified in virus family *Orthomyxoviridae*, genus *Isavirus*).
- First reported outbreak outside Norway was in Canada (New Brunswick) in 1996.
- First reported outbreak in USA was in Maine in 2001.
- First reported outbreak in the Southern Hemisphere was in Chile in 2007.
- This year (as of April 2012), only Norway and Canada (Nova Scotia) have reported ISA outbreaks.
- Primarily a disease of "farmed" Atlantic salmon
- No ISAV confirmed cases in "wild" Pacific salmon

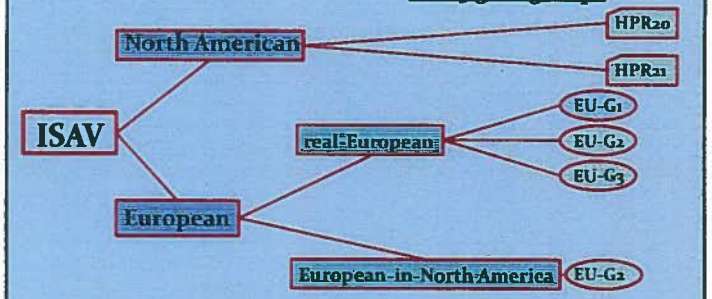
OIE Reported First-time Outbreaks of ISA



ISAV Strains

2 basic genotypes/ serotypes

2-to-3 genogroups



Kibenge *et al.*, 2001 Kibenge *et al.*, 2007 Nylund *et al.*, 2007

ISAV Strain identification

- ISAV strain designation is mostly based on sequence deletions/insertions in a 35-amino acid highly polymorphic region (HPR) of the HE protein (Nylund *et al.*, 2003, 2007; Plarre *et al.*, 2005)
- ISAV without any deletion/insertion in HPR is designated HPR0 to indicate "full-length HPR"
- All ISAV isolated to date from clinical disease have deletions in HPR relative to HPR0
  - are categorized numerically from HPR1 to >30 presently.

Susceptible host species

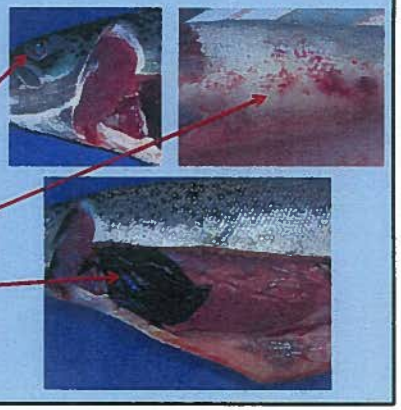
- **Natural clinical disease**
  - Farmed Atlantic salmon (*Salmo salar*)
- **Experimental clinical disease**
  - Rainbow trout (*Oncorhynchus mykiss*)
- **Natural asymptomatic infection** (detected by RT-PCR)
  - Rainbow trout (Ireland)
  - Coho salmon (*O. kisutch*) (Chile)
  - Feral Atlantic salmon
  - Feral brown trout (*S. trutta morpha fario & S. trutta morpha lacustris*)
  - Feral sea trout (*S. trutta morpha trutta*)
  - Feral Pollock (*Pollachius virens*)
  - Feral Atlantic cod (*Gadus morhua*)
- **Experimental asymptomatic infection** (detected by RT-PCR)
  - Arctic char (*Salvelinus alpinus*)
  - Herring (*Clupea harengus*)

Transmission & Risk Factors

- **Transmission**
  - Disease is spread horizontally by water-borne transmission
  - Main infection route is most likely through gills and/or intestinal tract
  - Virus shedding by infected fish may be through natural excretions/secretions.
  - Sea lice (*Lepeophtheirus salmonis*) may serve as mechanical vectors.
  - Reservoir for ISAV:
    - Recovered farmed Atlantic salmon can become carriers
- **Risk factors**
  - Linked to husbandry practices in aquaculture & horizontal transmission.
  - Geographical or hydrological proximity (<5-10 kms) to farms with ISA outbreaks or slaughterhouse processing plants
  - Sharing of staff & equipment.

ISA - Typical clinical manifestation

- **ISA clinical signs**
  - Anorexia
  - Lethargy
  - Anemia
  - Mortality (slow increase; variable)
- **Gross lesions**
  - Pale gills
  - Ascites
  - Exophthalmia
  - Petechial hemorrhages on abdomen, visceral adipose tissue & eyes
  - Congestion and enlargement of liver & spleen



Godoy *et al.*, 2008

Pacific NW ISAV Project Strategy "A National Approach"

- **Enhanced Surveillance**
  - The proposed strategy builds on existing State. Tribal. Federal and industry health infrastructures and activities
  - Current focus is Alaska and Washington due to proximity to BC. Canada
  - Geographically-distributed biannual sampling (Fall and Spring)of Pacific salmonids native to the Pacific Northwest for 2 years
- **Coordinated Screening and Testing**
  - Screening and testing tools (molecular and culture) are being evaluated and calibrated
- **Parallel Research Efforts**
  - genetics of "ISA-like" virus
  - Develop proper diagnostic tools
  - Risk assessment

Project Partners

Investigators

- Alaska State Dept. of Fish and Game
- Northwest Indian Fisheries Commission
- Washington State Department of Fish and Wildlife
- USDA APHIS Veterinary Services
- DOC NOAA National Marine Fisheries Service
- DOI USFWS and U.S. Geological Survey

Laboratories

- **Diagnostic/Screening Testing:**
- Idaho Fish Health Center USFWS, the Fish Pathology Laboratory
- Alaska Dept. of Fish and Game
- Washington Animal Disease Diagnostic Laboratory, Washington State University
- **Confirmation testing:**
- USDA APHIS National Veterinary Services Laboratories

Enhanced Surveillance

- Natural and enhancement population sampling includes all six species of native salmonids, although steelhead stocks (susceptible to known ISAV strains) are to be sampled most extensively.
- Emphasis is on salmonid life stages with marine exposure and greatest susceptibility to viral infection (e.g., adult spawners and net-penned juveniles)
- **Enhanced sampling of commercial Atlantic salmon if possible**

Enhanced Surveillance

- **Collection:** SOPs for collection methodology will be developed in advance of the surveillance period and will generally follow those described in Maine's ISA Program Standards (USDA APHIS VS et al., 2010) with some modifications.

Screening and Confirmation

- This Surveillance Plan is focused on detection of ISAV pathogens of regulatory concern (including HPR0).
- Surveillance samples will be tested in accordance with established and approved SOPs
  - Tribal, State and Federal laboratories approved by APHIS-VS for this surveillance effort may contribute to virus isolation testing.
- Confirmation testing preformed by USDA National Veterinary Services Laboratory

Research

- Research the "ISA-like" virus –genetics
- Parallel research may identify novel viruses or alternative diagnostic tests of potential relevance to the region.
  - To support research efforts, a subset of samples will be split upon collection for concurrent use in surveillance and research. Research samples (identified in the Research Plan) will be archived in RNALater.
- Depending on results of first 2 stages, asses the risk of a west coast strain in Pacific salmon

Reporting and Response

- Similar scheme as Maine Program
  - During field season (Fall and Spring), biweekly/monthly reporting of activities
- **Reporting:** Suspect and positive immediately reported to State and APHIS
  - Competent Authority for U.S.
  - Reports to OIE
- **Response:** At the request of the State, APHIS can assist with instituting a management strategy to eliminate ISAV from aquatic animal facilities
  - Enhanced biosecurity measures and audits, continue surveillance, data sharing and reporting, veterinary support

Communication and Outreach

- Agency partners are working together on communication plan
- Communications protocol (likely actions should we confirm a positive)
  - Message consistency among the task force agencies will be critical – APHIS lead public affairs coordination
  - APHIS public affairs can "field" calls when participating labs receive media calls
  - Development of specific information resources (fact sheets and updates) in advance of a positive finding is difficult since there are many possible scenarios.

Budget

- USDA APHIS provided start up funding support
- USFWS existing support
- NOAA existing support
- Funding request by USDA APHIS - President's 2013 Budget

Adaptive Management Approach

- Future surveillance and research results may indicate a need to adjust the surveillance plan based on new and emerging information. As new information becomes available, the *Task Force* will engage State and tribal partners to jointly address any needed modifications to this surveillance plan.
  - Funding dependent
  - Possibly expand geographic area to include Oregon, Idaho and California

Infectious Salmon Anemia Surveillance & Control

Great Lakes Fish Health Committee Meeting
30 July 2012
La Crosse, WI

Bill Keleher

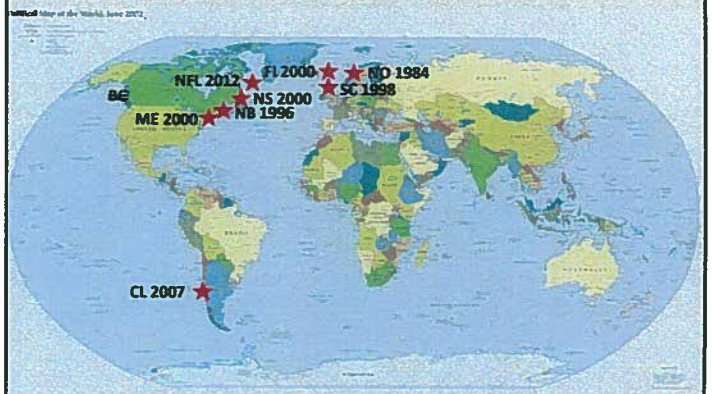
KENNEBEC RIVER BIOSCIENCES

Infectious Salmon Anemia

Infectious Salmon Anemia Virus

- *Orthomyxoviridae*
- 45-140 nm
- RNA virus
- 8 segments (1- 2.2 kb)

Distribution



USDA ISA Program Standards (2001)

- *Surveillance*
- *Control Measures*

Surveillance

- *V-C-P w/ USDA Accredited Veterinarian*
  - *Clinical signs*
  - *Mortality record*
- *10 moribund fish per site (negative) per month*
- *Sent to Approved Laboratory (VS mem. 567.2)*
- *Primary screening by PCR w/ IFAT secondary*
- *Cell culture on follow-up testing*

ISA Program Category	Category Description
Category 1 (presumed negative)	ISAV has not been detected at a cage or site participating in active ISAV monthly surveillance testing, considered negative for ISA.
Category 2 (suspect)	ISAV has been detected by at least one diagnostic test in at least one fish; considered suspect needing further evaluation and testing within 7 days.
Category 3 (infected)	A pathogenic genotype of ISAV has been detected by at least two diagnostic tests in at least two fish from the same cage. For subsequent cages on an infected site, a cage may be deemed Category 3 based on two fish found positive by one test (PCR, IFAT, gross pathology) accompanied by a positive virus isolation from individual samples or single cage pools.
Category 4 (diseased)	As for Category 3 above, plus clinical disease is present (as diagnosed by a veterinarian).
Category 5 (diseased)	As for Category 4 above, plus mortality consistent with ISA is present at the average rate of 0.05% per cage population per day over one week.
Category 6	Cage or site previously classified as Category 2 through 5 has been harvested or fallowed.

Testing Protocols *PCR*

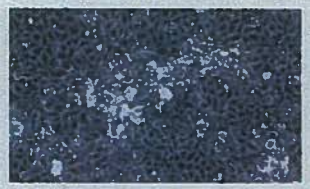
- *Kidney (RNA Later)*
- *Primer 1D/2 (Segment 8)*
- *Additional primers HPR0 differentiation*
- *NVSL Real-time rt-PCR*

Testing Protocols
IFAT

- *Kidney imprints*
- *Cocktail of 2 monoclonal antibodies (Norway)*
- *Low Sensitivity*
- *Subjective rating lower levels*

Testing Protocols
Cell Culture

- *CHSE & ASK (alternative SHK)*
- *Titers: CHSE 5.3 & ASK 6.3*
- *28 day incubation period*



Additional Testing

- *Virology (CHSE & ASK) all FW production lots and SW broodstock sites (2X per year)*
- *Broodstock (Freshwater)*
  - *10 moribund (September)*
  - *First 30 spawned of each lot (PCR/IFAT/Culture)*
  - *Generally 60 fish tested (FHPR/BB)*
- *Broodstock (Saltwater)*
  - *First 30 spawned of each lot (PCR/IFAT/Culture)*
  - *100% lethal sampling (Culture w/ archive PCR & IFAT all fish)*

Control

- *Risk Identification & Mitigation*
- *Biosecurity*
- *Vaccination*
- *Best Management Practices (Industry)*

Initial Response

- *Depopulation infected sites*
- *Cleaning & Disinfection*
- *Biosecurity Measures*
- *Indemnification*

Biosecurity

- *Mort Disposal*
- *Processing Plant Effluent*
- *Movement of people & boats, divers, etc.*
- Disinfection protocols

Best Management Practices

- *Single year class stocking*
- *Broodstock screening & Egg quarantine*
- *Site fallowing & rotation*
- *Stocking densities*

Sea Lice Control

- *Lepeophtheirus implicated in transmission*
- *Use of SLICE at hatcheries & marine sites*

Vaccination

- *Whole killed virus preparation (US & Canada)*
- *Used since early 2000 (ME and Canada)*
- *Laboratory trials 70-90% RPS*
- *Moderate usage w/ industry (Cost/Benefit)*
- *Subunit/DNA/Live Attenuated vaccines*

ISA Program

ISA Program Stats by Calendar Year

	2002	2003	2004	2005	2006	2007	2008	2009	2010	Totals
Samples (# of fish)	1,983	3,187	3,933	1,453	807	900	1,104	985	949	15,291
Site inspections	189	369	387	178	95	95	119	110	106	1,848
Site audits	22	21	13	11	12	16	9	13	9	126
Vessel audits	8	11	0	2	0	0	0	1	9	31
Cages confirmed positive	0	5	17	19	1	0	0	0	0	42
Confirmed cages removed	0	5	17	19	1	0	0	0	0	42
Newly confirmed sites	1	2	6	0	1	0	0	0	0	11
Previously confirmed sites	0	0	1	5	0	0	0	0	0	NA
Sites in water (throughout the year)	20	23	21	12	13	12	15	15	12	NA

Where We are Today

- *Last detection in ME - 2006 (pathogenic)*
- *Detections in NS & NFL 2012*
- *BMP & Biosecurity standard practice*
- *Surveillance continues*
- *BMP/Biosecurity measures have been effective*
- *Role of HPR0 (non-pathogenic) strains*

Abigail Fusaro
<abigail.fusaro@noaa.gov>
07/20/2012 08:04 AM

To Christina Haska <chaska@glfc.org>
cc Kenneth_Phillips@fws.gov, John Dettmers <jdettmers@glfc.org>
bcc
Subject GLANSIS fish pathogen impact assessments and management options

Good morning Christina,

When I last spoke with Ken Phillips, he mentioned that he might be able to add a request for GLANSIS fish parasite/disease fact sheet reviews to the July 30th GLFC Fish Health Committee meeting. I will be out of town next week (back 7/30), but by the end of today, we should have about 10 updates ready for external review (species list below). We would be grateful for any feedback the FHC could provide on our assessments of environmental, socioeconomic, and beneficial effects (realized or potential) of these species, as well as our summaries of available management/control options. These components will serve as enhancements to the searchable online GLANSIS fact sheets at http://www.glerl.noaa.gov/res/Programs/glansis/glansis.html.

Would you recommend that the documents for review be distributed prior to or following the meeting? As I mentioned, we will have one batch ready for distribution today. A second batch will be ready by August 3. I will pass along background information about these products, as well.

Thank you for any assistance you might be able to provide.

Best,
Abigail Fusaro

Ready for Review:
Aeromonas salmonicida
Bothriocephalus acheilognathi
Dactylogyrus amphibothrium
Dactylogyrus hemiamphibothrium
Dugesia polychroa
Myxobolus cerebralis
Neascus brevicaudatus
Novirhabdovirus sp.
Scolex pleuronectis
Timoniella sp.

Ready by Aug 3:
Acineta nitocrae
Glugea hertwigi
Heterosporis sp.

Ichthyocotylurus pileatus
Piscirickettsia cf. *salmonis*
Psammonobiotus communis
Psammonobiotus dziwnowi
Psammonobiotus linearis
Ranavirus sp.
Renibacterium salmoninarum
Rhabdovirus carpio
Sphaeromyxa sevastopoli
Trypanosoma acerinae

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