

State of Maine Department of Marine Resources Cooperative Agreement # CT-13A-20090126*4283 August 2010 Executive Summary

Objective: The primary objective of the cooperative agreement was to perform fish health screening in order to characterize pathogen presence endemic to sea-run rainbow smelt (*Osemerus mordax*) and Atlantic sturgeon (*Acipenser oxyrinchus*) in the Gulf of Maine (GOM). Improving understanding of fish health status, abundance, geographic distribution, and vectors of pathogens of sea-run rainbow smelt and Atlantic sturgeon is one of several areas of study necessary to develop and implement conservation strategies designed to protect and restore the two fish species listed in this project.

Deliverables: The University of Maine Animal Health Laboratory (UMAHL) was responsible for executing the following activities and tasks:

- a) Participate in regular planning projects
- b) Assist in the development and implementation of fish health monitoring protocols
- c) Perform fish health services on coastal fish species for infectious and noninfectious diseases and parasites
- d) Train principal investigators in field sample collection techniques as required
- e) By June 30, 2010, submit a final report that includes relevant information, including an itemized list of infectious and non-infectious diseases and parasites to host species and frequency of occurrence Report completed in August 2010

Fish Health Screening Design and Application:

The fish health screening for each species was designed very differently based on species population availability. The opportunity to work with Atlantic sturgeon was based on opportunity. Because of the UMAHL's willingness to participate, samples of collected sturgeon mortalities were submitted to the lab for necropsy through a federal program. These samples were submitted by Gayle Wippelhauser, DMR. The UMAHL was required to obtain a permit through the Department of Commerce, NOAA in order to be able to work with submitted sturgeon and distribute samples to federal and state agencies. The UMAHL is now recognized as a cooperating laboratory for endangered sturgeon (Permit no. 1614-01). Included with this report is the diagnostic case work-up performed.

The remainder of this report will serve as the results of the second year of health screening on rainbow smelt. Sampling took place March 2010 through May 2010.

<u>Overview of rainbow smelt screening</u>: As with most disease surveillance strategies, focus was placed on screening the specified species population for known documented pathogens of the particular species, pathogens of regulatory concern (state and national) affecting many species possibly carried by the specified species, as well as, general observable gross pathology. A literature search was performed in order to determine pathogens that have been documented in rainbow smelt. With this information and considering pathogens of regulatory concern for the state of Maine, screening protocols and diagnostic assays were performed according to the OIE and Blue Book ^(1,2) for the following bacterial, viral, and parasitic pathogens:

Bacterial:

Bacterial selective screening included all general aerobic bacteria, such as Aeromonas (ie: Aeromonas salmonicida), Vibrio (fresh and saltwater), Pseudomonas, Edwardsiella, Flavobacterium, and many others.

Viral:

Cell lines selected and temperature ranges of incubation allowed for screening for a wide variety of viral agents. The following list includes specific viruses that could be detected or ruled out as not detected by the screening procedures employed:

IPNV, infectious pancreatic necrosis virus IHNV, infectious hematopoietic necrosis virus ISAV, infectious salmon anemia virus VHSV, viral hemorrhagic septicemia virus VEN, viral erythrocytic necrosis virus (blood smear was performed for VEN) Aquareovirus Herpesviruses Other nonspecific CPE producing agents

Parasites:

General necropsy allowed for gross observation and classification of various parasites such as nematodes, trematodes, cestodes and protozoa. 10 % of rainbow smelt submitted had skin and gill examined by microscopic exam for parasites.

Sampling:

In 2010, samples of rainbow smelt were collected from 14 population survey sites throughout the GOM. These geographical sites were spawning areas where rainbow smelt congregate. These sites were considered to represent subset populations of the target species. It is assumed that this subset population increases the ability to detect pathogen presence due to congregation of fish into larger groups, and the physiological stress experienced during spawning.

The project goal was to collect 60 rainbow smelt for fish health screening at each of the 14 sites. Each site was considered a distinct point of sampling. The testing level at each site was based on an unknown overall population number but assumed a large population

(>1,000) at each site. Sampling was then performed at an assumed 5% pathogen prevalence level with 95% probability of detecting a pathogen.

The 14 sites and numbers of rainbow smelt tested are presented in Table 1.

Acces-		#			Water Temp			
sion	Site	Fish	Site Name	Date Sub.	°C	pН	Dis. Ox.	Conductivity
11377	1	60	Schoppe Brook ME	4/30/2010	6.2	n/a	9.4mg/L 76%sat	n/a
11405	2	56	Pleasant Brook ME	5/12/2010	n/a	n/a	n/a	n/a
11311	3	60	Deer Meadow ME	4/8- 5/5/2010	9.83	6.7	10.98mg/L 96.6%sat	29 mS/cm
11353	4	37	Mast Landing ME	4/22/2010	8.25	7.13	11.3mg/L 97%sat	133 mS/cm
11417	6	6 histo	Winnicut River ME	5/18/2010				
11418	7	2 histo	Squamscot t River	5/18/2010	11.38	7.55	10.84mg/L 99.2%sat	0.095 mS/cm
11320	8	6 histo	Parker River	53/27/09	4.95	7.22	14.2mg/L 107.2%sat	0.261 mS/cm
11321	8	6 histo	Parker River	3/24/2010	7.96	6.5	12.5 mg/L 105.7%sat	0.146 mS/cm
11371	9	35	Crane River	4/29 & 30/2010	9.09	7.12	11.89mg/L 104.9%sat	0.967 mS/cm
11319	12	60	Fore River	4/9/2010	15.2	6.8	10.1mg/L 100%sat	0.345 mS/cm
11332	13	60	Jones River	4/14/2010	10.7	6.2	9.4mg/L	0.170 mS/cm
11334	15	17	Oyster River NH	4/15/2010		7.49	12.04mg/L 103.4%sat	0.175 mS/cm
11350	16	41	Long Creek ME	4/20&22/ 5/6/2010	7.13/ 12.36	7.13/7.36	11.57 mg/L 95.9% sat/ 10.57mg/L 99.4%sat	380/247 mS/cm
11416	17	60	Brook ME	5/18/2010				
11419	18	60	i annery Brook	5/19/2010				

Table 1: Rainbow Smelt Index Sites Tested

Results of pathogen screening by site were as follows:

Site 1: Schoope Brook ME (Acc# 11377)

Gross observations:

Skin and gill microscopic exam revealed low to moderate Black Grub cysts.

Bacteriology: No bacterial growth was observed from 54 fish.

<u>Virology</u>: Virology was performed in five fish pools of selected tissues and plated on two cell lines with a broad range of viral susceptibility. Cell lines inoculated with samples were incubated at 15C and monitored for 21 days for any visible cytopathic effect (CPE). No viral agents were detected in fish from Site 1.

Individual fish blood smears were prepared and stained by Giemsa for VEN screening. Observations consistent with VEN were not detected in fish from Site 1.

<u>Histology</u>: Histology was performed on 10% of the collected fish (6 of 60). Moderate post mortem changes were seen at this site. A few larval cestodes were seen in the gut. The myocardial tissues of some fish appeared to have hemorrhaged, and focal myocardial intrafascial monocytic infiltrates were noted. The gills showed low numbers of *Icthyobodo spp*. protozoa, epithelial hyperplasia, and a few emboli. Renal interstitial nematode larva were noted in one fish. The liver showed moderate glycogen, congestion, and nuclear heterogeneity/basophilia. In summary, fish from this site were mildly parasitized, and some evidence for erythrocytic disease was present.

Site 2: Pleasant Brook ME (Acc# 11405)

<u>Gross observations</u>: Skin and gill microscopic exam revealed low Black Grub cysts on most fins, Trichodina was observed in low numbers, and nematodes were observed in a few fish.

<u>Bacteriology</u>: 1 of 50 fish sampled had rare bacterial growth observed. The organism was identified as a *Pseudomonas species*. This organism is ubiquitous to marine and freshwater environments.

<u>Virology</u>: Virology was performed in five fish pools of selected tissues and plated on two cell lines with a broad range of viral susceptibility. Cell lines inoculated with samples were incubated at 15C and monitored for 21 days for any visible cytopathic effect (CPE). No viral agents were detected in fish from Site 2.

Individual fish blood smears were prepared and stained by Giemsa for VEN screening. Observations consistent with VEN were not detected in fish from Site 2.

<u>Histology:</u> Histology was performed on >10% of the collected fish (6 of 50). Moderate post mortem changes were seen at this site. Two female fish with numerous *Philometra spp.*-like nematodes and rare microsporidia in the ovarian tissue, and spawning males were seen. Coccidia and acanthocephalan parasites were seen in the gut. The myocardial tissues of one fish contained a focal granuloma. Multifocal renal tubular luminal mineral deposits were present. The liver did not appear to contain glycogen, and extensive nuclear heterogeneity and basophilia were noted. The spleen contained some microsporidia, and hemosiderin deposition. In summary, fish from this site were relatively heavily parasitized, showed renal mineral deposition, and some evidence for erythrocytic disease was present.

Site 3: Deer Meadow Brook ME (Acc# 11311)

<u>Gross observations:</u> Skin and gill microscopic exam revealed moderate Black Grub cysts on most fins and Trichodina was observed in rare numbers on 1 of 6 fish gill samples screened.

<u>Bacteriology</u>: 4 of 54 fish sampled had rare bacterial growth observed. The organisms were identified as *Pseudomonas fluorescens biotype G, Chryseobacterium scophthalmum, Micrococcus luteus* and a probable Actinomyces species. These organisms are ubiquitous to marine and freshwater environments.

<u>Virology</u>: Virology was performed in five fish pools of selected tissues and plated on two cell lines with a broad range of viral susceptibility. Cell lines inoculated with samples were incubated at 15C and monitored for 21 days for any visible cytopathic effect (CPE). No viral agents were detected in fish from Site 3.

Individual fish blood smears were prepared and stained by Giemsa for VEN screening. Observations consistent with VEN were not detected in fish from Site 3.

<u>Histology</u>: Histology was performed on 10% of the collected fish (6 of 60). Tissues were in relatively good condition. The cortical tissues of the brain showed multifocal lymphocytic aggregates, nematode-like larvae in cross-section, and nematode cuticle and melanomacrophage aggregates within ventricles. In the periocular tissues, periocular larval nematodes were seen. Two females were seen from this site; both had large numbers of *Philometra spp.*-like nematodes in the gonadal tissues. The rest of the fish were spawning males. There were low numbers of larval cestodes present in the gut. The renal interstitium contained high numbers of melanomacrophage aggregates, and some microsporidia. The liver contained moderate amounts of glycogen; 1 fish showed focal hepatic necrosis, many melanomacrophage aggregates, and congestion. The spleen was congested, with focal hemorrhage, large hemosiderin deposition, and many melanomacrophages. In summary, a heavy parasite burden (*Philometra spp.*-like nematodes primarily), and some evidence for erythrocytic disease was present.

Site 4: Mast Landing, ME (Acc# 11353)

<u>Gross observations:</u> Skin and gill microscopic exam revealed moderate Black Grub cysts on most fins.

<u>Bacteriology</u>: 3 of 31 fish sampled had rare bacterial growth that was identified as *Burkholderia glumae, Aeromonas jandaei DNA group 9*, and *Vibrio aestuarianus*. These organisms are ubiquitous to marine and freshwater environments.

<u>Virology</u>: Virology was performed in five fish pools of selected tissues and plated on two cell lines with a broad range of viral susceptibility. Cell lines inoculated with samples were incubated at 15C and monitored for 21 days for any visible cytopathic effect (CPE). No viral agents were detected in fish from Site 4.

<u>Histology</u>: Histology was performed on >10% of the collected fish (6 of 37). Tissues were in good condition. Focal cortical hemorrhage of the brain, and periocular hemorrhage was seen. The gills showed mild postmortem change, some clubbing and emboli. Two of the fish were females and large numbers of *Philometra spp.*-like nematodes were seen in the gonadal tissues. The rest of the fish at this site were spawning males. A few coccidia were seen in the gut mucosa. Moderate glycogen was seen in the liver, and the spleen appeared red blood cell depleted, with moderate hemosiderin deposition. In summary, fish from this site were moderately parasitized (primarily nematodes) and some evidence for erythrocytic disease was present.

Site 6: Winnicutt River, NH (Acc# 11417)

<u>Histology:</u> Samples were submitted for histology only.

Tissues were in good condition. Cerebral focal cortical hemorrhage and congestion were noted. Mild epithelial hyperplasia and clubbing of the gills was present. All sampled fish were post-spawn males. Moderate numbers of intraluminal larval cestodes and nematodes were seen in the gut. Multifocal renal tubular luminal mineral deposits were present. The liver contained moderate glycogen. The spleen was congested, with mild focal necrosis. In summary, fish from this site were moderately parasitized, gill changes suggested water quality problems, and some evidence for erythrocytic disease was present.

Site 7: Squamscott River, NH (Acc# 11418)

Histology: Samples were submitted for histology only.

Tissues were in good condition. Cerebral focal cortical hemorrhage was noted. Mild epithelial hyperplasia and clubbing of the gills was present. All sampled fish were postspawn males. A few coccidia, but no other parasites were noted in the gut. The liver contained moderate glycogen and the spleen was congested. In summary, fish from this site were very lightly parasitized, gill changes suggested water quality problems, and some evidence for erythrocytic disease was present.

Site 8: Parker River, MA (Acc# 11320 and 11321)

<u>Histology:</u> Samples were submitted for histology only. Biologist submitted 6 formalin fixed fish from 2010 and 6 formalin fixed fish from 2009 (the previous year). 11320 [2009]: Moderate postmortem change was present in these tissues. The gills contained a few emboli. Many larval cestodes and postmortem change (sloughing of mucosa) was seen in the gut. There was mild focal interfascicular hemorrhage of the skeletal muscle. Multifocal hepatic necrosis was seen, consistent with postmortem change. Microsporidia were detected in the pancreas. The spleen was congested. In summary, moderate parasitism (primarily enteric cestodes) was seen in fish from this site.

11321 [2010]: Tissues were in good condition. There were encysted nematode larvae in the subchondral / episcleral tissues of the eye. Moderate chronic bronchitis, with epithelial bridging, emboli, and melanomacrophage aggregates were present in the gills. One fish showed focal myocardial intrafascial monocytic infiltrates. Hepatic glycogen was moderate; one fish showed multifocal hepatic necrosis and perivascular

melanization. The spleen was congested. In summary, mild parasitism (primarily nematode) was seen in fish from this site.

Site 9: Crane River, MA (Acc# 11371)

<u>Gross observations:</u> Skin and gill microscopic exam revealed low Black Grub cysts on 2 fish observed.

Bacteriology: No bacterial growth was observed from 29 fish.

<u>Virology</u>: Virology was performed in five fish pools of selected tissues and plated on two cell lines with a broad range of viral susceptibility. Cell lines inoculated with samples were incubated at 15C and monitored for 21 days for any visible cytopathic effect (CPE). No viral agents were detected in fish from Site 9.

Individual fish blood smears were prepared and stained by Giemsa for VEN screening. Observations consistent with VEN were not detected in fish from Site 9.

<u>Histology</u>: Histology was performed on >10% of the collected fish (6 of 35). Post mortem changes in the tissues from this site were severe. Focal cortical hemorrhage was seen in the brain. Three females were seen at this site, and all 3 females gonadal tissue was normal. The males were spawning. A few larval cestodes were seen in the gut. Multifocal renal tubular luminal mineral deposits were present. In summary, fish from this site were lightly parasitized, and showed renal mineral deposition.

Site 12: Fore River MA (Acc# 11319)

Gross observations: Skin and gill microscopic exams were not performed on this site.

Bacteriology: 13 of 54 fish had rare/ few mixed bacterial growth observed. The organisms were identified as *Aeromonas eucrenophila DNA group 6*, *Aeromonas sobria DNA group 7*, *Clavibacter michiganensis*, *Chryseobacterium scophthalmum*, *Curtobacterium citreum*, *Pseudomonas fluorescens biotype G* and various *Micrococcus* species. These organisms are ubiquitous to marine and freshwater environments. The level of bacterial isolation was higher in this group then most but likely can be attributed to the fish being held for 12 hours prior to testing.

<u>Virology</u>: Virology was performed in five fish pools of selected tissues and plated on two cell lines with a broad range of viral susceptibility. Cell lines inoculated with samples were incubated at 15C and monitored for 21 days for any visible cytopathic effect (CPE). No viral agents were detected in fish from Site 12.

Individual fish blood smears were prepared and stained by Giemsa for VEN screening. Observations consistent with VEN were not detected in fish from Site 12.

<u>Histology:</u> Histology was performed on 10% of the collected fish (6 of 60). Tissues were in good condition. The brain showed focal cortical hemorrhage, and larval nematodes were seen in the subretinal and periocular tissues. A few emboli and some microsporidia were seen in the gills. One female was seen; the ovaries were normal. Of the males, all were spawning; 1 showed focal hemorrhage of the testicular tissue. Moderate numbers of luminal nematodes and larval cestodes were seen in the gut. Two fish showed focal myocardial intrafascial monocytic infiltrates. Some postmortem change and congestion was seen in the liver. The spleen was red blood cell depleted, with some microsporidia and many melanomacrophage aggregates. In summary, fish from this site were lightly parasitized; although microsporidia were detected in tissues, no xenomas were seen. Some evidence for erythrocytic disease was present.

Site 13: Jones River, MA (Acc# 11332)

Gross observations: Skin and gill microscopic exams were not performed on this site.

<u>Bacteriology</u>: 6 of 54 fish sampled had rare bacterial growth observed. The organisms were identified as *Aeromonas hydrophila-like DNA group 2, Pseudomonas alcaligenes, Micrococcus species* and 2 non-enteric species that did not ID by Biolog. These organisms are ubiquitous to marine and freshwater environments.

<u>Virology</u>: Virology was performed in five fish pools of selected tissues and plated on two cell lines with a broad range of viral susceptibility. Cell lines inoculated with samples were incubated at 15C and monitored for 21 days for any visible cytopathic effect (CPE). No viral agents were detected in fish from Site 13.

Individual fish blood smears were prepared and stained by Giemsa for VEN screening. Observations consistent with VEN were not detected in fish from Site 13.

<u>Histology:</u> Histology was performed on 10% of the collected fish (6 of 60). Tissues showed severe postmortem change. Larval cestodes and coccidia were seen in the gut tissue. All fish were mature males. The spleen was congested. In summary, although tissues were severely affected by postmortem degeneration and interpretation is therefore limited, parasitism appeared to be relatively light and evidence for disease was not seen.

Site 15: Oyster River, NH (Acc# 11334)

Gross observations: Skin and gill microscopic exams were not performed on this site.

Bacteriology: No bacterial growth was observed from 11 fish.

<u>Virology</u>: Virology was performed in five fish pools of selected tissues and plated on two cell lines with a broad range of viral susceptibility. Cell lines inoculated with samples were incubated at 15C and monitored for 21 days for any visible cytopathic effect (CPE). No viral agents were detected in fish from Site 15.

Individual fish blood smears were prepared and stained by Giemsa for VEN screening. Observations consistent with VEN were not detected in fish from Site 15.

<u>Histology</u>: Histology was performed on >10% of the collected fish (6 of 17). Tissues showed mild to moderate postmortem change. An intraocular nematode was detected. The gills showed many emboli, and moderate postmortem change. Both coccidia and larval cestodes were seen in the gut. Multifocal renal tubular luminal mineral deposits were present. The liver contained moderate glycogen and multifocal melanomacrophage aggregates. The spleen was congested. In summary, fish from this site were mildly parasitized, and showed renal mineral deposition.

Site 16: Long Creek, ME (Acc# 11350)

Gross observations: Skin and gill microscopic exams were not performed on this site.

<u>Bacteriology</u>: 5 of 35 fish sampled had rare bacterial growth that was identified as *Burkholderia glumae. Pseudomonas* species, *Vibrio aestuarianus*, and *Vibrio* species, . These organisms are ubiquitous to marine and freshwater environments.

<u>Virology</u>: Virology was performed in five fish pools of selected tissues and plated on two cell lines with a broad range of viral susceptibility. Cell lines inoculated with samples were incubated at 15C and monitored for 21 days for any visible cytopathic effect (CPE). A CPE producing agent was detected in 3 of 8 five-fish pools from Site 16. The CPE producing agent is filterable at 0.22 μ m. Two attempts at TEM microscopy have come back negative for observable virion particles. IPNV, IHNV, ISAV, and VHSV have been ruled out by PCR techniques. This CPE producing agent is frozen and archived in the UMAHL repository.

Individual fish blood smears were prepared and stained by Giemsa for VEN screening. Observations consistent with VEN were not detected in fish from Site 16.

<u>Histology</u>: Histology was performed on >10% of the collected fish (6 of 41) Tissues were in good condition. Congestion was noted in brain tissues. Epithelial fusion and emboli were noted in gills. Two females in the study had *Philometra spp.*-like nematodes in the gonadal tissues. The remainder of the fish from the site were spawning males. Larval cestodes were noted in the gut. Moderate hepatic glycogen was seen. The spleen was red blood cell depleted. In summary, fish from this site were moderately parasitized, and gill changes suggested water quality problems.

Site 17: East Bay Brook ME (Acc# 11416)

<u>Gross observations:</u> Skin and gill microscopic exam revealed rare *Trichodina spp.* and one nematode was observed.

<u>Bacteriology</u>: 4 of 54 fish sampled had rare bacterial growth that were identified as *Arthrobacter species*(3 fish) and *Acidovorax* species. These organisms are ubiquitous to marine and freshwater environments.

<u>Virology</u>: Virology was performed in five fish pools of selected tissues and plated on two cell lines with a broad range of viral susceptibility. Cell lines inoculated with samples were incubated at 15C and monitored for 21 days for any visible cytopathic effect (CPE). No viral agents were detected in fish from Site 17.

Individual fish blood smears were prepared and stained by Giemsa for VEN screening. Observations consistent with VEN were not detected in fish from Site 17.

<u>Histology:</u> Histology was performed on 10% of the collected fish (6 of 60). Moderate post mortem changes were seen at this site. The gills contained microsporidia, and showed clubbing and melanomacrophage aggregates. All were spawning males. The gut contained larval cestodes. Myocardial tissues of one fish contained a focal granuloma. The liver contained moderate glycogen, was mildly congested, and showed moderate nuclear heterogeneity and basophilia. The pancreas was congested, and contained microsporidia. The spleen contained hemosiderin deposition. In summary, fish at this site were moderately parasitized and some evidence for erythrocytic disease was present.

Site 18: Tannery Brook ME (Acc# 11419)

<u>Gross observations</u>: Skin and gill microscopic exam revealed low Black Grub cysts on most fins and Trichodina was observed in moderate numbers on 1 of 6 fish gill samples screened.

<u>Bacteriology</u>: 4 of 54 fish sampled had rare bacterial growth observed. The organisms were identified as *Pseudomonas putida* and *Micrococcus species*. These organisms are ubiquitous to marine and freshwater environments.

<u>Virology</u>: Virology was performed in five fish pools of selected tissues and plated on two cell lines with a broad range of viral susceptibility. Cell lines inoculated with samples were incubated at 15C and monitored for 21 days for any visible cytopathic effect (CPE). No viral agents were detected in fish from Site 18.

Individual fish blood smears were prepared and stained by Giemsa for VEN screening. Observations consistent with VEN were not detected in fish from Site 18.

<u>Histology:</u> Histology was performed on 10% of the collected fish (6 of 60). Moderate post mortem changes were present in these tissues. Microsporidia were seen in the gills. The one female had normal gonadal tissue; the remainder of the fish were spawning males. Intraluminal nematodes were seen in the gut. The liver contained moderate glycogen and was congested. In summary, fish from this site were very lightly parasitized.

Conclusion: The 2010 spring rainbow smelt sampling consisted of screening 14 defined sites (Gulf of Maine Index Sites) for a total of 566 fish. Seven of these sites were in Maine, 3 from New Hampshire and 4 from Massachusetts. The target number of fish for each site was 60, most Maine sites achieved this number but NH and MA testing was far below the target number with many sites submitting for histology only.

General conclusion for the 2010 pathogen screening and are as follows:

- 1) No pathogens of regulatory concern (State of Maine DMR Chapter 24, USDA APHIS OIE) were detected at the 14 sites screened.
- 2) No bacterial pathogens were detected at the 11 sites screened (4 sites submitted histology only). Bacteria isolated were most likely extraneous to the testing and ubiquitous in nature.

- 3) Virology results did reveal a CPE producing agent in Maine waters at one site, Long Creek. This is one of two sites that were positive last year for this CPE producing agent. Attempts at performing TEM electron microscopy have not revealed viral particles (this is not an uncommon result). The CPE is filterable through a 0.22 µm filter suggesting a viral agent. There is a slight probability of this CPE producing agent fitting into the class *Mollicutes* (mycoplasma). It is difficult to place any significance to this agent at this point in time but levels detected are reflective of carrier states in the rainbow smelt population at this site and in particular with the repeated year detection. IPNV, IHNV, ISAV, and VHSV have been ruled out by PCR techniques.
- 4) Parasitological results are typical to wild fish populations with various trematodes, cestodes, nematodes and protozoa observed at all sites. Black Grub is a very common parasitic infection in many finfish species. The microsporidian parasite detected in various tissues of many individuals in this study was not identified as to species, but is consistent with *Glugea hertwigi* that has been confirmed from Site 12, the Fore River in Massachusetts. This parasite has been documented extensively and can be detrimental to spawning populations. The observation of large numbers of *Philometra spp.*-like nematodes in the gonads of the majority of female fish in the study is also consistent with reports of this parasite as an opportunistic pathogen of spawning female fish of other species (3).

In conclusion, the two year sampling did not detect pathogens of regulatory concern but did detect endemic parasites well documented in literature for these species. Testing numbers in Maine offer a good deal more confidence in the overall results based on sample numbers then from NH and MA where numbers are much lower. The two year testing can be used as a baseline for Rainbow smelt in regards to microbial health. The histology slides and tissue blocks are archived and can be used for future analyses if desired.

References:

- 1. USFWS and AFS-FHS (U.S. Fish and Wildlife Service and American Fisheries Society-Fish Health Section) 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.
- 2. OIE, Manual of Diagnostic Tests for Aquatic Animals, 5th Edition, 2006
- 3. Moravec1 F., de Buron I. New data on three gonad-infecting species of Philometra (Nematoda, Philometridae) from estuarine fishes in South Carolina, USA. Acta Parasitologica, 2009, 54(3), 244–252; ISSN 1230-2821