

**Second Maine Atlantic Salmon Technical Advisory Committee
Research Forum
7 January 2004
D.P. Corbett Business Building
University of Maine, Orono**

Program

- 8:00 a.m. *Registration, coffee*
- 8:30 a.m. **Welcome, announcements**
Michael Kinnison
University of Maine, Department of Biological Sciences, Orono, ME
Sharon A. MacLean
NOAA, Northeast Fisheries Science Center, Narragansett, RI

Session 1

Sharon MacLean, Moderator
*NOAA, Northeast Fisheries Science Center
Narragansett, RI*

- 8:40 a.m. **Update on coastal Maine river Atlantic salmon smolt studies: 2003**
James Hawkes, John Kocik and Gregory Mackey
- 9:00 a.m. **Changes in the proportion of naturally reared Atlantic salmon smolts to hatchery smolts emigrating from the Penobscot River, ME, during 2000-2003**
Christine Lipsky, James Loftin, Ed Hastings and Russell Brown
- 9:20 a.m. **Penobscot River adult Atlantic salmon migration study**
Kenneth Beland and Dimitry Gorsky
- 9:40 a.m. **Estimation of gamete viability, timing of sexual maturity, and fecundity of river-specific marine net pen reared Atlantic salmon in Maine**
Gregory Mackey, Michael Loughlin, Nick Brown and Ernie Atkinson
- 10:00 a.m. **Streamside incubation: a low tech, low cost approach to Atlantic salmon restoration**
Paul Christman, Kevin Dunham and Daniel McCaw
- 10:20 a.m. *Break*

Session 2

Michael Kinnison, Moderator
University of Maine
Department of Biological Sciences
Orono, ME

- 10:40 a.m. **Consequences of movement on Atlantic salmon (*Salmo salar*) survival estimates**
Gregg E. Horton and Benjamin H. Letcher
- 11:00 a.m. **Evolution of population structure in Maine's Atlantic salmon**
Adrian P. Spidle and Timothy L. King
- 11:20 a.m. **Scale pattern analysis discriminates Atlantic salmon by river-reach rearing origin**
Ruth Haas-Castro, Timothy Sheehan, Steve Cadrin and Joan Trial
- 11:40 a.m. **Evaluation of adult Atlantic salmon scales to determine the origin of Atlantic salmon recovered in Maine**
Michael Pietrak, Christopher Legault and Kenneth Beland
- 12:00 p.m. *Lunch*

Session 3

Michael Kinnison, Moderator
University of Maine
Department of Biological Sciences
Orono, ME

- 12:50 p.m. **Reduced genetic diversity and effective population size in an endangered Atlantic salmon population: Connecting past to present**
Christopher Lage and Ira Kornfield
- 1:10 p.m. **Loss of molecular and trait variation in six populations of endangered Maine salmon**
Nathan Wilke, Michael Kinnison and Timothy King
- 1:30 p.m. **Genetic parentage analysis of Sheepscot River Atlantic salmon: survival and distribution of stocked individuals**
Meredith Bartron, Jerre Mohler and Timothy King
- 1:50 p.m. **Nonlethal measurement of recent growth in Atlantic salmon smolts using RNA-DNA ratios**
Sharon A. MacLean and Jeanne St. Onge-Burns

2:10 p.m. **Comparison of lethal versus nonlethal sample sources for the detection of infectious salmon anemia virus (ISAV)**
Cem Giray, H. Michael Opitz, Sharon A. MacLean and Deborah Bouchard

2:30 p.m. *Break*

Session 4

Sharon MacLean, Moderator
*NOAA, Northeast Fisheries Science Center
 Narragansett, RI*

2:45 p.m. **Latent infectious salmon anemia virus infection in experimentally infected Atlantic salmon in saltwater and freshwater**
H. Michael Opitz, Sharon A. MacLean, Cem Giray, Deborah Bouchard, Sharon Clouthier, Wei Young-Lai, Dawna Beane and Sharon Blake

3:05 p.m. **Environmental persistence of infectious salmon anemia virus**
Cem Giray, Deborah A. Bouchard, Keith A. Brockway and Peter L. Merrill

3:25 p.m. **Evidence of episodic acidification in the Downeast Maine salmon rivers during the spring and fall of 2003**
Mark C. Whiting, Tracey Gamache and William Otto

3:45 p.m. **Evaluation of water alkalinity enhancement at the Craig Brook National Fish Hatchery on Atlantic salmon growth and survival**
Terry Haines, Benjamin Spaulding, Kenneth Beland and Barnaby Watten

4:05 p.m. **Ecology of the Atlantic salmon during the transition from maternal dependence to independent feeding: Research and management implications**
Keith H. Nislow, Benjamin H. Letcher, Sigurd Einum and John D. Armstrong

4:25 p.m. **Endocrine disruption in Atlantic salmon (*Salmo salar*) exposed to pesticides**
Benjamin Spaulding, Terry Haines, Rebecca Holberton and Rebecca Van Beneden

4:45 p.m. **Closing**
John Kocik
NOAA, Northeast Fisheries Science Center, Orono, ME

ABSTRACTS
ORAL PRESENTATIONS

Session 1

8:40 a.m.

Update on coastal Maine river Atlantic salmon smolt studies: 2003

James P. Hawkes¹, John F. Kocik¹ and Gregory Mackey²

¹NOAA, Northeast Fisheries Science Center, Orono, ME, ²Maine Atlantic Salmon Commission, Jonesboro, ME

The goal of our research is to quantify and compare Atlantic salmon smolt production across several of Maine's rivers. These comparisons are undertaken to (1) develop a better understanding of overwinter parr to smolt survival, population dynamics, and outmigration timing; and (2) strengthen stock assessments and population viability analyses. Atlantic salmon populations in Maine's rivers are critically low and recent survival estimates from juvenile to adult stages are well below replacement levels. Beginning with the deployment of a single rotary screw trap on the Narraguagus River in 1996, NOAA Fisheries and the Atlantic Salmon Commission have been investigating production, survival and migration of Atlantic salmon smolts in rivers of coastal Maine. Today, the salmon smolt research program operates 11 rotary screw traps on five rivers: four traps deployed in the Narraguagus, three traps in the Penobscot, one trap each on the Sheepscot, Pleasant and Dennys Rivers. These platforms support the field operations for the smolt production research, as well for mass marking and ultrasonic telemetry studies aimed at elucidating hatchery smolt movement patterns and survival rates. Findings of each of these field programs are summarized and briefly discussed.

Session 1

9:00 a.m.

Changes in the proportion of naturally reared Atlantic salmon smolts to hatchery smolts emigrating from the Penobscot River, ME, during 2000-2003

Christine Lipsky¹, James Loftin¹, Edward Hastings¹ and Russell Brown²

¹*NOAA, Northeast Fisheries Science Center, Orono, ME,* ²*NOAA, Northeast Fisheries Science Center, Woods Hole, MA*

Beginning in 2000, NOAA Fisheries has operated rotary screw traps in the lower Penobscot River to capture/recapture emigrating Atlantic salmon (*Salmo salar*) smolts. One objective of this program is to determine the relative proportion of stocked smolts to naturally reared smolts, and to assess the annual variability in this ratio. During 2000-2003, smolts were captured in the rotary traps between April and June. Fin scores were assigned to each fish based on the degree of erosion, with a fin score of 0 indicating no erosion, and a score of 3 indicating almost complete erosion of the fins, commonly seen in hatchery reared fish. Fish with a fin score of 0 or 1 that had no tags or marks were sampled for scales, which were subsequently analyzed to determine age and life history. The proportion of stocked smolts to naturally reared smolts has remained relatively stable over the past four years. The 2003 smolt season produced a slightly higher percentage of naturally reared smolts than in previous years.

Session 1

9:20 a.m.

Penobscot River adult Atlantic salmon migration study

Kenneth Beland¹ and Dimitry Gorsky²

¹*Maine Atlantic Salmon Commission, Bangor ME,* ²*University of Maine, Orono, ME*

The year 2002 marked the beginning of a cooperative research project between the Maine Atlantic Salmon Commission, U.S. Geological Survey (at Conte Anadromous Fish Research Center), University of Maine, U.S. Fish and Wildlife Service, National Marine Fisheries Service, and the Penobscot Indian Nation. This project investigates the temporal and spatial movements of Atlantic salmon during their upstream migration in the Penobscot River basin using PIT (Passive Integrated Transponder) tags. Antenna arrays and data loggers were installed at the entrance and exit of fishways at five Penobscot main stem dams (Veazie, Great Works, Milford, West Enfield, and Mattaceunk) and three Piscataquis drainage dams (Howland, Dover-Foxcroft, and Browns Mills). PIT tags were inserted into the dorsal musculature of approximately 400 salmon each year upon release to the river following capture at the Veazie Dam fishway trap. Remote fishway PIT tag antenna data loggers were downloaded weekly and data imported into a Microsoft Access relational database. These data allow assessment of salmon movements relative to date of capture, photoperiod, river flows, and temperature and of the final detection locations of salmon released as tagged smolts in the Penobscot basin.

*Session 1**9:40 a.m.***Estimation of gamete viability, timing of sexual maturity, and fecundity of river-specific marine net pen reared Atlantic salmon in Maine****Gregory Mackey¹**, Michael Loughlin¹, Nicholas Brown² and Ernie Atkinson¹¹*Maine Atlantic Salmon Commission, Jonesboro, ME*, ²*University of Maine, Franklin, ME*

As a means to supplement populations in several Downeast salmon rivers, NOAA's National Marine Fisheries Service, Maine Atlantic Salmon Commission, and U.S. Fish and Wildlife Service stocked river-specific Atlantic salmon adults in 2000 and 2001 that were reared in marine net pens by the aquaculture industry. Attempts to evaluate the reproductive success of these adults suggest that these fish achieved relatively low reproductive success. However, the methods used to evaluate this were either indirect or provided results with relatively low interpretive power. To test the hypothesis that poor gamete quality may be limiting reproduction by these fish, 26 pen reared adults were moved to the Center for Cooperative Aquaculture Research, Franklin, Maine, in October 2001, spawned as they matured, and the eggs were incubated in standard Heath tray stacks. These fish were of the same cohort that was stocked into the rivers and were moved to freshwater at the same time as river stocking. The fish matured slowly and many had not matured by December 12 when spawning efforts ceased. Of those that did mature, eight females and two males were spawned. Fertilization was greater than 95% in all spawnings, with the exception of one female that achieved 85% fertilization with both males. Survival to the eyed-egg stage was 83.8%. These results suggest that gametes are not the limiting factor in reproduction by these fish. However, the low rate of sexual maturation could explain poor reproductive performance if this occurred in the rivers.

*Session 1**10:00 a.m.***Streamside incubation: A low tech, low cost approach to Atlantic salmon restoration**

Paul Christman, Kevin Dunham and Daniel McCaw
Maine Atlantic Salmon Commission, Sidney, ME

During the winter of 2002 – 2003, staff from the Sidney office of the Maine Atlantic Salmon Commission tested the feasibility of streamside incubation as a method for volunteer groups to participate in Atlantic salmon restoration. Two types of flow-through incubators were constructed from discarded refrigerators. Three incubators were designed to hold egg filled Whitlock-Vibert boxes placed within an artificial channel and three were designed to hold eggs between layers of poultry nesting material. Prior to receiving eggs, incubators were deployed at three sites on two tributaries to the Sandy River. In February of 2003, a total of 43,496 eyed Atlantic salmon eggs at approximately 38% development were divided equally into each of the six incubators. At approximately 95% development, fry were removed from the incubators and enumerated to obtain hatching success. Success ranged from 85% to 98% with an average of 90% for all six incubators. Total operational cost for the project was \$2,351. Operational costs for a second year are estimated at approximately 30% of the initial investment. Total time spent on this project, not including traveling time, amounted to 1,355 hours. Total time for a second project year is also estimated at approximately 30% of the time spent during the initial year. High hatching success, time expended, and low cost makes this streamside incubator system a feasible approach for volunteers. Additional studies to test capacity and improve incubator efficiency are recommended.

Session 2

10:40 a.m.

Consequences of movement on Atlantic salmon (*Salmo salar*) survival estimates

Gregg E. Horton and Benjamin H. Letcher

U.S. Geological Survey, Silvio O. Conte Anadromous Fish Research Center, Turners Falls, MA

An implicit assumption common in estimating abundance and survival of juvenile Atlantic salmon populations is that of no (or restricted) movement during sampling events (geographical closure). Although valid for sampling individual sites when a section of stream can be blocked off with nets during the course of a day's sampling, this assumption may be invalid for sampling that takes place over extended time and space. The ability of individuals to move between stream reaches can bias estimates of survival through unequal capture probabilities, which is one of the most severe forms of bias in capture-mark-recapture models. When the study aim is to compare survival among segments of the population (e.g., ages, sexes) or across seasons or years, movement is confounded with survival. Comparison of these "apparent survival" rates may be valid unless a) emigration rates are different for different segments of the population, or b) there are annual or seasonal differences in movement. Further, a valid study objective may be to uncover the degree to which differences in emigration rates and real survival (both alone and in combination) are operating to explain production patterns from a certain system, the outcomes of competition, responses to changes in habitat capacity, etc. These problems are best addressed by estimating real survival and emigration separately. In Shorey Brook, a tributary to the Narraguagus River, Maine, a multiyear study is underway to evaluate the interaction of movement with survival of pre-smolt Atlantic salmon. PIT (Passive Integrated Transponder) tag technology, multiple samples per year, and passive PIT tag detection gear has allowed new insights by accounting for emigration. Survival estimates that failed to incorporate emigration information were negatively biased, potentially leading to incorrect conclusions regarding size-dependent processes. This problem could be affecting parameter estimates at many different scales of study.

Session 2

11:00 a.m.

Evolution of population structure in Maine's Atlantic salmon

Adrian P. Spidle and Timothy L. King

U.S. Geological Survey, Leetown Science Center, Kearneysville WV

Following the closure of commercial fisheries for Atlantic salmon in Maine in 1947, populations of Atlantic salmon in Maine were supplemented with broodstock of Maine origin through the 1950s. Broodstock of Canadian origin was used through the 1960s, before reverting to broodstock of Maine only origin from the 1970s onward. Present day Atlantic salmon population structure appears to reflect the relatedness of most Maine salmon back to the rivers used for supplementation in the 1950s, the Machias and Narraguagus, rather than the Miramichi, the Canadian river primarily used in the 1960s. We present a comparison of Atlantic salmon scales collected from the Machias and Narraguagus rivers in the 1950s and 1970s to present day Atlantic salmon populations, and infer the history of population structure in Maine's Atlantic salmon over the period examined (1952 through the present).

Session 2

11:20 a.m.

Scale pattern analysis discriminates Atlantic salmon by river-reach rearing origin

Ruth Haas-Castro¹, Tim Sheehan¹, Steven Cadrin¹ and Joan Trial²

¹*NOAA, Northeast Fisheries Science Center, Woods Hole, MA*, ²*Maine Atlantic Salmon Commission, Bangor, ME*

Anadromous populations of Atlantic salmon (*Salmo salar*) spawning in Maine's rivers are at historic low numbers. Knowing the relative contribution of each individual river reach to overall production could provide insight into the dynamics of a particular watershed. We evaluated the use of scale pattern analysis to discriminate among Atlantic salmon parr reared in different reaches of the Narraguagus River basin. Measurements of parr scales collected during 1990-1999 were used in a principal component analysis and to create linear discriminant functions for seven geographic strata and three river basin strata groups reflecting natural habitat breaks and Atlantic salmon management considerations. Discriminant functions were calculated using both annual and pooled data. Our results indicate there is enough differentiation among rearing habitats of different reaches (or between geographically grouped reaches) in the Narraguagus River basin to allow discrimination to a degree useful for management. Applying our models to scales from emigrating smolts and returning adult salmon could further improve our understanding of the contributions of specific rearing habitats to Atlantic salmon populations.

*Session 2**11:40 a.m.***Evaluation of adult scales to determine the origin of Atlantic salmon recovered in Maine****Michael Pietrak**¹, Christopher Legault² and Kenneth Beland³¹*Maine Aquaculture Association, Hallowell, ME,* ²*NOAA, Northeast Fisheries Science Center, Woods Hole, MA,* ³*Maine Atlantic Salmon Commission, Bangor, ME*

Recently issued discharge permits for the salmon aquaculture industry in Maine require a phased in marking of all Maine farmed salmon. The Maine Aquaculture Association in cooperation with federal and state agencies, industry, and nongovernmental organizations has been examining potential marking technologies that could be used to mark Maine's farmed salmon. Through this process, it has become clear that for most marking technologies a two-step marking system will be required that includes 1) an external mark for easy, reliable streamside identification of aquaculture origin at a minimum and 2) an internal tag that provides additional information on the origin of the fish. Scales have potential as an external marker due to the ease of detection in the field, low cost to apply and detect mark, and 100% mark retention rate. Preliminary studies using smolt scales to differentiate river and hatchery origin showed over a 95% accuracy of correctly identifying fish. The current study examined adult scales of known origin to determine their value in streamside identification of fish origin. Scales were collected from aquaculture fish at the processing plant, marked restoration fish, and historical wild scale collections predating 1982. The scales were digitally imaged and distributed around the country to 30 scale readers of varying experience levels. Readers were asked to classify the freshwater origin as hatchery, river reared or unknown and the marine origin as net pen, ocean or unknown. Based on the responses, fish were classified as industry, restoration, or river reared. Readers were asked to classify the origins of two test sets containing 100 fish each. The first test set was completed with no training or reference scales. The readers were given a primer on scale reading and a labeled reference set of scales for use with the second test set. Analysis focused on the accuracy of readers to distinguish the origin and classification of the test scales. The effects of reader experience and the use of the labeled reference set on accuracy also were examined.

*Session 3**12:50 p.m.***Reduced genetic diversity and effective population size in an endangered Atlantic salmon (*Salmo salar*) population: Connecting past to present****Christopher Lage¹** and Ira Kornfield²¹*University of Maine, Department of Biological Sciences, Orono, ME;* ²*University of Maine, School of Marine Sciences, Orono, ME*

Optimal conservation strategies should incorporate both present and historical knowledge of genetic variation, gene flow, ecological stability, and local adaptation. This study examined genetic diversity at seven microsatellite loci in wild and in aquaculture-origin Atlantic salmon from the Dennys River, Maine, from 1963 to 2001 using DNA extracted from archival scale and tissue samples. Composite haplotypes of the mitochondrial ND1 gene were generated using polymerase chain reaction primers designed for degraded mitochondrial DNA (mtDNA). An ND1 mtDNA haplotype previously thought diagnostic for European and Newfoundland stocks was discovered within this population. Additional cryptic genetic diversity, yet unrecognized, may be present in endangered Maine salmon populations and should be evaluated and maintained through conservation efforts. Overall temporal trends of diversity and effective population size (N_e) in wild fish show strong reductions from 1963 to 2001. Significant within-population differentiation was observed between 1995 and 2001 that, if generated through introgression, did not increase N_e estimations. Significant differentiation was observed between the aquaculture sample and all wild samples. Genetic diversity of aquaculture-origin fish from 1992-1998 was greater than that of contemporary wild samples and more representative of historic wild diversities. Observed temporal reductions in genetic diversity and N_e of the Dennys River salmon population raises the possibility that current restoration efforts may be impacted by historical loss of diversity critical to adaptation.

Session 3

1:10 p.m.

Loss of molecular and trait variation in six populations of endangered Maine salmon

Nathan Wilke¹, Michael Kinnison¹ and Tim King²

¹*University of Maine, Department of Biological Sciences, Orono, ME;* ²*U.S. Geological Survey, Leetown Science Center, Kearneysville, WV*

One of the primary concerns surrounding population declines in species at risk is the loss of potentially adaptive trait variation and its implications for fitness and population persistence. Variation at molecular markers has at times been used as an indicator for potential loss of adaptive trait variation. However, theoretical predictions for the fate of quantitative trait variation under population decline are variable, and empirical associations between molecular and adaptive trait variation have often proved elusive. Until now microsatellite variation has been the primary data assessed for genetic variation in populations of Maine Atlantic salmon (*Salmo salar*). This study compares patterns of microsatellite variation, phenotypic trait variation, and demographic history in six endangered populations of Maine Atlantic salmon. All of these populations are closely related and most were reared to maturity under similar captive conditions, allowing for better control of potential confounding effects. The study suggests that the loss of microsatellite variation in Maine endangered Atlantic salmon populations indicates a loss in phenotypic trait variation and, in turn, adaptive potential.

Session 3

1:30 p.m.

Genetic parentage analysis of Sheepscot River Atlantic salmon: Survival and distribution of stocked individuals

Meredith L. Bartron¹, Jerre Mohler¹ and Tim King²

¹*U.S. Fish and Wildlife Service, Northeast Fishery Center, Lamar, PA*, ²*U.S. Geological Survey, Leetown Science Center, Kearneysville, WV*

Survival and dispersal of stocked juvenile Atlantic salmon (*Salmo salar*) are vital components to the restoration and recovery efforts for the Distinct Population Segment (DPS) rivers in Maine. Recovery and restoration efforts focus on the stocking of fry and smolts into the DPS rivers. Stocked fry are recaptured as parr and a portion are sent to Craig Brook National Fish Hatchery to serve as broodstock upon maturation. Smolts are rarely recaptured due to the subsequent emigration to the ocean. Therefore, in-river assessment of survival, habitat usage, and stocking practices are best conducted using the stocked fry. In 2001, calcein marked Atlantic salmon fry of known parentage were stocked into the Sheepscot River, Maine. Offspring of 21 families, grouped into 6 stocking batches, were stocked into three locations in the Sheepscot River: Trout Brook, Choate Brook, and four locations in the West Branch of the Sheepscot. Approximately half of the stocked juveniles were marked with calcein to compare the effects of the mark on survival. A disproportionate number of unmarked individuals were recovered compared to the number of marked individuals (potentially due to a number of factors). To determine if the unmarked individuals were actually the stocked individuals, we used genetic parentage analysis based on genotypes from 11 microsatellite loci to identify origin. For individuals unable to be assigned parentage, the relatedness among the offspring was examined to determine the number of potential family groups represented and the potential of contribution of natural reproduction was evaluated. Due to the ability to determine parentage, the effects of stocking location and family group on survival were able to be evaluated to provide a better understanding of factors that contribute to restoration and recovery efforts.

Session 3

1:50 p.m.

Nonlethal measurement of recent growth in Atlantic salmon smolts using RNA-DNA ratios

Sharon A. MacLean¹ and Jeanne St. Onge-Burns²

¹*NOAA, Northeast Fisheries Science Center, Narragansett, RI,* ²*ETI Professionals, Inc., Amesbury, MA*

RNA-DNA ratios, reflecting protein synthesis, have been used as an index of growth in fishes at various life stages from larvae to adults. This technique was evaluated for its potential use in field assessments of feeding/growth of hatchery released Atlantic salmon (*Salmo salar*) smolts. Various tissue samples taken from smolts for RNA-DNA analysis were evaluated in a study conducted at the University of Maine Center for Cooperative Aquaculture Research in 2003. Tissue samples were collected nonlethally from the dorsal muscle, caudal fin, scales, and gills of 110 Passive Integrated Transponder (PIT) tagged smolts that were individually weighed and measured. After a one-month feeding period, the fish were re-measured and re-weighed, and tissue samples again were taken for nucleic acid analysis. Of the 110 fish in the experiment, 88 fish gained weight. Dorsal muscle RNA-DNA ratios were strongly correlated with growth in weight ($R^2=0.76$), whereas RNA-DNA ratios from scale, fin, and gill tissue samples were not. One month after sampling, all fish appeared healthy and showed no overt signs of distress or infection. Although the nonlethal sampling was highly successful, field experiments are needed to assess the utility of this technique for assessing the growth of smolts in the wild.

Session 3

2:10 p.m.

Comparison of lethal versus nonlethal sample sources for the detection of infectious salmon anemia virus (ISAV)

Cem Giray¹, H. Michael Opitz², Sharon A. MacLean³ and Deborah Bouchard¹

¹*Micro Technologies, Inc., Richmond, ME,* ²*University of Maine, Department of Animal and Veterinary Sciences and Cooperative Extension, Orono, ME,* ³*NOAA, Northeast Fisheries Science Center, Narragansett, RI*

The emergence of infectious salmon anemia virus (ISAV) in Canada and the USA has led to establishment of ISAV surveillance programs for cultured Atlantic salmon (*Salmo salar* L.) and wild fish species including Atlantic salmon. Current testing procedures for ISAV consist of viral culture, reverse transcription polymerase chain reaction (RT-PCR) and indirect fluorescent antibody test (IFAT) and have thus far employed the use of lethal sampling. In this study, blood samples were evaluated as a nonlethal way to test for ISAV using viral culture and RT-PCR. Kidney, spleen, blood, and mucus samples were tested from Atlantic salmon survivors of ISAV infection trials, representing potential ISAV-carriers, and from moribund individuals from ISA clinical sites. Blood compared well to tissue samples for viral culture and produced a greater number of positives than did kidney samples for the detection of ISAV by RT-PCR. Direct tissue RT-PCR using both kidney and blood samples was determined to be a more sensitive assay than viral culture which utilized kidney and spleen tissues. Mucus did not perform well in either assay compared to the other sample sources. Blood appears to be a reliable nonlethal sample source for the detection of ISAV by both viral culture and RT-PCR in both moribund and asymptomatic fish.

Latent infectious salmon anemia virus (ISAV) infection in experimentally infected Atlantic salmon in saltwater and freshwater

H. Michael Opitz¹, Sharon A. MacLean², Cem Giray³, Deborah Bouchard³, Sharon Clouthier⁴, Wei Young-Lai⁵, Dawna Beane¹ and Sharon Blake⁶

¹University of Maine, Department of Animal and Veterinary Sciences and Cooperative Extension, Orono, ME, ²NOAA, Northeast Fisheries Science Center, Narragansett, RI,

³Micro Technologies, Inc., Richmond, ME, ⁴Maine BioTek, Inc., Winterport, ME, ⁵Huntsman Marine Science Center, St. Andrews, NB, Canada, ⁶University of Maine, Department of Biochemistry, Microbiology and Molecular Biology, Orono, ME

A 63-day experimental study was conducted to determine if clinically normal ISAV-infected Atlantic salmon (*Salmo salar*) remain infectious with ISAV, and whether subclinical infection is re-activated during transition from marine to freshwater. Seventy Atlantic salmon smolts which had survived 39 days after intraperitoneal injection with 10^7 TCID₅₀ ISAV were divided into two equal groups. The first group (Group A, SW/SW) was kept in seawater throughout the study, while the second group (Group C, SW/FW) was gradually re-adapted to freshwater over a 3-week period. Fifteen naïve fish were added to both Groups A and C, and designated as Groups B (SW/SW) and D (SW/FW), respectively. Five naïve fish (designated as Group E, FW/FW) were kept in freshwater as isolated negative controls. Individually Passive Integrated Transponder-tagged fish were bled on days 1, 28 and 63 and the blood samples tested using reverse transcription-polymerase chain reaction (RT-PCR), virus isolation on SHK-1 cells, and antibody enzyme-linked immunosorbent assay (ELISA). Kidney tissues from all fish that died during the study and all fish that survived to the end of the experiment were also tested by RT-PCR and virus isolation. Re-activation of the ISAV infection did not occur in the 70 original ISAV-infected subclinical fish in Groups A or C, and there were no ISAV-related mortalities. In both groups, the number of RT-PCR positive fish declined until all fish were negative at day 63. However, subclinical infection of ISAV was detected in two fish in Group C and in one fish in Group A. Antibodies against ISAV were present in all but one fish in Groups A and C detected by ELISA on days 1 and 28, and 92% were still ELISA positive on day 63. ISAV was isolated from the naïve fish that died in Groups B (100%) and D (70%). RT-PCR, virus isolation, and ELISA remained negative in all fish in Group E (negative control). The results demonstrate that subclinical ISAV-infected fish can remain carriers of infection that can spread to susceptible fish. The stress associated with transition from saltwater to freshwater did not re-activate ISAV infection. While RT-PCR declined to zero 102 days after initial infection, ELISA antibodies were still detectable at a high rate.

Session 4

3:05 p.m.

Environmental persistence of infectious salmon anemia virus

Cem Giray, Deborah A. Bouchard, Keith A. Brockway and Peter L. Merrill
MicroTechnologies, Inc., Richmond, ME

The causes and mechanisms of infectious salmon anemia virus (ISAV) transmission among populations at aquaculture sites are not fully understood. It is presently unknown how long ISAV can survive outside its host in the environment, i.e., in seawater and on suspended particles, once it is shed. This study examined the ability to detect the presence of ISAV in the environment and the ability of ISAV to survive in seawater. Viral culture and reverse transcription-polymerase chain reaction (RT-PCR) were used to test seawater, surfaces, sediments and invertebrates collected from the field. ISAV was detected by RT-PCR in recently collected seawater samples from affected sites following concentration using an inexpensive seawater filtration system. The system was sensitive enough to detect ISAV by RT-PCR at levels of less than 30 virus particles per liter of seawater. Testing by cell culture required approximately a 1,000-fold increase in ISAV concentration in seawater for viable virus to be detected. ISAV was also detected from pontoon and boat surfaces, and from invertebrates such as sea lice (*Lepeophtheirus salmonis*) and blue mussels (*Mytilus edulis*). Seawater samples were inoculated in the laboratory with ISAV at 10^4 TCID₅₀/ml final concentration, and virus loss over time under varying conditions of temperature and microbial activity was examined. ISAV was detectable by RT-PCR after incubation in nonsterile seawater for up to two weeks at 16°C and up to 5 weeks at 4°C. No cytopathic effect (CPE) was observed by viral culture after 24 hours incubation at 16°C and after incubation for 1 week at 4°C. Although reduced by two orders of magnitude, ISAV CPE was still observed after incubation for six weeks in sterile seawater at 16°C and 4°C. ISAV was also detected by RT-PCR in both sets of samples. Observations will continue until ISAV can no longer be detected by either method.

Session 4

3:25 p.m.

Evidence of episodic acidification in the Downeast Maine salmon rivers during the 2003 field season

Mark C. Whiting¹, Tracey Gamache² and William Otto³

¹*Maine Department of Environmental Protection, Bangor ME,* ²*Narraguagus River and Pleasant River Watershed Councils, Columbia Falls, ME,* ³*University of Maine, Machias, ME*

Maine Department of Environmental Protection and the watershed councils collaborate in water quality monitoring of the Maine salmon rivers. Electronic pH meters were used by watershed council volunteers within 24 hrs of rain events in spring and fall 2003 to measure potential episodic acidification events during high water flows. Weather data for 2003 show that spring precipitation was normal, summer was relatively dry, and fall was relatively wet. In the spring, pH values of Tunk Stream and the Pleasant, Narraguagus, Machias, and East Machias rivers were 4.7 to 5.8. In the fall, the pH of Tunk Stream and Pleasant and Narraguagus rivers measured 4.5 to 5.8. For Tunk Stream and Pleasant River, pH values in the low fives lasted for a month in the spring (late March to late April) and again in the fall (late October to late November). A pH of 5.6 was observed for two consecutive weeks in the lower Narraguagus River in the fall. Some tributaries were more or less acidic than their respective river main stem. East Little River was less acidic and West Little River more acidic than the main stem of the Pleasant. Cranberry Brook and Great Falls Branch were less acidic than the Narraguagus, while the West Branch of the Narraguagus and Lawrence Brook were more acidic. Schoodic Brook was similar to the Narraguagus in acidity and sensitivity to stormwater events. Published data indicate low pH, low calcium and high aluminum work synergistically to create conditions that can be harmful for salmon and other sensitive species. The lasting low pH values observed in 2003 could contribute to harmful conditions. Similar conditions were observed in 2002 but not in 2001, Maine's worst drought year.

*Session 4**3:45 p.m.***Evaluation of water alkalinity enhancement at the Craig Brook National Fish Hatchery on Atlantic salmon growth and survival****Terry Haines¹**, Benjamin Spaulding¹, Kenneth Beland² and Barnaby Watten³*¹University of Maine, Department of Biological Sciences, Orono, ME, ²Maine Atlantic Salmon Commission, Bangor, ME, ³U.S. Geological Survey, Leetown Science Center, Kearneysville, WV*

The Craig Brook National Fish Hatchery, East Orland, Maine, is a key component of the Atlantic salmon conservation and recovery program. However, the hatchery is located in an area that receives acidic precipitation and where the geologic material is low in acid-neutralizing (buffering) capability. The prime water source, Craig Pond, is very low in dissolved minerals and is below the optimum alkalinity level (20 mg/L as CaCO₃) for rearing Atlantic salmon. This project evaluated the efficacy of a system to add limestone (calcium carbonate) to the hatchery water source to increase alkalinity, and the effects of rearing in higher-alkalinity water on fitness of the fish produced. The system was designed to increase water alkalinity from the average of 7 mg/L normally to approximately 30 mg/L (“medium alkalinity water”) and 50 mg/L (“high alkalinity water”). The limestone dissolution system was able to maintain reasonably consistent water chemistry with minimal attention and few apparent adverse effects to the fish, and was effective in raising the alkalinity and pH of the hatchery water to levels believed to be optimal for Atlantic salmon culture. There was a nonsignificant trend to higher mortality of embryos in the treated water that may have been caused by washout of fine limestone particles from the system to the hatching and rearing containers. Sand filters greatly alleviated this problem. The limestone treatment significantly improved survival of the fish in the riverine environment. Two years of stocking fry in the Narraguagus River demonstrated improved survival of fish from the treated water after five months as fry, after a year and five months as parr, and after two years as parr. Fish from the medium and high alkalinity treatments were recovered at significantly greater rates than expected if there had been no effect of the treatment on survival.

Session 4

4:05 p.m.

Ecology of Atlantic salmon during the transition from maternal dependence to independent feeding: research and management implications

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As salmon fry transition from dependence on maternally derived yolk reserves to independent feeding, they experience a highly dynamic biotic and abiotic environment, which often results in high mortality rates. Field experiments were conducted in North America and Europe to investigate the importance of fine scale spatial (scale of meters) and temporal (scale of weeks) variation in environmental (stream discharge, temperature) and biotic (population density, prior residence) factors for survival, growth, and life history. Results of these studies indicate that survival and growth during this period result from a complex interaction between abiotic and biotic factors in combination with maternal effects (egg size) and may have longer term and larger scale population and life history consequences. The implications of these results for management, specifically in the context of restoration efforts involving stocking programs will be discussed.

Endocrine disruption in Atlantic salmon (*Salmo salar*) exposed to pesticides

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Previous research has shown that smolts from the Narraguagus River, a Distinct Population Segment (DPS) river, have low gill Na^+/K^+ -ATPase activity and did not tolerate saltwater challenge tests (Magee et al., 2001). Commercial cultivation of lowbush blueberries is a common activity in the watersheds of many of the rivers in the DPS, and chemicals registered for use on blueberries have been detected in the Narraguagus and Pleasant rivers (Dill et al., 1998). Some of these pesticides are endocrine disruptors that may affect osmoregulatory ability (Fairchild et al., 1999). To determine if pesticides may contribute to osmoregulatory failure of Atlantic salmon smolts in Maine, chemicals registered for use on blueberries were tested for estrogenic activity in a cell culture assay (E-SCREEN). Mixtures of the highest-scoring E-SCREEN chemicals then were experimentally exposed to Penobscot strain smolts obtained from the Green Lake National Fish Hatchery. Fish in the first trial were exposed to Velpar (hexazinone), Orbit (propiconazole) and SuperBK (2,4-dichlorophenoxyacetic acid; 2,4-D) and in the second trial to Orbit, Sinbar (terbacil) and Imidan (phosmet). Pre-smolts were subjected to a total of five weekly, 24-hour pesticide mixture exposures and were then subjected to a 24 hour saltwater challenge (SWC). Gill tissue Na^+/K^+ -ATPase (McCormick, 1993) and blood plasma chloride, 17 beta-estradiol and vitellogenin concentration analyses were done. There was no significant difference in Na^+/K^+ -ATPase values between control and exposed fish in trial 1, but significant differences in trial 2 were found. Plasma chloride values did not show significant differences in either trial. The 17 beta-estradiol and vitellogenin assays are nearing completion with the data and results to follow.

References:

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