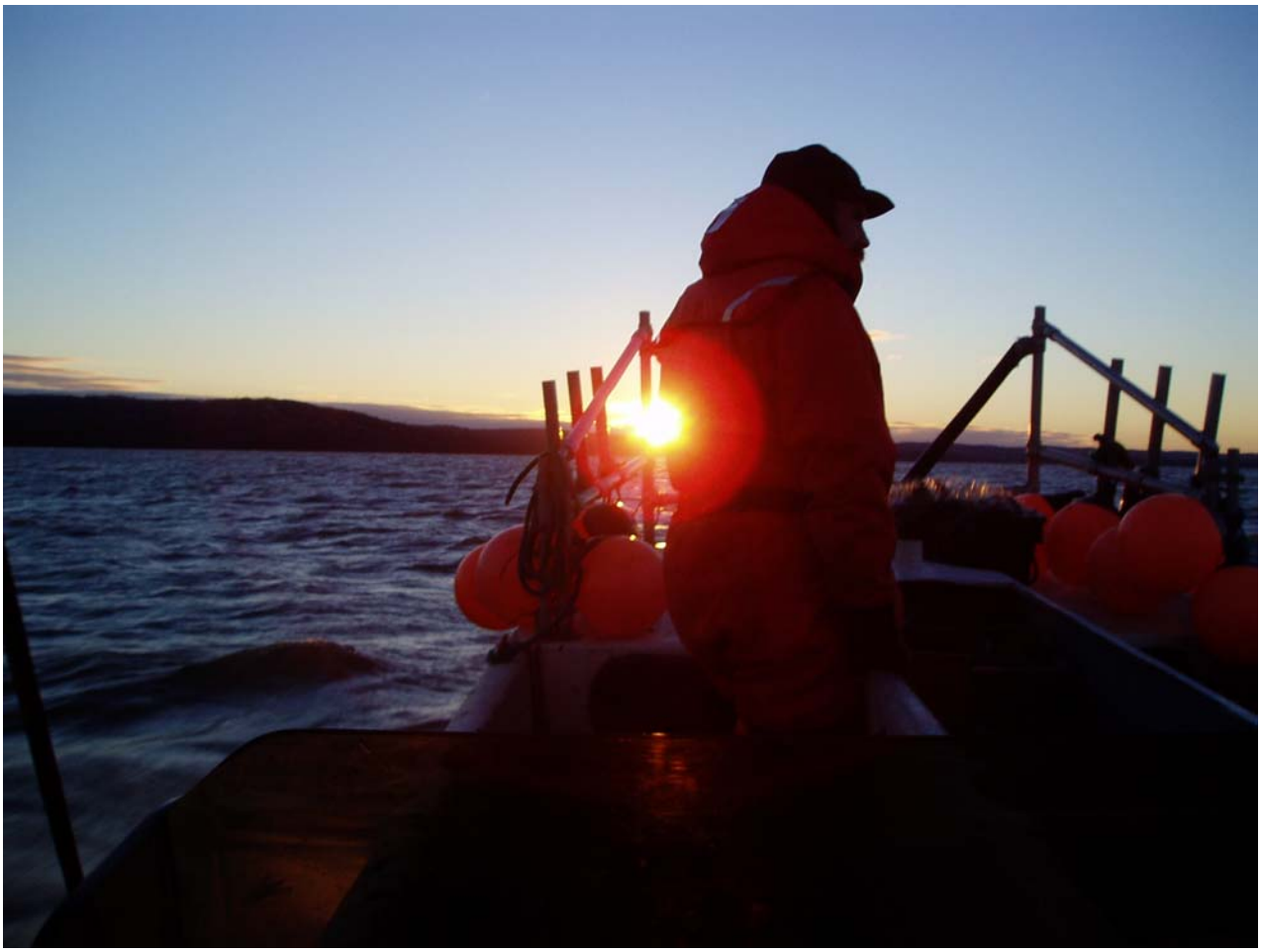
U.S. Fish & Wildlife Service

Northeast Fishery Center
Lamar, Pennsylvania

Annual Report of Biological Activities 2005



Sunrise expedition in December on the Hudson River – in search of Atlantic sturgeon

As we reflect back on projects undertaken and tasks accomplished in 2005, we are once again reminded how brief is the time which encompasses a year. Director, Mike Millard again led the staff of the Northeast Fishery Center - Lamar, PA (NEFC) through 2005 with no shortage of projects geared toward improving our knowledge and stewardship of the aquatic resources to which we are committed.

To accomplish our mission, NEFC is comprised of 3 inter-related components or sections: (1) Fish Technology (2) Fish Health and (3) Population Ecology and Genetics. Therefore, projects were diverse, ranging from a genetic-based restoration stocking study with endangered Sheepscot River, Maine Atlantic salmon, to experimental incubation and bacterial assessment of hatchery lake trout eggs. It was also the final year of field work on the Hudson River to develop a scientifically-sound juvenile Atlantic sturgeon sampling strategy under a contract agreement with the State of New York. Other interesting work included various projects with freshwater mussels, horseshoe crabs, striped bass, rainbow trout, and wild brook trout These and other projects are more fully described in the pages that follow which we hope you find informative.

In support of our mission, a major construction project was initiated with an expansion of the existing Fish Health Center's Laboratory complex. This facility is now in use and houses numerous specialty lab rooms for parasitology, virology, bacteriology, polymerase chain reaction (PCR) technology, and eventually, histology. NEFC welcomes the addition of Dr. Gavin Glenney, who will work as a Fish Health Biologist in the new laboratory complex. In addition, we welcome Tom Kehler as a term employee who will primarily work on a calcein marking study using funds from a Science Support Partnership (SSP) agreement with the USGS – Northern Appalachian Research lab in Wellsboro, PA. Sorely missed from the staff next year will be Anthony Carta, former Head of Maintenance who retired in 2005 and was known throughout Region 5 as one who came up with innovative ideas and had tremendous fabrication skills which came to the aid of many Project Leaders. Former Fish Health Biologist, Kimball Selmer-Larsen will also be missed, having retired early in 2005. In addition, we wish the best for another of our Fishery Biologists, Wade Jodun, who departed NEFC for a position as Region 5 Fisheries Regional SAMMS (Service Asset Maintenance Management System) Coordinator in Hadley, MA.

Finally, we are appreciative of the support and cooperation we received from our State and Federal partners as well as the continued interest in our programs shown by Congressman John Peterson, PA 5th District. We are pleased to report the following Biological Activities for 2005:

STUDIES PERFORMED

Study Number and Title:

LM-02-01 Relative abundance sampling of juvenile Atlantic sturgeon of the Hudson River (study finalized in 2005)

LM-05-01 Effects of handling and disinfection on eye-up of Lake Trout eggs at Allegheny National Fish Hatchery (ANFH)

LM 05-02 Evaluation of Atlantic salmon kelt broodstock diets

LM-05-03 Detection of calcein marks in juvenile Eliptio complanata mussels planted in their natal stream

LM-05-04 The effect of artificial substrate in incubation trays on the size and yolk condition of emerging Atlantic salmon *(Salmo salar)* fry.

LM-05-05 Assessment of watershed scale habitat features on the survival of juvenile Atlantic salmon

LM-05-06 Annual genetic characterization of Atlantic salmon broodstock at Craig Brook National Fish Hatchery (CBNFH)

LM-05-07 2005 pedigree line for the Pleasant River broodstock at Craig Brook National Fish Hatchery

LM-05-08 Genetic characterization of an isolated population of Northern Plymouth Red-bellied Cooter (*Pseudemys rubriventris*) in Massachusetts

LM-05-09 Genetic characterization of two northern riffleshell (*Epioblasma torulosa rangiana*) populations in the Allegheny River

LM-05-10 Evaluation of genetic diversity and relatedness for Atlantic sturgeon (*Acipenser oxyrinchus*) captively held by Maryland DNR

LM-05-11 Genetics assessment of Susquehanna River shad

LM-05-12 Characterization of the brook trout population in a degraded North-Central Pennsylvania stream and assessment of the population response after stream rehabilitation

LM-05-13 Removal of phosphorous using pelletized acid mine drainage sludge

OTHER BIOLOGICAL INVESTIGATIONS AND RELATED ACTIVITIES PERFORMED:

LM05A Congressional contacts

LM05B Visit from the Director

LM05C Salvage and refuge of mussels

LM05D Genetic characterization and marking of Merrimack River Atlantic salmon

LM05E Genetic characterization of “wild” Penobscot River Atlantic salmon

LM05F Participation in the National Wild Fish Health Survey

LM05G Region 5 Fisheries Project Leaders meeting

LM05H Fish Health Inspection/Monitoring/Diagnostic Services

LM05I Incidence and prevalence of infectious salmon anemia virus (ISAv) in sea run Atlantic salmon held at Service Nat’l Fish Hatcheries as broodstock

LM05J Fish health extension services

OTHER BIOLOGICAL INVESTIGATIONS AND RELATED ACTIVITIES PERFORMED: (continued)

LM05K U.S. Fish and Wildlife Service Title 50 Revision Committee (Fish Health)

LM05L Vaccination of pre-release Connecticut River smolts using multi-valent vaccine

LM05M Partnership with the Pennsylvania Fish and Boat Commission for pond culture of juvenile walleye and striped bass

LM05N Effluent management at hatcheries through nutrition

STUDIES / PUBLICATIONS IN WHICH THE CENTER COOPERATED:

Passive inducive transponder (PIT) tag quality control / quality assurance study. James Henne, Vince Mudrak – USFWS Warm Springs Fish Technology Center, and Adam Fuller – USFWS Mora Fish Technology Center (NEFC contact, **J. Mohler**)

Bay-wide tagging study to assess spawning migration and population size of horseshoe crabs in Delaware Bay. David R. Smith, U.S. Geological Survey - Biological Resources Division, Leetown Science Center, WV. Year 3 of 3. (**M. Millard**, USFWS Project officer)

Spawning optimization software for Craig Brook National Fish Hatchery. Ben Letcher, U.S. Geological Survey /Univ. of Massachusetts, Conte, MA (**M. Bartron**, USFWS Project Officer)

Hartman, K.J., M.D. Kaller, J.W. Howell, and **J.A. Sweka**. 2005. How much do valley fills influence headwater streams? Hydrobiologia. 532: 91-102.

P. Nerette, I. Dohoo, L. Hammell, N. Gagne, **P. Barbash**, S. MacLean, and C. Yason. Estimation of the repeatability and reproducibility of three diagnostic tests for infectious salmon anaemia virus. 2005. Journal of Fish Diseases 28:101-110.

McDaniel, Nichole K., Shozo H. Sugiura, **Thomas Kehler, John W. Fletcher**, Relicardo M. Coloso, Peddrick Weis and Ronaldo P. Ferraris. 2005. Dissolved oxygen and dietary phosphorus modulate utilization and effluent partitioning of phosphorus in rainbow trout (*Oncorhynchus mykiss*) aquaculture. Environmental Pollution 138:350 -357

Smith, D.R., **M.J. Millard**, S. Eyler. In press. Abundance of adult horseshoe crabs in Delaware Bay estimated from a bay-wide mark-recapture. Fishery Bulletin.

STAFF PUBLICATIONS:

Jodun, W. A and J. W. Fletcher. Accepted. Growth and survival of first-feeding Atlantic salmon fry in response to various starter diet regimens. North American Journal of Aquaculture

Jodun, W. A., M. K. King, P. R. Farrell, W. Wayman, and G. Looney. Accepted. Methanol as a potential cryoprotectant of Atlantic salmon spermatozoa. North American Journal of Aquaculture

Millard, M.J., J. W. Mohler, A. Kahnle, A. Cosman. 2005. Mortality Associated with Catch-and-Release Angling of Striped Bass in the Hudson River. North American Journal of Fisheries Management 25:1533–1541

STAFF PUBLICATIONS: (continued)

Mohler, J. W. Accepted. Mussels of Muddy Creek on the Erie National Wildlife Refuge. Northeastern Naturalist.

Mohler, J. W. Accepted for 2006. All that Atlantic sturgeon fry need are plenty of food, space and clean tanks. World Aquaculture Magazine.

Sweka, J.A. and K.J. Hartman. *In press*. Effects of large woody debris addition on stream habitat and brook trout populations. Hydrobiologia 00:00-00.

Sweka, J.A. and K.J. Hartman. *In press.* Contribution of terrestrial invertebrates to yearly brook trout prey consumption and growth. Transactions of the American Fisheries Society 00:00-00.

Sweka, J.A., C.M. Legault, K.F. Beland, J. Trial, and M. J. Millard. *Accepted pending revision*. Evaluation of removal sampling for basinwide assessment of Atlantic salmon. North American Journal of Fisheries Management 00:00-00.

Sweka, J.A., D.R. Smith, and M.J. Millard. *In review.* An age-structured population model for horseshoe crabs in the Delaware Bay area to assess harvest and egg availability for shorebirds. Estuaries 00:00-00.

TECHNICAL INFORMATION LEAFLETS:

LM-04-03 Growth evaluation of sub-adult Atlantic sturgeon offered one of 3 commercial diets

TECHNICAL REPORTS:

Bartron, M. L. 2005. Pleasant River parr and smolt parentage analysis for Craig Brook National Fish Hatchery. U.S. Fish and Wildlife Service, Lamar, PA.

Julian, Shannon, and Meredith Bartron. 2005. Fine-scale analysis of genetic population structure among horseshoe crab spawning beaches in Delaware Bay. U.S. Fish and Wildlife Service, 2005.

Sweka, J. A., J. Mohler, and M. J. Millard. 2006. Relative abundance sampling of juvenile Atlantic sturgeon in the Hudson River. Final Report to the New Your State Department of Environmental Conservation, Hudson River Fisheries Unit, New Paltz, NY, 44 pages.

FORMAL PRESENTATIONS:

Barbash, Patricia - Atlantic salmon health management: summary and update. Connecticut River Atlantic Salmon Commission Forum. February 9, 2005, Hadley, Massachusetts The Technical Committee of the Connecticut River Atlantic Salmon Commission is comprised of senior staff biologists from the fisheries departments of Massachusetts, Connecticut, New Hampshire, Vermont, plus the U.S. Forest Service, National Marine Fisheries Service, and the U.S. Fish and Wildlife Service.

Barbash, Patricia - Cooperative investigations of suspect disease cases in Atlantic sturgeon. June 14-17, 2005. Eastern Fish Health Workshop, Shepherdstown, West Virginia.

FORMAL PRESENTATIONS: (continued)

Barbash, Patricia - Findings from diagnostic cases from Atlantic sturgeon at the Northeast Fisher Center. August 29, 2005. Technology and information exchange held at the Northeast Fishery Center, Lamar, Pennsylvania.

Bartron, Meredith - Genetic assessment of Atlantic salmon captive broodstock at Craig Brook National Fish Hatchery. American Fisheries Society Annual Meeting, September 14, 2005. Anchorage, Alaska

Bartron, Meredith. 2005. Genetic evaluation of Atlantic salmon captive broodstocks at Craig Brook NFH. Atlantic salmon hatchery workhop. June 28, 2005. Orono, ME

Bartron, Meredith - Genetic considerations in broodstock management. June 8, 2005. Eastern Brook Trout Workshop, Lock Haven, PA.

Bartron, Meredith - Conservation genetics considerations in hatchery and mussel management. Freshwater Mussel Workshop. August 2, 2005. White Sulphur Springs, WV.

Coll, John - The need and direction for revision of the U.S. Fish and Wildlife Service Title 50 CFR 16.13 Importation Regulations. March 4, 2005. U.S. Fish & Wildlife Service Fish Health Centers and Fish Technology Centers joint national meeting, Sacramento, CA.

Coll, John - The Role of hazard analysis and critical control point (HACCP) evaluations as part of fish hatchery biosecurity. March 29-30, 2005. USFWS-Great Lakes Sea Grant Network's Aquatic Invasive Species HACCP Program for Aquaculturists, Hatchery Managers, and Fisheries Biologists, Northeast Fishery Center -Lamar, PA.

Coll, John - Introduction to fish health management. September 13, 2005. The Northeast Conference of the Native American Fish and Wildlife Society, South Kingston, RI.

Fletcher, John - Evaluation of Atlantic salmon kelt broodstock diets. April 17-20, 2005. Sixty-first Annual Northeast Fish and Wildlife Conference, Virginia Beach , VA

Fletcher, John – Wage grade conversion proposal. July 19-20, Region 5 Project Leaders meeting, Lamar, PA

Jodun, Wade - SAMMS accomplishments and the future of real property management. July 19-20, 2005. Region 5 Fisheries Project Leaders Meeting, Lamar, PA

Jodun, Wade - The practical application of a commercially available software application to track real property maintenance. April 17-20, 2005. Sixty-first Annual Northeast Fish and Wildlife Conference, Virginia Beach, VA.

Jodun, Wade - The use of saline and theophylline as activator solutions during the fertilization process using cryopreserved Atlantic salmon sperm. April 17-20, 2005. Sixty-first Annual Northeast Fish and Wildlife Conference, Virginia Beach, VA.

Jodun, Wade - Cryopreservation of Atlantic salmon milt. February 9, 2005. Connecticut River Atlantic Salmon Bi-Annual Research Forum, Hadley, MA.

FORMAL PRESENTATIONS: (continued)

Kehler, Thomas - Year-class composition of juvenile Atlantic sturgeon in the lower Hudson River. April 17-20, 2005. Sixty-first Annual Northeast Fish and Wildlife Conference, Virginia Beach, Va.

Millard, Michael - Contaminant loads in salmonid fish in the National Fish Hatchery System, northeast region. April 19, 2005. Sixty-first Annual Northeast Fish & Wildlife Conference, Virginia Beach, VA.

Millard, Michael - Contaminant loads in salmonid fish in the National Fish Hatchery System, northeast region. Sept. 12, 2005. American Fisheries Society Annual Meeting, Anchorage, AK. (poster presentation).

Millard, Michael - Mortality associated with catch-and-release angling of striped bass in the Hudson River. Sept. 12, 2005. American Fisheries Society Annual Meeting, Anchorage, AK. (poster presentation).

Mohler, Jerre - Mussels of Muddy Creek on the Erie National Wildlife Refuge. April 17-20, 2005. Sixty-first Annual Northeast Fish and Wildlife Conference. Virginia Beach, VA.

Julian, Shannon and Meredith Bartron - Analysis of fine-scale genetic diversity among Delaware Bay horseshoe crab (*Limulus polyphemus*) spawning beaches: implications for management. September 14, 2005. American Fisheries Society Annual Meeting, Anchorage, AK (poster presentation).

Sweka, John - Asssment of negative bias in removal estimators for fish population estimation at two spatial scales. U.S. Atlantic Salmon Assessment Committee Meeting. Woods Hole, MA (oral presentation).

Sweka, John - Status of USASAC juvenile abundance database. U.S. Atlantic Salmon Assessment Committee meeting. Woods Hole, MA.

Sweka, John - Influence of timber harvest activities on the trophic dynamics of streams: Usefulness of bioenergetics models. Feb. 2, 2005. Guest lecture at Penn State University (Paola Ferrerri - Fisheries Management Course)

Sweka, John - A modeling framework for maximizing the number of returning adult Altantic salmon from hatchery production. June 28 - 29, 2005. Interagency waorkshop titled “The Science Behind Maine's ATS Program - Past Accomplishments and Future Challenges”, Orono, ME.

Sweka, John - Juvenile Atlantic sturgeon habitat use in Newburgh and Haverstraw Bays of the Hudson River, NY. Sept. 12, 2005. American Fisheries Society Annual Meeting, Anchorage, AK. (poster presentation).

NATIONAL COMMITTEE PARTICIPATION:

Barbash, Patricia - Served on the U.S. Fish and Wildlife Service annual National Wild Fish Health Survey Manual Procedures Revision Committee.

Coll, John - Served as Chairman of the U.S. Fish and Wildlife Service Title 50 CFR 16.13 Importation Regulations Revision Committee.

NATIONAL COMMITTEE PARTICIPATION: (continued)

Coll, John - Served on the U.S. Fish and Wildlife Service annual National Fish Health Policy Revision Committee.

Millard, Mike - Served on the U. S. Atlantic Salmon Assessment Committee to provide information and recommendations to the U.S. NASCO delegation.

Millard, Mike - Served on an ad hoc national contaminants committee, representing the Service's Region 5 Fisheries program, tasked with the development of a draft national Service policy regarding monitoring of contaminants in fish from the National Fish Hatchery System, and the distribution of these fish. This group met in Arlington VA in February 2005, and remains in place to complete the draft policy.

Millard, Mike - Served on the Service's Publications Subcommittee, a group of Service and USGS scientists assembled under the under the aegis of the Service Science Committee, chaired by the Science Advisor Dan Ashe. The Publications Subcommittee was tasked with developing a publications policy that would apply to all scientific publications authored or co-authored by Service employees and establishing outlets that would ensure that Service scientific publications are consistent in appearance, meet consistent standards, and are made available to the Service and the public via consistent means.

OTHER SIGNIFICANT COMMITTEE PARTICIPATION:

Barbash, Patricia - Served on the Maine Fish Health Advisory Board to make recommendations to the Maine commissioners relative to fish health issues impacting wild Atlantic salmon populations and commercial aquaculture.

Barbash, Patricia - Served on the New England Salmonid Health Committee to make recommendations to the New England Atlantic Salmon Commission (NEASC) relative to fish health issues impacting the New England states. To reflect the increase in scope of guidelines to non-salmonids, the committee name has changed to the New England Fish Health Committee. Fish health inspection protocols for LMBV, SVCV and Heterosporosis are being considered for adoption.

Barbash, Patricia, John Coll, and Gavin Glenney, - Took part in the newly formed Northeastern Black Bass Committee conference call on August 4, 2005.

Bartron, Meredith - Served as Chair for the Craig Brook National Fish Hatchery (CBNFH) Broodstock Management Plan Committee. The committee created and submitted for TAC review a broodstock management plan for CBNFH.

Bartron, Meredith - Served on the Atlantic Salmon Biological Review Team, responsible for writing an updated Status Review for Atlantic salmon.

Coll, John - Served as committee member on the Design and Location Committee for the Penobscot River Atlantic Salmon Broodstock Building for Craig Brook National Fish Hatchery.

Coll, John - Served on the Great Lakes Disease Committee to represent Region 5 relative to disease issues affecting the Great Lakes. John replaced Kimball Selmer-Larsen in this activity.

OTHER SIGNIFICANT COMMITTEE PARTICIPATION: (continued)

Fletcher, John - Served as chairman on the Design and Location Committee for the Penobscot River Atlantic Salmon Broodstock Building for Craig Brook National Fish Hatchery.

Jodun, Wade - Selected as a trainer for the Service and Maintenance Management System (SAMMS)

Millard, Mike - Organized and served as co-chair of radiotelemetry symposiuim at 2005 American Fisheries Society Annual Meeting, Anchorage, AK. Sept. 13, 2005. Symposium entitled: *Advances in Telemetry: Opportunities and Challenges for Fisheries and Aquatic Ecology*

Mike Millard, John Fletcher, and John Coll - Served on Allegheny National Fish Hatchery Review Committee to consider short and long term designs to incorporate biosecurity and enhanced fish production capabilities into the Region 5 lake trout program.

Mohler, Jerre - Served as a member of the Atlantic States Marine Fisheries Commission Atlantic sturgeon Technical Committee.

Sweka, John - Served as a technical advisor on the Northeast Black Bass Technical Committee reviewing the status of largemouth bass virus and the impacts of seasonal spawning regulations on sport fisheries.

Sweka, John - Served on the U.S. Atlantic salmon Assessment Committee maintaining databases for stocking, adult returns, and juvenile abundance data.

Study Number: LM-02-01 (study finalized in 2005)

Title: Relative abundance sampling of juvenile Atlantic sturgeon of the Hudson River

Principal Investigator: John Sweka, Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Mike Millard and Jerre Mohler (NEFC); Andy Kahnle and Kathy Hattalla (NY Dept. of Environmental Conservation)

Background and Justification

The early juvenile stage offers the best opportunity for monitoring Atlantic sturgeon (ASN) populations over time and for evaluating the population response to the current fishery moratorium. At the early juvenile stage, ASN spend the entire year in the river and have attained a size that can be effectively sampled by gill nets. A practical approach for long term monitoring of ASN population trends would be via annual estimates of relative abundance, such as mean catch per unit effort (CPUE), from a standardized sampling design.

Study Objectives

This 2-year study was aimed at developing the sampling methodology that will make assessment of trends in annual CPUE of juvenile Atlantic sturgeon possible at a practical level of field effort.

Materials and Methods

Sampling was conducted in Newburgh and Haverstraw Bays of the Hudson, as these were known areas of juvenile Atlantic sturgeon concentrations. We sampled spring (March – April) and fall (October – November) seasons beginning in October 2003 and ending in November 2005. Both bays were generally stratified according to bottom type and depth: hard/deep, hard/shallow, soft/deep, and soft/shallow. Juvenile Atlantic sturgeon were sampled using anchored gill nets of 76, 102, and 127 mm (3, 4, and 5 inch) stretch mesh deployed in a block pattern at randomly chosen sites within habitat strata. We set the nets perpendicular to the shore in Haverstraw Bay, but experimented with parallel vs. perpendicular sets in Newburgh Bay to determine if the orientation of the nets influenced catches of juvenile Atlantic sturgeon. We also compared catches during slack tides vs. running tides.

Results

A total of 562 juvenile Atlantic sturgeon were caught during the course of this study. The majority of these fish were caught in Haverstraw Bay. Frequency of occurrence and CPUE of juvenile Atlantic sturgeon was consistently highest in soft/deep areas of Haverstraw Bay. Seasonal comparisons showed CPUE within soft/deep areas of Haverstraw Bay was greatest during mid to late spring.

Although there was a high degree of overlap in the size distributions of juvenile Atlantic sturgeon caught in each gill net mesh size, 127 mm stretch mesh tending to catch larger fish than 76 or 102 mm stretch mesh. Tidal state (slack vs. running) and net orientation (parallel vs. perpendicular) did not influence catches of juvenile Atlantic sturgeon.

For future monitoring of relative abundance of juvenile Atlantic sturgeon in the Hudson River we recommend sampling in soft/deep areas of Haverstraw Bay during mid to late spring seasons when water temperatures exceed 4^{o}C using 76, 102, and 127 mm stretch mesh anchored gill nets. Sample sizes required to detect a given rate of CPUE change over time were also calculated and may be used by the Hudson River Fisheries Unit to decide how much effort to expend during future monitoring.

Study Number: LM-05-01

Title: Effects of handling and disinfection on eye-up of Lake Trout eggs at Allegheny National Fish Hatchery (ANFH)

Principal Investigator: John Coll, N.E. Fishery Center (NEFC)
Co-Investigators: Tracey Copeland and Dave Blick (ANFH); John Sweka (NEFC); P. Barbash (NEFC); J. Mohler (NEFC)

Background and Justification

The primary fish production program at the Allegheny National Fish Hatchery (ANFH) is directed towards restoration of Lake Trout (LKT) for the Great Lakes. Two strains of trout are spawned at the hatchery to produce progeny for the restoration stocking program: Seneca strain (SLW) and Superior strain (SMD). Total egg eye-ups are typically measured at 68– 80% (D. Blick, USFWS, personal communication). In both 1998 and 2003 eye-ups were much lower than expected for SLW at 34 and 24%, as well as SMD at 37 and 54%, respectively. Temperature abnormalities were recorded during both years: In 1998, incubation temperatures reached about 8° F higher than normal due to a chiller malfunction and in 2003, just before egg take, ambient water temperature in broodstock rearing units reached an all-time high of 56.6° F before slowly declining through the egg take period (end of Oct. through end of Nov). Nonetheless, in order to discover procedures which may improve egg survival, 4 treatments of fertilized eggs were established at the ANFH using eggs from fall-spawned LKT.

Study Objectives

We utilized about 240K fertilized eggs from LKT spawned at ANFH in November, 2004 to discover whether any of 3 alternative egg handling / disinfection techniques resulted in improved eye-up over a control group of normally-processed eggs.

Methods.

Eggs from 12 females were pooled and gently mixed then equal portions were placed into each of 12 basins (4 treatments X 3 replicates each). Milt was obtained from about 20 males and kept in separate vials until just prior to fertilization at which time it was divided equally into 6 containers at a volume of 12 ml each. One-half of the volume of each container was then used to fertilize each of the 12 basins of eggs. The 4 treatment groups were: (1) Treatment **(H)** - To minimize the possibility of handling eggs at a period of time critical to early mitotic divisions, eggs were water-hardened using iodophor in the incubation tray as soon as possible after fertilization. After 30 minutes exposure to iodophor, each tray was pushed into the stack and allowed to rinse via the incubation flow. Trays positioned below were then filled with other production eggs from the top down. (2) Treatment **(S)** – To minimize bacterial infestation, eggs were rinsed with a 0.69% saline solution both before and after fertilization to remove bacteria, and organics which could block the egg miccropyles (3) Treatment **(HS)** - A combination of treatments H and S and (4) Control **(C)** – Fertilization and disinfection procedures as normal performed at Allegheny NFH. Each treatment was established in triplicate trays placed at tray position #2 among 12 stacks of incubation trays. Egg survival as % eye-up was compared via ANOVA between treatments. Bacterial colony forming units was obtained from each replicate pre-and post disinfectionand at eye-up. Yellow pigmenting isolates were confirmed as *Flavobacterium psychrophilum* (coldwater disease) by polymerase chain reaction methods.

Results

Mean % eye-up was considered low for all treatments but ranged from 30.9 in the **(C)** group to a high of 39.6 in the **(HS)** group which was significantly higher than other treatments (P=0.001). However, these differences may not be truly significant since there were infrastructure problems at ANFH which may have caused differential environmental parameters between treatments during incubation.

Low levels of *Flavobacterium psychrophilum* were detected in isolates from some egg samples but they were not judged to be high enough to cause important amounts of egg mortality.

Study Number: LM 05-02

Title: Evaluation of Atlantic salmon kelt broodstock diets

Principal Investigator: John Fletcher, Northeast Fishery Center (NEFC)
Co-Investigators/Cooperators: Dale Honeyfield, Northern Appalachian Research Laboratory; George Ketola, Tunison Laboratory of Aquatic Science; Ann Gannam, Abernathy Fish Technology Center; Larry Lofton and Fred Yost, North Attleboro NFH; John Sweka, NEFC

Background and Justification

Reproduction from Atlantic salmon (ATS) kelts represents valuable genetic and numeric contributions to restoration fry stocking. However, survival, maturation and gamete quality of kelts has been inconsistent. Nutritional variability and seasonal availability of raw ingredients found in the standard formulation are viewed as potential problems.

Study Objectives

The present study examines the nutritional effect of two diets (standard *vs* USGS) upon kelt reproductive success.

Method and Materials

Biochemistry of the standard diet was examined by comparing mineral and lipid profiles of eggs from wild sea-run ATS and hatchery rejuvenated kelts collected in 2000. The analyses showed that kelt eggs were deficient in copper and selenium and contained excessive amounts of manganese relative to sea-run eggs. The standard diet was found to contain high levels of copper, zinc, manganese and selenium. A five fold reduction of mineral premix was recommended to correct the mineral imbalance found in the standard diet at study inception in 2001. An alternative USGS diet formulation based upon advances in nutritional research moved to processed meals and lower levels of minerals in readily absorbed chelated form. Merrimack and Connecticut River ATS kelts at North Attleboro NFH received standard and USGS diets from rejuvenation in 2001, 2002 to spawning in 2003 and 2004. Evaluation of gamete quality as measured by survival to eye-up was determined for each mature female from a diet-river group by fertilization with milt from all males representing that group; viability of each male from a diet-river group was measured against a pooled composite of cohort eggs.

Results

Variation in individual performance within trial groups was evident (Std. Dev. ~ 24 %). Although increase in kelt age ($P = 0.04$) negatively impacted gamete viability, differences were not detected for river of origin ($P = 0.67$) or between standard (73%) and USGS diets (66%), ($P = 0.31$). Lipid and mineral analyses are to be conducted in FY 2006 upon trial diets and 2003- 2004 kelt eggs and analyses will be examined for potential correlation to reproductive success.

Study Number: LM-05-03

Title: Detection of calcein marks in juvenile Eliptio complanata mussels planted in their natal stream

Principal Investigator: Jerre W. Mohler-USFWS NE Fishery Center, Lamar, PA
Cooperators: Jeff Cole – USGS Northern Appalacian Research Lab, Wellsboro, PA

Background and Justification

As a result of the magnitude and immediacy of the nationwide threats to native freshwater mussel fauna, the National Native Mussel Conservation Committee (NNMCC) produced a conservation document titled: National Strategy for the Conservation of Native Freshwater Mussels which contains various strategies for reaching conservation goals. Included are recommendations to determine the viability of artificially-propagated juveniles to determine their suitability for release in restoration and recovery plans. This necessitates some type of marking technique to permit differentiation between hatchery-reared and wild mussels. Previous research with oxytetracycline (OTC) and calcein to batch mark juvenile mussels via immersion showed that calcein produced brighter and more recognizable marks on mussel shells. The USFWS-Northeast Fishery Center in Lamar, Pennsylvania (NEFC) has been performing experimental fish marking with calcein since 1995 and has patented a calcein mark detector which allows the operator to immediately discern between marked and non-marked fish by the presence or absence of a visible green fluorescence in the fin rays and other calcified structures. In this study we will collect wild juvenile Eliptio complanata mussels and use them to evaluate the utility of the calcein detection device to differentiate between calcein-marked and unmarked individuals transplanted back into their natal stream.

Objectives

Beginning in the summer of 2005, we will use approximately 60 juvenile E. complanata collected from wild to determine the utility of the calcein detection device for differentiating marked vs. un-marked individuals after 1 year of growth in the wild.

Methods

In August 2005, a minimum of 60 juvenile E. complanata mussels will be collected from the Conodoguinet Creek in Cumberland County, PA. Once collected, mussels will be transported to NEFC, where they will either receive a SE-MARK^{TM} calcein treatment or remain untreated. After marking, mussels will be observed for one week to monitor mark uptake and mortality. After a week-long monitoring period for mark detection and mortality, each mussel will be tagged with a uniquely number floy tag attached to the shell via superglue. Test animals will be measured for length with equal numbers from each treatment returned to three specific locations marked with rebar driven into the substrate near their place of capture. After 1 year, mussels will be recovered and evaluated as before. Marks will be scored via photographs using color analysis functions in commercial software. Numeric values of mark brilliance and mussel size will be compared statistically between treatments using T-tests. Alpha levels will be 0.05. The null hypotheses to be tested are:

> H_o 1= Calcein marks are not detectable in mussels with the hand held detection device after one year in the wild
>
> H_o2 = There is no difference in mark brilliance or growth between treatment groups.

Results

Only a small number of adult E. complanata were found and no native juvenile mussels were found at the Conodoguinet Creek site. Numerous field trips to this site over the last 5 years have shown the native mussel species are being displaced by the Asian clam Corbicula fluminea an invasive species which was found in abundance during the 2 field trips to the site in 2005. The study will be performed in 2006 using mussels from another source.

Study Number: LM-05-04

Title: The effect of artificial substrate in incubation trays on the size and yolk condition of emerging Atlantic salmon *(Salmo salar)* fry.

Principal Investigator: Patrick Farrell, Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: John Sweka and Wade Jodun (NEFC)

Background and Justification

The NEFC incubates from 3000 to 30000 Atlantic salmon eggs annually using standard stacked incubation units (Heath Tecna Corp., Kent, Washington USA) that have factory installed fine mesh screen bottoms in the trays. Kelt eggs from North Attleboro National Fish Hatchery were incubated at NEFC. Four of the six groups were incubated in trays that had 8mm square by 2mm deep plastic grids installed over the factory screen bottoms. Mean length and weight of swim-up fry from trays with the extra grids were 24.0mm & 0.21g respectively; those without the extra grids were 23.4mm & 0.16g. No formal analysis was conducted, but the observed differences indicated that a controlled study would be in order. Therefore, we formalized this study to measure differences in the size of Atlantic salmon fry incubated with and without substrate.

Study Objectives

In 2005 we will incubate 8 trays containing Atlantic salmon eggs to test for differences in length, weight and survival between different substrates.

Materials and Methods

A total of 8 trays, four each from the 2 stacks that are available at NEFC, were divided in half length-wise with perforated sheet PVC cut to fit and glued in place. A double layer of 8mm square by 2mm thick plastic grid will be placed on the left side of 4 trays & the right side of the remaining 4 for a 8mm square by 4mm deep grid. The North Attleboro NFH Kelt eggs were pooled and equal amounts were placed in each of the 16 compartments. In an attempt to minimize tray effect the trays were placed in drawers starting with #2 and descending to #5 with the grid sides being placed so that one will not be directly below the one above. The second stack will mirror the first. Flows through the stacks were maintained at 15.2 L/min and any treatments will follow NEFC standard protocols. Individual lengths and weights were taken from either 50 or 10% of the population, dependant on the population size at the end of the study. Data analysis was performed using SAS/STAT software (SAS, 1990). To avoid problems associated with pseudoreplication, tank means were used as experimental units in all analyses (Hurlbert 1984). Percent survival data was arcsine square root transformed prior to all analyses to stabilize non-constant variance (Zar 1984). Data were checked for normality (Kolmogoroff-Smirnoff's test) and equality of variance (Levene median test). To test for differences in length, weight and survival among substrates data were analyzed as a randomized complete block design with tray position as the blocking factor. Tukey's Honestly-Significant-Difference test was used for all posthoc comparisons. All statistical analyses were performed at an alpha level of $P<0.05$.

Results

There was a significant difference in the mean lengths and weights of fry reared with substrate versus no-substrate at 29.4 vs. 28.8 mm and 24.9 vs. 22.9 g, respectively. However, survival was found to be higher in the no-substrate treatment, perhaps due to a problem with the artificial substrates coming loose in some of the trays and trapping fry beneath them, resulting in an increased mortality. Only one informal observation was made for deformed yolk-sacs (10 for substrate versus 72 for no-substrate. This may be an indication that incubating fry with substrate could reduce abnormalities in yolk-sac development.

Study Number: LM-05-05

Title: Assessment of watershed scale habitat features on the survival of juvenile Atlantic salmon

Principal Investigators: Meredith Bartron and John Sweka, Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Joan Trial and Paul Christman (Maine Atlantic Salmon Commission)

Background and Justification

Atlantic salmon populations are currently at all time low levels throughout New England and eight rivers in Maine were listed as an endangered species by the USFWS and NOAA Fisheries in December 2000. Present recovery efforts rely heavily on the stocking of juvenile Atlantic salmon with the majority of fish being stocked as fry. In order for recovery efforts to be successful there is a need to identify areas of the watershed which yield the greatest fry to parr survival and contribute most to the outmigrating smolt population. Identification of such areas will allow managers to refine fry stocking practices to increase survival to the parr stage and optimize the number of outmigrating smolts per the number of fry stocked. Also, identification of critical juvenile Atlantic salmon production areas will help guide future salmon habitat enhancement and restoration efforts.

Study Objectives

(1) Determine quantitative relationships between inter-stage survival of juvenile Atlantic salmon and macrohabitat variables such as watershed area, temperature, pH/Alkalinity, stream gradient, abundance of non-salmon species, and abundance of predatory species.
(2) Use genetically marked fry to identify the rearing locations of outmigrating Atlantic salmon smolts and assess relative survival to the smolt stage from various stocking locations.

Materials and Methods

Sheepscot River 2004 broodstock at Craig Brook National Fish Hatchery were genotyped using highly polymorphic microsatellite DNA markers. Using genetic parentage analysis as a “mark”, we will be able to identify stocking location, and use the recapture and abundance of the marked fish to evaluate survival. Within a given river reach, a single genetic group of fry was stocked in May 2005. Estimation of survival to parr stages began in the September 2005. Age-0 parr survival was assessed at 27 electrofishing sites throughout the watershed. A subsample of parr had a fin clip taken for genetic analysis to determine the degree of immigration from fish stocked in other river reaches. Abundance of non-salmon species was also estimated at each electrofishing site. Survival of age-0 parr to the age-1 parr stage will be assessed during the early fall of 2006 in the same manner. Survival to the smolt stage will be assessed using a rotary screw trap near the Head Tide Dam on the mainstem of the Sheepscot River in the spring 2007. Upon collection, smolts will have a fin clip taken for parentage analysis which will identify the location of stocking as fry. Multiple regression analysis will be used to determine relationships between site level survival to each lifestage and macrohabitat features such as watershed area, stream gradient, temperature (proportion of time within the range for positive salmon growth), minimum and mean pH/Alkalinity, and biological components such as the abundance and biomass of non-salmon species and predatory species.

Results

Data entry and QAQC measures for initial data from September 2005 were not yet completed by the time of this report.

Study Number: LM-05-06

Title: Annual genetic characterization of Atlantic salmon broodstock at Craig Brook National Fish Hatchery (CBNFH)

Principal Investigators: Meredith Bartron-USFWS Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Tom King and Denise Buckley-USFWS CBNFH

Background and Justification

Atlantic salmon broodstock management incorporates the use of genetic characterization of all potential broodstock. Genetic characterization is used to track changes in estimates of genetic diversity, monitor for evidence of artificial selection, and manage the future spawning stock to maintain genetic diversity over time. Genetic data is used to identify optimal spawning pairs, assess recovery of stocked juveniles, and screen for potential aquaculture escapees. Characterization is completed annually for parr collections for future use as broodstock from the six populations maintained at CBNFH, and also for adult salmon returning to the Veazie Dam on the Penobscot River. Genetic characterization and monitoring of the broodstocks follows recommendations described in the CBNFH Broodstock Management Plan, and the Recovery Plan for Atlantic salmon.

Study Objectives

(1) Genetically characterize all Atlantic salmon incorporated into the broodstock programs at Craig Brook National Fish Hatchery. Samples include parr collected from DPS populations and adults from the Penobscot River.
(2) Use estimates of genetic diversity to monitor for changes in genetic diversity, determine familial representation and recovery of stocked families in parr collections, and screen for aquaculture escapees.

Materials and Methods

Eleven microsatellite loci are used to genetically characterize the broodstock. Results are used to examine levels of heterozygosity, number of alleles per locus, alleleic diversity, relatedness, and subsequently used for parentage analysis and during spawning.

Results

Broodstock characterization of the DPS populations was completed for the capture year up to and including 2003. Results from the analysis were presented at the Hatchery Workshop in June, 2005 in Bangor, Maine. Samples from the 2004 capture year from the DPS populations were received in the fall of 2005 and will be analyzed in the spring of 2006. All samples were characterized from adult Atlantic salmon returning to the Veazie Dam on the Penobscot River and subsequently spawned at Craig Brook, including the years between 2002 and 2005, thereby completing years for which data had not been analyzed. Results from these analyses will be included in a report to be appended to the Broodstock Management Plan.

Study Number: LM-05-07

Title: 2005 pedigree line for the Pleasant River broodstock at Craig Brook National Fish Hatchery

Principal Investigators: Meredith Bartron-USFWS Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Tom King and Denise Buckley-USFWS CBNFH

Background and Justification

Due to a small broodstock population size and poor recovery of stocked hatchery juveniles, a pedigree line was established for the Pleasant River. To ensure adequate numbers of individuals in the broodstock, the use of a pedigree line was implemented for the offspring from the 2003 spawning year for the Pleasant River. A pedigree line is composed of two parts, where fry from all families are mixed, and half of the juveniles are retained in the hatchery (domestic portion), and the other half are stocked into the river according to stocking practices (captive portion). Following collection of the captive portion from the river as parr, all individuals from both portions are genotyped to determine the familial representation within each group. Individual familial contribution is equalized using individuals from both groups, and then surplus individuals (to broodstock needs) are stocked into the river as smolts.

Study Objectives

1) Ensure a sufficient captive effective population size is available for use as broodstock,
2) Reduce the potential for loss of genetic diversity if only a few parr from a few families were recovered,
3) Reduce the threat of inbreeding when the effective population size is low.

Materials and Methods

Tissue samples were provided to the FWS Region 5 Conservation Genetics lab from individuals from both the domestic and captive portions of the pedigree line. All tissue samples correspond to an implanted PIT tag in each juvenile for future identification. Eleven microsatellite loci are used to genetically characterize the broodstock, and parentage analysis will be completed to determine familial representation.

Results

-Tissue samples have been received from 98 juvenile Atlantic salmon as the captive portion, and 1579 juveniles that were retained in the hatchery (domestic portion).
-Genotyping and parentage analysis will be completed prior to the end of March 2006 to allow for stocking of surplus juveniles as smolts.

Study Number: LM-05-08

Title: Genetic characterization of an isolated population of Northern Plymouth Red-bellied Cooter (*Pseudemys rubriventris*) in Massachusetts

Principal Investigator: Meredith Bartron, Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Michael Amaral (USFWS-New England Field Office); Shannon Julian (NEFC)

Background and Justification

The Northern Red-bellied cooter is currently listed as endangered in Massachusetts. There is some discussion regarding whether or not the Massachusetts population is a subspecies, or represents a single isolated population. Because the Massachusetts population is isolated and its status is supplemented by a head-start hatching program, there are additional concerns about the genetic diversity and long-term viability of the population, particularly through high levels of inbreeding. Information about the genetic diversity observed in the Massachusetts population will be used by agencies to revise management and conservation strategies as needed.

Study Objectives

The goal of this project is to characterize the genetic diversity observed in an isolated turtle population in Massachusetts relative to populations observed in other parts of its range. Due to the isolated nature of the population, there is an increased potential for inbreeding to occur in the population, which could result in decreased fitness of individuals in the population, increasing the potential for localized extinction.

Materials and Methods

Samples will be obtained from juvenile turtles in the head-start program run by Massachusetts Department of Wildlife to characterize the isolated population. In addition, samples are being obtained from populations in Maryland, Pennsylvania, Virginia, New Jersey, and North Carolina for comparison to the Massachusetts population. Multiple microsatellite markers will be used to obtain genotypes used to characterize individuals from each population, and data will be analyzed to determine the partitioning of genetic variation observed within and between populations.

Results

This study is ongoing and is estimated to be completed in the summer of 2006.

Study Number: LM-05-09

Title: Genetic characterization of two northern riffleshell (*Epioblasma torulosa rangiana*) populations in the Allegheny River

Principal Investigator: Meredith Bartron, USFWS-Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Catherine Gatenby (USFWS-White Sulphur Springs)

Background and Justification

The northern riffleshell (*Epioblasma torulosa rangiana*) is currently listed as an endangered species. Two isolated populations are thought to be the only populations of northern riffleshell remaining in the Allegheny River, a tributary to the Ohio River. The two populations in the Allegheny River are currently threatened by road construction projects. To prevent potential harm to the population due to increased sedimentation due to bridge construction, both groups of mussels will be temporarily removed from the area and housed at the USFWS White Sulphur Springs (WSSNFH) mussel culture facility.

Study Objectives

The use of refugia and captive propagation for mussel culture presents unique opportunities to obtain genetic material from mussels. We propose to characterize estimates of genetic variation in each of the populations to identify relative isolation of the populations, estimate inbreeding coefficients, and determine genetic viability of each population. Additionally, captive breeding protocols will be developed to ensure long-term maintenance of genetic diversity.

Materials and Methods

Tissue samples will be obtained non-lethally from the captive northern riffleshells held at WSSNFH. Variable microsatellite markers will be used to characterize genetic diversity of the two captive populations. Genetic information for each population will be used to estimate gene flow between populations and levels of genetic diversity within and between the two populations.

Results

Initial sample collection experiments were completed in the summer of 2005. Sample collection and analysis are expected to be completed by fall of 2006.

Study Number: LM-05-10

Title: Evaluation of genetic diversity and relatedness for Atlantic sturgeon (*Acipenser oxyrinchus*) captively held by Maryland DNR

Principal Investigator: Meredith Bartron, Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Brian Richardson (Maryland DNR)

Background and Justification

Hatchery supplementation has often been used as a tool in fisheries management to restore declining populations, supplement existing populations, or introduce new species. An important component of hatchery supplementation is broodstock management. The primary goals of captive broodstock management for restoration or recovery of declining populations include minimizing inbreeding potential and maintaining genetic diversity. To minimize inbreeding and maintain genetic diversity, the primary broodstock techniques focus on the number of adults used for spawning and the mating strategy used for propagation. Maryland Department of Natural Resources (MDDNR) currently maintains a captive broodstock for Atlantic sturgeon. Offspring resulting from captive broodstock propagation would be incorporated into a stocking program for the greater Chesapeake Bay area. Juvenile Atlantic sturgeon were obtained by MDDNR from the Chesapeake Bay.

Study Objectives

In this report, we used microsatellite markers to estimate measures of genetic diversity for individuals from both the wild caught and captively bred groups of Atlantic sturgeon by MDDNR for use as broodstock. Information including genetic variation (heterozygosity), relatedness (proportion of shared alleles), and genetic distance were used to quantify each group.

Materials and Methods

Samples were provided for a total of 66 fin tissue samples were taken from wild Atlantic sturgeon. Allele frequencies, observed heterozygosity, number of individuals per locus were calculated using GENEPOP (Raymond and Rousset 1995), and GDA (Lewis and Zaykin 2001). Pairwise relatedness values (R_{xy}) for individuals was calculated using SPAGeDi (Queller and Goodnight 1981).

Results

- A total of 66 individual Atlantic sturgeon were genotyped using 15 microsatellite loci.
- The wild-caught population had an average of 13 alleles per locus for 15 loci, observed heterozygosity of 0.827 (H_o), and fixation index (f) of 0.035 (Table 1).
- Mean pairwise relatedness was -0.017 (R_{xy}), similar to values observed in wild-caught juveniles from Chesapeake Bay in a previous study.
- This information will be used in conjunction with analyses from previous years to develop a broodstock management plan and spawning design for the Maryland captive sturgeon.

Study Number: LM-05-11

Title: Genetics Assessment of Susquehanna River Shad

Principal Investigator: Meredith Bartron, FWS Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Shannon Julian, FWS Northeast Fishery Center (NEFC)

Background and Justification

Hatchery propagation and stocking of American shad also represent a large component of the restoration efforts. In a subsample of the returning adult population at Conowingo Dam West Fish lift in 2003, approximately 26% of the adults were of wild origin, and 74% were of hatchery origin (Hendricks 2003a). The Susquehanna River Anadromous Fish Restoration Committee (SRAFRC) collects shad eggs from two locations on the Susquehanna River, and from the Hudson and Delaware River for stocking in the Susquehanna River. If possible, determination of the source of wild origin shad in the Susquehanna that successfully returns as adults (as the marked individuals) which then successfully produce offspring that return as adults (unmarked) will aid in restoration efforts, through the targeting of those stocks for stocking efforts.

Study Objectives

We propose to conduct genetic investigations of adult shad returning to the Lapidum area and to Conowingo Dam in the spring of 2005, to identify the source of wild-origin adult shad. Comparisons of estimates of genetic diversity between shad of hatchery and wild origin may identify which introduced stocks were successful in establishing reproducing populations. Additionally, characterizing shad utilizing different sections of river may aid in the understanding of life history characteristics and behavior.

Materials and Methods

We will use a combination of molecular markers to quantify genetic diversity, estimate of diversity and the partitioning of genetic variation for shad returning throughout the run. Additionally, in combination with otolith analysis to identify hatchery or wild origin, genetic comparisons can be made between individuals of different origin. To conduct these analyses, we are developing additional microsatellite markers to aid in population resolution.

Results

So far, we have developed microsatellite libraries for American shad, and sequenced the shad DNA inserts obtained from bacteria used in the marker development process, resulting in 1200 sequences that have potential microsatellite loci. Tissue samples were collected from adult shad in the Hudson, Delaware, and Susquehanna rivers, and otoliths have been analyzed by PFBC. Work remaining includes completion of marker development, genetic analysis of the collected shad samples, and data analysis and report preparation. Completion of this project is expected in the spring of 2007.

Study Number: LM-05-12

Title: Characterization of the brook trout population in a degraded North-Central Pennsylvania stream and assessment of the population response after stream rehabilitation

Principal Investigator: Jerre W. Mohler; Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: John Sweka – NEFC, Dave Keller – PA Fish & Boat Commission, Andy Eldred - PA Dept. of Environmental Protection; Kevin McJunkin – Lycoming County Planning Comm.; Lycoming Creek Watershed Association

Background and Justification

Aquatic resources in the U.S. are in decline with habitat loss or alteration being one of the principal reasons. Habitat alteration is a major contributing factor to 75 percent of all fish extinctions during the past 75 years and 91% of fish listings under the Endangered Species Act. To address this problem, national conservation leaders have developed the National Fish Habitat Initiative (NFHI). Currently, a geographic model for implementing the NFHI known as the Eastern Brook Trout Joint Venture (EBTJV) is being jointly developed by the U.S. Fish and Wildlife Service, U.S. Forest Service, the International Association of Fish and Wildlife Agencies, thirteen state fisheries agencies, Trout Unlimited, the Izaak Walton League, and the National Biological Information Infrastructure (NBII). One focus area of the EBTJV is to restore impaired populations of brook trout which have been impacted by these changes.

Approximately 800 feet of lower Wolf Run, a tributary to Lycoming Creek in Lycoming County, PA. downstream of the existing SR0015 limited-access highway has been degraded by gravel extraction. Currently, this section of stream is a candidate for rehabilitation by PAFBC using fish habitat improvement structures under the Adopt-a-Stream program. Proposed stream improvement structures are designed with natural materials to enhance the stream channel. In addition, they involve only minor disturbance to the stream, blend in with their surroundings, and often create stream bank stability as a secondary benefit. To evaluate the response of the BKT population to changes brought about by stream rehabilitation, electro-fishing surveys of the treatment stream (Wolf Run) and a reference stream (Daugherty's Run) before and after stream rehabilitation will be performed.

Study Objectives:

We will determine the baseline numbers and biomass of wild BKT in the study area during October, 2005/06 and measure changes in those population parameters for a minimum of 3 years subsequent to implementation of stream rehabilitation devices.

Materials and Methods:

Two tributaries of Lycoming Creek will be used in the evaluation: Wolf Run and Daugherty's Run. Wolf Run will be the treatment (manipulated) stream with 800 lineal feet undergoing rehabilitation. In order to verify that natural population variation is not responsible for changes which are measured in the rehabilitated stretch, an additional 800-foot reference section immediately upstream of the manipulated reach will also be evaluated. Additionally, a section of a nearby similar tributary known as Daugherty's Run will serve as a reference stream. Baseline BKT data will be collected during October, 2005/06 to coincide with spawning season. Backpack electro-fishing equipment will be used to perform multiple-pass removal of BKT. Numbers of BKT captured on successive passes will be used to calculate the maximum likelihood estimate of population size for each 800-ft. stream section. Subsequent to stream rehabilitation, the BKT populations will be assessed as before and on similar calendar dates for a minimum of 3 years. The null hypothesis to be tested is: Number and biomass of brook trout in the treatment stretch of Wolf Run will remain unchanged after stream rehabilitation. This hypothesis will be tested using a "Before-After-Control-Impact" (BACI) study design which compares the ratio of treatment to reference populations from before manipulation to after manipulation using t-tests.

Results:

The fall 2005 baseline survey resulted in 11 BKT trout captured during 3-pass removal in the degraded section of Wolf Run while the undisturbed upstream section yielded 89 BKT. The reference stream section of Daugherty's Run yielded 47 BKT. This study will be on-going for a number of years.

Study Number: LM-05-13

Title: Removal of phosphorous using pelletized acid mine drainage sludge

Principle Investigator: Thomas Kehler, USFWS Northeast Fishery Center
Co-Investigator/Cooperator: Phillip L. Sibrell, USGS Leetown Science Center (USGS LSC)

Background and Justification

Phosphorous is usually found to be the critical limiting nutrient within aquatic systems. Because it acts as a limiting nutrient, the health of the system may depend upon the quantities available. Excess amounts of phosphorus inputs contribute to eutrophication, which alters chemical and biological components within an aquatic system. The discharge of phosphorous into aquatic systems from fishery facilities is a concern. Although several forms of phosphorous may occur within an aquatic environment, the most readily available form to aquatic organisms are inorganic phosphates, also know as orthophosphates. The ability to decrease input loads of orthophosphates would be an important step in decreasing the incidence of eutrophication; thus, protecting our natural systems.

Study Objectives

Determine the effectiveness of pelletized acid mine drainage sludge (AMDS) for orthophosphate removal from a small fish rearing unit.

Materials and Methods

This study is in operation at the USFWS Northeast Fishery Center, Lamar, Pennsylvania. Thirty-six kilograms of rainbow trout are being reared in a 1.5-meter fiberglass circular tank. Fish are fed 0.5% of their biomass daily using a 12-hour belt feeder. An onsite spring provides an inflow of 7.56 l/m. An external pump creates a current within the tank, assisting in solid waste removal. Two flow-through columns containing pelletized AMDS receive an effluent of 3.78 l/m from the circular tank in alternating twelve-hour intervals by use of an electronic flow control device and pump. Supplementary oxygen supply is provided by an oxygen generation system.

Effluent collection and analysis- Water samples are collected twice a day, two days a week from three different sources: Inflow, the column which is discharging, and within the circular tank (representing tank effluent). Water samples are processed using the Ascorbic Acid method for orthophosphates and analyzed by a spectrophotometer. Dissolved oxygen concentration and temperature are monitored weekly.

Results

-Columns #1 and #2 removed a total average of 53.4% and 47.4%, respectively, of orthophosphates from their effluent from September 20th to November 10, 2005.

-From the sampling period December 5th to January 20, 2006, Column #1 removed 46.9%, while column #2 removed 45 % of orthophosphates from their effluent. The study is ongoing.

OTHER BIOLOGICAL INVESTIGATIONS AND RELATED ACTIVITIES PERFORMED:

LM05A Congressional contacts.- Mike Millard met with Congressman John Peterson (R, PA-5th) and his staff in Washington DC twice during FY05 to discuss the Center's programs and update the Congressman on fisheries issues in and around the district. Contact: Mike Millard

LM05B Visit from the Director.- In October 2004, the Center enjoyed a visit from Service Director Dr. Steve Williams, in what we believe is the first personal visit by a standing Director in the history of the facility at Lamar. Director Williams spent almost a full day touring the facility and meeting with staff.

OTHER BIOLOGICAL INVESTIGATIONS AND RELATED ACTIVITIES PERFORMED: (continued)

LM05C Salvage and refuge of mussels.- In August the Center assisted White Sulphur Springs National Fish Hatchery with transporting mussels removed from the East Brady, PA bridge replacement site to refugia at White Sulphur Springs National Fish Hatchery, WV as part of the mitigation for the bridge replacement project. The site contained federal and state-listed species as well as more secure species. Contact: Patrick Farrell

LM05D Genetic characterization and marking of Merrimack River Atlantic salmon.- Atlantic salmon were extirpated from the Merrimack River in the mid-1800's when dams constructed for hydropower blocked salmon from returning to upstream spawning habitat. The objective of this study is to determine the effectiveness of the hatchery stocking and breeding program on the Merrimack River. This project characterized sea run broodstock, the kelt broodstock, and returning adults to the river. Parentage analysis will be used to identify parents of returning adults, which will allow determination of parental source (sea-run or kelt), and stocking location of the family group to evaluate stocking habitat on the survival to adult stage. Samples have been obtained from the broodstocks, and have been genotyped. Future returns of adults to the Merrimack River will also be analyzed as part of this study. Project is in conjunction with Nashua National Fish Hatchery. This project is ongoing. Contact: Meredith Bartron

LM05E Genetic characterization of "wild" Penobscot River Atlantic salmon. – Atlantic salmon that are "wild" can either be the product of natural spawning by adult salmon, or result from stocking as fry from hatchery-spawned adults. A small number of wild adults returning to Veazie Dam on the Penobscot River, and passed upstream (so therefore not previously genotyped as part of broodstock management efforts) were identified as of wild origin by Maine Atlantic Salmon Commission. If these fish are the result of fry stocking, their parentage could be determined due to the known paired matings for all hatchery-spawned Penobscot adults. If the product of river spawning, then their parentage could not be determined because their parents would be unknown. The FWS Region 5 Conservation Genetics Lab is conducting the analysis of these fish for the Maine ASC as part of their management efforts, and represents an annual assessment by the lab. Contact: Meredith Bartron

LMO5F Participation in the National Wild Fish Health Survey.- This project, launched in 1997, continues to involve all nine Service Fish Health Centers nationwide, incorporating standardized diagnostic techniques and data management methods to ensure comparability. In fiscal year 2005, the Lamar Fish Health Center initiated 14 cases for the Survey, in which 395 fish (11 different species) from a total of 12 sites were examined and efforts continued to enter completed cases into the NWFHS database. Species of major interest this year included Atlantic salmon, brook trout, and brown trout for management programs; and largemouth bass, smallmouth bass, redbreasted sunfish, and pumpkinseeds for prevalence of largemouth bass virus (LMBV). The National Wild Fish Health Survey Database which is capable of single and double queries based on either fish species or fish pathogens, is now accessible via the internet on the Service website. The National Wild Fish Health Survey is partnership driven. In FY2005 the Lamar Fish Health Unit conducted cooperative work with West Virginia Department of Natural Resources, Vermont Fish and Wildlife Department, West Virginia Department of Natural Resources, and the Pennsylvania Fish and Boat Commission on assessing the prevalence of largemouth bass virus in bass residents of several bodies of water. Another new partner to the Survey, the U.S.Geological Survey National Office in Reston, Virginia, provided fish health samples from fish being collected in the Charles River in Massachusetts for contaminants. Outreach activities to increase awareness of the National Wild Fish Health Survey and involve other Service and partnering programs continue. Contact: John Coll

OTHER BIOLOGICAL INVESTIGATIONS AND RELATED ACTIVITIES PERFORMED: (continued)

LM05G Region 5 Fisheries Project Leaders meeting.- In July, 2005, the Center hosted the Region 5 Fisheries Project Leaders meeting, which was also attended by Geoffrey Haskett, acting Regional Director and Rick Bennett, Deputy Regional Director. Approximately 32 project leaders, staff, and Regional Office personnel assembled in the new Conference Room in the Genetics Lab to exchange ideas and formulate plans regarding the future of the R5 Fisheries program. Tom Busiahn from the Washington Office Fisheries and Habitat Conservation program and Kathi Bangert from R5 External Affairs also provided some new perspectives for the group. A catered BBQ dinner was held on the grounds one evening and was enjoyed by all. Contact: Mike Millard

OTHER BIOLOGICAL INVESTIGATIONS AND RELATED ACTIVITIES PERFORMED: (continued)

LM05H Fish Health Inspection/Monitoring/Diagnostic Services - The Lamar Fish Health Center processed 315 laboratory cases in 2005. Region 5 has an extensive fish health monitoring program to enhance the fish health inspections, allowing continual surveillance of the health of the stocks, some of which have been identified as limited distinct population segments (DPS) which the Service has listed under the Endangered Species Act. This surveillance consists of screening mortalities for parasites, bacteria, and viruses, and accounted for over 250 fish tested from 5 Service broodstock facilities throughout the year. Another strategy that enhances the screening of Service stocks is the collection and testing of spawning products for those pathogens that are passed on to offspring. In 2005, reproductive fluids from over 2200 fish spawned at 9 Service facilities and 2 partnering fish hatcheries were examined. The Fish Health Center had 30 lot by lot fish health inspection cases which are conducted to allow interstate transfer as well as release of fish. Ten federal (or cooperating with a federal program) facilities received these on-site statistically based investigations and another 11 were conducted, as outlined in the Service Fish Health Policy, as virology lab services only for non-Service entities. These exams are essential to prevent the spread of fish diseases through fish and/or egg transfers and are necessary to enable facilities to comply with regulations on transporting and releasing fish. In addition to the 165 monitoring cases involving examination of fish, 2 Service facilities provided 61 water monitoring cases, where water from rearing units was examined by the water filtration method, an effective proactive protocol for diagnosing furunculosis before an epizootic occurs. In 2005, 26 laboratory cases were diagnostic exams from 8 facility/agencies, where fish were sampled to determine the cause(s) of mortalities and other problems and recommendations for resolution were provided. During these diagnostic workups, a serious pathogen, infectious pancreatic necrosis virus (IPNV), was isolated before causing large scale, catastrophic losses. In some diagnostic cases, protozoan parasites were identified and proper control treatments were recommended. Contact: John Coll

LM05I Incidence and Prevalence of Infectious Salmon Anemia virus (ISAv) in Sea Run Atlantic salmon held at Service NFHs as Broodstock. –The non-lethal ISAv surveillance protocol for screening sea-run Penobscot River Atlantic salmon as they are captured and brought to U.S. Fish and Wildlife National Fish Hatcheries to determine incidence was continued in 2005. At Craig Brook NFH in Maine, a sub-sample (60) of fish were sampled non-lethally (blood)and tested by reverse transcriptase-polymerase chain Reaction (RT- PCR) and cell culture on SHK-1 and ASK cells. All fish tested negative by both PCR and cell culture techniques. As a tool for managing this virus at the facility, the entire population (n=476) was similarly screened, following cohabitation and prior to spawning. The Connecticut River sea-run Atlantic salmon broodstock held at Cronin National Salmon Station in Massachusetts (n=163) were also non-lethally screened for ISAv. All tested negative for the virus and no isolation / quarantine of eggs was deemed necessary. Contact: John Coll

LM05J Fish Health Extension Services -The Lamar Fish Health Center continues to provide extension services to all federal, state, tribal and private inquiries in the area of fish health. Services provided include technical consultations, provision of supplies for fish necropsies, treatment recommendations and calculations, antibiotic injections, vaccinations, development of biosecurity plans, review of international and interstate fish importations, and furnishing procedural protocols**.** Participation within the Service's National Investigational New Animal Drug program administered by the Aquatic Animal Drug Approval Partnership (AADAP) Program in Bozeman is an integral requirement for providing treatment recommendations that are both safe and effective, and thereby approved by U.S. Food and Drug Administration. Contact: John Coll

LM05K U.S. Fish and Wildlife Service Title 50 Revision Committee - The Lamar Fish Health Center continued in the Service-wide effort to revise the Service's national importation regulations for fish, egg, and gamete importation, 50CFR16.13. Work will be cooperative with the newly developed National Aquatic Animal Task Force (NAAHTF) established through the Joint Sub-committee on Aquaculture (JSA). Contact: John Coll

OTHER BIOLOGICAL INVESTIGATIONS AND RELATED ACTIVITIES PERFORMED: (continued)

LM05L Vaccination of pre-release Connecticut River smolts using multi-valent vaccine – The Lamar Fish Health Center, in cooperation with partners involved with the Connecticut River Atlantic Salmon Committee, coordinated and administered injectable vaccine to 75,000 Atlantic salmon smolts the Pittsford NFH in 2005. This is the fourth year of administering this vaccine. The vaccine will protect the fish during their stay at the hatchery, as well as up to a year after they are released into the river from the bacterial pathogens that cause furunculosis and vibriosis. Evaluation of the efficacy of the vaccine in improving salmon survival in the wild will be based on adult sea run returns.). Contact: John Coll

LM05M Partnership with the Pennsylvania Fish and Boat Commission for pond culture of juvenile walleye and striped bass - A Memorandum of Understanding established between the Pennsylvania Fish and Boat Commission and the Service in 2003 to provide use of five Northeast Fishery Center ponds for culture resulted in the production of 107,000 striped bass Phase I fingerlings for stocking of Commonwealth waters in 2005. Contact: John Fletcher

LM05N Effluent Management at Hatcheries through Nutrition - Abernathy, Mora, Lamar and Bozeman Fish Technology Centers and USGS, Northern Appalachian Research Laboratory, initiated a National FONS Effluent Study. The study will develop and test diet formulations to reduce levels of total suspended solids and phosphorus (P) from hatchery effluents to address new and more stringent EPA and State standards. Regionally significant fish species will be fed low P diets both with and without guar gum binder. Levels of pollutants in waste stream and growth response of fish will be measured. It is anticipated that non-friable fecal matter created in response to diet additives may more readily be removed from the water column. Trials are to be conducted in FY 2006. Contact: John Fletcher

LM05O Aquatic Invasive Species Hazard Analysis and Critical Control Point Workshop - Pennsylvania Sea Grant and the NEFC – Fish Technology Center sponsored a two day workshop at the NEFC Population Ecology Branch – Conservation Genetics Conference Room, in March, 2005. Over 30 scientists representing federal, state, and commercial interests participated in training to identify risks and focus upon procedures to prevent the spread of nuisance species through natural resource management pathways. Contact: John Fletcher

LM05P Atlantic Sturgeon Biological Information and Technology Exchange - On August 29 - 31, 2005, the NEFC hosted a Region 4 / Region 5, meeting to review existing culture knowledge for Atlantic Sturgeon and review potential future lines of research. Contact: John Fletcher

LM05Q Fish Technology Center Construction 2005 - Cyclical Maintenance force accounts were used to purchase materials for closed formalin treatment delivery systems for Hatchery Building and Dissolved Gases Laboratory. The systems utilize variable speed multi-channel roller pumps to deliver differential doses to experimental hatching and rearing units. Contact: John Fletcher

LM05R Application of calcein as a dietary component for fish marking to enhance product evaluation and management capabilities. - This 3 year study is part of the Science Support Partnership Program with partners from the U.S.G.S. Leetown Science Center Northern Appalachian Research Laboratory (NARL), U.S.F.W.S. Aquatic Animal Drug Approval Partnership, Northeast Fishery Center (NEFC), and Bozeman Fish Technology Center. Recently, calcein has been approved as an Investigative New Animal Drug via immersion delivery on fish weighting less than 2 grams. However, because these restrictions make it impractical for some research and fish management situations, another form of delivery warrants investigation. The initial objective is to determine and compare scale luminosity between brook trout and rainbow trout using six different dosages of encapsulated and powdered calcein via extruded feed as a delivery method and to use these results to determine marking potential in other fish species. The brook trout component of this study was conducted at the NARL by feeding 2% of biomass for 5 consecutive days both encapsulated calcein and powdered calcein at dosages of 0, 0.5, 1.0, 2.0, 4.0, and 8.0 grams of calcein per kilogram of extruded feed. Qualitatively, scale luminosity was greater in fish that consumed feed containing the powdered form of calcein. However, encapsulated calcein was found not to perform to specified manufacturing conditions resulting in reevaluation of encapsulation process. Rainbow trout from the NEFC were found not to consume feed containing powdered calcein. Contact: John Fletcher, Jerre Mohler, or Tom Kehler

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