

# ANNUAL REPORT OF BIOLOGICAL ACTIVITIES

2000



U.S. Fish and Wildlife Service

**Northeast Fishery Center**  
**Lamar, Pennsylvania**

**570.726.4247**

**STUDIES PERFORMED.-** In fiscal year 2000, the Northeast Fishery Center (NEFC) continued at an aggressive pace toward conducting applied research in many areas of fish culture technology with inter-jurisdictional species such as American shad, Atlantic salmon, and Atlantic sturgeon. In cooperation with state, private, and other federal agencies, advances were made in fish marking technology, Atlantic sturgeon culture, and in understanding how handling stress may impact tank-spawning American shad spawning. Considerable effort and ingenuity also went into performing a study of mortality associated with recreational angling of striped bass and American shad on the Hudson River in cooperation with the state of New York.

Study Number and Title:

(Previously unreported results from 1999 experiments):

LM-99-01      Investigations relative to poor reproductive success of landlocked Atlantic salmon *Salmo salar* reared at Allegheny National Fish Hatchery - incubation density and cold water disease.

(Current fiscal year, 2000 studies):

LM-00-01      Winter feeding strategies for optimal growth, survival, and feed conversion of hatchery-reared Atlantic sturgeon

LM-00-02      Detection of fluorescing marks in Age 5 Atlantic salmon immersed as sac-fry in calcein solutions

LM-00-03      Two-color fluorescent labeling of larval Atlantic salmon otoliths using oxytetracycline and calcein.

LM-00-04      Determination of sexual maturity in age-6 domestic Atlantic sturgeon.

LM-00-05      Development and refinement of tank spawning procedures for adult American shad captured at Conowingo Dam.

LM-00-06      Comparative efficacy and an evaluation of the stress response of Atlantic sturgeon to three fish anesthetics.

LM-00-07      Differential predation on calcein-marked and non-marked Atlantic salmon parr by captive wild brook trout.

LM-00-08      Selected review of literature pertinent to impacts of incubation temperature on Atlantic salmon fry.

LM-00-09      Effects of short-term light deprivation upon milt production of feral Atlantic salmon.

**OTHER BIOLOGICAL INVESTIGATIONS PERFORMED:**

- LM00A Fish Health Inspection/Monitoring/Diagnostic Services
- LM00B Participation in the National Wild Fish Health Survey
- LM00C Cooperative work on a newly found virus of Atlantic salmon.
- LM00E Ongoing Participation in Maine Fish Health Advisory Board concerning Infectious Salmon Anemia virus (ISAv) Issues
- LM00F U.S. Fish and Wildlife Service Fish Health Procedures Handbook
- LM00G Patent application for calcein detection devices.

**STUDIES IN WHICH THE CENTER COOPERATED:**

Effect of dietary phosphorus and vitamin D3 on phosphorus levels in effluent from experimental culture of rainbow trout. - *Reli Coloso, Department of Pharmacology and Physiology, UMDNJ - New Jersey Medical School, Newark, NJ*

Screening of thiamine levels in Penobscot, Merrimack, and Connecticut River Atlantic salmon sea run brood stock. - *Dale Honeyfield, U.S. Geological Survey, N. Appalachian Research Lab, Wellsboro, PA.*

Estimating freshwater survival and environmental influence on survival for several Yukon River chum salmon stocks: Preliminary testing of calcein-marking of smolts. - *James Finn & Scott Maclean, U.S. Geological Survey, Alaska Biological Science Center, Anchorage, AK.*

The bioavailability of methylmercury to aquatic vertebrates. - *Joy Leaner, University of Maryland, Chesapeake Biological Laboratory, MD.*

Detection of calcein induced via ultrasound into rainbow trout. - *Greg Kindschi, U.S. Fish and Wildlife Service, Bozeman Fish Technology Center, MT*

Induction and detection of calcein in rainbow trout at French River Hatchery. - *Mary Negus, Fred Tureson, Minnesota Dept. of Natural Resources.*

Assessing the efficacy of sedatives in transport water for suppressing stress associated with handling and transport of adult American shad. - *USGS/BRD-Conte Anadromous Fish Research Center, Susquehanna River Coordinator, Chesapeake Bay Ecoteam, and Normandeau Associates.*

Gillnet selectivity for the spring weakfish fishery near Delaware. *G. Swihart, J. Galvez, Gloucester Office of Fisheries Assistance, VA.*

## **STUDIES IN WHICH THE CENTER COOPERATED** (continued)

Spring 2000 horseshoe crab spawner survey in Delaware Bay. - *Limuli Labs, Inc, USGS-Leetown Science Center, Delaware Dept. Nat. Resources & Environmental Conservation,, and New Jersey Dept. Fish, Game, and Wildlife.*

## **PUBLICATIONS:**

Jodun, W.A. and M.J. Millard. In progress. Effect of iodophor concentration and duration of exposure during water-hardening on survival of Atlantic salmon eggs. *North American Journal of Aquaculture.*

Jodun, W.A., M.J. Millard, and J.W. Mohler. In progress. The effect of rearing density on growth, survival, and feed conversion of juvenile Atlantic sturgeon. *North American Journal of Aquaculture.*

Mohler, J.W., M.K. King, and P.R. Farrell. 2000. Growth and survival of first-feeding and fingerling Atlantic sturgeon under culture conditions. *North American Journal of Aquaculture* 62:174-183.

Mohler, J.W., M.J. Millard, and J.W. Fletcher. Submitted. Predation by captive wild brook trout on calcein-marked and non-marked Atlantic salmon fry. *North American Journal of Fisheries Management.*

Mohler, J.W. In progress. Early culture of Atlantic sturgeons *Acipenser oxyrinchus oxyrinchus* (Mitchell) and preliminary stocking trials. *Publicaciones Especiales de Instituto Espanol de Oceanografia.*

Smith, D.R. B,L. Swan, S.F. Michels, W.R. Hall, P.J. Himchak, and M.J. Millard. In press. Spatial and temporal distribution of horseshoe crab *Limulus polyphemus* spawning in Delaware Bay: implications for modeling. Submitted to *Estuaries*, August 2000.

## **TECHNICAL INFORMATION LEAFLETS:**

LM-00-08. Selected review of literature pertinent to impacts of incubation temperature on Atlantic salmon fry.

## **TECHNICAL REPORTS:**

Millard, M.J., S. Welsh, J. Skjeveland, W. Fletcher, J. Mohler, M. Hendrix, A. Kahnle, and K. Hattala. 2000. Mortality associated with catch and release of American shad and striped bass in the Hudson River. Final Report: 1999 Project Year to: NY State Department of Environmental Conservation, Hudson River Fisheries Unit, New Paltz, NY

Millard, M.J. The horseshoe crab stock assessment process; searching for clues. *In* Proceedings of the Delmarva Coastal Bays Conference III: Tri-State Approaches to Preserving Aquatic Resources. EPA/620/R-00/001. US EPA National Health and Environmental Effects Research Lab, Narragansett, RI. June 2000.

**TECHNICAL REPORTS** (continued)

Swihart, G., M. Millard, N. Lazar, and C. Wenner. 2000. Gill net selectivity for the spring weakfish fishery near Delaware. A report to the Atlantic States Marine Fisheries Commission Weakfish Technical Committee, ASMFC, Washington, DC.

**FORMAL PRESENTATIONS:**

Barbash, Patricia - Implementation of the National Wild Fish Health Survey in the Northeast: Partnerships, Findings, Goals. 25th Annual Eastern Fish Health Workshop. April 10-14, Plymouth, Massachusetts.

Coll, John - The National Wild Fish Health Survey - Concept and Recent Findings/ Applications. East Coast Trout Management and Culture Workshop III. June 6-8, Frostburg, Maryland.

Millard, Michael - Relationship between stocking history and subsequent fecundity trends in Merrimack River Atlantic salmon. Presented at the annual US Atlantic Salmon Assessment Committee meeting, Gloucester MA, March 2000.

Millard, Michael - A framework for the stock assessment of horseshoe crabs. Presented to the ASMFC Management Board at the ASMFC Annual Meeting, Clearwater Beach FLA, Sept 2000.

Millard, Michael - Mortality associated with hook and release of striped bass in the Hudson River. Striped Bass Working Session sponsored by Univ. Maryland-Eastern Shore and USGS/BRD. August 2000, Baltimore, MD.

**National Committee participation:**

Millard, Michael.- Served on the U.S. Atlantic Salmon Assessment Committee, the technical body which responds to Atlantic salmon assessment tasks defined by the U.S. section to North Atlantic Salmon Conservation Organization (NASCO).

Millard, Michael.- Serves a member of the American Fisheries Society national Continuing Education Committee.

**Study Number: LM-99-01**

**Title: Effect of two broodstock diets upon rainbow trout reproductive success**

**Investigators:** Bill Fletcher - Northeast Fishery Center (NEFC) and Dale Honeyfield - Biological Resources Division (BRD), Wellsboro and Co-investigators - Mike Hendrix and Jerre Mohler - NEFC; Bill Krise -BRD, Wellsboro; Kari Duncan-White Sulphur Springs NFH

### **Background and Justification**

The mission of Ennis, Erwin, and White Sulphur Springs (WSS) National Fish Hatcheries is to produce adequate numbers of disease-free, genetically distinct strains of trout eggs to support the National Fish Hatchery System as well as other federal agencies, researchers, and cooperators.

Erwin strain rainbow trout at Ennis and White Sulphur Springs NFH have shown poor eye-up (about 75%) for a number of years. Although many environmental variables are present, diet formulation can be examined without major modification of facility operations and has been demonstrated to impact egg and fry quality.

### **Study Objectives**

The objective is to determine the effect of diet on reproductive performance of about 600 WSS NFH rainbow trout (Erwin strain) broodstock fed the current standard pellet diet (GR7-30, double vitamin pack) or a nutritionally updated extruded diet (RBT-5) in 1998.

### **Methods**

**Diet.-** The experimental RBT-5 diet was prepared using modern extrusion technology which has been shown to improve nutrient availability including addition of ingredients which may effect reproductive performance. The mineral supplements for the RBT-5 diet, was provided in an organic matrix, metal - proteinate form, which are biologically available at an increased level, compared to formulations used in GR 7-30. The RBT-5 diet contained a vitamin premix at 2.5 times more than listed requirements including vitamin C in a protected form at eight times the NRC recommended level of 50 mg/kg. Perdue Feed Inc. produced the diets.

**Culture.-** The study commenced at WSS NFH in Feb. 1998, to provide a five month minimum diet period. Four raceways containing a total of 4,000 Erwin strain rainbow trout were used in the study. A total of 600 were Floy-tagged and measured at start and just prior to spawning. Two raceways each were fed either RBT-5 diet or GR7-30 diet.

**Spawn. -** A total of 120 spawns were evaluated, 60 from each diet treatment. Each spawn was enumerated and incubated in partitioned Heath trays. Egg samples each group were collected prior to fertilization and frozen for nutrient analysis.

### **Results**

Fish offered the experimental RBT-5 diet had greater growth than those offered standard Government-contract GR7-30 trout diet but there was no difference detected in reproductive performance between the two treatment groups. Eggs as well as flesh produced by fish offered the experimental RBT-5 diet had greater pigmentation than that produced by fish fed the standard GR7-30 diet.

**Study Number:** LM-00-01

**Title:** Winter feeding strategies for optimal growth, survival, and feed conversion of hatchery-reared Atlantic sturgeon

**Principal investigator:** Wade Jodun; Northeast Fishery Center (NEFC)

**Co-investigators/Cooperators:** Jessica Kelligher; Penn State University  
Mike Millard; Northeast Fishery Center  
Kim King; Northeast Fishery Center

#### **Background/Justification:**

In accordance with recommendations from the Atlantic States Marine Fisheries Commission's (ASMFC) Atlantic Sturgeon Aquaculture and Stocking Committee, and the U.S. Fish and Wildlife Service's "The Framework for the Management and Conservation of Paddlefish and Sturgeon Species in the United States" NEFC undertaken the challenge of developing the technology necessary to advance and refine Atlantic sturgeon culture techniques with the goal of producing and disseminating hatchery guidelines for the species. Currently, a portion of sturgeon being reared as potential captive broodstock at the NEFC are overwintered in low water temperatures ( $< 10^{\circ}\text{C}$ ). In response to these cooler water temperatures, feed rate is often reduced by as much as 50%. However, no studies have ever been conducted to examine optimal feed rates at low temperatures for this species. In its attempt to restore depleted stocks of sturgeon through an expanded aquaculture effort, the Atlantic states Marine Fisheries Commission (ASNUC) has listed establishing environmental tolerances for factors such as temperature at different life stages and exploring sturgeon diet and nutrient requirements as some of its highest research priorities.

#### **Study Objectives:**

Therefore, this study was performed to define the optimum feeding rate which will produce the greatest growth, survival and feed conversion in Atlantic sturgeon juveniles at low water temperatures.

#### **Methods:**

Eighteen month-old Atlantic sturgeon juveniles produced at NEFC from Hudson River broodstock were employed for this study. 225 sturgeon were randomly distributed into fifteen circular tanks at a density of 15 fish per tank. Water levels were maintained at a constant depth of 41.90 cm via adjustment of external standpipes, resulting in volumes of 459 liters. Flows were maintained at 16 L/min. Fish were sampled following their distribution to establish a baseline mean weight and length. Initial biomass of each tank was adjusted to within 5% of the mean tank weight. Each tank was randomly assigned one of the five following feeding rates (offered as percent body weight per day) and consisting of three replicates each:

(1) 0.25 (2) 0.50 (3) 0.75 (4) 1.0 (5) 1.25

Feed was delivered via automatic feeders continuously over 24 hours for a period of 56 days. Whole body weight (BW) and total length (TL) will be measured on each fish bi-weekly to track growth over the duration of the study.

#### **Results:**

After 56 d of rearing, percent body weight increase (%BWI) for sturgeon reared in low ( $< 7^{\circ}\text{C}$ ) water temperatures was not affected by feeding rate ( $P>0.5$ ). Temperature, however, exerted a significant impact upon %BWI even with feeding severely inhibited ( $P<0.0001$ ). Since initial analyses showed that feeding rate treatment elicited no discernable influence on %BWI or mean survival and a subsequent test of equality of variances between treatment groups showed no significant difference ( $P > 0.5$ ), this effect was removed from the final analyses and %BWI data were pooled and subjected to regression analysis to elucidate temperature's impact upon growth rate. A significant positive correlation was found to exist between water temperature and %BWI. Mean Feed Conversion Rate (FCR) ranged from 30.3 to 37.9 with no significant difference between treatments. Survival in all treatments was 100%.

**Study Number:** LM-00-02

**Title:** Detection of fluorescing marks in age 5 Atlantic salmon immersed as sac-fry in calcein solutions

**Principal Investigator:** Jerre W. Mohler-NEFC

#### **Background and Justification:**

Since 1995, NEFC has experimented with calcein to produce a non-lethally detectable mark in fin tissue of Atlantic salmon. Calcein is dissolved into solution and fish are immersed for a period of time similar to techniques for batch-marking fish with oxytetracycline. However, calcein has been shown to be detectable in fin tissues up to 2 years post-immersion unlike oxytetracycline which has only been detected in fish otoliths for extended periods of time. If calcein is present in fin tissues of adult salmon marked as fry, the mark could theoretically be detected without sacrificing the fish and as such would be a valuable tool for use in Atlantic salmon restoration programs. Concurrent with experimentation on calcein immersion techniques, work at NEFC has also progressed on developing devices which can be used to detect calcein marks in fish under field conditions. These prototype devices have been used successfully to detect marks in two-yr-old salmon but are not likely powerful enough to detect the marks in 5-year-olds unless specific locations of calcein marks in mature fish are identified. Therefore, this study is dual purposed: (1) to locate calcein concentrations in adult fish marked as fry and (2) to test currently available detection prototypes on those locations if they are found. To accomplish this, salmon which were immersed in calcein solutions in 1995 as sac-fry and reared to adulthood will be the subjects of examination.

#### **Study Objectives**

In February, 2000, we sacrificed at least six 5-yr-old Atlantic salmon which were immersed in calcein solutions as sac-fry and section various fin tissues to attempt location of fluorescing marks using microscopy as well as hand-held and benchtop detection devices.

#### **Materials and Methods**

Initially, 4 fish were examined; 3 which were immersed in 125 mg/L and 3 immersed in 250 mg/L calcein for 48 hours, 5 years prior. Fish were sacrificed and fins were removed for dissection. Removal of fins included excision of tissue associated with fin/body articulation sites since some tissue growth may have encased calcein marks over time. A Stadie-Riggs tissue slicer and scalpel were used to attempt sectioning of un-fixed tissues since fixation or other chemical expose may destroy the calcein marks if present. Initial examination was performed using fluorescence microscopy and later examination of live fish was performed with hand-held and benchtop devices.

#### **Results:**

The tissue slicer used for the processing of fin tissues was not able to cut through the intended tissues due to the size and texture of the fin rays on an adult salmon. Therefore a scalpel was used to attempt tissue sectioning. No calcein marks were found in this manner via fluorescence microscopy on the small number of fish sacrificed for this procedure. Given this, a diversion from the original study plan was decided where a number of 2½-yr-old salmon which had been immersed in calcein as fry were examined with the hand-held and benchtop detection devices to verify the location and presence of calcein marks since these fish had been marked with improved techniques (Study L98-04). Calcein marks were readily visible on 100% of the 2½-yr-old fish and were located at the base of the pelvic and pectoral fins when viewed with the detectors. We conclude that the 5-yr-old fish were no longer marked such that practical detection means could uncover a positive calcein mark. Two possible reasons are hypothesized: (1) The calcein mark will not be retained in Atlantic salmon for a 5 year period or (2) The initial marking techniques (static immersion for 48 hours in cold water) did not induce a reliable mark which could be detected at a five-year period.

**Study Number:** LM-00-03

**Title:** Two color fluorescent labeling of larval Atlantic salmon otoliths using oxytetracycline and calcein.

**Principle investigator:** Jerre Mohler, NEFC

**Co-investigator:** John Fletcher, NEFC

#### **Background and Justification:**

U.S. Fish and Wildlife Service and its state fishery agency partners in the Northeast rely largely on fry stocking in restoration programs for migratory fish populations such as Atlantic salmon (ATS) and American shad (AMS). Success of fry stocking hinges upon the ability of biologists to assess the effectiveness of such a management strategy with fish marking techniques. Otoliths are among the most common tissues targeted for chemical marks and are used extensively in evaluation of American shad restoration programs. Shad incubated in these programs are stocked as both non-feeding and feeding fry and receive a chemical immersion baths in oxytetracycline at about 250 mg/ L at various daily intervals to produce a series of otolith marks according to stocking location. Some fry receive as many as six sequential oxytetracycline immersions at various daily intervals for their identification. This can introduce a level of uncertainty when otoliths are being examined from returning adult fish due to the complexity of the marking scheme. Since 1995, NEFC has experimented with calcein to produce a non-lethally detectable mark in fin tissue of Atlantic salmon, and found that the compound marks salmon otoliths as well. Other researchers found that teeth and bones of mice and rabbits were able to be double-labeled with contrasting fluorescent colors when oxytetracycline (yellow fluorescence) and purified calcein (DCAF) (green fluorescence) were injected intra-peritoneally four days apart. In fish restoration programs, the ability to use two distinguishable chemical marks would provide fisheries managers with greater marking flexibility and perhaps lead to greater confidence in relating fry stocking site to adult returns. Currently, calcein is not FDA-approved for use on potential food fish, but testing must go forward to provide information needed for obtaining an Investigative New Animal Drug (INAD) approval by determining the efficacy of this compound to mark fish tissues.

#### **Study Objectives:**

In February, 2000, we immersed 1800 Atlantic salmon eggs and/or non-feeding fry in sequential calcein (CLN) and oxytetracycline (OT) solutions to attempt induction of two-color fluorescent labels on otoliths. Evaluation of mortality, mark readability, and growth was compared between four treatment groups up to 30 days post-immersion.

#### **Materials and Methods:**

Immersion trials consisted of 7-min osmotic induction treatments; 3½ min in a 5% (50 g/ L) non-iodized salt solution followed by 3½ min in a buffered, 1 % solution of the selected chemical. After 7 days, each replicate was immersed in the opposite marking solution to induce a two-color mark on the otolith. Each treatment had 3 replicates; non-feeding fry holding units consisted of 9-liter plastic rectangular tanks equipped with a screened standpipe; eyed egg holding units were 6½-L acrylic hatching jars. Each replicate contained 100 ATS non-feeding fry or eggs of Connecticut River domestic parental origin. Control replicates received the same treatment as immersed fish/eggs but no chemical immersion. At about 24 hours post-immersion, sub-samples of 10-20 fish/replicate were examined for mark uptake via fluorescence microscopy.

#### **Results:**

Oxytetracycline marks were not visible on otoliths when viewed under fluorescent microscopy, however, calcein marks were clearly visible. Two-color labeling using these two fluorochromes does not appear to be a practical marking strategy. It is possible that the calcein marks simply overpowered the weaker fluorescence of oxytetracycline marks given only a seven-day interval between immersions. Fifty-day post-immersion inventory showed that mean survival of individuals was similar between calcein-marked fish and controls ( $183 \pm 1.3$  vs.  $178.3 \pm 5.3$ , respectively). In addition, mean biomass of replicates was also similar between calcein-marked and control fish at ( $43.6 \pm 0.3$  grams vs.  $41.0 \pm 1.2$  grams, respectively).

**Study Number:** LM-00- 04

**Title:** Determination of sexual maturity in age-6 domestic Atlantic sturgeon

**Principal Investigator:** Jerre W. Mohler-NEFC

**Co-investigators:** Bill Fletcher - NEFC

**Background and justification:**

From 1993 to 1998, NEFC successfully spawned Atlantic sturgeon from the Hudson River resulting in production of 5 year-classes of domestic stock. The 1993 year-class currently numbers about 70 individuals as 6-yr-olds. As part of the long-term commitment to refine culture techniques for this species, NEFC is developing domestic broodstock populations at the Lamar, PA facility. This broodstock program is important for two reasons: (1) application of culture technology to potential commercial aquaculture ventures and (2) maintenance of a pool of broodstock available for use in restoration stocking programs should the necessity arise. Among other factors required in a domestic broodstock program is the ability to determine fish gender ratios and stage of reproductive maturity present in captive fish. Currently, it is not possible to accurately determine these factors in sub-adult Atlantic sturgeon by external characteristics, therefore internal examination is necessary. It is also important to identify gender in potential broodstock because there is no information available concerning the influence of environmental conditions on sexual maturation/ovulation in domestic Atlantic sturgeon broodstock. Therefore, once gender has been determined in domestic stocks, replicated experimentation is possible to determine the best course of action for controlling female maturation and spawning.

Gonadal sex differentiation is complete in farm-reared white sturgeon by age 18-months at mean body weight of 1.1-2.3 kg. Further examination at 3-4 years showed that domestic white sturgeon exhibited a 1:1 sex ratio. Gonadal development is more a function of sturgeon size rather than age (J.P. Vaneenennaam, UC-Davis, personal communication) and at weights of 6 - 9 kg it may be possible to determine gender without histology. Currently, FY93 year-class sturgeon weigh from about 5 - 8 kg and therefore should be candidates for gender determination through biopsy in the summer of 2000.

**Study Objectives:**

In June, 2000, we will examine gonadal development via biopsy in at least twelve FY93 yr-class Atlantic sturgeon and permanently tag individuals (PIT tag) as to gender if possible.

**Materials and Methods:**

Twelve of the largest FY93 yr-class Atlantic sturgeon at NEFC will be sequestered and undergo biopsy for gonad sampling in June, 2000. Each fish will be anesthetized using metomidate at 15 ppm then placed ventral side up on a stretcher and sustained with a water-tube during the biopsy. An incision of the necessary length will be made to extensively view the gonad and to extract gonad tissue samples. Tissue samples will be preserved in 10% formalin upon extraction. Tissue samples will be taken from both of these locations for microscopic examination. A portion of the sample will be sent to UC-Davis for analysis of the stage of sexual development for all samples taken. Fish will be weighed and measured and PIT-tagged for future reference. If possible males and females will be placed into separate culture tanks. Depending upon the outcome of these first 12 biopsies, the balance of the FY93 yr-class fish may or may not be processed similarly. All biopsied fish will receive Liquimycin antibiotic at a dosage of 20 mg/ kg active terramycin.

**Results:**

The study is on-going and has been re-designated as LM-01-01 to include all year classes of hatchery-reared sturgeon maintained at NEFC.

**Study Number:** LM-00-05

**Project Title:** Development and refinement of tank-spawning procedures for adult American shad captured at Conowingo Dam.

**Principal Investigators:** (experiment A -Michael Millard) (experiment B - John -Fletcher)

**Co-investigators/ Cooperators:** Jerre Mohler - NEFC; Steve McCormick, USGS-BRD; Richard St. Pierre, USFWS; Chesapeake Bay/Susquehanna River Ecoteam; Susquehanna Electric Company; PA Fish and Boat Commission.

#### **Background/Justification:**

Efforts for restoration of American shad in the Susquehanna River are enhanced by artificial spawning of wild adult fish and stocking of larvae. Programs that include the use of stocked progeny of wild fish are dependent upon acquiring suitable brood stock and being able to spawn the fish either streamside or in a holding facility. The USFWS and several state agencies are experimenting with hormone-induced tank spawning of American shad to increase egg production and to reduce costs associated with strip spawning. However, fish that are trapped, sorted, sexed, and transported to the hatchery from the Conowingo Dam on the Susquehanna River are heavily stressed and related high mortality rates have reduced efficiency of tank spawning. Spawning hormone type and dosage also needs refinement therefore this study is composed of two sub-projects, or experiments.

#### **Study Objectives:**

Experiment A proposes to identify causes and suggest means to reduce stress impacts on broodfish used for tank spawning. Experiment B proposes to assess the relative effectiveness of two different hormones (LHRHA and GnRHa) as inducers of ovulation, plus assess the efficacy of hormone implants in males to increase fertilization rates.

#### **Materials and Methods:**

This work was conducted under several Investigational New Animal Drug Permits (INADs): Metomidate (trade name Marinil) ; sGnRHa (trade name Ovaplant 95S150); and LHRHA (trade name Ovaplant 95LI50) . Throughout the American shad migration at the Conowingo Dam, fish were removed from the west lift for study purposes. Four transport tanks were utilized to transport 40 shad each. **Experiment A (sedative evaluation):** Using blood chemical analysis, we tested effects of sedative treatments to transport water (metomidate, clove oil, and salts) in replicated transport trials. Tank spawning efficiency and egg production from each treatment was monitored at NEFC, as further indicators of a response to improved handling and transport techniques. Blood samples were analyzed by USGS-BRD; Conte Anadromous Fish Research Laboratory. **Experiment B (spawning hormone evaluation):** Captured American shad were used to investigate the efficacy of LHRHA and sGnRHa implants while producing eggs and fry for restoration stocking. In weeks two, four and six no hormones were implanted in the males. Males in weeks one, three, and five received the same type and level of hormone implant as females in respective tanks. The primary response variable was the mean number of eggs collected per female per day.

#### **Results:**

1. There was no transport survival difference between controls and metomidate or clove oil sedated groups with blood chemistry revealing increased levels of stress-related parameters in all groups.
2. Average % survival of shad held 7 days was greater in 2-4 ppt salinity as compared to fresh water.
3. There was no difference in reproductive performance between fish given LHRHa vs. sGnRHa.
4. There was no difference in survival or reproductive performance between fish held in circular vs. cross- flow tanks.
5. 3 million eggs were collected, 2 million were incubated, and 328,000 marked fry were stocked into the West Branch of the Susquehanna River.
6. Gas Bubble Disease was present in fry (Nitrogen gas =102.5 % and total gas pressure = 101.3 %).

**Study Number:** LM-00-06

**Study Title:** Comparative efficacy and an evaluation of the stress response of Atlantic sturgeon *Acipenser oxyrinchus* to three fish anesthetics.

**Principal Investigators:** Wade Jodun and Kim King - Northeast Fishery Center

**Co-investigators:** Bruce Barton - University of South Dakota; Mike Millard, Jerre Mohler, - NEFC; Jessica Kelligher, Tonia Barton - Penn State University.

#### **Background and Justification:**

The development of captive propagation and culture technology for Atlantic sturgeon *Acipenser oxyrinchus* is currently underway at the United States Fish and Wildlife Service's Northeast Fishery Center (NEFC), Lamar, PA with the goal of producing and disseminating a hatchery manual for the species. Many experimental and field investigations require handling fish for a wide array of procedures from tagging to recording length and weight. Anesthetics have routinely been employed to subdue fish during these procedures and to alleviate stress and reduce handling injury. Metomidate is an anesthetic which has been successfully employed for experimental purposes on both Atlantic sturgeon juveniles and adults (Jerre Mohler, personal communication). The compound has been shown to have few adverse side effects, has been cleared for certain fisheries applications in Canada and work is presently underway to license it for use in fisheries work in the United States. Information pertaining to anesthetizing Atlantic sturgeon is not available in the published literature, therefore the need exists to determine safe and effective concentrations of anaesthetics that produce rapid induction, quick recovery and which are non-toxic.

#### **Study Objectives:**

1. Assess the suitability of clove oil, MS-222 and metomidate as potential anesthetics for Atlantic sturgeon.
2. Determine the concentrations of clove oil, MS-222 and metomidate that will minimize time to induction and time to recovery.
3. Assess the suitability of these anaesthetics for prolonged exposure.
4. Determine if exposure to specific anaesthetic conditions mitigates or induces physiological stress.

#### **Materials and Methods:**

In order to facilitate tracking individual fish, all fish were tagged using numbered floy tags one week prior to the initiation of the study. In **Phase 1 (Range Testing)** approximately 200 sturgeon (FY98 yr-class) were pooled and groups of 12 fish, selected at random, were exposed to Clove oil (100 - 500- ppm); MS-222 (25-300 ppm) ; and Metomidate (5-25 ppm). Times to anesthesia, recovery, and 48-hour mortality was recorded. Fish were given a one week recovery period and the experiment was replicated. In **Phase 2 (Prolonged Exposure)** three lots each consisting of 48 juvenile sturgeon were randomly assigned one of the three previously tested anesthetics at the most effective dose at intervals of 10, 15, 20 and 30 minutes. Subsequently, 12 fish were removed and returned to fresh water where recovery times and mortality was monitored for 48 hours. Fish were given a one week recovery period and the experiment was replicated. For **Phase 3 (Stress Assessment)** 150 sturgeon will be randomly pooled from which lots of 25 fish will be randomly selected, netted, and exposed to one of the five following treatments at the most effective concentration determined from Phase 1: (1) Clove oil. (2) MS-222 un-buffered. (3) MS-222 buffered to a pH of 7.8 with sodium bicarbonate. (4) Metomidate. (5) Water as the control. Following treatment, 1 mL of blood will be drawn from the caudal vein using a heparinized needle. Hematocrit will be determined immediately by centrifugation. Plasma cortisol will be measured using radioimmunoassay and plasma glucose will be determined colorimetrically using Sigma Diagnostics ortho-toluidine reagent.

#### **Results:**

The study is on-going

**Study Number:** LM-00-07

**Title:** Differential predation on calcein-marked vs. non-marked Atlantic salmon parr by captive wild brook trout

**Principal Investigator:** Jerre W. Mohler-NEFC

**Co-investigators:** Mike Millard; Mike Hendrix; John Fletcher - NEFC

#### **Background and Justification:**

U.S. Fish and Wildlife Service and its state fishery agency partners in the Northeast rely largely on fry stocking in restoration programs for Atlantic salmon. Success of fry stocking hinges upon the ability of biologists to assess the effectiveness of such a management strategy, therefore fry marking techniques are necessary. Since 1995, NEFC has experimented with calcein to produce a non-lethally detectable mark in fin tissue of Atlantic salmon applied to batches of non-feeding fry. NEFC has also developed a non-lethal means of calcein mark determination via a detection device. The mark has been detected up to 2 years post-immersion in calcein by viewing the whole fish to examine fins for fluorescent green marks indicative of a past calcein treatment. Though calcein marks appear to be invisible to the naked human eye, it is not known whether a typical fish predator such as brook trout would not preferentially select calcein-marked parr over non-marked parr. Brook trout were selected as the predator due to their presence in most tributaries where Atlantic salmon fry are stocked in both the Connecticut River and Maine River restoration programs (Joseph McKeon and Jerry Marancik, personal communication). Experimentation such as this must go forward to provide information needed for obtaining an Investigative New Animal Drug (INAD) approval by determining the efficacy of using this strategy to mark fish tissues.

#### **Study Objectives**

In July, 2000, we will introduce about 600 calcein-marked and 600 non-marked Atlantic salmon parr to 12 brook trout predators in flow-controlled indoor raceways to examine differential predation on the two treatment groups. Evaluation of predation will be performed through recovery and examination of surviving fish for a calcein mark along with stomach content analysis of brook trout.

#### **Materials and Methods**

**Experimental units** . - Trials were conducted in indoor concrete raceways supplied with some cover for fry and predators. Flow was adjusted similarly in all raceways and temperature was ambient . Raceways were screened on all sides with black plastic polyfilm to minimize human disturbance during trials and lighting were natural photoperiod. A trial consisted of one raceway containing the same predators and prey for 4 consecutive days. **Predators**.- Each trial consisted of two wild brook trout per raceway as predators. Predators were collected in NEFC drainage facilities and acclimated to their respective raceways prior to introduction of prey. At the termination of each 4-day trial, predators were sacrificed and stomach contents were scanned with the calcein detector as one evidence of predation upon marked or un-marked fry. Naive predators were used for subsequent trials. **Prey**.- Prey were feeding Atlantic salmon fry of Connecticut River domestic origin which were calcein-marked as sac fry at a Developmental Index of about 85. Each trial consisted of 100 marked and 100 un-marked fry per raceway. At the end of 4 days, predators were removed and remaining fry were captured and scanned for a calcein mark. The entire experiment consisted of two 4-day trials. Numbers of marked and un-marked fry survivors were compared with chi-square test of independence to determine whether differential predation occurred:

#### **Results:**

1. Pooled data from the 4 trials showed equivalent survival of calcein-marked and non-marked fry at 256 and 254, respectively, indicating that calcein-marked Atlantic salmon fry are not preferentially captured or preyed upon by captive wild brook trout.
2. Two brook trout were able to consume between 20 -99 salmon fry over a 3-day period

**Study Number:** LM-00-08

**Study Title:** Selected Review of Literature Pertinent to Impacts of Incubation Temperature upon Atlantic Salmon Fry

**Principal investigator:** John W. Fletcher - NEFC

**Background/Justification:**

The Connecticut River Fish Culturist Sub-Committee requested a review of literature regarding negative impacts of early incubation temperatures greater than 9 °C for Atlantic salmon. Five papers, along with the manual, Atlantic salmon culture for restoration (Gaston 1988) were chosen as representative works concerning Atlantic salmon incubation. The abstract of each paper was included in total. Additionally several tables which presented information relative to survival response or temperature incubation impacts were transcribed. To supplement this information, annotations were drawn from each paper and included.

**Representative works:**

Gaston, P.B. 1988. Atlantic salmon culture for restoration. U.S. Department of Interior. Fish and Wildlife Service, Newton Corner, Massachusetts.

Gunnes, K. 1979. Survival and development of Atlantic salmon eggs and fry at three different temperatures. *Aquaculture* 16(3) 211-218.

Hamor, and E.T. Garside. 1976 Development rates of embryos of Atlantic salmon, Salmo salar L., in response to various levels of temperature, dissolved oxygen, and water exchange. *Canadian Journal of Zoology* 54: 1912-1917.

Pavlov, D.A. 1985. Effect of temperature during early ontogeny of Atlantic salmon, Salmo salar L. 1. Variability of morphological characters and duration of development of Atlantic salmon under different temperatures. *Journal of Ichthyology* 24 (6) 30-38.

Petersen, R.H., C.E. Spinney, and A Sreedharan. 1977. Development of Atlantic salmon, Salmo salar eggs and alevins under varied temperature regimes. *Journal of the Fisheries Reserve Board of Canada* 34:31-43.

**Results:**

Information excerpted from the papers generally fell within three areas: (1) recommended incubation temperature(s) (2) temperatures where problems were encountered and (3) adaptive significance of variable responses to incubation profiles. Relevant results from all referenced works were summarized and

presented as a Technical Information Leaflet (LM-00-08).

**Study Number:** LM-00-09

**Study Title:** Effects of short term light deprivation upon milt production of feral Atlantic salmon

**Principal Investigator:** John W. Fletcher

**Co-investigators:** Mickey Novak, R. S. Cronin National Salmon Station (NSS); Jerre Mohler, Mike Millard and Wade Jodun, NEFC

**Background/Justification:**

Joseph Ravita, Manager Whittemore Salmon Station (SS), reported to the Connecticut River Fish Culturist Sub-Conunittee observations in the fall of 1999 concerning milt production from sea-run Atlantic salmon which had been held in covered tanks excluding light for 10 days immediately prior to spawning season. As a result of a construction project, Atlantic salmon were held in covered tanks from September 27 to October 7. Milt was first stripped from the 22 males in the population on October 13, and was collected twice per week until November 9. During this spawning interval only one male failed to yield on one occasion. This 1999 performance appeared to differ from previous seasons in being briefly delayed in onset of milt production and in terms of greater quantity, less failures to produce, and thicker consistency, which could indicate elevated spermatocrits. Historically there has been difficulty with sea-run Atlantic salmon held at Cronin NS S in terms of dependable frequency of milt production and possibly in quality as measured by resultant eye-up. It was of general consensus at the Sub-Committee Post Spawn Meeting, February 2000, that a controlled photoperiod test would be of value.

**Study Objectives:**

To determine whether an eleven-day light deprivation holding environment will change milt production characteristics of feral sea-run Atlantic salmon held at Cronin NS S, the Northeast Fishery Center will establish test holding units and evaluate potential changes in milt production onset, duration, quantity and quality.

**Materials and Methods:**

Approximately 12 sea-run males Atlantic salmon were collected from returns to the Holyoke Fish Lift and held for spawning at the Cronin NS S. These salmon were tagged with pit tags. From capture until the beginning of the current trial, the males were held in a common tank with 60 females. A distribution trailer with four 1500 L circular fiberglass tanks supplied with flow-through water was used to hold experimental fish. Three males were placed in each tank; two tanks had a gasket sealed lid which excluded light for an eleven-day treatment period while the other two tanks had a plexiglass lid to provide natural photo period. After light manipulation treatment, all salmon were returned to the common holding tanks with the females. Milt was taken from experimental fish once/week for 7 weeks until all females had spawned. On each spawning date the following information was taken: (1) tag number, (2) quantity of milt (3) milt motility recorded as % motile, (4) spermatocrit, and (5) milt viability as determined by a eosin-nigrosin smear where dead spermatozoa are stained red and live cells appear clear.

**Results:**

There were no significant differences in quantity of milt produced, sperm cell volume, or sperm motility in male salmon held in a dark environment vs. those exposed to natural photoperiod. Milt production profiles over the 7-week experiment were similar between treatment groups except that fish kept in the dark gave slightly greater amounts of milt earlier than fish under natural photoperiod. Maximum milt production for both groups peaked at the fourth week.

#### **OTHER BIOLOGICAL INVESTIGATIONS PERFORMED:**

- LM00A Fish Health Inspection/Monitoring/Diagnostic Services.** - The Lamar Fish Health Unit processed 359 laboratory cases in fiscal year 2000. Region 5 has a very extensive fish health monitoring program to enhance the fish health inspections, allowing continual surveillance of the health status of the stocks, some of which have been identified as very limited distinct population segments (DPS) which the Service has just recently proposed for listing under the Endangered Species Act (ESA). The Fish Health Unit had 31 inspection cases, which included 15 that were conducted, as outlined in the Service Fish Health Policy, as virology lab services only for non-Service entities. These statistically based fish health examinations 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 181 monitoring cases involving examination of fish, 4 Service facilities provided 26 water monitoring cases, where water from rearing units is examined by the water filtration method, a very effective proactive protocol for diagnosing furunculosis before an epizootic occurs. In fiscal year 2000 twenty-six laboratory cases were diagnostic examinations, where moribund fish were examined and tested to determine the cause(s) of mortalities and other problems and recommendations for resolution were provided.
- LM00B 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 2000, the Fish Health Unit initiated 95 cases for the Survey, in which 3,171 fish from a total of 65 sites were examined and efforts continued to enter completed cases into the NWFHS database, soon to be accessible to all via the Internet. Interesting findings in fiscal year 2000 include the detection of bacterial pathogens *Renibacterium salmoninarum*. from several sites sampled in waters of the state of Vermont and *Yersinia ruckeri* from brook trout from a site sampled in Virginia's Shenandoah National Park. Infectious pancreatic necrosis virus was confirmed from brook trout in Vermont and Virginia, as well as in blacknose dace in another site in Virginia. Outreach activities to increase awareness of the Survey included demonstrations of the database on the Internet at various meetings and conferences as well as development of a NWFHS Outreach Committee coordinated out of the Washington Office.
- LM00C Cooperative work on a newly found virus of Atlantic salmon.** - In fiscal year 1999, work with the new retrovirus (salmon swimbladder sarcoma virus) discovered in Atlantic salmon continued. A non-lethal PCR test for detection of this virus developed by Cornell University was used for screening Atlantic salmon in the spring and fall of 1999 at Craig Brook NFH, in Maine, where it was found that additional salmon stocks were also positive, but no clinical signs have ever been observed there. Clinical signs, including irregular skin patches, swimbladder tumors, and mortalities, were only observed at one of three facilities, where the environment and the diet were unique. Without any additional disease signs in subsequent research or production environments, Rivers Postulates have yet to be fulfilled. Though evidence to date indicates the virus probably came from wild parr taken from the Downeast Rivers of Maine, much more is needed to be understood about the virus's virulence, contagion, range, or general epidemiology. Work this fiscal year consisted of review of a draft manuscript "Identification of a retrovirus associated with swim bladder fibrosarcoma in Atlantic salmon, *Salmo salar*" co-authored with Drs. Paul Bowser and Jim Casey of Cornell University.
- LM00E Ongoing Participation in Maine Fish Health Advisory Board concerning ISAv Issues.**- The Maine Fish Health Advisory Board serves as a scientific advisory board to the state Commissioners. The group, containing a representative from the Fish Health Unit, is very heavily involved with Infectious Salmon Anemia virus (ISAv), presently an exotic to the United States, but with a geographical proximity right up to the border, as well as other fish health issues related to private aquaculture and wild resources. The Service, at the Lamar Fish Health Unit, has established a monitoring program for all sea-run Atlantic salmon mortalities as has the Maine salmon industry initiated an intensive ISAv

monitoring program. Due to the low numbers of the distinct population segments (DPS) of Atlantic salmon, it is vital that the impacts of this and other diseases are held to a minimum.

**LM00F U.S. Fish and Wildlife Service Fish Health Procedures Handbook** - In cooperation with all eight other fish health centers, a procedures handbook to assist in national consistency has been initiated. This fiscal year Lamar developed chapters for several of the specific tests for fish pathogens, e.g. ELISA, PCR. as well as assistance on committees to determine the manual's scope and use.

**LM00G Patent application for calcein detection devices**.- As part of the continuing development of fry marking and mark-detection techniques, NEFC has submitted a patent application for both a bench-top and hand-held calcein detection device which will make it feasible to quickly and efficiently detect fluorescent marks on individual fish under rigorous field conditions without the need for a microscope.