INTRODUCTION

The Northeast Fishery Center presently consists of the Lamar National Fish Hatchery, the Fish Technology Center, and the Regional Fish Health Unit. Legislative authority for establishing the Fish Hatchery is contained in 46 Statute 371 (White Act), dated May 21, 1930. The station was officially established in 1933 and in the ensuing years has produced and distributed to Federal, State, and private waters, largemouth bass, smallmouth bass, bluegill, catfish, walleye, muskellunge, rainbow trout, brook trout, brown trout, and striped bass. Water to operate the facility is supplied by Washington Spring and Big Fishing Creek. The Fish Hatchery has taken a lead role in supporting the Technology Center programs dealing with Atlantic sturgeon and Atlantic salmon as well as Hatchery Product Evaluation. Production of catchable rainbow trout was greatly reduced at the Fish Hatchery beginning in 1994, but a small number are still produced for the annual fishing derby which remains a valuable public outreach program.

The Lamar Fish Cultural Development Center was created in 1965 on the grounds of the Lamar Fish Hatchery. In 1984, the name was changed to the Fish Technology Center and its mission was modified. The mission was again modified in 1993 and current emphasis is on: (1) developing new cultural and management technology for threatened and endangered (T&E) aquatic species and species of special concern in the nation's interjurisdictional rivers, (2) implementing a hatchery product evaluation program, and (3) developing aquatic population dynamics capability. The Northeast Fishery Center is the lead facility in development of culture technology for Atlantic sturgeon, which has been identified by the Atlantic State Marine Fisheries Commission as a species of concern. In addition, the Center takes a progressive role in Atlantic salmon restoration efforts underway in New England.

The Northeast Fishery Center is located 0.5 mile south of Highway 64 on the Tylersville Road in Lamar, Porter Township, Clinton County, Pennsylvania.

Facilities at the Center include an Intensive Culture building for striped bass and sturgeon, an accessible office building, hatchery building complex, technology laboratory, fish hatchery laboratory and office building, coldwater experimental building, maintenance shop, two garages, a fish storage building, twenty concrete raceways, five hypalon-lined earthen ponds, and ten other earthen ponds in need of rehabilitation.

STATION OPERATIONS

In FY96, the Northeast Fishery Center (NEFC) received and fulfilled many requests for technical assistance and information from fisheries units and scientists across the U.S. as well as internationally. Results of experimental culture work with Atlantic sturgeon was published and widely distributed, and headway was made in hatchery product evaluation as it relates to the restoration of Atlantic salmon and sturgeon. NEFC was also active in the ecosystem approach to managing our fish and wildlife resources by taking leadership roles on the Ohio, Delaware, Hudson, Chesapeake and Gulf of Maine eco-teams. Though the work force was further reduced by the departure of an animal caretaker, NEFC was able to mitigate the loss through the part-time labor of graduate student, Kim King, and by Wade Jodun, who has volunteered over 2,000 hours to NEFC. Major Center activities in FY96 were as follows:

1. Fourth year in a row - Captured and spawned adult Hudson River Atlantic sturgeon, Acipenser oxyrhynchus oxyrhynchus, and successfully hatched 15,000 fry.
2. Cooperated with private commercial fishermen, Cornell University, the National Biological Service, states of New York, New Jersey, Delaware, and Pennsylvania to obtain tissue samples from Atlantic sturgeon of the Hudson and Delaware Rivers for use in determining genetic differences between rivers.
3. Searched for adult Atlantic sturgeon in the Delaware River, and Chesapeake Bay.
4. Performed culture experiments with larval and juvenile Atlantic sturgeon.
5. Actively engaged in public outreach/advocacy activities.
6. Continued construction of water degassing and tempering system to be installed at the cold water experiment lab (holding house) building.
7. Conducted field experiments related to improving percent hatch of Atlantic salmon eggs at other federal fish hatcheries.
8. Cooperated with the National Biological Service on development of technology for mass marking Atlantic salmon fry as a tool for better hatchery product evaluation.
9. Cooperated with National Biological Service - Leetown on fish health issues.
10. Served as Regional aquaculture coordinator.
11. Provided recreational fishing opportunities.
12. Provided fish feed inspections and technical assistance to the Region on issues related to Regional fish food contract.
13. Conducted a volunteer program.
14. Served on ecosystem management teams and chaired U.S. Assessment Committee Subteam on Cultural Related Activities.
15. Reviewed technical papers.
16. Provided fish culture information worldwide.
17. Shipped Atlantic sturgeon to various groups for research on culture, disease, management, and fish passage.
18. Conducted field experiments on Atlantic salmon from fry through smolt stage to compare effects of menhaden vs. herring oil in fish feed.
19. Submitted a manuscript to North American Journal of Fisheries Management on mass-marking Atlantic salmon fry with a non-lethally detectable mark.
20. Began writing the Culture Manual for Atlantic sturgeon.
21. Coordinated community assistance projects with Rockview State Prison.

Other activities involving the Technology Center are summarized as follows:

"STURGEON - ON THE ROAD AGAIN"
NEFC used the combination dual-wheel pickup and custom hauling trailer/tank unit to exhibit live sturgeon and salmon at four public outreach events this year: (1) Family camp at Camp Susque, Lycoming County, PA (2) Summer festival, downtown Lock Haven, PA (3) Clinton County conservation days, Clinton County fairground, PA (4) Beech Creek and Liberty elementary schools, Clinton County, PA.. Children and adults were able to get very close to a live sturgeon and salmon, many for the first time. Young and old alike were amazed at the sight of the "prehistoric" sturgeon and were supportive of our restoration efforts.

SPAWNING OF HUDSON RIVER ATLANTIC STURGEON:
Three female Atlantic sturgeon from the Hudson River near Norrie Point State Park, New York were captured and transported to NEFC. Hormone injections of Common Carp Pituitary (CCP) were used to induce two of the females to spawn. Both females gave partial spawns to yield approximately 15,000 fry. The third female was not spawned but is being held at NEFC for future use. Milt for fertilizing the eggs was obtained from Hudson River male sturgeon which were released unharmed.

Fry from the resulting hatch were used in a density study to discover at what point density begins to adversely affect growth and what the effect of increased ration has on growth and survival in crowded conditions during early rearing.

In FY96, excess Atlantic sturgeon fry, 1, 2, and 3-year old juveniles were shipped to the following organizations:

NBS-Conte lab, MA.
NBS-Leetown lab, WV.
State of Maryland
Maryland Fisheries Resource Office
U.S. Army Corps of Engrs., Waterways Exp. Station, Vicksburg, MS.
Maritime Center, Norwalk, CT.
New Jersey State Aquarium
New York City Aquarium
Hofstra University, NY
Science Fair Research Project, Charleston, SC

The shipped sturgeon will be involved in diet, behavior, culture, and disease experiments as well as public display. Approximately 150 individuals of the FY96 hatch remain at NEFC for use in additional experiments or as future broodstock.

WETLAND PLANTING AND HABITAT CONSTRUCTION: A wetland construction initiated in FY95 was completed with the help of a group of community service students from the Bellefonte High School. A seed bed was prepared and large logs for wildlife perches were placed in the wetland. A rock/rubble platform was also constructed to create additional microhabitat and sunning areas for amphibians. The area will be used for public enjoyment as well as hatchery effluent treatment.

ATLANTIC STURGEON STOCKING WORKSHOP: NEFC biologists attended a one-day workshop on the problems and goals of a restoration stocking program for Atlantic sturgeon. The meeting was hosted by the Hudson River Foundation in their New York City office. Main points of discussion centered around preservation of genetic diversity both inter and intra-river, potential for competition with endangered shortnose sturgeon, and monitoring of success.

ATLANTIC STURGEON RESEARCH/RECOVERY EFFORTS IN THE CHESAPEAKE BAY COORDINATION MEETING: NEFC biologists attended and presented culture information at this meeting hosted by the USFWS-Maryland Fisheries Resource Office in Annapolis, Md.. Highlights were: (1) discussion of partnerships, research funding sources, and development of a "Chesapeake Bay Sturgeon Recovery Plan (2) Consensus that a carefully designed experimental stocking program committed to long-term monitoring would be important (3) USFWS will solicit a no-jeopardy opinion from NMFS to allow Maryland DNR to proceed with Chesapeake Bay sturgeon stocking in early summer, 1996.

FISH FEED INSPECTIONS: As in previous years, the Center has taken responsibility for quality control of production fish diets for Region 5. Quarterly feed inspections were coordinated and performed by biologist Jerre Mohler and biotechnician Pat Farrell. Only minor quality control problems were encountered during the year and the feed contract was awarded to a new company (Perdue Specialty Feeds, Catawissa, PA) during the 4th quarter of the year.

CHINESE SCIENTISTS VISIT: The Susquehanna River Coordinator, Richard St. Pierre hosted six scientists from China and brought them to NEFC for a tour of our facility. At least two of the scientists were somewhat familiar with sturgeon culture and had many questions concerning our work with Atlantic sturgeon. The visitors seemed very appreciative of the hospitality they received.

INFORMATION DISSEMINATION AND PUBLIC RELATIONS: The Center received and responded to the following requests for information and other technical assistance during FY96:

1. NEFC sent an abstract for display at the sturgeon symposium held in San Francisco, CA.

2. Columbia River Inter-tribal Fish Commission, WA requested information on NEFC's experimental Atlantic sturgeon mark-release-and recapture of hatchery-produced Atlantic sturgeon in the Hudson River to apply results to white sturgeon recovery efforts in the Columbia River.

3. Jeff Hamilton, high school student from Belmont, MA requested and received information on the effects of phosphorus on fish.

4. Jill Stevenson, graduate student at the University of Maryland requested information on Atlantic sturgeon culture

5. Iyanda Morufu, college student from Nigeria, requested information on aquaculture.

6. Ed Schupp, a private individual from Kingston, NY, requested various technical leaflets produced at Lamar.

7. Violet Tolosta, a private individual from Washington, DC, requested information on tank culture of fish.

8. The following individuals and organizations requested reprints of the NEFC Atlantic sturgeon article which appeared in the July 1996 Progressive Fish Culturist:

   S.E. Robb, Biology Dept., Montreal, Quebec
   Museum of Natural History, Paris, France
   Phillipe Jatteau, CEMAGREF, France
   Mark Collins, S. Carolina DNR
   Gray Bass, Florida Game and Fresh Water Fish Commission
   Guy Verreault, Ministry of Environment and Wildlife, Quebec
   Ann Foster, US Geological Survey, Gainesville, Florida
   Stephen McCormick, Conte Anadramous Fish Lab, Turners Falls, Mass.
   Boyd Kynard, Conte Anadramous Fish Lab, Turners Falls, Mass.
   Alex Haro, Conte Anadramous Fish Lab, Turners Falls, Mass.
   Eric Rochard, CEMEGREF, France
   Doug Bush, commercial fisherman, Catskill, New York

9. The Regional Office (R5) requested all information available on Atlantic sturgeon management plans.

N.P.D.E.S PERMIT COMPLIANCE: The Center is responsible for monthly reporting of flows and various effluent parameters to state and federal agencies for purposes of compliance with the National Pollution Discharge Elimination System permit (N.P.D.E.S.). Water samples from designated discharge points on the facility are taken to a nearby laboratory each month to undergo analysis for: B.O.D., Suspended Solids, Phosphorus, and Ammonium. Monthly reporting forms were completed and sent to the appropriate agencies.

COLLECTION OF GENETIC SAMPLES FROM ATLANTIC STURGEON: Thirty Atlantic sturgeon from each of the Hudson and Delaware Rivers were captured and sampled by NEFC with help from commercial fisherman Doug Bush of Catskill, NY. Tissue samples were preserved in alcohol and shipped to Dr. Tim King, NBS-Leetown for subsequent analyses using DNA markers to determine whether stocks are genetically unique to each river system. This is one discovery which is necessary before restoration or enhancement efforts using hatchery-produced fish could proceed.

SURPLUS ATLANTIC STURGEON GIVEN TO THE STATE OF MARYLAND: Approximately 175 fish (age 2 and 3) were picked up by the State of Maryland in order to provide Maryland state fish culturists practical experience in culture of the species. A total of about 245 two and three-year olds still remain at NEFC, the largest of which is about eight pounds and three feet in length. These fish were hatched at NEFC in 1994 and 1993.

SIDE-SCAN SONAR OBSERVED IN FIELD USE ON THE DELAWARE RIVER: In cooperation with the States of Pennsylvania and Delaware, NEFC was able to observe and direct operation of a side-scan sonar unit to examine it's potential use in locating large Atlantic sturgeon. It was found that the unit was not practical for use in this manner, but detailed river bottom maps were recorded that show substrate condition in an area where a number of juvenile Atlantic sturgeon are consistently captured by the state of Delaware. The sonar unit is owned by the state of Pennsylvania and was operated by Aquatic Systems Corporation, Pittsburgh, PA.

STUDIES PERFORMED: Fiscal year 1996 provided the time frame for NEFC to perform challenging and interesting biological work. The majority of studies performed by Center biologists and our partners were related to Atlantic sturgeon and Atlantic salmon restoration and are identified below:

Study Number and title:

L-96-01 Atlantic salmon egg transport studies, fall 1995, a re-examination of eye-up between *Salmo salar* eggs transported to incubation facilities unfertilized with those fertilized prior to transport.

L-96-02 Feasibility of using side-scan sonar to locate mature Atlantic sturgeon as indication of spawning areas in the Delaware or other east coast rivers.

L-96-03 Comparison of growth and fin condition in Atlantic salmon fed U.S. government contracted feeds manufactured with herring oil or menhaden oil (stage 2; parr to smolt).

L-96-04 Using nuclear and mitochondrial DNA from Atlantic sturgeon of the Hudson and Delaware Rivers to determine whether the populations are genetically distinct.

L-96-05 Comparison of growth and fin condition in Atlantic salmon fed U.S. government contracted feeds manufactured with herring oil or menhaden oil (stage 1; fry to parr).

L-96-06 The effects of diet and seasonality upon growth, tissue composition, and survival of juvenile Atlantic sturgeon.

L-96-07 An annotated bibliography for fry marking

L-96-08 Inducing fluorescent marks in larval Atlantic salmon using calcein green and calcein blue

L-96-09 Study of larval rearing density with hatchery-produced Atlantic sturgeon *(Acipenser oxyrhynchus)*.

L-96-10 Time of optimum milt yield after injection of common carp pituitary (CCP) in 2+ domestic Atlantic salmon *(Salmo salar)*

STUDIES IN WHICH THE CENTER COOPERATED:

Mechanical shock sensitivity of activated Atlantic salmon eggs during early embryonic development - *Dr. William Krise, Research and Development Lab - Wellsboro, PA*

Development of immunological marking methods for tagging of Atlantic salmon fry - *Dr. William Krise, Research and Development Lab - Wellsboro, PA ; Dr. John Sternick, Mansfield University, PA*

Fertilization of Atlantic salmon eggs using cold-stored sperm held up to six hours - *Dr. William Krise and Dale Honeyfield, Research and Development Lab - Wellsboro, PA*

Study Number: L-96-01

Title: Atlantic salmon egg transport studies, fall 1995, a re-examination of eye-up between (*Salmo salar*) eggs transported to incubation facilities unfertilized with those fertilized prior to transport

Principal Investigators: Bill Fletcher, Mike Hendrix and Jerre Mohler; Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Bill Krise, Dept. Interior -Wellsboro
Larry Lofton; North Attleboro NFH
Bruce Jenson; White River NFH
Vic Segarich, Bob Groton; Nashua NFH

Background and Justification

The Atlantic Salmon Program relies heavily upon fish cultural facilities to produce fry, parr, and smolts for restoration stocking. In practice many of the production eggs are spawned at one station and transported for incubation at another hatchery. Eye-up for Atlantic salmon eggs, transported green and fertilized in gallon jugs, have reached levels exceeding 90 percent; however, in recent years eye-ups have at times, been below 60 percent. In 1993 and 1994, the Northeast Fishery Center began a series of studies to evaluate Atlantic salmon egg transport methods (Fletcher, et.al. in progress). Based upon the dramatic improvement observed for delayed fertilization egg transport in 1993, trials were undertaken to evaluate this shipping protocol on a production scale in the fall of 1994 (Study L-95-02). Overall the eye-up for green Atlantic salmon eggs fertilized after transport to incubation facilities was improved over those eggs fertilized at spawning sites prior to transport, but statistically, results were not strongly conclusive.

Study Objectives:

The objective of the present study is to repeat and expand the Merrimack River domestic brood and Connecticut River kelt portion of Study L-95-02. Again, comparison of eye-up on a production scale between green Atlantic salmon eggs fertilized prior to transport with those fertilized after transport to incubating facilities will be performed.

Methods:

Work will be performed at the following facilities with the indicated number and type of eggs: Nashua NFH (100,000 Merrimack River domestic broodstock eggs); North Attleboro (100,000 Connecticut River kelt eggs and incubation site); and White River NFH (incubation site). From each female one half of the eggs spawned will be: Dry fertilized with two ml of pooled milt per take, rinsed, water hardened, transported in insulated one gallon jugs, disinfected at the receiving station, and placed into Heath incubators - the traditional method. The second half of the eggs will be: collected into plastic bags; oxygenated and placed into coolers on a tray that rests on ice; milt will be transported in cooled, oxygenated test tubes. These eggs will then be fertilized, water hardened in iodophor and disinfected a second time at the receiving station after transport. Eggs will be enumerated and placed in incubators at 8,000 per tray. Success of eye-up between fertilized green Atlantic salmon eggs transported in jugs vs. eye-up with eggs undergoing delayed fertilization will be evaluated with 2 sample t-tests.

Results:

1. Nashua NFH domestic ATS eggs with delayed fertilization had significantly better eyeup than immediate fertilized eggs ($P \# 0.10$) incubated at North Attleboro NFH.
2. There was no difference in eyeup between delayed and immediate fertilization in North Attleboro NFH kelt eggs incubated at White River NFH

Study Number L-96-02

Title: Feasibility of using side-scan sonar to locate mature Atlantic sturgeon as indication of spawning areas in the Delaware or other east coast rivers.

Principal Investigator: Northeast Fishery Center biologists

Co-Invest/Cooperators: Jorgen Skeveland, MD Fisheries Res. Office State of Delaware Dept. of Nat. Res. (DNR) State of New Jersey Dept. of Environ. Protection (DEP) State of New York Dept. of Environ. Conservation (DEC)

Background and Justification:

Commercial records of Atlantic sturgeon (ASN) landings from the late 1800's to the present indicate a severe decline in the fishery. This problem has been addressed in the form of management plans for restoration of this species throughout its range by the Atlantic States Marine Fisheries Commission (ASMFC). Specific management objectives of the management plan include improvement of knowledge and enhancement and restoration of the Atlantic sturgeon stock. There are significant gaps in our knowledge of this species. For example, information on specific locations of spawning sites is needed to better protect these habitats. The culture and stocking group, which is comprised of all ASMFC states, the U.S. Fish and Wildlife Service, and the National Marine Fisheries Service, has made various recommendations including: "Standardized baseline data on habitat and population status for important sturgeon rivers should be obtained and that data should include assessment of spawning habitat.

Using echo-sounding equipment in 1995, segments of the Delaware River, Chesapeake Bay, and Hudson River were examined by NEFC and Maryland FRO staff. No mature sturgeon were located in the Delaware River or Chesapeake Bay, but as a result of examining a known spawning area on the Hudson River, workers became confident in relating echo signal characteristics to identification of mature sturgeon. A limitation of echo sounders is that multiple passes must be made to cover wide areas. Conversely, much wider areas can be searched in a single pass using side-scan sonar.

Study Objectives:

Objectives are to use side-scan sonar technology to locate individual sturgeon in the Delaware River at a location where juveniles are known to congregate at low tide.

Materials and Methods:

The State of Pennsylvania will be contacted to coordinate use of their Imagenex, model 855 color-imaging sonar system. The unit will be operated by the consulting group, Aquatic Systems Corp.. NEFC will direct the search by recommending search areas. Should ASN be identified with the equipment, gill nets will be set to attempt capture for the purpose of determining gender and to obtain a tissue sample for genetic analysis.

Results

The area just upstream from Artificial Island in the Reedy Island Range of the Delaware River was scanned and video-taped with the sonar equipment. No individual fish of any size were positively identified, but a highly detailed map of bottom topography was obtained in an area known to be frequented by juvenile ASN. Results show that this equipment is not capable of locating single fish, but could be valuable for discovering what constitutes suitable habitat for various life stages of ASN.

Study Number: L-96-03

Title: Comparison of growth and fin condition in Atlantic salmon fed U.S. government contracted feeds manufactured with herring or menhaden oil - Part 2 (parr to smolt)

Principal Investigator: Northeast Fishery Center biologists

Co-Invest/Cooperators: Paul Gaston, Green Lake NFH

Background and Justification:

Currently, in Region 5 of the U.S. Fish and Wildlife Service (USFWS), Atlantic salmon restoration is a high priority program. In addition to producing and stocking non-feeding fry, the USFWS also supports smolt and parr stocking from production at Green Lake National Fish Hatchery (GLNFH) in Ellsworth, Maine. Additionally, Atlantic salmon (ATS) broodstock are produced and maintained at several USFWS hatcheries in the northeastern U.S. for egg and milt production. The current USFWS formulation for Atlantic salmon diet specifies use of herring oil as a diet ingredient. However, supplies of herring oil are limited due to decreased demand and spoilage is common since inventories are slow moving and the cost is becoming prohibitive (personal communication, Tim Markey, nutrition services manager, Zeigler Brothers, Inc.). Menhaden fish oil is the commonly used oil in aquaculture feeds by most major aquaculture feed companies in the USA because it is palatable and readily available (personal communication, Zeigler Brothers, Inc.). Before changing USFWS-Region 5 feed specifications to favor use of menhaden oil, it would be wise to review existing information concerning the potential effect of such a change and to conduct field evaluation of Atlantic salmon fed contract ASD2-30 feed manufactured with menhaden oil.

Study Objectives:

This study will compare growth performance, fin condition, and skin condition of approximately 80,000 Atlantic salmon hatched and maintained at GLNFH where they will be fed either a diet containing menhaden oil or herring oil. The study will begin at early parr stage and continue for approximately one year. Fish from yolk-sac to parr will be studied at NEFC (see study plan L-96-05)

Materials and Methods:

Zeigler Brothers, Inc., Gardners, PA. will manufacture two diets for use in this study with one containing menhaden oil instead of herring oil. Green Lake domestic strain of Atlantic salmon will be used in the experiment. Fry hatched in the spring of FY96 will be pooled from a variety of families and stocked into inside circular tanks. At yolk-sac absorption, feeding will commence using Biodiet feed (Bioproducts, Inc., Warrenton, Oregon), followed by gradual conversion of the fish to Region 5's ASD2-30 herring oil formulation. In June, 1996, fish will be moved to outside circular tanks and stocked at production densities of 25,000 individuals/tank. At this time, eight experimental tanks will be established (*four replicates X two treatment groups*) and experimental diets will be introduced. In September, 1996, parr will be separated from potential smolts and enumerated for each tank. Fish remaining in the study will be fed experimental diets through the winter of 1996/97. In Fall of 1996 and again in the Spring of 1997, (prior to stocking study fish) 150 fish will be randomly evaluated for: total length, weight, skin condition, and fin condition.

Results

Study completion is scheduled for the Spring of 1997 with subsequent data evaluation in the Fall of 1997.

Study Number: L-96-04

Title: Using nuclear and mitochondrial DNA from Atlantic sturgeon of the Hudson and Delaware Rivers to determine whether the populations are genetically distinct.

Principal Investigator: Northeast Fishery Center biologists

Co-Invest/Cooperators: Tim King, NBS-Leetown Science Center, WV

Background and Justification:

Historically, the Delaware River was a principal sturgeon fishery which exceeded all others in the United States. In 1890, the average was 60 sturgeon per net and by 1899 it dropped to as low as 8 fish per net. In the mid-Atlantic region, the Hudson River in New York is currently the only reliable source of spawning adult ASN. No spawning sturgeon from the Delaware River have been verified in recent years. The collapse of ASN populations has been addressed in the form of management plans for restoration of this species throughout its range by the Atlantic States Marine Fisheries Commission (ASMFC). The goal of the ASMFC plan is to restore ASN to a fishable abundance of 700,000 pounds per year (10% of recorded 1890 landings). Recent efforts to supplement wild populations with hatchery-produced ASN were suspended due to management concerns that introduced fish might compromise the genetic integrity of the recipient population.

Study objectives

The U.S. Fish and Wildlife Service-Northeast Fishery Center-Lamar, PA proposes to work with the NBS-Leetown Science Center, WV to develop microsatellite markers in Atlantic sturgeon DNA using current technology and to determine stock differences by screening 30-50 individuals from each river system using primers developed for these DNA regions.

Materials and Methods:

Genomic DNA of 30 fish from each population (i.e., Hudson and Delaware Rivers, and as many as possible from the Chesapeake Bay) will be isolated from fin tissue by the CTAB method. Selected regions of the mitochondrial DNA molecule will be amplified and prepared for restriction fragment length polymorphism analysis using the polymerase chain reaction (PCR) employing conserved primers. DNA fingerprinting using commercially available probes will be used to identify and quantify genetic diversity among and within Atlantic sturgeon populations. Initially, DNA from six individuals from the Hudson and Delaware Rivers will be digested with four restriction enzymes. The DNA will be run on a gel then transferred to MagnaGraph nylon transfer membrane (MSI, Westboro, MA) filters following the manufacturer's protocol. Band sizes will be estimated by comparing each band to a DNA marker (Life Technologies, Gaithersburg, MD) and analyzed with computer software (Scanalystics, Billerica, MA). DNA will be amplified using RAPD technology whereby products will be visualized by ethidium bromide staining. Statistical analysis of multilocus DNA fingerprinting and RAPDs will be performed to determine genetic differences.

Results

Microsatellite markers were developed for Atlantic sturgeon. Results revealed a great deal of variability within and between systems with common alleles of different frequencies observed between fish from the Delaware and Hudson. However, some alleles present in the Chesapeake Bay fish were not present in the Hudson and Delaware fish. This could be the result of gene flow from a southern influence or inadequate sample size from the Hudson and Delaware. A follow-up study is expected in FY97 to resolve this matter.

Study Number: L-96-05

Title: Comparison of growth and fin condition in Atlantic salmon fed U.S. government contracted feeds manufactured with herring or menhaden oil - Part 1 (yolk-sac to parr).

Principal Investigators: Northeast Fishery Center biologists

Co-Investigators/Cooperators: Paul Gaston, Green Lake NFH

Background and Justification: Currently, in Region 5 of the U.S. Fish and Wildlife Service (USFWS), Atlantic salmon restoration is a high priority program. In addition to producing and stocking non-feeding fry, the USFWS also supports smolt and parr stocking from production at Green Lake National Fish Hatchery (GLNFH) in Ellsworth, Maine. Additionally, Atlantic salmon (ATS) broodstock are produced and maintained at several USFWS hatcheries in the northeastern U.S. for egg and milt production. The current USFWS formulation for Atlantic salmon diet specifies use of herring oil as a diet ingredient. However, supplies of herring oil are limited due to decreased demand and spoilage is common since inventories are slow moving and the cost is becoming prohibitive (personal communication, Tim Markey, nutrition services manager, Zeigler Brothers, Inc.). Menhaden fish oil is the commonly used oil in aquaculture feeds by most major aquaculture feed companies in the USA because it is palatable and readily available (personal communication, Zeigler Brothers, Inc.). Before changing USFWS-Region 5 feed specifications to favor use of menhaden oil, it would be wise to review existing information concerning the potential effect of such a change and to conduct field evaluation of Atlantic salmon fed contract ASD2-30 feed manufactured with menhaden oil.

Study Objectives:

NEFC will compare survival, growth performance, fin condition, and skin condition of approximately 3000 ATS hatched and maintained at NEFC where they will be fed either a diet containing menhaden oil or herring oil. The study will begin at yolk-sac absorption and continue until early parr stage, approximately 120 days. The relative performance of ATS fed diets containing the herring or menhaden oil will be determined for parr and smolts at GLNFH during part 2 of the experiment (see study plan 96-03).

Materials and Methods:

Zeigler Brothers, Inc., Gardners, PA. will manufacture two diets for use in this study. Experimental diets will be manufactured under USFWS Region 5 fish food contract specifications for ATS except that one will contain menhaden oil instead of herring oil. Connecticut river domestic strain of ATS will be used in the experiment. During mid-March, eyed-eggs will arrive at NEFC from White River National Fish Hatchery. Just prior to yolk absorption, fry will be placed into six, two-foot indoor circular (40L) tanks at 200 per tank. Feeding will commence using Biodiet feed (Bioproducts, Inc., Warrenton, Oregon), followed by gradual conversion to the experimental diets. Three tanks will be fed herring oil diet and three will be fed menhaden oil diet,(Zeigler Brothers, Inc.). Time of initial feeding, feed size, and conversion to ASD2-30 will, as close as possible, follow standard hatchery procedures and be at the discretion of NEFC biologists. Mortalities will be recorded daily and feed amounts will be adjusted monthly. At early parr stage, July 1996, this study will terminate. During FY97, growth, fatty tissue/skin/fin condition, blood cholesterol and hematocrit will be compared between the two groups of fish.

Results:

Data evaluation is expected to be completed during FY97.

Study Number: L-96-06

Title: The effects of diet and temperature upon growth, tissue composition and survival of juvenile Atlantic sturgeon.

Principal Investigators: Kim King; Bucknell University
Co-Investigators/Cooperators: Northeast Fishery Center biologists

Background and Justification:

Decline in the wild Atlantic sturgeon population has prompted the Atlantic coast states and the federal government to provide for its protection. In November 1990, the Atlantic States Marine Fisheries Commission adopted a Fishery Management Plan to restore the Atlantic sturgeon resource to fishable abundance throughout its range. The plan encourages aquaculture efforts the species and evaluation of the potential for stock restoration/enhancement through the release of cultured fish. Identification of suitable commercial diets will facilitate research on specific nutritional needs for the species. In addition, fundamental information on Atlantic sturgeon culture will result.

Study Objectives:

A generalized growth rate curve will be described for year-class 0-1+ Atlantic sturgeon subjected to naturally occurring fluctuations in temperature. Also, effects of diet and temperature fluctuations on growth and the nutritional condition of this species will be investigated. Finally, fish performance will be evaluated using six commercial feeds as grower diets.

Materials and Methods:

One thousand eighty Atlantic sturgeon fingerlings will be provided by the NEFC at Lamar Pennsylvania. Fingerlings will be distributed into 18 - 400 L circular tanks with 60 fish/tank and acclimated. After acclimation, biomass will be adjusted equally for each tank. Six different diets will be assigned randomly to tanks with three replicates per treatment: Biodry 4000; Biokyowa; Extruded Sturgeon diet; Zeigler's high energy-high fat diet; ASD2-30 and GR7-30 (US Fish and Wildlife formulations). Weight and length inventories will be conducted at monthly intervals and feed amounts adjusted accordingly. Body measurements will be collected from individual fish at monthly intervals and at the beginning and end of the experiment. Seasonal variations of specific growth rate will be calculated. Proximate composition of the diets and fish will be determined and gross energy measured with a Parr calorimeter. Blood plasma samples will be collected at the time of proximate composition sampling. Blood glucose and protein concentrations will be determined by glucose oxidase using a Sigma kit and Lowry methods. Collected data will undergo statistical analyses using Analysis of Variance and General Linear Models procedures described in SAS version 6.04.

Results:

The study will terminate during the summer of 1997 with subsequent data evaluation during FY97.

Study Number: L-96-07

Title: An Annotated Bibliography of the Marking and Tagging of Salmonid Fry

Principal Investigator: Kim King - Bucknell University

Background and Justification:

Stocks of Atlantic Salmon *(Salmo salar)* have dramatically declined in New England rivers due to over fishing, dam construction, and degradation of spawning and rearing habitats. Since the late 19th century, federal and state agencies have been involved in Atlantic salmon recovery efforts, the goal of which is to restore self-sustaining populations of the species to native waters. In recent years, emphasis has been placed on stocking fry rather than parr or smolts. Critical to the evaluation of fry stocking for salmon restoration is a viable marking system that would allow managers to determine stock contribution, migration, growth, survival and population estimates.

In 1991, a Hatchery Product Evaluation Program (HPEP) was developed to improve achievements of both National Fish Hatchery and overall Fisheries Activity goals. The program "monitors both the quality of fish throughout the hatchery rearing cycle and maintains follow-up evaluations on their post-release performance relative to the appropriate management goals and objectives.". Some of the current evaluations identified as important by the HPEP for Atlantic salmon fry include:

1) Development of better marking techniques to differentiate between hatchery and wild fry

2) Methods for marking feeding and non-feeding fry with non-lethal method of detection

3) Development and assessment of a non-lethal batch mark for fry to provide a means of identifying origin (river or tributary)

Objectives:

A bibliography will be prepared to provide fishery biologists and resource managers an overview of sources that can be used to make informed decisions on potential fry marking and tagging methods available for Atlantic salmon.

Methods and Materials:

A literature search on marking and tagging methods was performed through the former National Biological Service (NBS) Information Transfer Center in Fort Collins, Colorado and the former NBS Research and Development Lab-Wellsboro, PA. using keywords: Marking, tagging, salmon, and trout.

Results:

Through this search, 721 citations were obtained along with an additional 20 sources obtained *via* cross reference. Abstracts and or parts thereof were substituted for annotation where deemed appropriate.

Two appendices were included in the bibliography. Appendix A provides three tables which summarize the characteristics of marks and tags. The original list of 721 was narrowed to 113 articles directly pertaining to tagging methods which are listed unconventionally by title in Appendix B.

The bibliography was also published as Lamar Informational Leaflet 96-07 titled: "An Annotated Bibliography of the Marking and Tagging of Salmonid Fry"

Study Number: L-96-08

Title: Inducing fluorescent marks in larval Atlantic salmon using calcein (green) and calcein (blue)

Principal Investigator: Jerre W. Mohler-NEFC

Background and Justification:

Fry stocking by the U.S. Fish and Wildlife Service has become an increasingly important part of the Atlantic salmon (ATS) restoration program in the Northeastern U.S.. Allowing nature to select those which are fit for their environment is currently viewed as an important management tool to promote establishment of self-sustaining populations over their historic range. Paramount to the success of fry stocking in achieving management goals is the ability to assess the effectiveness of such a management strategy. Therefore, a need exists within region 5 of the U.S. Fish and Wildlife Service for a technique of marking non-feeding Atlantic salmon fry (sac-fry) with a readily recognizable tag or mark capable of being detected in returning adult fish. In 1995, NEFC biologists tested calcein on larval ATS for effects on short and long term mark retention, health, and growth *(Study L-95-04)*. Calcein-treated fish received a mark detectable as brilliant green fluorescence in all fin ray structures when viewed under long wave fluorescent microscopy. Marks were non-lethally detected in 88 - 95% sampled at 5 months post-immersion. No difference in 10-day mortality was found between treatments (P>0.05).

Study Objectives

This study will compare 12, 24, 36, and 48 hours as immersion durations for successful marking with calcein (green). Mortality and mark induction will be compared between calcein (green) at 200 and 250 mg/L. In addition, marking capabilities for calcein (blue) will be explored. It will be determined whether there is a difference in mark induction or mortality between ATS larvae immersed in NEFC vs White River NFH water (where most eggs are incubated for Region 5 hatcheries). Lastly, the efficacy of marking larvae in Heath trays by recirculating a calcein solution will be tested.

Materials and Methods:

Immersion treatments - Immersion trials will consist of calcein static baths. Each immersion trial will have 3 replicates containing 200 non-feeding ATS fry. Calcein immersions will be at concentrations of 200 and 250 mg/L. Thirty fish from each replicate will be transferred into separate hatching jars containing fresh flow-through water at 12, 24, and 36 hours into the trial in triplicate. Immersion treatments of calcein (green) for 48 hours will be performed in both NEFC spring water and White River NFH water. Three control replicates will receive the same treatment as immersed fish but without an added chemical. **Heath tray treatment** - The immersion will take place in a heath tray incubation stack at NEFC by recirculating the calcein solution over a 48 hour period. Fresh water flow will be introduced back into the trays after 48 hours. Larvae will be pooled into a culture unit just prior to the onset of exogenous feeding and subsequently scored under fluorescent microscopy for mark detection.

Results

It was found that fish in the high concentration of calcein green for 48 hr had a significantly higher score for mark readability than other treatments except for those immersed in heath trays or using White River NFH water (P#0.05). Mortality was significantly higher in 48-hour treatments of calcein blue and heath trays than all other treatments (P#0.05). Mortality was not significantly different in White River water vs. NEFC water. Evaluation of mark uptake in fish immersed in calcein blue is scheduled for completion by May, 1997.

Study Number: L-96-09

Title: Study of larval rearing density with hatchery-produced Atlantic sturgeon *(Acipenser oxyrhynchus)*.

Principal Investigator: Jerre W. Mohler; Northeast Fishery Center

Co-Invest/Cooperators: NEFC Biologists

Background and Justification:

Commercial records of Atlantic sturgeon landings from the late 1800's to the present indicate a severe decline in the fishery. This problem has been addressed in the form of management plans for restoration of this species throughout its range by the Atlantic States Marine Fisheries Commission (ASMFC). The ASMFC recommended formation of a culture and stocking group to develop guidelines for culture and restoration activities for ensuring consistency with goals and objectives of the ASMFC's Fishery Management Plan for Atlantic Sturgeon, November, 1990. The culture and stocking group, which is comprised of all ASMFC states, the U.S. Fish and Wildlife Service, and the National Marine Fisheries Service, has made various recommendations in Special Report No. 22 titled: Recommendations Concerning the Culture and Stocking of Atlantic Sturgeon. Recommendation 1.4 states: "Basic cultural experiments should be undertaken at appropriate federal and state facilities to provide information on .....fry production techniques, rearing and holding densities...". Relationships between culture stocking density, rearing unit substrate area, rearing unit volume, and feed item density are unknown for this species. It is important to discover these relationships for efficient hatchery operation and for obtaining greatest fry survival whether rearing fish for restoration stocking purposes or for commercial aquaculture. Knowledge gained from this study will be beneficial to future restoration efforts.

Study Objectives:

A total of 14,100 larval ASN will be used at five different initial stocking densities and two feed rates to compare effects on mortality and growth over a 20 day period.

Materials and Methods:

Larvae from Atlantic sturgeon eggs incubated at NEFC will be used in the experiment. Prior to yolk sac absorption, larvae will be pooled and randomly selected for inclusion into 60 L tanks with the following densities: 200, 600, 800, and 1000 per tank. One set of replicates having 1000 per tank will receive a double ration of feed daily. At 12 d post-hatch, Artemia will be introduced to all replicates 3 times daily by hand. Feed amounts will remain equal for all tanks over the duration of the 20 day experiment. Data collected on each treatment will be analyzed using Analysis of Variance Procedures in the SAS statistical software program.

Results

1. Through termination of Artemia feeding (study day 20), mortality ranged from 48% in the low density group to 90% in the 800/tank group with a significant difference between the low density group and all others ($P \# 0.05$).
2. In general, elevated mortalities for all treatments suggest either poor quality larvae or insufficient food supply.
3. In high density tanks receiving a double ration of feed, mortality was lower (78 vs 88%) than those receiving a single ration of feed, suggesting that feed ration may have been a limiting factor at that density of fish.

L-96-10 Time of optimum milt yield after injection of Common Carp Pituitary (CCP) in 2+ domestic Atlantic salmon *(Salmo salar)*

Principal Investigators: NEFC Biologists

Background and Justification:

Synchronization of Atlantic salmon (ATS) gonadal development is sometimes necessary to ensure that sufficient male gametes are available at the appropriate time to permit desired paired matings in USFWS Region 5 ATS restoration efforts. A study was performed in 1994 by the Northeast Fishery Center (NEFC) at Nashua NFH using sixty three (63) 2+ year male domestic Atlantic salmon which were producing little or no milt. The experiment was performed to determine whether time of spermiation, milt volume, and sperm counts were different in three groups of male ATS which received either LHRHa, CCP, or injection of saline solution (controls). Additionally, percent hatch was compared in eggs fertilized with milt produced from males receiving the three treatments. Results of this study showed that spermiation can be manipulated in 2+ male Nashua domestic ATS broodstock but more work was needed to determine the "window" of time for maximum milt yield after hormone injection.

Study Objectives

The 1994 study only addressed milt production at 7 and 14 days post-injection. Objectives of this study are to **1)** determine a more narrow range of time for optimum milt yield post-injection of CCP solution in a group of 30 domestic male ATS at Nashua NFH over a ten day period, **2)** determine whether milt quality as measured by motility and cell count is correlated to time elapsed post-injection in individual fish.

Materials and Methods

Sixty fish producing little or no milt will be selected and separated into two groups by Nashua NFH for the study. Thirty fish will be injected with CCP and 30 will be injected with sterile saline solution as a control group. CCP dosage will be 10 mg/Kg mixed in a carrier of sterile saline solution and administered in one cc aliquots per fish into the dorsal musculature. Each fish will be anesthetized, tagged with a numbered floy tag, and weighed prior to injection. Each fish will be examined for milt production at 48 hours post-injection and every other day thereafter until milt volume obviously decreases. Milt expressed during each examination will be measured volumetrically for each fish and individuals will be tracked throughout the study. Milt quality will be determined by percent motility and cell count.

Results:

1. At two days post-injection, 8 of the 30 fish injected with CCP produced a total of 46 mls of milt. Only 2 control fish (which received a sham injection) produced a trace amount of milt.
2. At four days post-injection, milt production was similar to the previous inspection for the same CCP-injected fish and 4 control fish produced a small amount (total = 3 milliliters) of milt.
3. At 6 days post-injection, total milt volume decreased for CCP-injected fish (total = 27 mls) while control fish milt production increased slightly (total = 9 mls).
4. Only males which showed secondary sex characteristics (kype formation, color change) responded to hormone injections.
5. In CCP-injected fish, sperm motility was >90% until day 6 when a decrease was seen. In general, cell counts were lower at day 6 than prior and CCP-injected fish had higher cell counts than controls.
5. It is suggested that at Nashua NFH, workers separate age 2+ males showing secondary sex characteristics two weeks before spawning time. If scarcity of milt requires hormone usage, CCP at 10 mg/Kg should be injected in males two-three days prior to egg take.