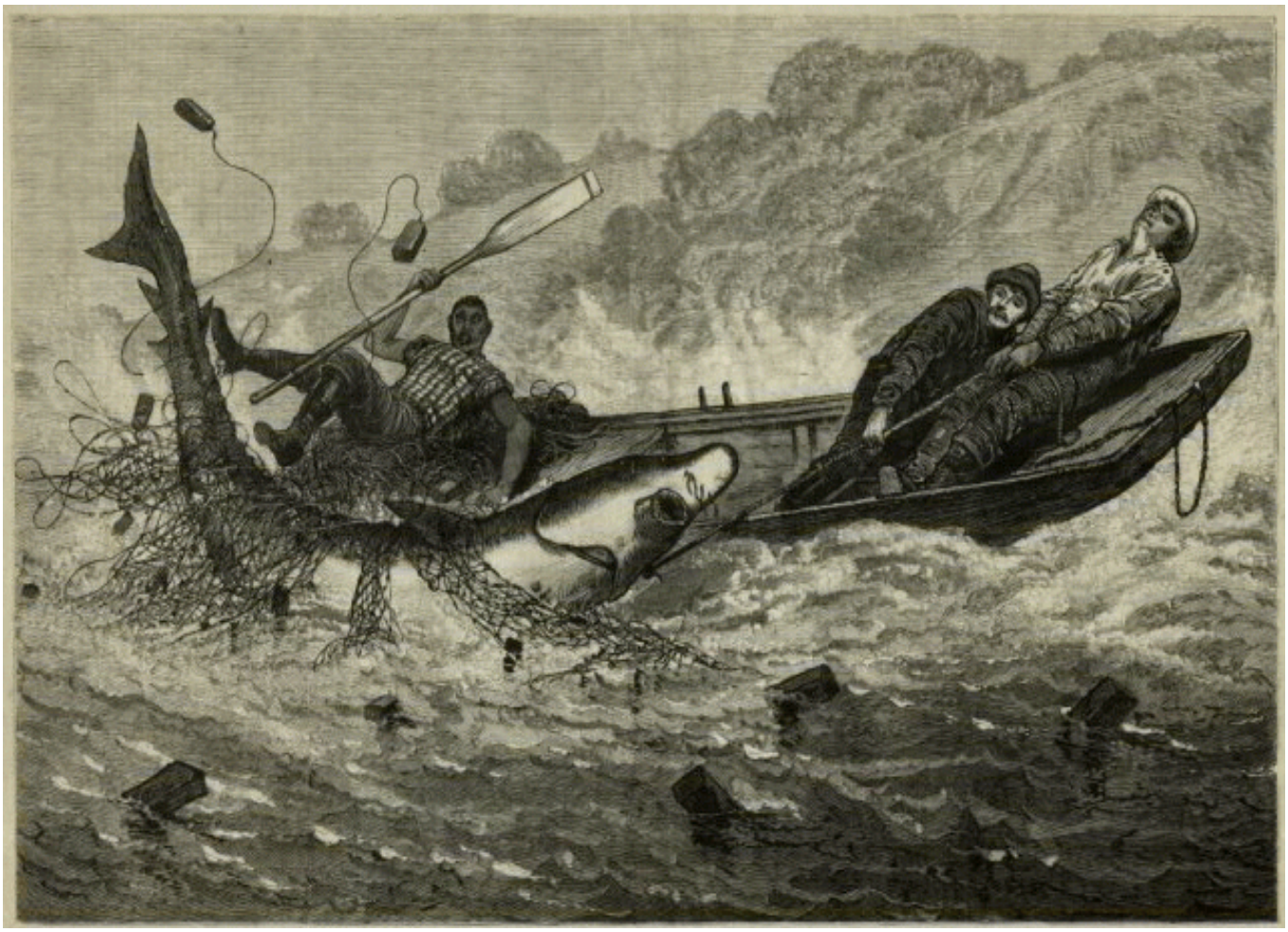
Culture Manual for the Atlantic sturgeon

Acipenser oxyrinchus oxyrinchus



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PREFACE

"The sturgeon passed their boats in such vast numbers that in a little while the occupants had killed and secured eleven. This was as many as they could take home and, as the run continued, they slew many more on the principle that it was a fish not only scarcely of any value, but was actually a nuisance in the river....." (In Fish, Fishing, and Fisheries of Pennsylvania, by William E. Meehan) as reported by Cobb (1899).

Such colorful anecdotes written about Atlantic sturgeon near the end of the 19th century can lead to a desire to revert back in time to witness such natural phenomena first-hand. Even though it is likely that many such stories have been somewhat embellished by their originator, in the case of the Atlantic sturgeon, respected naturalists of that era lend credence to those reports. Through published "Bulletins of the U.S. Fish Commission" by Bashford Dean (1893), John Ryder (1900), and John Cobb (1899), we glimpse the magnitude of the early Atlantic sturgeon fishery and it's rapid decline in the Delaware River, which once yielded harvests exceeding all others in the United States. These accounts, coupled with the recent absence of spawning adult sturgeon in the Delaware River, are a much too familiar commentary on human stewardship of aquatic natural resources. Fortunately, the literature produced over 100 years ago by agents of the U.S. Fish Commission laid a solid foundation of reference upon which others could build the current knowledge base of Atlantic sturgeon biology. As we blew the dust off the century-old documents and began to search for additional literature, it soon became evident that little had been published concerning culture of this species. Nevertheless, all relevant information was compiled and relied upon extensively by the author and supporters of this manual who saw value in conservation of the species not simply for it's economic worth, but for it's intrinsic value and role in preserving the integrity of our aquatic ecosystems. Hopefully others will continue to build upon our knowledge of Atlantic sturgeon culture which was shaped over the years by a combination of trial and error, assistance from a myriad of cooperators, the scientific method, and some good fortune (but not necessarily in that order).

Sturgeon and paddlefish populations worldwide have declined and their diversity is currently threatened due to human influences (Wirgin et al. 1997) . Likewise, Atlantic sturgeon populations have been depleted over most of their natural range. As a result of these declines, all sturgeon and paddlefish species worldwide were included in Appendix II of the Convention on the International Trade of Endangered Speciesof Wild Fauna and Flora (CITES) regulations on April 1, 1998. This provided a regulatory mechanism for import and export of sturgeon and their products thereby helping to control the illegal caviar trade and its detriment to wild populations.

Depletion of sturgeon stocks world-wide has increased interest in aquaculture-based restoration programs (Secor, et al. 2000). Some success was reported by Smith et al. (1980; 1981) in Atlantic sturgeon culture with wild broodstock from South Carolina but most fry did not survive past about 30 days post-hatch. Likewise Parauka et al. (1991) reported successful spawning of the subspecies Acipenser oxyrinchus desotoi, but with poor fry survival. In light of serious declines in populations of Atlantic sturgeon, the Atlantic States Marine Fisheries Commission (ASMFC) formulated a management plan for the species seeking to restore populations to a harvestable level (Taub, 1990). Part of this management plan called for development of reliable artificial propagation techniques as a potential tool to supplement wild populations. Updated information and recommendations for management of the species were presented in Amendment 1 to the Interstate Fishery Management Plan for Atlantic Sturgeon (ASMFC,

1998). One of the most important elements of Amendment 1 is adoption of a series of recommendations concerning the culture and stocking of Atlantic sturgeon (ASMFC, 1992). This, in turn, led to development of a Breeding and Stocking Protocol for Cultured Atlantic Sturgeon (ASMFC , In Press) which provides guidance to maximize genetic diversity and minimize inbreeding depression in the event that cultured sturgeon are needed for population enhancement or restoration.

Faced with the potential for losing yet another valuable species and part of our national heritage, the U.S. Fish and Wildlife Service-Northeast Fishery Center at Lamar, Pennsylvania (NEFC) assumed responsibility for taking the lead role in developing guidelines to hold and propagate Atlantic sturgeon before wild populations dwindled to the point where no broodstock could be found. This task was no small commitment, given the difficulties encountered and special equipment needed to capture, transport, hold, and spawn broodstock. The time commitment was also considerable given the estimation that it may take 12 - 15 years for offspring to become mature adults in the hatchery.

Simply stated, it would not have been possible to produce this manual were it not for the countless hours each staff member at the Northeast Fishery Center (NEFC) dedicated to the project. Whether working away from home for weeks at a time on the water attempting to locate and capture sturgeon, or working at the Center in a support role, all had an equally important part in this production. This project represents one of the rare fragments in the course of history where an extraordinary combination of people found themselves assembled together. Each offered up his own special talents, which alone would not have sufficed, but when combined represented a complete package of abilities able to meet the formidable task of producing this Atlantic Sturgeon Culture Manual. Working with such a large and unusual species presented challenges not only from a biological perspective, but also from that of equipment availability and engineering. Much of the gear needed for field and laboratory work was not commercially available; therefore, the fabrication skills and ingenuity of all were called upon. We were all fortunate to have been given the chance to be involved with this project.

When presented with the challenge of coordinating this project, I learned that I had been functioning most of my life under the false notion that "someone else out there" is taking care of this or that particular problem or species in decline. When I realized that we were that "someone else", the responsibility for stewardship of this valuable resource became less a job and more of a joy. At times our tasks seemed surrealistic, and we got a glimpse of what it must have been like for that famous group of fishermen in the first century who cast their nets into the Sea of Galilee to bring up a bounty from the depths. At times the rain sliced out of the sky horizontally while we retrieved our nets. Equally strange was the backdrop of a skyscraper canyon while "wrestling" an adult sturgeon on the Plaza of Rockefeller Center in Manhattan accompanied by naturalist Jim Fowler for all of America to see on NBC's Today Show.

All those memories are more than enough reason to acknowledge the following individuals for their part in this production: **Michael A. Hendrix** - NEFC Director during the project, who believed in the abilities of those under his command and allowed them to pursue their ideas, and who almost went down with the ship on a sturgeon expedition; **Dr. Michael J. Millard** - Subsequent NEFC Director who reviewed and prioritized the publication of this manual; **John W. Fletcher** - NEFC Tech Center Unit Leader, who was tireless in his efforts to obtain success on the project, and who always made sure our boat "Recovery One" was outfitted with the best gear for the task at hand; **Anthony Carta** and **Tom Bryerton** - NEFC

Maintenance staff, who always devised novel ways to construct the customized equipment needed and who continually modified Recovery One; **Kim King**, **Pat Farrell**, and **Wade Jodun** - NEFC Biological Staff, who performed investigative studies, had the pleasure of interfacing daily with the sturgeon, and came up with ways to "tame the wild beasts" in captivity; **Christine Eisenhower** - NEFC Administrative Assistant, who always managed to get us paid every two weeks and even helped spawn sturgeon on occasion; **John Coll**, **Patricia Barbash**, **Kim Selmer-Larsen**, and **Rich Gunsallus** - NEFC Fish Health Unit staff, who diagnosed disease and helped us treat our sick sturgeon friends; **Richard St. Pierre**- Susquehanna River Coordinator (U.S. Fish & Wildlife Service) who made sure we had the necessary permits and all our ducks were in a row; **Mike Mangold** and **Jorgen Skjeveland** - Maryland Fisheries Resource Office**,** for assistance with boats, manpower, and evaluation of experimental stockings; **Bill Krise** and **Martin DiLauro-** U.S.G.S. Research Lab at Wellsboro, PA (formerly of the U.S. Fish & Wildlife Service) for physical and technical help in the pioneering stages of spawning and culture; **Kathy Hattalla** and **Andy Kahnle** - New York Department of Environmental Conservation, who allowed us to borrow and sometimes harvest valuable and scarce wild sturgeon broodstock from the mighty Hudson; **Bill Andrews** - New Jersey Department of Environmental Protection, who was always willing to cooperate and lined us up with some very interesting commercial sturgeon fishermen out of Belmar, NJ; **Craig Shirey** - Delaware Department of Natural Resources, who guided us to the "hang out" of juvenile sturgeon near the Salem nuclear reactor; **Joel Van Eenennaam**, **Serge Doroshov**, and all the white sturgeon researchers at the University of California-Davis, who showed us the ropes concerning spawning techniques and always provided technical help when we needed it, and **Craig S. Kennedy**, for helpful editorial comments. Finally, a special acknowledgment is proper for one-time commercial sturgeon fisherman, **Doug Bush** who departed in 2001 but not before he befriended us and passed on the endangered art of gill-netting mature sturgeon on the Hudson River while teaching us the curious ways of that prehistoric inhabitant of the murky depths - the Atlantic sturgeon.

Jerre W. Mohler

Contents

Introduction

Historically, Atlantic sturgeon Acipenser oxyrinchus oxyrinchus were reported in most major rivers on the eastern seaboard of North America ranging from the Hamilton Inlet on the Atlantic coast of Labrador to the St. Johns River in Florida. A sub-species known as the Gulf sturgeon, Acipenser oxrinchus desotoi is limited historically to the Gulf of Mexico, the northern coast of South America, and possibly Bermuda (Murawski and Pacheco 1977). North American Atlantic sturgeon are closely related to the European Atlantic sturgeon, Acipenser sturio , and were once referred to with the same scientific name by Dean (1893), Ryder (1900), and Cobb (1899). However, they were subsequently classified as separate species morphologically by Vladykov (1955) and genetically (Ong et al. 1996; Birstein and DeSalle 1998).

Through biological classification, this species is placed in the family Acipenseridae, a category of ancient bony fishes which have been able to survive as a group in contemporary environmental conditions due to adaptative advantages such as: wide range of spawning temperatures, longer retention of fertilizability by sperm and eggs in water, early tolerance of juvenile fish to wide variations in water salinity, broad spectrum of feeding, protection from predators afforded juvenile fish by their scutes (i.e., external bony plates) , etc. (Detlaff, et al. 1993). Atlantic sturgeon are late-maturing anadromous fish that may live up to 50 years, reach lengths up to 14 feet (4.3 m), and weigh over 800 pounds (364 kg). They are distinguished by armor-like plates and a long snout and are opportunistic benthic feeders, filtering quantities of mud along with their food which consists of aquatic insects, amphipods, and oligochaete worms in fresh water, while in salt water their diet consists of marine gastropods, shrimps, amphipods, and isopods (Vladykov and Greely, 1963). Johnson et al. (1997) found that sand and organic debris were a major component of the stomachs of offshore New Jersey Atlantic sturgeon with polycheates and isopods being the prevalent prey items.

Mature fish migrate from the sea to fresh water in advance of spawning with females first maturing at ages ranging from 7-19 years old in South Carolina to 27-28 years in the St. Lawrence River; males can be somewhat younger at first spawning. Spawning occurs in flowing fresh or estuarine waters with a hard bottom (U.S. Departments of Commerce and Interior 1998). Shed eggs are 2-3 mm in diameter and become sticky when fertilized, frequently becoming attached to submerged detritus until hatching in several days. After hatching occurs, juveniles remain in fresh water for several years but have been documented to out-migrate to coastal areas in their 3rd year. Out-migrating Atlantic sturgeon are known to frequent distant estuary systems; tagged age-0 fingerlings stocked in the Hudson River in 1994 were found in the Chesapeake and Delaware Bays in 1997 (Bain, 1998) .

Mature Atlantic sturgeon have great commercial value for both flesh and roe, the latter being known as caviar. It is clear that Atlantic sturgeon underwent significant range-wide declines from historical abundance levels due to overfishing in the late 1800s (U.S. Departments of Commerce and Interior 1998). Populations did not rebound to any appreciable extent except in the Hudson River, which in the 1980's and 1990's once again became the target of a directed harvest of mature sturgeon during annual spawning migrations. Evidence of poor population recruitment in the Hudson River eventually led to the closure of that fishery in 1997 by the State of New York. In 1998, a moratorium on Atlantic sturgeon harvest in all U.S. waters was adopted by the Atlantic States Marine Fisheries Commission, enforceable under the provisions of 1993 amendments to the Atlantic Coastal Fisheries Cooperative Management Act

(P.L. 82-721) . This moratorium is anticipated to remain in place until there are at least 20 protected age classes of females in each spawning stock. For the Hudson River, the moratorium duration is anticipated to be approximately 41 years from its initiation. Supplementation of wild populations through use of hatchery-reared fish has been suggested as a restoration strategy but genetic concerns must first be addressed. These include the possibility of inbreeding through use of insufficient numbers of broodstock and the possibility of inter-population transfer of genetic material to adjacent wild stocks. Two experimental stockings of Atlantic sturgeon were conducted at the request of the states of New York and Maryland for the purpose of obtaining baseline information on survival, population recruitment, out-migration, and growth. The first stocking of fingerlings was performed in the Hudson River in 1994 and the second in the Nanticoke River of Chesapeake Bay in 1996 using fish hatched and reared at the Northeast Fishery Center - Lamar, PA (NEFC). Subsequent recapture of hatchery-reared individuals showed that survival was high and growth was comparable to that of their wild counterparts (Skjeveland et al. 2000) suggesting that prudent use of hatchery-reared fish for supplementation of wild stocks can be a useful tool.

The information contained in this manual represents the results of 11 years of effort by the Northeast Fishery Center and can be used as the best available information but untried methods, trials , and equipment may also be found useful in the culture of this species.

Trade name s and company names mentioned in this manual are for informational purposes only, and do not imply U.S Government endorsement of commercial products. All uses of fishery compounds must be registered by appropriate state or federal agencies. Only the uses described on the label are permitted, and only at the rates or dosages listed.

Chapter 1 Feral Broodstock Collection

The following information on broodstock collection is the result of experience gained from 1991 - 1998 by NEFC personnel on the Hudson River, Delaware River, and ocean waters off coastal New Jersey.

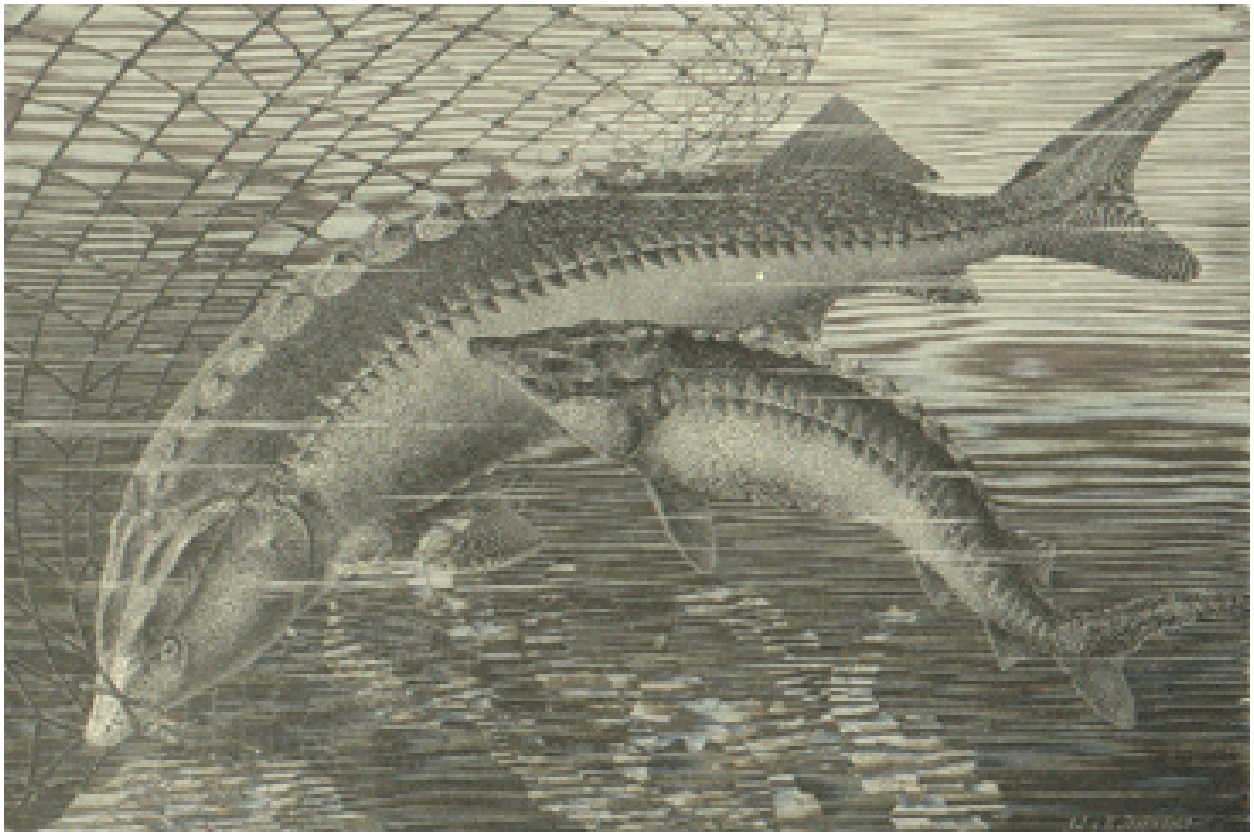
1.1 Spawning Areas

At this writing, the most reliable source of ripe Atlantic sturgeon broodstock in the U.S. is the tidal Hudson River in New York state. Out of 185 Atlantic sturgeon sampled in 1992 and 1993 from the New York Bight, all gravid females as well as the majority of ripe males were captured at Hudson River kilometer 136 or greater (Van Eenennaam et al. 1996). A well documented spawning area is located near Hyde Park, New York on the east side of Esopus Island in water depths of 18 meters or more. Active spawning areas on other rivers which historically supported migrations of this species are not well known. Historical accounts of commercial sturgeon landings from the Delaware River in the late 1800's by Cobb (1899) describe productive sturgeon capture areas many of which were likely spawning areas but sedimentation, dredging, and other hydrologic changes since then may have altered historical spawning habitat.

1.2 Capture Methods

Ripe Broodstock.- Typically, capture is performed with anchored gill nets having a mesh size of 28 to 38 cm diagonal stretch. Nets approximately 90 to 212 meters in length and 2 - 3.5 meters in height are anchored or "set" perpendicular to the river channel, preferably in a known spawning area, at periods of slack tide during spawning season (approximately May - July in the Hudson River). Water depths are often 18 meters or more in spawning areas. Once set, nets are kept in the water until the tide begins to recede or advance. Most fishermen use some sort of indicator such as an anchored buoy to determine ebb and flow of tides. The duration of the set depends not only on general river location but local position within the channel as the tide may be flowing mid-channel but slack near the shoreline. When the tide begins to flow after being slack for a period, nets are generally hand-retrieved by two persons in a john-boat or similar sized craft (Figure 1). Even before a netted sturgeon can be seen by fishermen, numerous bubbles often appear at the water surface upon net retrieval, indicating that a sturgeon is entangled. Most likely, expansion of the swim bladder due to reduced water pressure near the surface is responsible for this phenomenon. Upon capture, it is not unusual for the sturgeon to continue releasing air bladder pressure which is clearly audible as moaning or grunting sounds.

Captured sturgeon can be hoisted onto the boat using 2 simple rope nooses; one slipped over the head of the fish behind the large pectoral fins and one slipped over the tail in the caudal peduncle area. After removing sturgeon from the net, it is desirable to have some type of holding tank outfitted with an oxygen supply on board the vessel (Figure 2) if the sturgeon is to be kept alive for an extended period of time or transported by boat to a distant location. Sturgeon placed in the holding tank should be protected from direct sunlight by some type of tank cover. Occasionally, sturgeon become entangled



in a gill net in a manner which renders both gill plates (opercula) immobilized. A fish in this condition must be removed from the net as soon as possible to prevent mortality. Broodstock which are to be kept alive for short periods of time (up to one hour) can be sprayed with water or kept wet by bailing water from the river and continually pouring it over the gills and skin. Mature sturgeon, especially ripe females, undergo much stress as a result of gill net capture and afterwards may float upside-down for a number of hours when released into the river or placed in a holding tank. Ripe females have been observed to cease respiration upon or shortly after capture but can be resuscitated by a combination of heart massage and manual flexion of the gill plates using oxygenated river water. Long-term survival of females captured in this condition, especially if artificially spawned, is unlikely.

Figure 1.- Retrieving sturgeon gill net

Figure 2.- Atlantic sturgeon in holding tank on capture boat

Non-ripe broodstock.- Non-ripe broodstock Atlantic sturgeon can be captured in New Jersey coastal waters during late winter and early spring. One such area exists about 1.6 km off-shore from the mouth of the Shark River at Belmar , New Jersey. Capture techniques are slightly different in ocean areas as compared to rivers. Specifically, nets of greater length (up to 0.8 kilometers) are set for a longer durations (24 hours or more). Nets are set and retrieved by employing a large hydraulically-operated spooling device mounted on a sea-worthy vessel (Figure 3). In ocean netting, there is a greater chance for sturgeon mortality to occur since nets are not retrieved for long periods of time. Captured sturgeon should be placed in a holding tank or kept wet as described above depending on the desired situation. Biopsy procedures (Section 3.3) must be performed on these fish to determine sex and stage of gonadal maturity. Wild female Atlantic sturgeon captured in this manner can be subsequently induced to spawn in captivity (Section 3.6).

Figure 3. Commercial fishermen retrieving spooled gill net

Chapter 2 Broodstock Holding and Transport

2.1 Holding Tanks for Field Work

Boat-mounted tanks.- In designing field holding tanks for broodstock, consideration must be given to the safety and health of the sturgeon being held, the desired outcome of holding, and practicality of equipment. Tank length and width should be sized to enable a fish to straighten comfortably without being able to turn around. Broodstock are usually a minimum of 1.5 meters total length and can become injured by constantly changing orientation if tank width is excessive. Desirable options for short-term holding tanks include an oxygen supply and a lid to obstruct sunlight. A tank length of at least 0.6 meters greater than the expected fish length is helpful for loading and unloading purposes. A small 12-volt electric pump outfitted with a length of hose can be used to transfer water between the holding tank and river as necessary. Boat-mounted tanks for short-term holding (up to one hour) should be filled with water of similar temperature to that of the capture area before gill nets are retrieved. Holding tanks should also be fitted with some type of gravity-flow drain. Broodstock are under stress as a result of capture, therefore it is desirable to monitor dissolved oxygen in the holding tank in order to maintain levels of > 80% saturation. If oxygen levels become too low, increase water flow or oxygen flow as needed to improve the chance for survival and spawning success of captured sturgeon.

Dockside tanks.- Dockside tanks are not recommended for holding ripe female sturgeon for more than a few hours if the fish are to be spawned in the immediate future. Stress of capture coupled with the high degree of gonadal maturity in females captured during spawning season can result in premature shedding of eggs or *in vivo* ooycyte degradation when fish are held dockside in ambient temperature river water. Conversely, male sturgeon have been successfully held for up to three days in dockside holding tanks on flow-through ambient river water without adversely effecting hormone-induced spermiation. The same design considerations for boat-mounted tanks apply here with the exception that dockside tanks can be somewhat larger and have provisions for constant flow-through water (Figure 4). Additionally, dockside tanks should be located in a secure area and be equipped with lockable lids if left unsupervised. Dockside tanks containing sturgeon which are left unsupervised for long periods of time are an invitation for loss of fish life due to water supply failure or interruption of electric power. River intakes for pump pipelines or submersible pumps must be kept free of debris to insure a continuous flow of water to holding tanks. In addition, if supply pumps are used in tidal areas, the pump or it's intake line must be submerged below the expected low tide line. It is good practice to conceal or secure any exposed power connections such as plugs and power cords. Additionally, make sure that all wiring and power equipment associated with the water supply is properly matched with the amperage rating of the circuit breaker being used.

THE RIVER FISHERIES OF THE ATLANTIC STATES.

A sturgeon camp on Winyah Bay, South Carolina: catching sturgeon in gill-nets; the pound for keeping them alive; unheading; saving caviare. (Sect. v, vol. i, p. 625.)

Drawing by H. W. Elliott.

PLATE

Figure 4.- Dockside sturgeon holding tanks with flow-through water supply

Trailer-mounted tanks.- If properly designed, a trailer-mounted tank yields the most versatility and safety for field crews and fish when a riverside holding tank is necessary. Mobility is the greatest advantage with this type unit which not only reduces man-hours involved in sturgeon transport but also reduces fish handling and is instrumental in obtaining the desired outcome for captured broodstock. Loading and unloading individual fish which may weigh over 90 kilograms are critical maneuvers made much easier and safer for all involved if the trailer-mounted tank has a low profile with easy access (Figures 5 & 6). A properly designed trailer-mounted tank can also double as a maturation tank for ripe females (see Section 3.6 on induced spawning).

Figure 5.- Trailer-mounted sturgeon transport/holding tank with fold-down steps

2.2 Transport

Many sturgeon capture areas are located where the general public has free access to dockside facilities and small crowds of interested people often gather when workers are transferring sturgeon from capture boat to dockside tank. Therefore, fisheries workers should be prepared by having proper sturgeon handling equipment before embarking on a capture trip. It should be standard operating procedure to use humane and safe handling and transport techniques.

Transfer of individual fish over short distances (e.g. from boat to holding tank) can be safely performed by using a stretcher and four workers. The stretcher should be constructed of plastic-coated or rubberized canvas type material. Hypalon® material typically used for lining constructed ponds works well for this. The stretcher should be equipped with 3.5 cm diameter steel pipe or equivalent material for handles (Figure 7). Captured sturgeon often have extremely sharp scutes which can easily slice skin as well as stretcher material, thus it is recommended that gloves be used when handling sturgeon. Wounds received from sturgeon scutes should receive first aid treatment immediately due to possible infection.

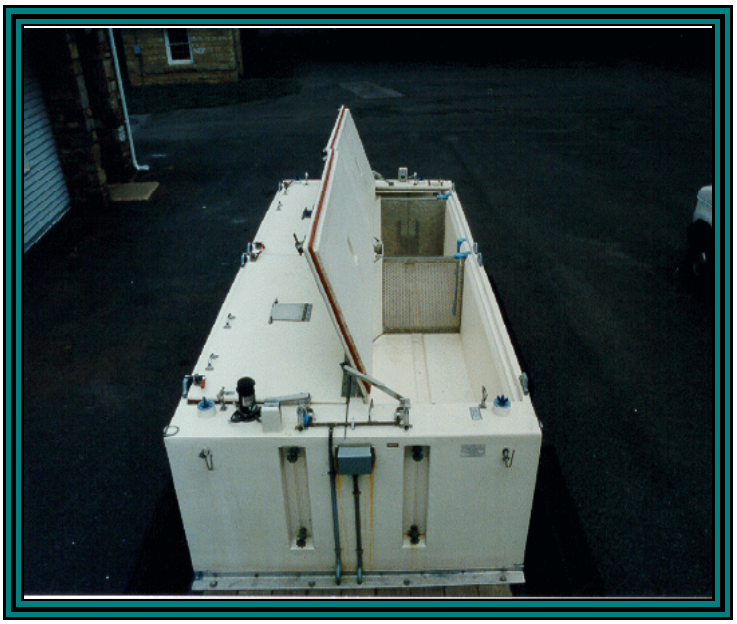


Figure 6.- Top view of tank showing screened compartments; equipped with oxygen injection, aerators, and top viewing ports

Figure 7.- Transferring a sturgeon back to the Hudson River with a heavy-duty stretcher

Typically, the Hudson River is about 23EC during the latter part of June when broodstock are captured during spawning migration. Detlaff et al. (1993), reported that long-term keeping of females at spawning temperatures causes the egg quality to deteriorate. Practical experience by NEFC has shown this to be accurate. However, good results have been obtained by transporting ripe broodstock (especially females) in fresh water maintained at a cool temperature (15-18EC) rather than ambient river water prior to administering spawning hormone injections. Use of salt in hauling tank water is not recommended for ripe Atlantic sturgeon broodstock which are to be spawned that season due to it's unknown effect on egg maturation.

2.3 Long-term Captivity

2.3.1 Culture Tanks and Rearing Conditions

It is recommended that broodstock sturgeon be held in circular tanks of at least 6 meters in diameter with a water depth of about 1 meter (Figure 8). At NEFC, fresh flow-through water has been successfully used to hold and grow captive broodfish for 11 consecutive years. Culture parameters during this time period were:

Temperature : 1E- 17EC annually
Dissolved Oxygen : > 6 mg/L
Hardness(Ca + Mg): about 70 mg/L
Biological Oxygen Demand (5-day): < 7 mg/L
pH: 7.5 - 8.0.

Figure 8.- Six-meter-diameter culture tanks used for broodstock sturgeon at NEFC-Lamar, PA.

A flow of 100 liters/minute is sufficient to maintain at least 10 broodfish under these conditions. Upon arrival at the holding facility, wild broodstock (except ripe females) should be treated with non-iodized salt at a concentration of 1% (10 grams per liter) for 24 hours to minimize fungal infestation. As a side benefit, this salt treatment also kills zebra mussel Dreissena polymorpha larvae which may be attached to the sturgeon if transported from infested waters. Subsequently, salt treatments should be administered for 7 consecutive days at a concentration of 0.25% (2.5 grams per liter) to replace electrolytes lost due to stress response. Stress of capture and transport along with abrasions received from gill nets, may cause a fungal infestation in freshly-captured sturgeon regardless of salt treatments, therefore daily flush-type formalin (Paracide F, Argent Chemical Laboratories, Redmond, Washington) treatments of 150 ppm should be administered to the culture tank for about 7 consecutive days after capture.

Typically, captive fish will circle the tank slowly and have periods of prolonged inactivity. However, it is not uncommon for a mature fish to suddenly bolt across the tank and break the surface of the water, therefore the holding tank should be equipped with plastic fencing or other acceptable material to a height of about 1 meter above the water level.

2.3.2 Conversion of Captive Fish to Formulated Feed

Offering feed to captive fish can commence as soon as fish are acclimated to an appropriate tank. It is common for mature and sub-adult wild fish brought into captivity to undergo an extended (months-long) period of starvation. In general, it is difficult to convert wild fish to captive feeding but most will eat frozen whole shrimp and bloodworms when thawed. Sturgeon have well-developed olfactory organs and are highly sensitive to food odors (Pyatkina 1991; Devitsina and Kazhlaer, 1993). They can actively patrol large areas and discover olfaction zones with high concentrations of food organisms (Kasumyan and Kazhlayev, 1993). One approach for conversion of wild fish to captive feeding is to offer

bloodworms or shrimp in conjunction with a formulated salmon or sturgeon diet. With this technique formulated pellets (Special Brood Diet, 10mm, BioOregon, Warrenton, Oregon) are soaked for about 10 minutes in a thawed bloodworm solution. Once saturated, the pellets are introduced 3-4 times daily into only one area of the holding tank. In this manner, fish can be observed for feeding behavior as they swim over the feed pellets. Feeding behavior is characterized as deliberate, irregular flaring of the gill plates when passing over food items and fish may appear to "bounce" slightly off the tank bottom as the mouth is protruded downward to capture food. Observations of feeding behavior are best performed from an elevated vantage point. Once regular feeding has been observed, the bloodworm solution can be gradually diluted and then eliminated completely. This process can be accomplished in 3-5 weeks (Wade Jodun, USFWS, personal communication).

An alternative method for converting fish from natural feed to pelleted feed involves mixing a preferred natural feed (bloodworms or shrimp) and target feed (pellets) into a matrix of gelling starch (Appendix I). Begin with about 90% preferred natural feed and 10% of the target feed ground up in a blender. This mixture can then be incorporated into the gelatinous matrix and allowed to set or gel prior to offering it to sturgeon. Introduce the diet and observe for feeding behavior as described above. As fish begin to eat the diet, the percentage of preferred natural feed can be reduced while increasing the content of the target feed until weaning to a pure diet of the target feed occurs. If neither of the above-described methods results in successful feeding of captive sturgeon, serious consideration should be given to returning the fish back to it's natural habitat.

In general, feeding behavior slows noticeably in Atlantic sturgeon once water temperatures drop below about 10EC, but at mean temperatures as low as 5.4°C minimal growth has been measured in juveniles offered a 0.25% body weight daily ration (Jodun and Kelligher, In progress). At temperatures above 10°C, an adequate feed ration for actively-feeding mature fish is 0.5% body weight per day. At times, captive fish may cease to feed due to seasonal fluctuations in water temperature therefore it may be necessary to re-initiate either of the two feeding strategies described above.

Chapter 3 Sexual Maturity

3.1 Wild Captive Sturgeon

Females transported immediately in season from a known spawning area have routinely been induced to spawn if proper measures are taken without delay. However, at this writing only 1 wild female Atlantic sturgeon has developed mature oocytes while held in long-term captivity and been repeatedly spawned. This particular female was first captured and spawned in 1995 then discovered to be gravid again in 2000. In June, 2002, biopsy revealed that this female had oocytes in Stage III of development (Van Eenennaam and Doroshov, 1998) which are likely 1-2 years away from final maturity.

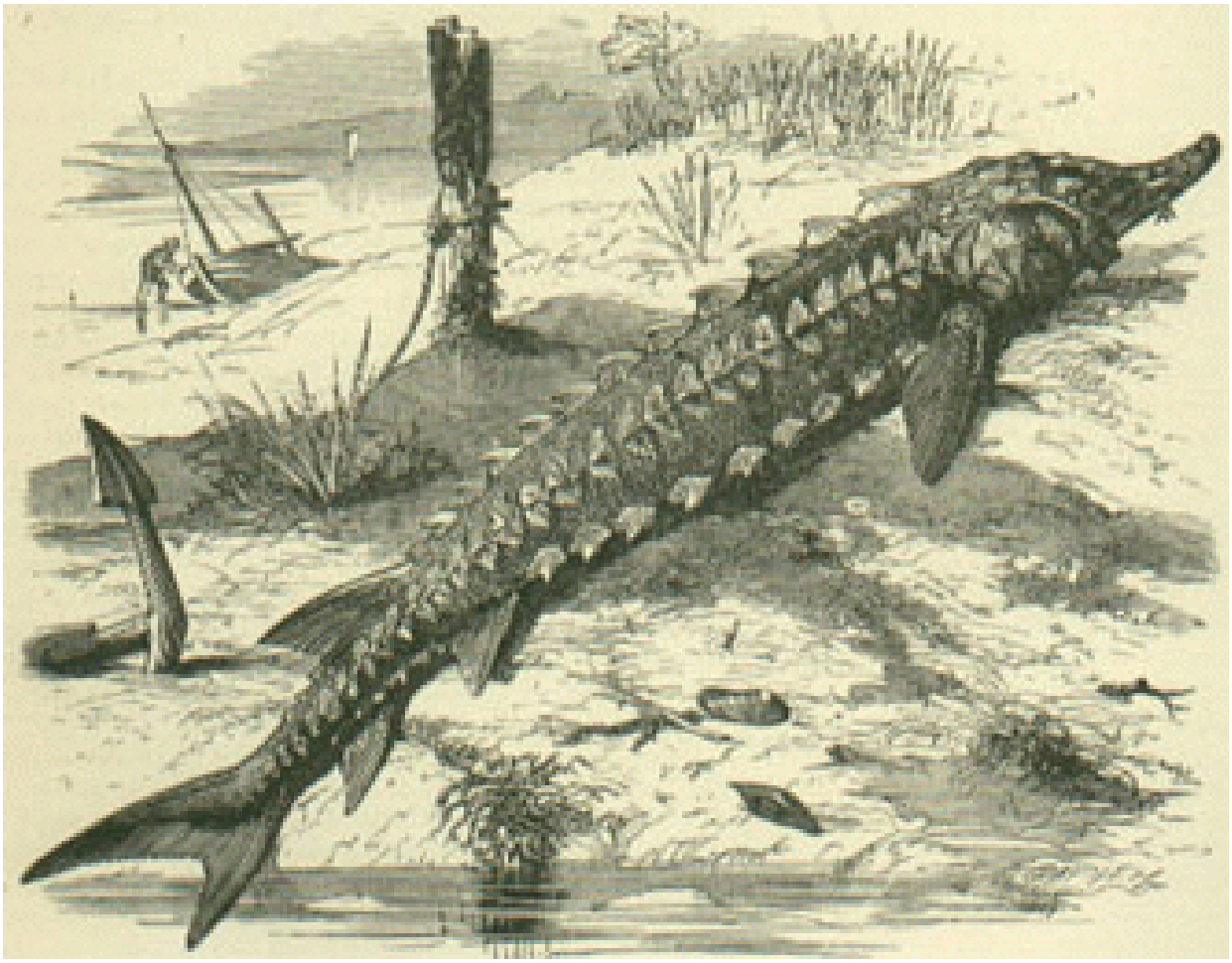
Male Atlantic sturgeon held in long-term captivity have been induced to spermiate with hormone injection once regular feeding occurs and condition factor increases. This has been achieved using common carp pituitary (CCP), luteinizing hormone analogue; des-Gly^{10},{D-Ala^{6}}-Luteinizing Hormone-Releasing Hormone Ethylamide, hereafter referred to as LHRHa, or a combination of both CCP and LHRHa (Mohler and Fletcher, 1999) (Table 1). In addition, males transported in season from a known spawning area and placed into large circular holding tanks have been induced to yield milt for up to five days after capture when injected with spawning hormones.

TABLE 1.—Hormone treatments and milt yield (Jun 19) of male Hudson River Atlantic sturgeons after 4 years of captivity at the Northeast Fishery Center in 1995. Hormones used were common carp pituitary (CCP) and luteinizing hormone-releasing hormone analogue (LHRHa).

Fish identification	Hormone	Dosage (mg/kg)	Injection schedule: Time (hours)	Injection schedule: Date	Milt yield (mL)
HDW-024	CCP	1.0	0930	Jun 18	320
HDW-021	LHRHa	0.01	1600	Jun 17	
	CCP	1.0	0930	Jun 18	260
HDW-023	LHRHa	0.03	0930	Jun 18	160

3.2 Hatchery-Reared Sturgeon

At this writing, no hatchery-reared Atlantic sturgeon have reached the size necessary for sexual maturity. It was found through biopsy and subsequent histology that gonads of 7-year-old domestic sturgeon (average fork length = 93.3 cm; average weight = 5.9 kg) were just beginning to sexually differentiate while those of 8-year-old fish (average fork length = 114.6 cm; average weight = 10.2 kg) showed greater development but oocytes of females were still pre-vitellogenic (Joel Van Eenennaam, University of California-Davis, personal communication)



3.3 Gender Determination

Currently, there is no reliable way to visually determine the gender of Atlantic sturgeon except when broodstock are being collected during spawning season. By sampling sturgeon from the Hudson River during spawning migrations in 1992 and 1993, Van Eenennaam et al. (1996), showed that females were larger than their male counterparts (Table 2) but size alone should not be used to determine gender. Measurements can be used to assist in gender determination along with observations of milt expression from ripe males. Experience gained by NEFC has shown that nearly all sexually mature males captured during spawning migration express milt easily when the abdomen is depressed or when milt extraction is performed through use of a syringe and tubing (See Section V - Milt extraction). If male sturgeons have been out of water (lying of the bottom of a boat) for approximately 10 minutes or more, it is sometimes difficult to extract milt even with the help of a syringe and tubing. These males must be placed back into water for a period of time (15-30 minutes) before milt can be easily taken once again.

Extreme turgidity or hardness of the abdomen can also be used as an indicator of female gender, but this condition does not always exist in ripe females upon capture. Inflation of the swim bladder due to a decrease in pressure a result of retrieving fish from deep water may cause abdominal hardness which can be mistaken for female ripeness. In the event that the above observations do not answer the question of gender, fish may be placed ventral side up on a stretcher and a biopsy can be performed.

TABLE 2.- Body size, gonadosomatic index, and age of Atlantic sturgeon. Data are means, standard deviation, and sample size in parentheses. All means between the sexes are significantly different ($p \leq 0.05$).

	Females	Males
Fork length (cm)	194.0 ± 14.9 (28)	161.7 ± 14.3 (66)
Total length (cm)	217.9 ± 15.8 (28)	181.5 ± 15.4 (66)
Body weight (kg)	72.7 ± 20.3 (22)	37.3 ± 7.5 (48)
Condition factor (%)	0.94 ± 0.11 (22)	0.83 ± 0.08 (48)
Gonadosomatic index (%)	13.84 ± 5.72 (16)	3.97 ± 1.13 (42)
Age (year)	20 ± 5 (27)	15 ± 2 (66)

Data courtesy of University of California - Davis

3.4 Determining Stage of Gonadal Development

In some situations it may be deemed necessary to perform a biopsy to determine gender or degree of gonadal development. After anesthesia is administered (see Spawning and Gamete Processing section), an incision can be made between the third and fifth ventral scutes anterior to the pelvic fins and offset from the ventral midline (Figure 9). It is helpful to use a retractor to spread the incision to facilitate examination (Figure 10). ***Males*** - Degree of gonadal maturity in males is determined by the size and color

of the testes. An immature testis is typically uniform in texture, consisting of smooth, yellowish, fatty tissue surrounding a strip of white testicular tissue which extends lengthwise through the gonad. A ripe testis is comprised mostly of large, white-lobed testicular tissue. In immature fish, the incision may have to be up to 6 cm long in order to view the paired gonads which are positioned adjacent to each side of the body wall. A blunt probe may be used to gently move internal organs for examination. An otoscope equipped with a long speculum can be inserted into the incision to view internal organs. If visual determination of the gonad is not conclusive, it may be necessary to perform histology on a tissue sample removed from the fish in question. The incision can be sutured as described in Appendix II.

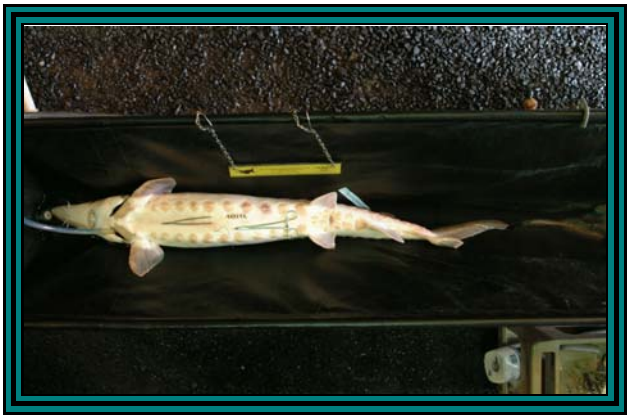


Figure 9.- Biopsied sturgeon showing water tube for life support, location of incision, series of single sutures, and instruments needed for closing the incision.

Figure 10.- Biopsy incision held open with a retractor to facilitate gonad examination

Females - Gonadal maturity in females can be determined through biopsy procedures as described above. In white sturgeon, ovarian tissue is somewhat grainy due to presence of many small oocytes and ovigerous folds or "ovarian grooves" (Doroshov et al. 1997) are present adjacent to the body wall. Van Eenennaam et al. (1996) also indicates the presence of ovarian grooves for Atlantic sturgeon. The gonad can be manipulated gently with a blunt instrument to either visually determine the presence of ovarian tissue or a tissue sample can be taken (see Surgical Procedures Section for biopsy techniques). Various stages of oocyte maturity have been described for white sturgeon by Conte et. al (1988) (Table 3) and similar stages described for Atlantic sturgeon (Van Eenennaam and Doroshov, 1998). Mature ovaries are

characterized by individually discernible dark-colored oocytes 2-3mm in diameter. If mature oocytes are present (stage IV), a few can be extracted through a small incision and withdrawn with suction using a 3mm (inside diameter) plastic tubing to determine whether they ready for induced ovulation. The incision can be sutured as described in Appendix II.

In season at known spawning areas, it is possible to capture ovulating females with eggs being actively extruded from the genital opening. Experience has shown that a female in this condition yields high quality eggs but provisions must be made for immediate spawning.

Table 3.- Gross characteristics of ovaries and testes of white sturgeon notable during surgical examination (Conte et al. 1988).

Stage	Ovaries	Testes
0	Gonad apparent only as undifferentiated tissue.	Gonad apparent only as undifferentiated tissue.
1	Small ovary: folded; no visible oocytes; tissue color white to yellowish.	Testis appears as thin strip of tissue; some adipose tissue; no pigmentation in tunica.
2	Moderate-size ovary: small 200-500 μm oocytes; profuse adipose tissue; sometimes "salt & pepper" -like particles present; "salt:" developing follicles; "pepper:" atretic follicles.	Small testis: beginning folds may be apparent; high adipose content; tunica with some "translucent smokey" pigmentation.
3	Large ovary: varies in color from white to yellowish to light gray; oocytes 1.5-2.5 mm; sometimes with "salt & pepper" appearance.*	Large testis: some adipose tissue; folds beginning to form lobes; some pigmentation in tunica still apparent.
4	Presence of large, dark oocytes, 3.0 + mm.	Large, lobular white testis.†

* The stages are relative, and as transitions are made between stages, gradations between stage characteristics may be apparent.
† Mature testis will not exhibit pigmentation in the tunica. Advanced Stage 3 testes have been used to obtain sperm, but sperm viability is often reduced.

At this writing, only one wild female Atlantic sturgeon has matured in captivity, displaying a 5-year spawning cycle. This particular fish was captured in the Hudson River as a ripe female in 1995 and successfully induced to spawn. Oocytes were removed via abdominal incision and successfully fertilized. The female survived this procedure and remained in captivity, being weaned to semi-moist formulated pellets (Special Brood Diet ,10mm, BioOregon, Warrenton, Oregon). A subsequent biopsy performed at 3 years post-spawn revealed immature oocytes. However, at 5 years post-spawn another biopsy revealed the presence of mature oocytes. This biopsy was performed in the month of June when a majority of Hudson River sturgeon normally spawn in the wild. Egg staging procedures revealed eggs were in a developmental stage acceptable for fertilization (Stage IV with a germinal vessicle index of 0.07 or less) (Figure 10). Subsequent hormone injections (See Section C - *Spawning Induction of Ripe Broodstock)* of common carp pituitary (CCP) induced ovulation but too much time (32 hours) passed between the final injection and initiation of egg removal. Eggs were over-ripe and had begun to breakdown into a grey

paste-like condition with no successful fertilization. This event emphasized the importance of timing when planning for artificial spawning of sturgeon.

3.5 Egg Staging

If biopsy of a wild captive or hatchery-reared female indicates mature oocytes, the fish should be considered a potential spawner and it may be necessary to determine whether oocytes will respond to gonadotropic hormones. Dettlaff et al. (1993) reports a quick method for the determination of gonad maturity in sturgeon where a small number of eggs are removed from potential spawners and boiled in water for about 2 minutes. Subsequently, oocytes are chilled and dissected along the animal-vegetal axis to determine the position of the germinal vesicle (GV). A polarization index (GVI) is then calculated based on the egg diameter and position of the GV in relation to the animal pole of the egg (Figure 10). In the Russian sturgeon Acipenser gueldenstaedti, a polarization index of 0.07 or less indicates that a normal response of follicles to spawning hormones is likely. A similar procedure is also reported for white sturgeon Acipenser transmontanus culture (Conte et al., 1988). Experience has shown that this procedure is also applicable to Atlantic sturgeon. Female Atlantic sturgeon whose eggs have the appropriate GV index can be induced to spawn with injection of gonadotropic hormones as outlined in Section 3.6 (Spawning Induction of Ripe Broodstock Using Hormones).

Williot et al. (1991) stated that use of the polarization index alone would lead to a high degree of failure with induced spawning of the Siberian sturgeon Acipenser baeri. Therefore, to be more certain that oocyctes will respond to injected spawning hormones, a progesterone assay can be performed by removing about 50 oocytes from the ovary via biopsy and incubating them in a progesterone solution to determine if the GV will migrate towards the animal pole and undergo breakdown. This procedure is described by Conte et al. (1988) and is summarized as follows:

3.5.1 Progesterone Assay for Egg Staging

1. Remove about 50 oocytes from the female and place in a beaker of chilled (16°C) Leibovitz solution held above (not in contact with) a bed of crushed ice. Leibovitz solution (L-15 medium) is a buffered incubation medium which supports development of embryonic cells in-vitro.
2. Prepare stock solution of 10mg of progesterone (4-Pregnene-3,20-dione) dissolved in 10 ml of 100% ethyl alcohol.
3. Place about 25 ooctyes from the female in a petri dish along with 20 ml of Leibovitz or Ringer's incubation medium.
4. Add 0.2 ml of the progesterone stock solution to the petri dish containing the oocytes and 20.0 ml of incubation medium and gently mix. (Final concentration is 10 ug progesterone per ml of solution)
5. Incubate the petri dishes for 24 hours at 15 -16° C.
6. Boil the oocytes in 150-ml beakers for 5-8 minutes then chill on crushed ice.
7. Dissect the oocytes along the animal-vegetal axis with a safety razor blade and determine whether GV breakdown has occurred (Figure 10).
8. Normally, suitable pre-ovulatory females exhibit GV breakdown in 80-100% of the oocytes incubated.
9. Non-ripe females will typically exhibit a GV breakdown range between 0 - 10%.

At this time there is no tested measurement for determining whether males will spermiate other than administering a spawning hormone during the season when water temperatures and photoperiods in the hatchery match those which naturally occur during spawning season in the wild. There is some indication that photoperiod may be more important than water temperature for spermiation in male sturgeon since milt with motile sperm cells has been obtained in the hatchery from long-term captive fish at water temperatures as low as 11° C after hormone injection (John W. Fletcher, U.S. Fish & Wildlife Service, Personal communication).

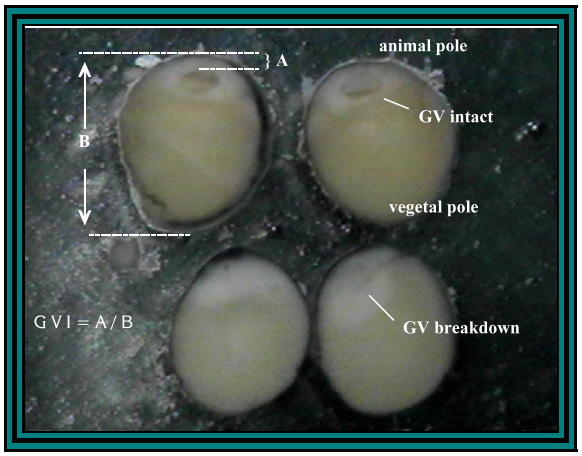


Figure 10.- Two boiled and bisected Atlantic sturgeon oocytes showing germinal vessicle (GV) and formula for determining GV index (GVI).

3.6 Spawning Induction of Ripe Broodstock Using Hormones

Both common carp pituitary (CCP) and Lutenizing Hormone Releasing Hormone analogue (LHRHa) injections have been used to induce spermiation and ovulation in wild-captured broodstock during spawning season. The hormones in CCP are gonadatropins; proteins which are transported via the circulatory system to the gonads for stimulation of target cells which control spermiation or ovulation (Turner and Bagnara, 1976). Conversely, releasing hormones such as LHRHa, are transported to target cells in the pituitary (Turner and Bagnara, 1976) of the fish which in turn release gonadatropins into the bloodstream to stimulate other target cells of the gonad. From analysis of Atlantic sturgeon harvested from the Hudson River commercial fishery from 1992-1995, VanEannannem et al. (1996) found that most females captured during spawning migration had oocytes in an advanced stage of maturity (GVI of 0.07 or less). Oocytes at this stage should respond to direct stimulation through use of CCP, therefore in all but one of the females spawned at NEFC, CCP was the treatment chosen. LHRHa has been used successfully to induce ovulation in female Atlantic sturgeon captured during spawning migration in the Saint Johns River in New Brunswick, Canada (M. Litvak, University of New Brunswick, personal communication). In 1993, NEFC biologists injected LHRHa into a female being held in a stream-side holding tank supplied with flow-through Hudson River water. This female did not respond to the hormone therapy; oocytes degenerated and into a grey paste-like material (Figure 11). This situation was not likely a result of using LHRHa but possibly from holding mature females at elevated temperatures prior to maturation. This can lead to oocyte degradation and failure to obtain viable eggs (Detlaff et al. 1993; Doroshov et al. 1997).

Borodin (1925) also commented on this subject when he categorized female sturgeon based on the quality of caviar they yielded. One such category was described as "pasters", females whose oocytes had broken down into a grey paste-like material.

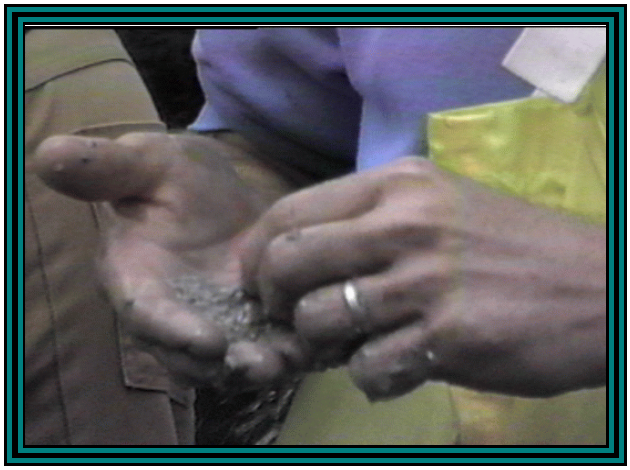


Figure 11.- Oocytes which have degraded into a grey paste-like condition due to over-maturity or improper holding temperature for female

3.6.1 Hormone Injection Procedures and Schedule

When working with wild-captured fish, hormone injection procedures depend upon the desired spawning location (stream-side or at some distant location). If the fish is to be spawned immediately at a stream-side or nearby location it is important to ensure that holding tank temperatures are not higher than that of the natural habitat from which the female was captured. If it is desired to spawn the female at a distant location, it is recommended that water temperature in the transport tank be approximately 8 - 10°C colder than river water to delay oocyte maturation until arrival at the desired location. NEFC has routinely transported ripe female Atlantic sturgeon over 6 hours by truck using cold water in the transport unit. For example, the Hudson River is normally about 23° C during the end of June when capture is initiated. The insulated transport tank is filled with 12° C water prior to embarking upon the capture trip and can warm up to nearly 16° C during the expedition. Experience has shown that under these conditions, females captured at 23° C and transported at 15-16° C, will be in good condition to receive hormone injections upon arrival at the desired location. No initial temperature acclimation period has been used. However, after transport in cold water, temperature is slowly elevated to about 21° C for the hormone injection schedule. Various attempts have been made to transport ripe females in ambient river water (about 23° C) for the 6-hour journey from the Hudson River to NEFC which resulted in premature release of entire spawns in the transport tank during travel. Once transport of the female is complete, it is desirable to have the fish in a dark and relatively quiet location for injection and oocyte maturation. At NEFC, this is accomplished by allowing the female to remain in the transport tank which serves a dual function as the maturation tank. This minimizes fish handling and facilitates water temperature manipulation and hormone injections.

Good results have been obtained with minimal stress to fish and workers by using underwater injection

techniques. Sturgeon are easily injected in a transport unit tank or other small holding unit and with a little patience, they can even be injected in a large long-term holding tank (Figure 12). Alternatively, the sturgeon can be captured in an elongated tube-style net (Figure 13) and loosely restrained at the edge of the culture tank until flexing ceases. ***Mature sturgeon are very powerful during flexing and it is virtually impossible to restrain them until they relax. Therefore, it is important to allow the fish to flex in the net without severe restraint as the fish will usually tire in about 5 minutes and workers as well as fish will be at less risk for injury.*** Once flexing ceases, a good injection site can be found by using a finger to feel for soft tissue areas located between the dorsal scutes. Attempting to make injections at random locations on the body can easily result in a damaged injection needle due to the numerous, small dermal denticles which form the matrix of Atlantic sturgeon skin. Once a soft spot has been found, the injection should be made intramuscular in a direction away from the spinal cord. If the fish becomes restless during an injection, the syringe can be withdrawn quickly or left in the tissue until the fish relaxes. This choice is dictated by the experience of the handler and the observed excitability of the particular fish being injected. It is good practice to depress the plunger slowly, not injecting more than about 2cc of material in one injection location due to possible tissue damage. When the needle is withdrawn, massage the area with a finger for 15-30 seconds to prevent excessive hormone leakage from the injection site. Needles of 20 gauge thickness and 3.8 cm (1½ inches) length work well for injection of mature broodstock. The capacity of the syringe needed depends upon which hormone is used. For LHRHa, a 1cc syringe is usually adequate while CCP requires use of a 5cc syringe. The suggested injection dosage and schedule is the same as that reported by Conte et al. (1988) for white sturgeon spawning and basically requires a primer dose (10% of total dose) followed 12 hours later by a resolving dose (90% of total dose) for females; males require only one dose (100% of total dose) (Table 4).

Figure 12.- Underwater injection of sturgeon broodstock

Figure 13.- Tube net used to capture and restrain sturgeon in culture tank (net is open on both ends)

Table 4.- Suggested spawning hormone total dosages for Atlantic sturgeon broodstock			
Common Carp Pituitary (CCP)		Luteinizing Hormone Releasing Hormone analog (LHRHa)	
Male	1 mg/kg	Male	0.03 mg/kg
Female	4 mg/kg **	Female	0.10 mg/kg **

** For females the total dose is divided into 2 separate injections:

primer injection = 10% of the total dose

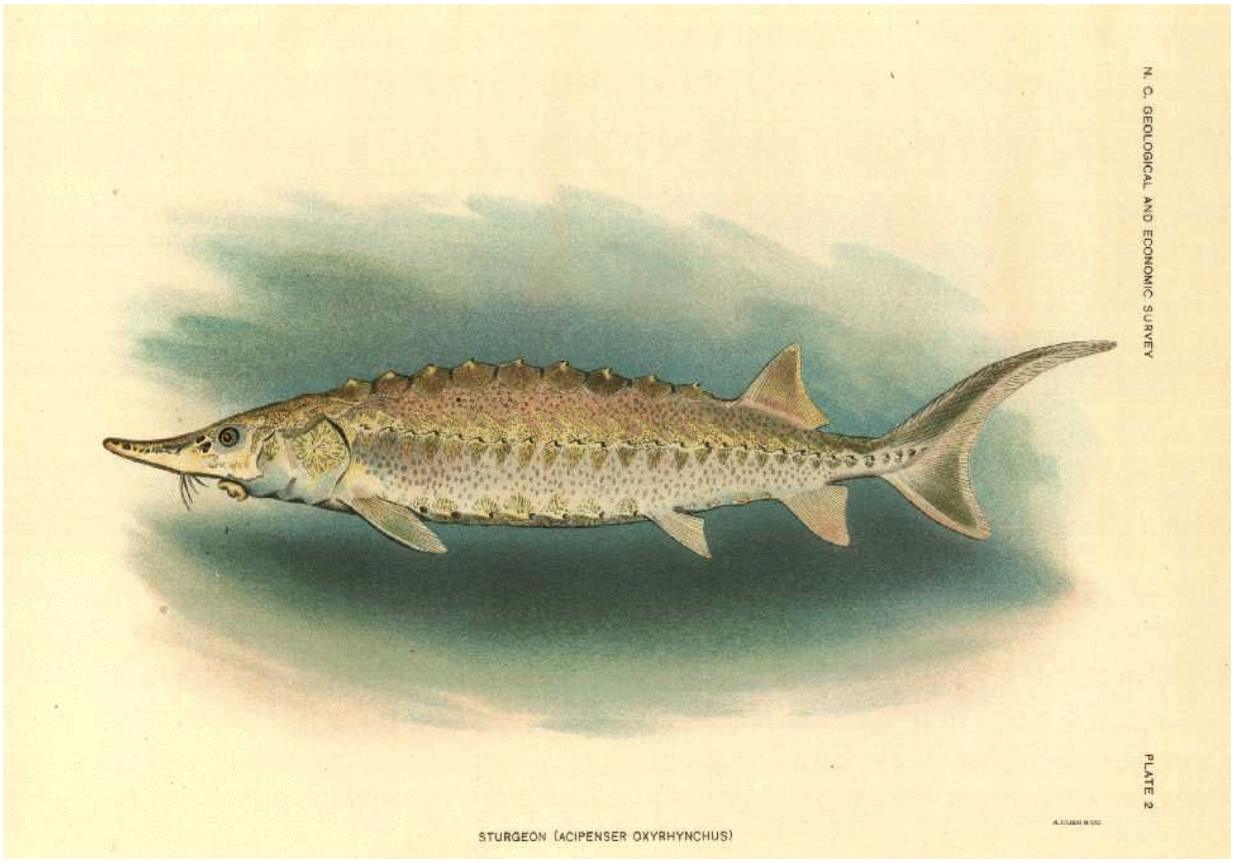
resolving injection = 90% of the total dose 12 hours after the primer injection

3.6.2 Hormone Response

For gravid female sturgeon, a normal response to administration of spawning hormone would be the release of a few hundred oocytes in the maturation tank from 18-22 hours after the resolving injection. Therefore, beginning at 16 hours after the last injection, the female should be checked every two hours for signs of released eggs. When checking is necessary during the night, a flashlight is helpful. If the bottom of the tank cannot be seen, a small dip net can be swept along the bottom to capture eggs which may have been shed. When hundreds of slightly sticky eggs are shed into the tank, it is time to begin egg removal procedures. NEFC has found that females which do not respond normally after 24 hours post-injection, usually do not give good quality eggs or never appear to respond to the hormone injections and oocytes

remain attached to the ovaries. One particular female artificially spawned at NEFC, produced ovulated eggs from only one ovary while eggs were still firmly attached to the other. Experience has shown if females are held at the preferred spawning temperature (20-21°C) and few or no eggs have been shed by 24-hours after the resolving injection, then obtaining fertilizable eggs is unlikely. If only a few eggs have been shed by the 24-hour mark, it may be desirable to begin egg removal procedures with a high likelihood of obtaining a small quantity of fertilizable eggs rather than waiting too long and obtaining none.

In mature male sturgeon, one hormone injection is usually adequate to induce spermiation. Wild Hudson River males captured in spawning season and transported for holding at NEFC have been induced to spermiate using LHRHa after as many as seven days post-capture. Additionally, wild males from both Delaware and Hudson Rivers have been held up to six years in captivity at NEFC and subsequently induced to spermiate using both LHRHa and CCP injections (Mohler and Fletcher, 1999) (Table 1). Milt from one of these long-term captives was used to successfully fertilize a number of eggs at NEFC in 1995. Behavior of injected males may be observed to change from slow swimming to a noticeable increase in swimming velocity and overall activity once the hormone takes effect. The two most important factors for successful milt production in captive males appears to be: water temperature and photoperiod. For wild captive fish, these factors must coincide with that which naturally occurs in the wild for the particular stock being used. Practical experience has shown that for captive Hudson River wild stock, if no milt has been obtained by the end of June, the likelihood of inducing spermiation is slim. No information is yet available for spermiation in hatchery-reared domestic males.



Chapter 4 Spawning and Gamete Processing

4.1 Sedation of Sturgeon

All surgical procedures including those related to spawning or other activities which require the sturgeon to be immobilized must be preceded by administering an appropriate sedative to the subject fish. Three sedatives have been tested on Atlantic sturgeon at NEFC: (1) Metomidate (trade name: "Marinil" by Janssen Pharmaceutica) (2) MS-222 or tricainemethane sulfonate (trade name: "Finquel" by Argent Chemical Laboratories, Redmond, Washington) and (3) 5% clove oil /95% ethanol mix (active ingredient in clove oil is eugenol). The optimal dosage for each of these compounds at water temperatures from 5-15° C (Table 5) was defined as the concentration necessary to sedate fish in 3-4 minutes and allow recovery in < 10 minutes with the exception of metomidate which required longer recovery times regardless of concentration tested (Unpublished data, U.S. Fish and Wildlife Service, Northeast Fishery Center-Lamar, PA).

Table 5. Optimal dosages for 3 sedatives used on 500 -1000-gram Atlantic sturgeon

Sedative	Concentration (ppm)	Knock out (sec.)	Recovery (sec.)
at 5° C :			
Metomidate	15	147	2283
MS-222	200	338	618
5% Clove oil mix *	200	205	930
at 15° C :			
Metomidate	15	181	785
MS-222	200	162	203
5% Clove oil mix *	100	178	254

* 5% clove oil/95% ethanol

In general, sturgeon took longer to recover at colder water temperatures. Metomidate-treated fish required the lowest dosage but took the longest to recover overall. Sturgeon (500-1000 g) were also exposed for 20 minutes to the given optimum dosages at water temperatures from 5-15° C with no mortality. For larger sturgeon (6-7 kg) the same optimal dosages apply as in Table 5 but recovery times can double or triple in some cases.

For sub-adult and juvenile fish, a simple water bath can be used to administer the sedative. For sedation

of individual fish or those too large to place into a water bath, the desired solution can be administered via a recirculation system . This is accomplished by placing the sturgeon onto a stretcher assembly and delivering solution to the fish via an electric pump and tubing (Figure 14). This stretcher assembly can be designed to allow the delivered anesthesia to drain back to a reservoir and be used continuously in a recirculating fashion. If the recirculating technique is used, one person should be assigned the task of constantly observing the fish for effects of the sedative so that over-exposure does not occur. With any sedative, risk of lethal over-exposure increases if gill movement stops for an extended period of time therefore it is prudent to switch the recirculating system to deliver fresh water once gilling frequency slows considerably. For the most part, fish remain relatively motionless once positioned ventral side up on a stretcher with flowing water applied to the gills. However, if the water flow is interrupted or otherwise mis-directed away from the gills, the sturgeon which has yet to be sedated may react by flexing. If gill movement ceases for an extended period of time during sedation, do not be to hasty in pronouncing death upon the subject. Complete cessation of gill movement in sedated fish has been observed for as long as 15 minutes with full recovery of the individuals.

Figure 14.- Stretcher assembly used for examination and surgery on Atlantic sturgeon

4.2 Milt Extraction

Whether obtaining milt from freshly-captured males or from captives which have been induced to spermiate through use of hormones, techniques for milt extraction are similar. Good results have been obtained using a 60cc syringe outfitted with about 10cm of plastic tubing for withdrawing milt from the uro-genital opening of the fish (Figure 15). Freshly-captured wild fish typically float upside down when placed in a boat-mounted tank. This is conducive to milt extraction where one worker can balance the fish ventral side up in the holding tank while another dries the uro-genital area with a paper towel to prevent accidental milt activation. Milt can then be extracted with the syringe apparatus. There are two openings into the body cavity in the uro-genital pore. The anus is located in the ventral position and separated

from the dorso-posterior genital opening by a fleshy septum. If freshly captured wild males remain out of water for a period of time, milt expression may become suppressed. These fish usually give milt after being permitted to recover in the live well for 15-30 minutes.

Figure 15.- Withdrawing milt from a hormone-treated male using a 60cc syringe and plastic tubing

In captive fish induced to spermiate, a stretcher assembly mounted between two braces can be fashioned to facilitate milt withdrawal (Figure 14). The fish is positioned ventral side up for the procedure and a water-tube is employed to flush the gills with fresh water and calm the fish while milt is taken. The fish may be less agitated if only the stream of water is directed into the mouth rather than placing the tube directly into the mouth. After the male is positioned and calm, milt can be withdrawn as previously described. As much as 320 ml of milt have been taken from one sturgeon during a single procedure. Alternatively, the male can be captured in an elongated tube-style net (Figure 13) and loosely restrained at the edge of the culture tank until flexing ceases. ***Mature sturgeon are very powerful during flexing and it is virtually impossible to restrain them until they relax. Therefore, it is important to allow the fish to flex in the net without severe restraint as the fish will usually tire in about 5 minutes and workers as well as fish will be at less risk for injury.*** Once flexing ceases, the animal can be rolled over, the genital opening dried with a paper towel, and milt extraction can proceed without removing the fish from the culture tank or the net.

4.2.1 Milt Storage

Milt which has been exposed to water will cause the sperm cells to be immediately activated. Once activated, the sperm cells will remain useful for fertilization for only slightly more than 2 minutes. For this reason, it is important to dry the genital opening of the male with paper towels before withdrawing milt intended for storage. Milt can be transferred from the withdrawal syringe to a small plastic bag or another type of dry container. The sample should then be oxygenated and placed on crushed ice to extend it's viability. Small plastic bags ("whirl-pak" or other similar product) make ideal on-site storage

containers since they can be filled with oxygen and sealed or resealed as necessary. If this method is chosen for storage, the bags must be re-oxygenated daily. An insulated cooler works well to keep milt samples cool and prevents sunlight from potentially damaging the sperm cells. Using the above techniques, milt has been stored for 5 days and used to successfully fertilize eggs. DiLauro et al. (1994) reported that milt samples exhibited at least 80% motility and 99% viability after short-term cold storage for 5 days on ice and replenished daily with oxygen. One sample exhibited 40% motility and 50% viability even after 17 days of storage. However, it is not wise to rely on aging milt samples if fresh milt is readily available. Short-term cold storage represents a viable alternative for extending the availability of milt when resources are not available to hold adult male sturgeon. At this writing, no Atlantic sturgeon eggs have been successfully fertilized using cryo-preserved sperm cells but Kopeika et al. (2000) successfully fertilized eggs of the sterlet sturgeon Acipenser ruthenus using cryo-preserved milt from the European Atlantic sturgeon Acipenser sturio, a close relative of the subject species.

4.2.2 Sperm Motility and Viability

Regardless of the length of storage time, each milt sample should be checked for motility of sperm cells prior to fertilization procedures. This factor is less critical if milt from numerous males is pooled prior to fertilization, but it is unwise to jeopardize fertilization for the small amount of time needed to examine milt samples. Motility is checked by placing a tiny drop of milt onto a glass microscope slide via a dry glass stirring rod. The sample is then placed under the microscope at 100 X and focus is adjusted at the edge of the milt sample. Once in focus, a small amount of water can be added to the milt sample with a glass rod and mixed briefly. Since the sample was already in focus, little manipulation should be necessary to focus the activated milt. Milt with good viability will exhibit sperm cells with vigorous forward motion for about 30 seconds with steadily reduced activity to about three minutes post-activation. After about three minutes, sperm cells will show little if any motion. Good motility of sperm is not a guarantee of good viability but is usually a good indicator. Determination of true sperm cell viability requires use of a specialized technique such as flow cytometry where fluorescent staining of sperm cells is used to measure the number of cells which are live, dead, and dying in a particular milt sample. Flow cytometry is an attractive technique because it allows the charactierization of each sperm cell in terms of cell function and integrity (Ogier de Baulny, 1997).

4.2.3 Transporting Milt

Extracted milt can be shipped virtually anywhere via express delivery service. If the samples have adequate oxygen and remain cold, they should maintain much of their viability for a 24-hour journey. It is best to transfer milt directly from the extraction syringe to the desired shipping containers and oxygenate and refrigerate immediately until shipped. Plastic centrifuge tubes of 50-ml capacity work fine for shipment of 10-20 ml samples as long as oxygen is injected into the container prior to sealing the lid. Plastic bags are not recommended for shipping milt due to high probability of leakage in transit. Oxygenated centrifuge tubes containing milt can be placed into a tube-holder rack and positioned on top of synthetic ice packs in an insulated container (such as styrofoam). Packing material should be placed on top of the tubes to stabilize them during transit. The container should then be packaged into a corrugated cardboard box and sealed with packaging tape for shipment.

4.3 Egg Extraction

In the event that there is no concern for further use of the fish as broodstock, the female can be sacrificed and the abdomen slit open to remove the entire spawn quickly once it has been determined that ovulation has occurred. However, at this writing capture of mature female Atlantic sturgeon is rare over the range of this species in the U.S., therefore non-lethal spawning techniques will be more attractive in most situations.

If it is desired to obtain fertilizable eggs non-lethally, the female must be positioned ventral side up with fresh water flushing over the gills during egg removal activities. At NEFC this is normally accomplished by placing the female on a stretcher with a stream of water directed into the mouth via a flexible water tube. If anesthesia is used, a separate reservoir of the chemical must be established with a dedicated water tube and pump apparatus (Figure 14). One individual should be assigned the responsibility of switching water supplies back and forth as necessary between fresh and anesthesia-containing water for the duration of the procedure.

There are three basic methods which can be attempted to obtain eggs non-lethally from a ripe female: (1) abdominal incision with sutures, (2) Repeated manual stripping to obtain small quantities of eggs, and (3) oviduct incision with manual stripping. Unlike salmonids and most other fish species, sturgeon have paired internal oviducts through which eggs must pass in order to exit the body cavity via the genital opening. It is for this reason that one of the following special techniques must be used to obtain fertilizable eggs.

4.3.1 Abdominal Incision with Sutures

Wild-caught female sturgeon are under stress due to spawning condition and stress response due to capture/handling. As a result, the female may expire prior to, during, or shortly after the operation is performed. This technique requires someone skilled in basic surgical techniques to perform the operation. Using a scalpel or single edged razor blade, an incision about 10 cm in length is made between the 3rd and 5th ventral scutes anterior to the genital opening. The incision should be offset from the body mid-line and located in a place with the minimum number of visible dermal denticles to facilitate closure with suture material. When performing the incision, care must be taken not to puncture the intestine or other internal organs. Tissue forceps are helpful in pulling the skin away from the intestine and other internal organs during the incision (Figure 16).

Figure 16- Using tissue forceps to help prevent puncture of internal organs during incision. Note the ovulated, dark-colored eggs which have flowed from the incision.

Once a small cut is completed through the body wall and ovarian fluid begins to flow out, a number of ovulated eggs may also appear. At this point the incision can be enlarged to accommodate a long-handled plastic spoon so that eggs can be scooped out and placed into a stainless steel or plastic bowl. It is not known how this type of trauma to internal tissues may affect the survival of the fish. To shorten the procedure and increase the chance of female survival, it may be necessary to leave some quantity of eggs in the female. Closing the incision with sutures is the most difficult part of the surgery due to the dermal denticles in the skin. These denticles deflect and bend surgical needles and can add much time to the closure procedure. At NEFC, the suture material of choice is PDS II monofilament equipped with an OS-4 curved cutting needle (Ethicon, Inc.). Suture material, a heavy-duty hemostat, and forceps are the tools used to close the incision with series of single sutures about 6mm apart (Figure 9). It is unknown whether disinfection of the skin and sutured areas with iodine compounds is the best course of action since both harmful and beneficial body flora are eliminated with their use. It is always preferred to maintain aseptic conditions as much as possible during surgery to minimize infections. At NEFC a terramycin antibiotic (Liquimycin LA-200, Pfizer, Inc.) is injected into the female intramuscularly at a 20 mg/kg dosage post-surgery to minimize systemic infection. Survival of gravid females undergoing this type of surgery is low with only one of seven spawned in this manner having survived the procedure at NEFC. The one female which survived was also repeatedly spawned with subsequent survival five years after undergoing the first spawning surgery. This suggests that elimination of handling associated with capture and transport of wild fish may lead to increased survival of female brood fish.

4.3.2 Repeated Manual Stripping to Obtain Small Quantities of Eggs

Once eggs are released from the ovaries, some will naturally be extruded through the oviducts and out the genital opening. Abdominal massage is helpful to obtain small quantities of eggs in this manner and repeated efforts may result in obtaining adequate numbers of eggs for a particular purpose but a large proportion will remain in the body cavity . This technique requires moving the female in and out of the holding tank numerous times but post-spawning survival may be higher than in fish which have undergone surgery.

4.3.3 Oviduct Incision with Manual Stripping

This technique has been successfully used to obtain eggs from Atlantic sturgeon. On the Hudson River in 1998, an NEFC crew was able to obtain 3 L of eggs using this technique on a wild sturgeon captured in the process of spawning. In the absence of having a stretcher on hand, the female was suspended ventral side up in the transport tank with the head underwater and genital opening above water. A scalpel outfitted with a #15 blade was inserted into the genital opening and up into the left oviduct about 4 cm. A slight lateral motion with the scalpel punctured the oviduct and caused eggs to flow freely from the genital opening (Figure 17). Additional eggs were easily collected with abdominal massage. Eggs were directed through a length of plastic tubing into a container or scooped with a plastic spoon as they exited the body cavity. This technique has been currently described and illustrated by Stech et al. (1999).

Use of this technique for egg removal is expected to increase the survival rate for female broodstock since it is less intrusive than making an abdominal incision. More oocytes will be retrieved using this method as opposed to repeated manual stripping only. At this time it is unknown what long-term effects on the female may result from puncturing an oviduct for egg removal.

Figure 17- Eggs flowing freely from genital opening after oviduct puncture.

4.4 Egg Enumeration

Enumeration should take place prior to fertilization to prevent unnecessary handling of fertilized eggs. At least three, 15 ml egg samples should be taken and hand counted to calculate the average number of eggs/ml. This figure can then be applied proportionally to the entire volume of the spawn for calculating the total number of eggs taken. After fertilization, eggs will become larger due to activation by sperm and associated water uptake, therefore egg enumerations performed prior to fertilization/de-adhesion can not be applied to egg volumes upon placement into incubation devices. A two- or three-fold increase in egg volume is possible from fertilization to placement in incubation devices. At NEFC, pre-fertilized eggs have been measured at 80 - 120 per ml while post-fertilized eggs were measured at 30 - 50 per ml upon placement into incubation devices. Necropsy of a mature pre-spawned female measured at 213 cm total length revealed that the fish contained 9.1 L of eggs at 83.3 eggs/ml for a total fecundity of about 758,000 eggs.

4.5 Egg Fertilization

Eggs which have been removed from a female sturgeon should be placed into a stainless steel bowl or other clean, dry container. Eggs should remain at a constant temperature through spawning, fertilization, and placement into incubation devices. Once eggs have been collected and enumerated, they should be fertilized as soon as possible. The first step is to pour off excess ovarian fluid from the eggs prior to adding milt since it interferes with fertilization (Detlaff et. al 1993). Milt is then added to hatchery water by making a 1:200 dilution (5ml milt in 1000ml water) and immediately pouring the solution onto the eggs. Enough milt solution should be used so that the quantity of eggs being fertilized is covered with the solution. Eggs should be gently mixed by hand for about 1 minute to disperse sperms cells throughout the spawn and allowed to sit motionless for 1- 2 minutes more. De-adhesion procedures must then be initiated immediately.

4.6 Egg De-adhesion and Disinfection

Fertilization procedures cause activation of the egg which triggers changes in the egg membranes. These changes cause egg membranes to swell and become sticky and will result in severe clumping of eggs unless this process is not counteracted. Egg stickiness is counteracted by either chemical or mechanical procedures known as de-adhesion. At NEFC, mechanical processing is preferred due to it's straight forward and simple design. The de-adhesion solution should be prepared and ready to use prior to egg fertilization. Using this method, a solution of fine sediment known as Fuller's earth (Sigma, St. Louis, Missouri) is prepared in a 19-L plastic bucket of water by mixing in several hands-full of the silt in water having the same temperature as the eggs. This solution is poured directly onto the freshly-fertilized eggs with enough volume to cover them (Figure 18). A gentle hand-mixing is then performed for about 30 minutes or until eggs no longer stick together; solutions should be changed at least once every 10-15 minutes to maintain a constant temperature. After 30 minutes or more, eggs will be coated by a thin film of Fuller's earth which will prevent clumping. The Fuller's earth solution is poured off and eggs can either be rinsed a number of times or placed directly into incubation devices.

For chemical de-adhesion, Conte et al. (1988) recommends using a series of treatments with a combination of urea and sodium chloride, with subsequent tannic acid washes. This procedure was attempted on sturgeon eggs at NEFC with some success, but it was found to take additional time to measure out chemicals with no obvious benefits over mechanical de-adhesion.

Fertilized Atlantic sturgeon eggs can be disinfected to remove surface pathogens by preparing a solution of 50 mg/L active iodine and immersing eggs for 30 minutes prior to incubation without adversely affecting the percent hatch. Temperature of disinfection solutions should be similar to egg temperatures.

4.7 Shipping Eggs

At this writing, no technique has been found to ship unfertilized eggs with the intention of successful delayed fertilization. However, it is possible to transport fertilized eggs. About 3 L of fertilized eggs were transported by automobile in a plastic, 5-gallon bucket supplied with 23° C, oxygenated water for 6

hours with a resulting survival of >80% (personal communication, John Fletcher, U.S. Fish and Wildlife Service). However, if the ultimate intention is to provide sturgeon to a distant location, it would be more desirable to ship newly-hatched larvae since shipping conditions may lead to unfavorable incubation temperatures or mechanical shock for fertilized eggs.

4.8 Egg Incubation

Once de-adhesion is complete, eggs can be placed into incubation devices. Clear, 6.5-L acrylic, McDonald-style jars have worked well at NEFC (Figure 19) . It is important to make sure that initial water temperature in incubation jars is similar to that of eggs. Experience at NEFC has shown that 20-21 °C temperature is favorable for incubation. Temperatures below 18°C prolong hatching and increase the incidence of fungal infestation of dead eggs which in turn can destroy viable eggs (John W. Fletcher, U.S. Fish and Wildlife Service, personal communication). Temperatures of 20-21°C are similar to, but somewhat lower than the temperature of the Hudson River at the time of year when broodstock are captured during their natural spawning migration. When fertilized/de-adhesed egg temperatures are lower than the desired incubation temperature, heated water must be slowly blended in with the ambient water until the target temperature (20-21°) is reached. This temperature ramping is done automatically at NEFC using a Universal Digital Controller (UDC 3000) and automatic mixing valve (Modutrol motor, Honeywell Inc., Washington, Pennsylvania). A prudent rate of ramping is to increase temperature 1 degree centigrade per hour until the target is reached. At 20-21° C, eggs should begin to hatch in about 60 hours with an increase in hatch time as temperatures decrease (Table 6). If water must be heated to reach proper incubation and rearing temperatures, provisions must be made to eliminate gas super-saturation conditions which may develop in the heated water. One common technique is to direct incoming heated water through a packed column. This provides a cascading effect which allows incoming water to be aerated and creates a condition conducive to the liberation of super-saturated gasses. If this is not done, hatched fry may develop gas-bubble disease which is characterized by formation of bubbles of gasses trapped between or within tissues, or in the circulatory system (Post 1987). This condition may cause direct mortality or indirect mortality by interfering with the ability of the fry to begin consuming feed.

Figure 18.- Mixing eggs gently in Fuller's earth solution to prevent clumping.

Table 6.- Time of initial hatch of Atlantic sturgeon eggs at various incubation temperatures at NEFC.	
Incubation temperature (°C)	Time of initial hatch (hours)
17	96
21	60
22	55
24	51

4.8.1 Egg Incubation Equipment

At this writing, 6.5-L capacity acrylic-plastic MacDonald-style jars are the preferred incubation containers (Figure 19). In principle, water flowing vertically down through the jar's center tube is deflected by a perfectly hemispherical bottom to produce an evenly-distributed up-welling flow which keeps all eggs gently rolling with a minimum of "dead" zones during incubation. Normally, no more than 500 ml of fertilized / de-adhesed eggs are placed into one jar unless incubation space is scarce. Assuming that eggs have a volume of 40 per ml at this stage and an 80% hatch rate occurs, one jar containing 500 ml of eggs would yield about 16,000 fry. Fry receiving tanks are placed adjacent to incubation jars in advance of expected hatching so hatched fry can flow freely into their larval rearing environment (Figure 19).

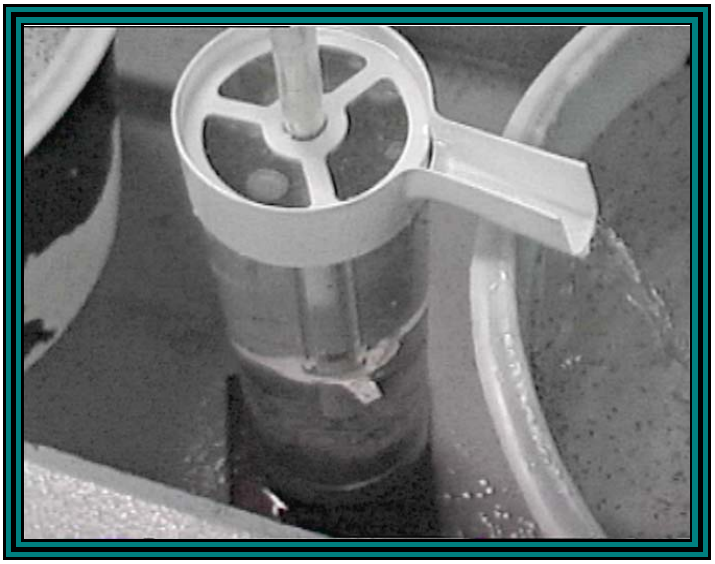


Figure 19- MacDonald-style incubation jar with top screen ajar allowing newly-hatched larvae to flow into culture tank.

4.9 Determination of Fertilization and Hatch Rate

Once fertilized, the oocyte cells begin to divide rapidly and embryonic development can be monitored at certain stages with the aid of a dissecting microscope to determine the success of fertilization, to estimate

the percent hatch, and also determine the number of deformities. Microscopic examination of developing embryos can also be used to some extent for evaluation of the environmental conditions present during incubation. Rate of embryonic development in sturgeon eggs is greatly temperature dependant and must be considered when deciding to take egg samples for evaluation. From past experience at NEFC, hatching for Atlantic sturgeon embryos begins somewhat more quickly, but approximate to those reported by Detlaf et al. (1993) for the stellate sturgeon, Acipenser stellatus. Therefore, we will use A. stellatus incubated at 21° C as a model for embryonic development in Atlantic sturgeon.

4.9.1 Fertilization Rate

For determining the fertilization percentage, samples should be taken at the 2nd cleavage division or 4 blastomere stage (Detlaf et al. 1993) (Figure 20). At 21° C, this should occur at about 3 hours post-fertilization. Place about 200 eggs taken from a well-mixed incubator into a petri dish and separate eggs which show cleavage (fertilized eggs) from those which do not (unfertilized). Hand-count the two groups and calculate the percent which were fertilized. At this point in the egg development, it is also possible to tell what percentage of eggs were fertilized by more than one spermatozoa (polyspermy). Normal eggs fertilized with one spermatozoa at the 2nd cleavage division show 4 blastomeres, whereas polyspermic eggs have 6 and more. If the insemination technique was correct, usually no more than 4-6% of polyspermic eggs are present in a batch of good quality eggs (Detlaff et al. 1993).

4.9.2 Hatch Rate

At 21°C incubation temperature, a favorable time for sampling eggs to determine percentage of egg losses is at about 36 hours post-fertilization. At this stage (neuralation) the central nervous system is under development and the neural tube is well defined (Figure 22). A random sample of about 200 eggs taken from a well-mixed incubator is used to make the determination of expected hatch by examination under a dissecting microscope. Those embryos which have no evidence of neuralation are separated from the rest to make the calculation of hatch rate. Eggs which will not hatch are typically marbled in appearance or may be arrested at a stage of earlier embryonic development.

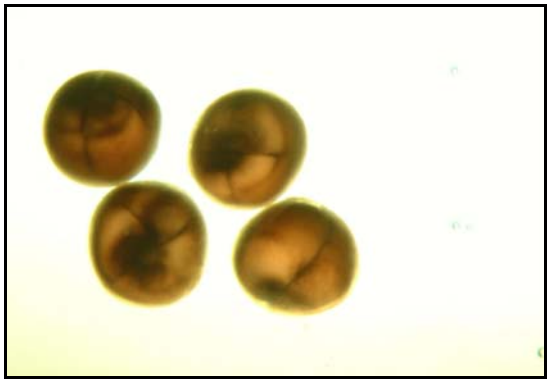


Figure 20.- Atlantic sturgeon embryos, 2nd cleavage, 4-cell stage (about 3 hrs post-fertilization at 21°C). Egg membranes have been removed to provide a clear picture.

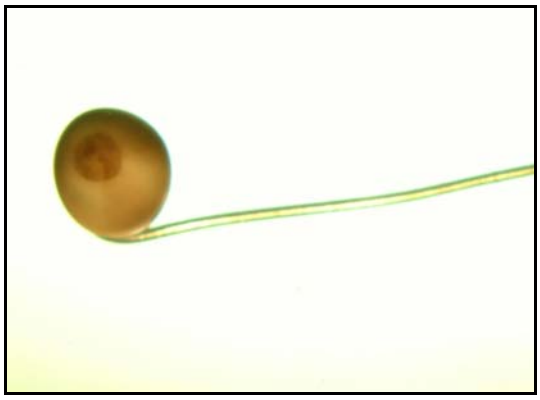


Figure 21.- Atlantic sturgeon embryo at developmental stage of yolk-plug formation (about 24 hours post-fertilization at 21°C). Egg membrane has been removed to provide a clear picture. The embryo is positioned on a wire holder.

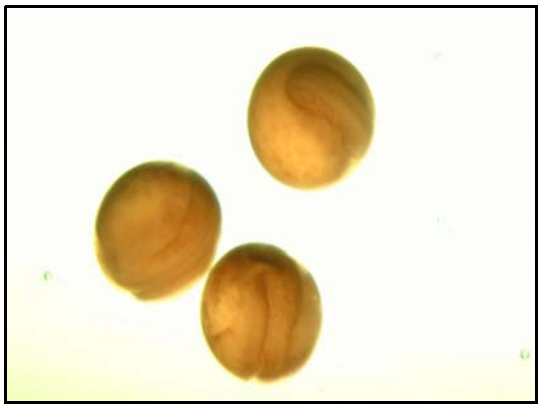


Figure 22.- Atlantic sturgeon embryos at the stage of neuralation showing some body definition (about 36 hours post-fertilization at 21°C). Egg membranes have been removed to provide a clear picture.

Chapter 5 Care and Rearing of Juvenile Fish

The following information on culture of juvenile Atlantic sturgeon was compiled from experience in hatching and rearing 5 different year-classes of fish obtained from spawning wild broodstock captured in the Hudson River, New York. These methods have been used successfully for fry culture and resulted in successful production of fingerling and sub-adult Atlantic sturgeon. In large part, these guidelines are the result of controlled experimentation but some observations and other practical information has also been included.

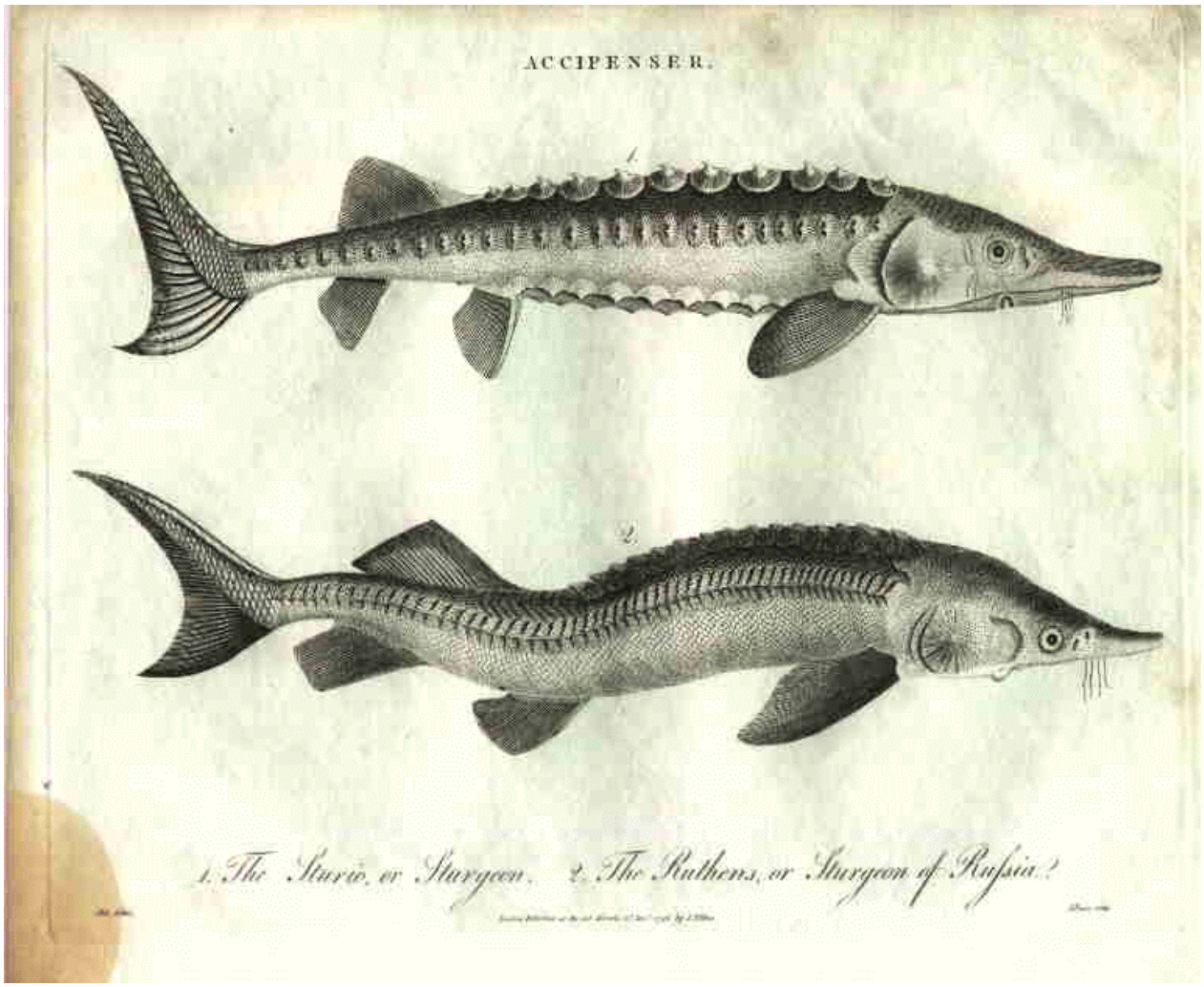
5.1. Fry Culture

Due to the difficulty in obtaining mature individuals of both genders to produce young, culture parameters given for fry may not be optimal since the results from many culture trials were based on the performance of progeny from single-female spawns. However, milt from multiple males was normally used to fertilize the eggs in an attempt to provide some genetic variability in the offspring. Overall, we found the most important parameters for fry culture are: initial stocking density, food type and supply, and culture tank cleanliness. Specific culture guidelines are summarized in Table 7.

5.1.1 Rearing Units

Circular tanks were found to be the best all-around design for rearing sturgeon fry. With a circular tank, in-flowing water can be directed to establish a circular flow pattern which is conducive to distributing food evenly throughout the tank. In addition, in-flowing water can also be directed perpendicular to the wall of the circular tank causing a flow pattern which facilitates separation of dead, weak, or deformed fry from healthy ones (Figure 23). The preferred configuration for circular tanks is: 0.6 - 1.2 meters in diameter, about 30 cm in depth, and equipped with a center drain screened with synthetic fabric having a mesh size opening of no more than 1mm. Both gray and light blue tank colors have been used successfully to rear Atlantic sturgeon fry.

Culture of non-feeding larvae was attempted in static, fertilized ponds without success at NEFC and Bowden National Fish Hatchery in Elkins, West Virginia. However, some success was attained in rearing feeding fry in a static, fertilized pond at NEFC where survival was 7%and survivors were robust, attaining a length of about 100 mm in 2 months.


ACCIPENSER.
1.
2.
1. The Sturio, or Sturgeon. 2. The Ruthens, or Sturgeon of Ruſsia?

5.1.2 Behavior

There are distinct phases of behavior in sturgeon as they mature from larvae to feeding fry. These phases can be used as signals to dictate how culture activities must progress. Immediately after hatching, larvae are mostly pelagic inhabiting all areas of the culture tank and exhibiting a "swim-up and drift-down" behavior. At this point fry are nourished endogenously from nutrients present in the yolk sac (Figure 24) which will be nearly depleted in about 9 days at a water temperature of 17° C . Fry exhibit a negative photo-taxis at this life stage. At 3 - 4 days post-hatch (water temperature = about 19° C) fry will begin to exhibit benthic clumping behavior and swim against the flow direction in the tank. This behavior is rather fascinating to observe as fry clump together on the tank bottom and form continually-evolving legions of individuals (Figure 23). Fry will remain benthic for about 4 days.

Figure 23.- Atlantic sturgeon larvae exhibiting benthic clumping behavior. Inflow is directed perpendicular to tank side to attract healthy individuals and isolate mortalities and cripples on the opposite side of center screen as shown

At about 9 days post-hatch, fecal plugs visible in the spiral valve region of the gut will begin to be extruded as the yolk sac is nearly used up. When shed, fecal plugs are usually visible as small dark extrusions on the tank bottom. At about 10 days post-hatch fry will begin inhabiting all areas of the tank again and are in full-feeding mode. At this stage, fry have been observed to be pelagic until live brine shrimp (Artemia sp.) is introduced at which time fry will move to the tank bottom to feed on sinking brine shrimp nauplii. It has been generally observed that fry do not actively search for food at this life stage but rather wait for nauplii to be swept toward them by water currents. (This is one reason why circular tanks

are preferred to rectangular ones for rearing Atlantic sturgeon fry). Feeding behavior can be observed as fry suddenly lunge forward or sideways to capture nauplii. Once they have reached the proper size for converting to formulated feed, fry can be observed capturing feed particles and will appear to "bounce" slightly off the tank substrate as the mouth is protruded downward. From this stage on, sturgeon will actively search for formulated feed as they navigate the tank.

Atlantic sturgeon (age 3 months) appear to possess fairly plastic feeding behavior as they have been observed to capture and consume live invertebrates such as Ephemeroptera sp. (mayfly) larvae and Gammarus sp. after being reared exclusively on formulated feed for two months prior.

Figure 24.- Newly-hatched Atlantic sturgeon fry with yolk-sac (note spiral valve structure in posterior gut region)

5.1.3 Fry Feeding

Through both formal and informal experimentation, no formulated diets were found to be effective for rearing first-feeding fry. Freeze-dried tubifex worms also yielded poor results when offered as a starter diet. Live Artemia sp. (brine shrimp) is currently the only feed documented to successfully rear 75% or more fry to the stage where conversion to formulated feed occurs. It is a good practice to introduce brine shrimp to tanks while larvae are still benthic (Table 6) so that food is available at the initiation of exogenous feeding. Keep in mind that sturgeon larvae will use up their yolk sac more quickly as water temperatures increase, thus requiring exogenous feed sooner. Though feed type and amount is important to fry growth and survival, initial fry density was also found to be a critical factor (Mohler et al. 2000). If fry densities are excessive to those recommended, conversion to formulated feed will be prolonged or may not occur, leading to excessive mortality. It is in the best interest of the fish-culturist to expedite conversion from live brine shrimp to formulated feed due to the amount of labor required for daily production and feeding of a live diet. If correct densities are maintained and adequate quantities of brine shrimp are offered (Table 7), fry should attain the Conversion Threshold Size (CTS) with less than 25% mortality. The CTS is defined as the mean minimum size at which Atlantic sturgeon fry will convert from brine shrimp to formulated feed; specifically identified as: 34.5mm total length and 0.18 grams (Mohler et al. 2000).

Table 7.- Suggested culture parameters for first-feeding Atlantic sturgeon fry.

Parameter	Value	Comments
Water temperature	15 to 19 °C	19°C gave higher growth rate (Kelly and Arnold 1999)
Flow rate	2-3 L/min (0.6 m dia tanks) 4-5 L/min (1.2 m dia. tanks)	Establish circular flow pattern
pH	7 to 8	Average values at NEFC-Lamar
Water hardness	60 to 80 mg/L as $CaCo_3$	Average values at NEFC-Lamar
Dissolved oxygen	8 mg/L or greater	Average values at NEFC-Lamar
Photoperiod	Natural	Supplemental overhead fluorescent from 0730 to 1600 hours is optional
Tank cleaning	Daily	Critical to control tank parasites
Initial stocking density for first-feeding fry	Maximum of 7.4 fry per Liter or 0.13 grams per Liter	More important may be number of fry per unit of tank bottom substrate area (recommended maximum = 0.3 fry /cm^2)
Begin introducing feed (Live brine shrimp)	Six days post-hatch	Larvae still benthic - yolk plug not extruded yet (*based on water temp. of **19°C** for 2 days post-hatch then **17°C** maintenance temperature*)
Number of brine shrimp nauplii per 54-Liter (0.6-m diameter) culture tank per day	4 - 6 X 10^5 nauplii per day Tank inflow = 2-3 L /minute (average = 1.5 nauplii /10 ml culture tank water)	Delivered automatically for 3 min at 30-min intervals over a 24-hour period (2700 - 4100 nauplii / minute of delivery)

In addition to stocking density and food type, daily ration is also important for successful fry culture. Atlantic sturgeon fry require a nearly constant daily supply of brine shrimp for about 20 - 26 days to attain the CTS. Hatching of approximately 9 kg of brine shrimp cysts was needed to rear 20,000 first-feeding Atlantic sturgeon to the CTS in 26 days (Mohler et al. 2000). This required a brine shrimp incubation unit with a 150-L capacity for daily production.

Ineffective diets for first-feeding fry cause excessive mortality to occur beginning approximately day 13-17 post-hatch (at about 18° C; Figure 25). This type of early mortality is likely a result of starvation due to: (1)

fish not accepting formulated diets and depletion of available energy reserves as depicted by line "A" or (2) type of feed is accepted but amount offered is insufficient to meet energy requirements and mortality somewhat delayed as depicted by line "B" (Figure 25).

An effective way to deliver brine shrimp is through use of a timer-controlled bellows pump which can be set to deliver the desired volume of brine shrimp culture from a 100-L, aerated hopper at regular intervals over 24 hours. The pump can be used to introduce feed into the water delivery system thereby feeding all tanks simultaneously (Figure 26). A schedule of feeding for three minutes every ½ hour of the day has been effective for rearing fry to the CTS in 20 - 26 days (Table 7). Use of peristaltic or other pump types to deliver brine shrimp may kill the nauplli upon delivery. Feeding dead nauplii has not been adequately tested on first-feeding Atlantic sturgeon.

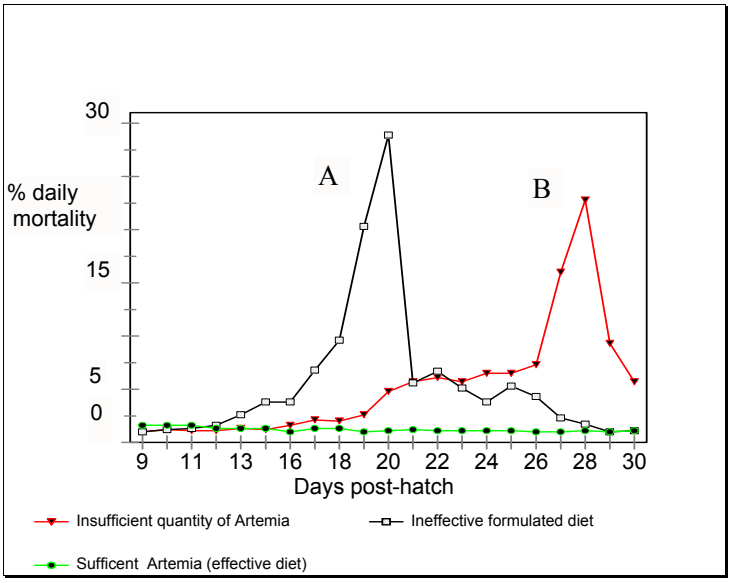


Figure 25.- Mortality patterns of first-feeding Atlantic sturgeon fry reared under similar conditions and offered ineffective vs. effective diets

Once the CTS has been attained, fry can be fed a commercially available formulated diet. Zeigler sturgeon diet (Zeigler Brothers, Inc., Gardners, Pennsylvania) (Mohler et al. 2000) as well as Biokyowa (Biokyowa Inc., Chesterfield, Missouri) (Mohler et al. 1996) have been used successfully as conversion diets for Atlantic sturgeon. At this life stage, diet particle size should be 200 - 400 microns and feed should be offered via automatic feeders at 3 - 7% body weight per day. Kelly and Arnold (1999) found that 0.3-g Atlantic sturgeon had higher maximum growth when offered 7% body weight in feed per day (at 19°C) but best feed conversion was obtained at a 3% feed rate.

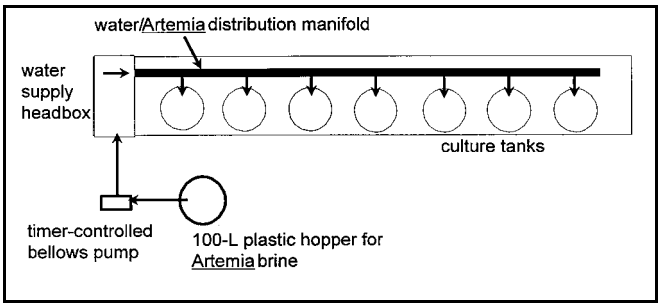


Figure 26.- Schematic for automatic-timed feeding of brine shrimp to Atlantic sturgeon fry

5.1.4 Shipping Larvae, Feeding Fry, and Fingerlings

Larvae, feeding fry, and fingerlings are somewhat tolerant of shipping conditions. All have been sent successfully by filling plastic fish-shipping bags with 1/3 water and 2/3 oxygen, placing the container into an insulated cooler/corrugated cardboard box system, and shipping via express delivery service. Two plastic bags should be used by placing one inside the other. The open end of each bag should be twisted closed and secured tightly with multiple rubber bands. Practical experience has shown that after a 24-hour shipment, fish recipients report dissolved oxygen at saturation and slightly depressed pH levels between 6 and 7; probably due to fish respiration. Survival is generally >90%. In warm weather, it is wise to place an artificial ice pack in the shipping container but not in direct contact with the transport bag. As many as 2100 larvae have been successfully shipped in about 5 Liters of water using this technique.

5.2 Fingerlings (about 0.3 grams to age-1 fish)

As with fry, circular, flow-through tanks are preferred for rearing fingerlings (Figure 27). However, unlike fry, fingerlings will survive and grow fairly well in rectangular raceways. If circulars are used, mesh size for drainage screens can be increased over that used with fry to facilitate tank cleaning. If circular tanks are used which do not have a center standpipe for drainage but rather a flat bottom drain with an external standpipe, excessive wastage of feed may occur. Drop some feed in the tank and observe how much goes to drainage uneaten. If the amount is judged excessive, adjust flows or flow patterns to minimize feed waste. Water flows which maintain dissolved oxygen levels of about 8 mg/L or more in culture tanks have been shown to be adequate for rearing fingerlings.

5.2.1 Feeding Fingerlings

At a water temperature of 19°C, Kelly and Arnold (1999) found that 0.3-gram sturgeon had the highest growth when offered feed at a 7% body weight per day ration vs. 3 and 5% rations. For 28-gram sturgeon reared at about 17°C, maximum growth occurred at 3% body weight per day while peak feed conversion occurred at a ration of 1.5% (Jodun In Progress). Kelly and Arnold (1999) studied effects of ration (0.5 - 1.5%) and temperature (15 - 19° C) on growth of 60-gram Atlantic sturgeon and found that fish had highest growth at 1.5% ration and 15°C at the rations and temperatures tested but cautioned that maximum growth most likely occurs at some ration beyond 1.5% since an upward trend in growth was noted. Similar to

culture of other fish species, as Atlantic sturgeon grow, the percent daily ration able to be consumed decreases, therefore ration offered is normally reduced over time to optimize feed conversion.

Figure 27.- Seven-month-old Atlantic sturgeon fingerlings

Young-of-year Atlantic sturgeon show a wide variability in growth response to different formulated diets (Mohler et al. 1996). Above average (relative) growth has been obtained with Biokyowa (Biokyowa Inc., Chesterfield, Missouri) and Zeigler (Zeigler Brothers, Inc., Gardners, Pennsylvania) sturgeon diets but many commercially-produced formulated diets have not been tested on Atlantic sturgeon. In general, excellent conversion ratios (<1.0) can be obtained in fish sized from 0.3 to 60 grams when offered formulated diets at rates of 7% to 1.5% body weight per day, respectively. Optimal nutritional requirements have not been adequately investigated for Atlantic sturgeon, however acceptable growth and survival for juvenile Atlantic sturgeon has been demonstrated using commercial formulated diets containing approximately 48 - 59% protein, 16% fat, and 7 - 12% ash content (Mohler at al. 1996; Jodun et al. 2002).

Culture tank loading density for fingerling Atlantic sturgeon has been investigated to some extent where it has been demonstrated that growth is inversely proportional to tank density (Mohler et al. 2000). Through experimentation in circular tanks, it was found that 0.8-g fingerlings which have converted to formulated feed can be stocked at initial densities up to 2.22 grams per liter and reared for 28 days with at least 90% survival and exhibit feed conversion ratios of about 0.50 (1 gram of wet weight gain for each 0.50 g of feed offered) (Mohler et al. 2000).

One of the most notable changes in behavior of cultured Atlantic sturgeon at fingerling-size is their tendency to be nearly always benthic and at times sedentary. However, when food is positioned upstream from fingerlings at rest on the tank bottom, the fish appear to sense the presence of food and move toward it. Encountering food and consuming it seems to trigger subsequent searching activity. If fish are sedentary and there are accumulations of uneaten feed on the tank bottom, fish are likely being overfed.

5.3 Juveniles - Age 1 or Greater

As with earlier life stages, circular tanks are preferred but both indoor and outdoor concrete raceways have been used successfully for juveniles up to about 92 cm total length (36") and 2.5 kg in weight. One problem with concrete raceways is the tendency for sturgeon to swim into the side walls with a burst of speed resulting in snout damage and other lacerations. This is mostly prevented when circular fiberglass or plastic tanks are used. Juvenile Atlantic sturgeon have a fair capability to jump therefore it is recommended that provisions be made to prevent escapement. From this life stage to maturity, a series of circular tanks from 2 to 6 meters in diameter is recommended with the latter size sufficient for maintaining mature broodstock.

Atlantic sturgeon appear to grow and survive much better in a flowing water environment but pond-rearing has been attempted. Culture of 150 yearlings in a static pond met with difficulties at NEFC. In one episode, over 100 2-yr-old juveniles died during a seasonal eruption in the population of toad tadpoles in the culture pond. Subsequent necropsies revealed that sturgeon had gorged themselves with tadpoles to the point where their stomachs and swim bladders were found to be full of the organisms. The feeding response of sturgeon is likely hard-wired and automatically triggered when a soft-bodied prey item is detected via barbels or other chemo-sensory organs. Though not verified by lab analysis it is likely that the sturgeon mortality was a direct result of tadpole ingestion since it well-documented that some species of toads are equipped with toxin-secreting glands in the skin. Therefore it follows that excessive tadpole ingestion may be lethal to fish. Also observed was an over-wintering phenomenon where contact with surface ice produced "frost-bite" lesions which became infested with Saprolegnia sp. fungus and led to a significant early spring mortality of about 25%. Ponds operated on a flow-through basis offer some potential for rearing Atlantic sturgeon during the growing season, but in mid-Atlantic and more northern latitudes ambient winter water temperatures can be as low as 1° C , therefore water depth and flow must be great enough to prevent excessive freezing. One pond configuration being evaluated for long-term sturgeon rearing at NEFC consists of conversion of an existing pond into a simulated riverine environment for long-term rearing of juveniles through adult life stages. In this configuration, a center island is constructed with incoming water directed in a manner that causes a circular flow pattern around the island (Figure 28).

Figure 28.- Simulated riverine sturgeon rearing pond with circular flow at NEFC-Lamar, PA

5.3.1 Feeding Juveniles - Age 1 or Greater

For some culture operations, it may not be economically feasible to continue supplying heated water to culture units once sturgeon become early juveniles, therefore fish may be subject to a seasonal water temperature regime. Once water temperatures drop to 10°C or less, a noticeable decrease in feeding activity usually occurs but minimal weight gains have been measured at mean water temperatures as low as 5.4°C with weight losses occurring at lower water temperatures. It was found that no growth benefit is derived from offering juvenile fish feed in excess of 0.25% body weight per day at water temperatures below 6.8°C (Jodun and Kelligher In Progress). At NEFC this age group Atlantic sturgeon is normally offered about 1.0% body weight as a daily ration until 18-24 months of age at which time feed rate is reduced to about 0.5%. When seasonal water temperatures decline to below 10°C rations are reduced to levels as low as 0.25%. After reaching age 2, Atlantic sturgeon juveniles have been successfully reared for 7 years under ambient water conditions ranging annually from 1 - 17°C, however growth is limited at temperatures below 10°C. Under seasonal water temperatures typical at Lamar, Pennsylvania (1- 15°C), an 8-yr-old Atlantic sturgeon could be expected to have a total length of about 118cm (46") and weigh about 10 kg (22.2 lbs) (Figure 29). Growth potential may be greater than shown in Figure 29 if heated water (>10°C) is supplied year-round.

Influence of season water temperatures upon growth and body composition was studied by King (In Progress) where one five different diets were offered to juvenile Atlantic sturgeon maintained at ambient water temperatures over the course of one year. Results indicated a seasonal influence on growth dependent upon which diet was offered.

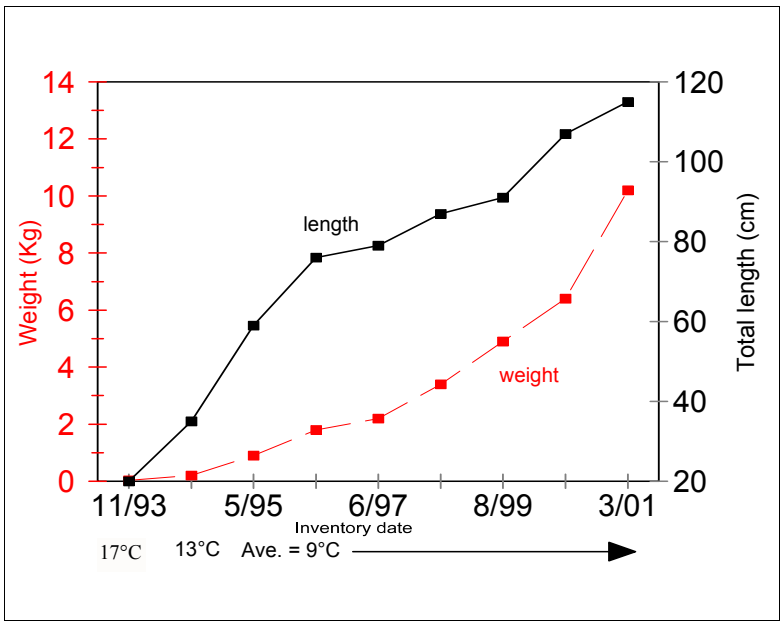


Figure 29.- Eight-year growth curve for hatchery-reared Atlantic sturgeon at NEFC-Lamar, PA

Effect of rearing density on growth of 368-g, age-1 sturgeon was investigated by Jodun et al. (2002) where it was reported that growth was inversely proportional to fish density. After 7 weeks of rearing, fish stocked initially at 3.6 kg/m^2 of tank substrate had greater growth than replicates stocked at higher densities (up to 16.3 kg/m^2) when replicates were fed to satiation.

5.3.2 Behavior of Juveniles

It is not uncommon for juvenile sturgeon to exhibit short bursts of swimming speed when startled by overhead motion or a sudden tank vibration; the startled fish may then trigger others to act likewise. Sturgeon do not have functionally well-developed visual reception (Kasumyan and Kazhlayev, 1993) and have often been observed to swim full speed directly into a tank wall or other obstacle when startled. For this reason it is wise to eliminate any unnecessary plumbing or other tank-related structures from the culture tank or immediately above it's water surface due to potential injury of startled fish. It is also common to observe juveniles swimming with their ventral surface against culture tank walls similar to the manner in which they swim against the tank bottom. In addition, juveniles have often been observed swimming with the snout above water or simply protruding the snout above the water surface for no apparent reason.

As water velocities approach 7 cm/sec cultured juveniles respond by orienting into the direction of flow (Wade Jodun, U.S. Fish and Wildlife Service, personal communication). At NEFC, flow velocity is normally maintained below this threshold and cultured fish swim in random directions most of the time. This flow level is sufficient to facilitate waste removal but not high enough to influence directional orientation. At higher velocities, it appears that sturgeon are able to manipulate their large pectoral fins so that water flowing over the surface pushes the fish downward to maintain a benthic position.

Very little or no aggression towards co-habiting individuals has been observed in juveniles; many times they can be seen lying on top of each other with little evidence of intentional displacement of other individuals for feed or tank position. If a hierarchy exists in cultured juveniles, it's manifestations are subtle and may require detailed observation to discover.

5.4 Experimental Stocking

The Fishery Management Plan (FMP) for Atlantic sturgeon adopted by the Atlantic States Marine Fisheries Commission (ASMFC) (Taub 1990) does not include a specific component which recommends production and stocking of hatchery-reared fish. One of the stated objectives of Amendment 1 to the FMP (ASMFC 1998), was to close the fishery for a sufficient time period to reestablish spawning stocks and increase numbers in current spawning stocks. This resulted in a long-term harvest moratorium on the species throughout it's range in the U.S. It is anticipated that the moratorium will remain in place until there are at least 20 protected age-classes of females in each spawning stock.

Two experimental stockings of Atlantic sturgeon have taken place along with some evaluation of the liberated fish (Mohler 2000). The stockings were performed to demonstrate the ability of hatchery-reared fish to survive and grow when released into two different drainages within the historical range of the species. Artificially-propagated progeny of wild Atlantic sturgeons gill-netted from the Hudson River, New York were used in the studies.

5.4.1 Hudson River Stocking

In October 1994, 4927 fish were marked with a pelvic fin amputation and a coded-wire tag under the first dorsal scute. Fish were then released into the Hudson River by the New York Department of Environmental Conservation to evaluate survival, growth, and estimate wild recruitment (mean length at release was 10.3 cm and mean weight was 4.1 g). Gill net sampling using stratified random sampling on the Hudson River in 1995 resulted in capture of 15 hatchery-reared fish and 14 wild fish which were estimated to be the same age as hatchery-reared fish. Assumed age-1 wild fish had greater total length than hatchery-reared fish (mean = 51.3 vs. 38.9 cm, respectively) (Petersen et al. 2000). Petersen (1998) also reported results of targeted gill-net capture for juveniles in 1996 and 1997, where nearly 50% of all juveniles captured were of hatchery origin (12 of 25) and (83 of 182), respectively.

Out-migration of hatchery-reared fish was documented as three marked fish (pelvic fin amputation + coded wire tag) released into the Hudson River in 1994 were captured in the Delaware River estuary in 1997 and re-tagged with visible floy tags. One month later, one of these re-tagged individuals was recaptured in the Chesapeake Bay estuary (Bain 1998).

5.4.2 Nanticoke River Stocking

In July 1996, an experimental stocking was performed with 3275 age-1 hatchery-reared fish. All were marked with either a coded-wire tag or a floy T-bar tag and were stocked at two sites on the Nanticoke

River, a tributary of the Chesapeake Bay. Evaluation of hatchery-reared sturgeon was achieved via a monetary reward program administered by the U.S. Fish and Wildlife Service - Maryland Fisheries Resources Office from 1996 through 1998. Commercial fishermen were compensated for holding any live sturgeon obtained as by-catch until verified by program administrators as either wild or of hatchery origin. Subsequent evaluation (Skjeveland et al. 2000) showed that equivalent numbers of hatchery-reared and wild Atlantic sturgeon (461 and 451, respectively) were captured in the Chesapeake Bay from 1996-2000) . In addition, length-weight relationships for sturgeon ranging from 44.5 - 99.5 cm were similar between wild and hatchery-reared fish but all sturgeon longer than 100 cm were wild fish. Even though stocked at only two sites on the Nanticoke River, hatchery fish showed wide dispersal, being captured throughout the Chesapeake Bay (Skjeveland et al. 2000; Secor et al. 2000a).

Both the Hudson and Nanticoke River experimental stockings demonstrated that hatchery-reared Atlantic sturgeon can survive in the wild for at least 2-3 years and increase in size. Additionally, some individuals stocked into the Hudson River began out-migration at about age 3 as demonstrated by their capture in the Delaware River and Chesapeake Bay. Long-term evaluation is needed to determine whether stocked fish have imprinted to the watershed of release and will eventually help to rebuild depleted populations through successful reproduction.

Chapter 6 Fish Health

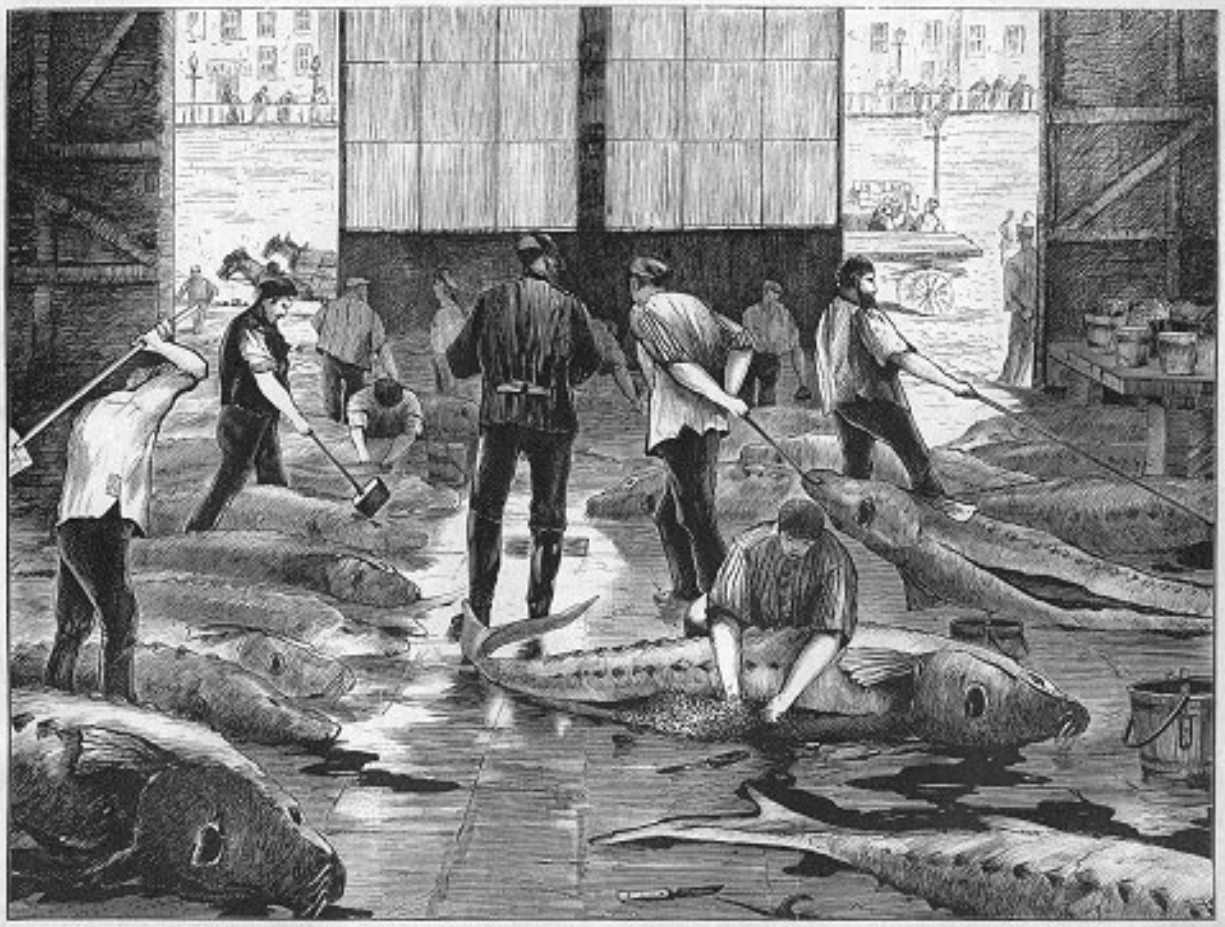
This section describes specific health concerns for Atlantic sturgeon and is not intended as a substitute for a comprehensive fish health textbook. However, all accepted fish health practices concerning disinfection of eggs and equipment to prevent the introduction or spread of disease pathogens are applicable to Atlantic sturgeon. One important concern when working with and transporting wild fish to a different location for spawning or rearing is the possibility of introduction of a non-indigenous pathogen or nuisance species on the transported fish or in transported water. Therefore one should always do a bit of homework to find out what potential exists for transfer of unwanted organisms from the capture site to the destination site and take appropriate precautionary measures.

6.1 Bacterial Diseases

Furunculosis.- Cultured Atlantic sturgeon are susceptible to Aeromonas salmonicida , the causative agent of furunculosis. The pathogen was transmitted horizontally and caused mortality among 40-gram Atlantic sturgeon that were tested by co-habitation with infected brook trout. However, it was found that sturgeon were more resistant to the pathogen than were brook trout (Rocco Cipriano, U.S. Geological Survey - Biological Resources Division, personal communication). Fishes with furunculosis become more lethargic as the disease progresses and may eventually show visible signs including redness at the base of fins and other tissues. There may be discrete furuncules in some organs and some furuncules may be visible through the skin (Post 1987) (Figure 30). In some instances, no visible furuncles are present.

Other symptoms of furunculosis observed at NEFC include inflamation (Figure 31) and protrusion of the uro-genital opening along with a bloody discharge upon mild pressure to the area. Infected fish may become discolored with a lighter-than-normal appearance especially noticeable in the dorsal scutes and cranial region. In addition, behavior of infected fish may include uncoordinated swimming, difficulty maintaining equilibrium, and remaining at or near the water surface (Wade Jodun, U.S. Fish and Wildlife Service, personal communication)

A certified fish health biologist should be contacted to diagnose this disease and to test whether the particular strain of bacteria is resistant to the drugs available for treatment of the disease. At NEFC, the disease has been treated using injectable oxytetracycline on individual fish (Liquimycin LA-200; Pfizer, Incorporated, NY, NY) . A typical injection schedule for treatment of a systemic infection would include the initial dose (40 mg/kg active ingredient) followed by a booster injection at the same dosage two days after the initial injection. No more than 2 cc of material is injected in one particular site during a treatment to prevent tissue damage in the immediate area of the injection.



Die Stör-Schlächterei in St. Pauli zu Hamburg. Originalzeichnung von Hans Petersen.

Figure 30.- Dermal lesion in adult Atlantic sturgeon from which Aeromonas salmonicida (causative agent of furunculosis) was obtained.

Figure 31.- Hemorrhagic area at the base of pelvic fin from which Aeromonas salmonicida, causative agent of furunculosis was isolated.

Hyper-inflated swim bladder syndrome (HISB).- When hatchery water temperatures fall below about 10°C, a small percentage of cultured Atlantic sturgeon may become afflicted with HISB. Symptoms include loss of equilibrium and inability to maintain normal benthic posture, distended abdomen (caused by over-inflated swim bladder), and at times floating upside-down at the water surface with occasional struggling to regain upright position (Figures 32 and 33). The causative agent for HISB has not been positively identified, however an anerobe (Bacteriodes sp.) which has been isolated from the spiral intestine of white sturgeon is suspect. This bacterium produces hydrogen gas as a metabolic waste which may inflate the swim bladder if produced in excess. The swim bladder is directly associated with the esophagus and separated from it solely by a sphincter, therefore it is conceivable that excess metabolic gasses associated with the bacterium or irritation of the sphincter could allow the swim bladder to become hyper-inflated. Adjusting water temperatures up to at least 10° C will usually relieve symptoms in about 48 hours if instituted immediately. When symptoms persist, some improvement may be achieved using injectable oxytetracycline (Liquimycin LA-200 Pfizer, Incorporated, NY, NY) administered intra-muscularly at a dosage rate of 40 mg/kg active ingredient. A few hours post-injection, it is not uncommon to observe rapid respiration of treated fish with some recovery after 24 hours. If recovered individuals are placed back into cold water, some may again develop HISB symptoms. Some individuals may never recover from the condition regardless of treatment employed. Mortality from HISB is generally a result of emaciation due to inability of the fish to maintain a benthic position for feeding.

Figure 32.- Juvenile Atlantic sturgeon showing symptoms of Hyper-inflated swim bladder (HISB) (Note slight bulge on the side of abdomen)

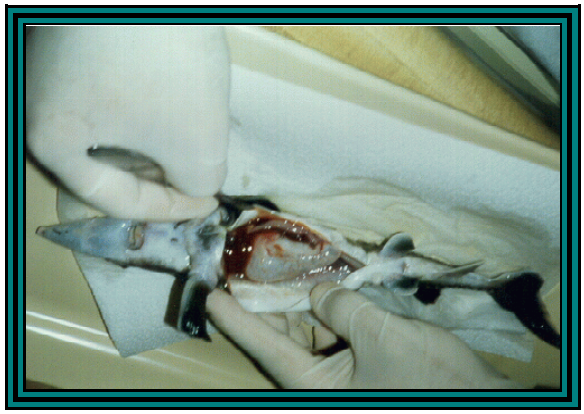


Figure 33.-Necropsy of sturgeon showing hyper-inflated swimbladder

6.2 Mycotic (fungal) Diseases

Saprolegniasis.- Saprolegniasis is a fungal disease of fish and fish eggs caused by a member of the family Saprolegniaceae. The name has been broadly accepted when the etilogy is a species of the genus Saprolegnia, Achlya, or Dictyuchus (Post 1987). These fungi require organic matter for growth and reproduction, liberating digestive enzymes into the surroundings to reduce organic material into absorbable nutrients for their continued survival. The basic structure of the fungi which have been observed on Atlantic sturgeon is a network of fine filaments called hyphae which in advanced stages are visible as patches of fluffy cotton-like white or greyish-colored growth on external surfaces of the fish including gills. A small sample of the material placed onto a microscope slide with some water can be viewed microscopically to verify the presence of branched, filamentous hyphae. Saprolegnia or other fungal infestation may be secondary to another type of environmental insult to the sturgeon such as handling stress, other parasitic infestations, external abrasions, nutritional deficiency, or bacterial and viral infection. Saprolegnia has been diagnosed on all life stages of Atlantic sturgeon including eggs.
Wild broodstock are susceptible to fungal infestations when brought into captivity. The combination of stress from capture and transport along with wounds and abrasions received during capture and handling creates conditions favorable for fungal infestation after only 2-3 days in captivity. Therefore, a non-iodized salt and formalin treatment regimen is recommended. The treatment schedule is as follows:

– Wild fish (except ripe females targeted for immediate spawning) should be treated with non-iodized salt at a concentration of 1% (10 grams per liter) for 24 hours to control parasites and minimize fungal infestation. Subsequently for 7 days, salt treatments should be given at a

concentration of about 0.25% (2.5 grams per liter). Concurrent with salt treatments, daily flush-type formalin (Paracide F, Argent Chemical Laboratories, Redmond, Washington) treatments of 150 ppm should be administered for about 7 consecutive days after capture as a preventative measure. Ripe females may be placed into this treatment schedule after spawning procedures are completed if the fish is to remain in captivity for any length of time.

6.3 Viral Infections

White sturgeon Herpesvirus type 2 (WSHV-2).- Atlantic sturgeon are susceptible to WSHV-2, a pathogenic virus isolated from juvenile and adult white sturgeon which is epizootic among captive white sturgeon populations in Northern California. When 17-gram Atlantic sturgeon were exposed to tissue culture infective doses of the virus, 3% died and about 17% of the survivors were carriers of the virus. Dying fish have clinical signs of infection including hemorrhagic lesions and ulcers on both dorsal and ventral surfaces and particularly around the mouth. Comparatively speaking, Atlantic sturgeon show greater resistance to infection than white sturgeon but do become infected (Ron Hedrick, University of California-Davis, personal communication). At this time, there is no known treatment for the disease.

6.4 Special Fish Health Concerns for Fry

Starvation and parasites.- Starvation and infestation by external parasites along with secondary fungal infestation have been identified as principle causes of Atlantic sturgeon fry mortality (Figure 34). Death from starvation is commonly preceded by a slow struggling motion when swimming is attempted along with a U-shaped posture when the fish is at rest. If prescribed feeding and culture criteria have been met as outlined in the section on "Care and Rearing of Juvenile Fish" but mortality continues to rise, fish must be sampled and microscopically examined. The following parasites have been observed on cultured Atlantic sturgeon fry: Chilodonella sp., Trichodina sp., Hexamita sp., Ichthyoboda sp. (Costia), and Icthyopthirius multifiliis (Ich). See Post (1987) or another text on general fish health for identification of specific parasites. If unchecked, infestation from these parasites can result in >50% mortality in as little as four days. Effective treatment for these parasites is: one standing formalin treatment (Paracide F, Argent Chemical Laboratories, Redmond, Washington) at 150 ppm for 1 hour with the exception of Ich which usually require multiple treatments. Accumulation of uneaten brine shrimp and fish waste in culture tanks promotes parasite infestation and must be avoided. Since Atlantic sturgeon fry spend much of their time on or near the tank bottom to feed, they are in direct contact with parasites which proliferate in organic waste material.

Figure 34.- Caudal fin of Atlantic sturgeon fry showing damage from Chilodonella sp. and secondary fungal infestation

An additional cause of mortality may be due to ingestion of multiple, unhatched Artemia cysts which may result in blockage of the digestive system. If ingested, unhatched cysts can be easily seen by examining fry under a dissecting microscope. It is unknown to what extent this may cause mortality but it is a good precaution to allow brine shrimp cultures to settle for about 20 minutes and then skim off the unhatched cysts prior to feeding. If mortality continues to rise despite the above, a qualified fish health laboratory should sample fry for possible viral or bacteriological infection.

6.5 Tolerance to Therapeutic Compounds

Tolerances to salt, chloramine-T, and formalin have been determined by King and Farrell (2002) for 1-gram fingerling sturgeon by determining the 24-hour safe exposure range and 96-hour LC50 values (concentration of the compound which causes 50% mortality at 96 hours) (Table 8) .

Table 8.- Tolerance of 1-gram Atlantic sturgeon fingerlings to salt, chloramine-T, and formalin.

Salt (non-iodized)	chloramine-T	formalin
96-HOUR LC50		
9.74 g/L	7.66 mg/L	31 ul/L
24-HOUR SAFE EXPOSURE		
7.13 - 11.88 g/L	6.7 - 11.2 mg/L	54.0 - 90.0 ul/L

6.6 Miscellaneous Health Concerns

Tadpole ingestion.- Further investigation is needed on the effect of toad tadpole ingestion by juvenile Atlantic sturgeon being reared in a pond environment. In some regions at certain times of year, ponds are used as breeding habitat for toads which can result in a prolific hatch of toad tadpoles. Though not thoroughly investigated for cause and effect, an episode of American toad tadpole Bufo bufo ingestion by 2-yr-old sturgeon at NEFC was associated with both abrupt and delayed mortality of >100 individuals being reared in a 0.1- hectare pond. Necropsy of sturgeon mortalities revealed that both stomachs and swim bladders were gorged with toad tadpoles. Some species of mature toads are equipped with glands which produce compounds that are highly toxic to some fish (Jacob et al. 1994). In addition, Crossland and Alford (1998) report that early life stages of toads (including tadpoles) also possess chemicals that are noxic or toxic to predators and that many aquatic predators appear to be unable to detect and avoid those toxins.

Sunburn.- Rearing Atlantic sturgeon in uncovered outdoor raceways or tanks where water depth is insufficient to block harmful radiation can lead to sun-damaged skin. Sturgeon which develop this condition often get patches of light discoloration on their dorsal surfaces which become susceptible to secondary fungal infestation. Fungal infestation can be treated with formalin as previously described but skin may be permanently scarred or discolored to some extent.

Appendix I Diet formulation for wild-captured Atlantic sturgeon broodstock

Ingredients for bloodworm diet:
1) frozen bloodworms
2) Special Brood Diet (Bioproducts, Inc. Warrenton, Oregon)
3) cold water gelling starch (Mira Gel 463, A.E. Staley Manufacturing Company , Decatur, Illinois, USA)

Instructions (For 1.5 Kg of feed):
- thaw 1.1 Kg frozen bloodworms in a sieve and save the liquid
- finely grind 0.375 Kg Special Brood Diet mix the above together in a blender
- add and mix in 150-300 grams "Mira Gel" (10 - 20 % of total feed amount) until a dry cookie dough-like consistency is achieved
- run the mixture through a food-mill with 1/4" holes or a cookie press (biscuit maker) with 1/4" holes
- if feed is too stiff add saved bloodworm liquid
- cut to desired size and feed at least twice daily to fish
(it is easier to observe feeding behavior if feed is placed in one area of the tank vs. broadcasting over the entire tank)

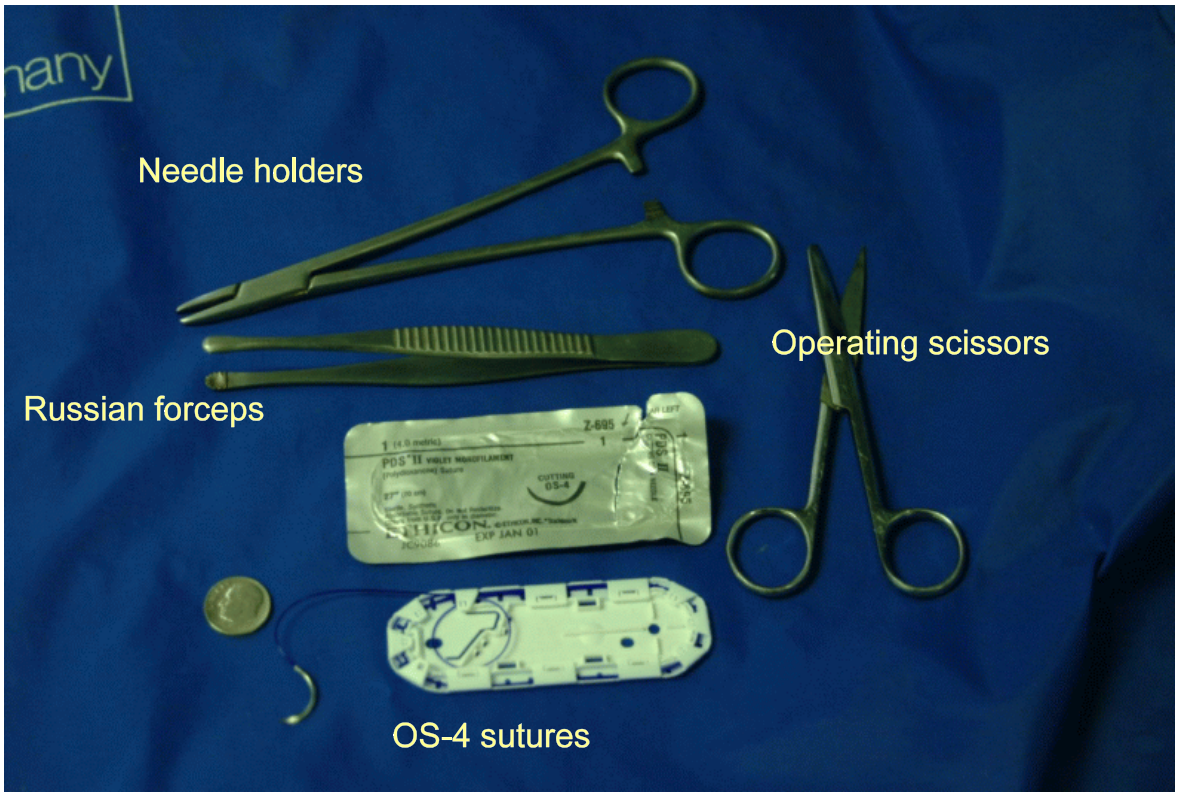
Offer only bloodworms or the preferred natural food for a few weeks prior to introducing your formulated diet mixture.

After routine feeding is observed, introduce the bloodworm/formulated feed mixture.

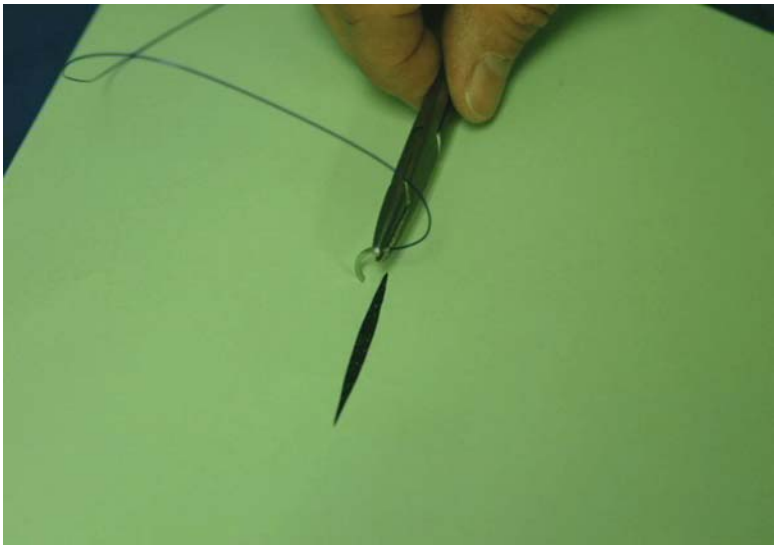
Gradually increase the percentage of Special Brood Diet and decrease the amount of bloodworms in the feed until the fish will feed on 100% Special Brood Diet.

You may wish to substitute other diets in place of Special Brood Diet in the above recipe or if the sturgeon species you are working with is known to prefer a natural food other than bloodworms, this can also be substituted.

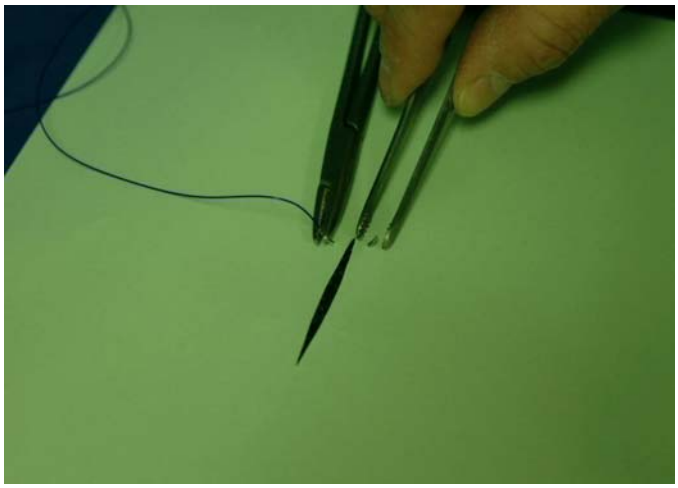
Appendix II Suture Technique



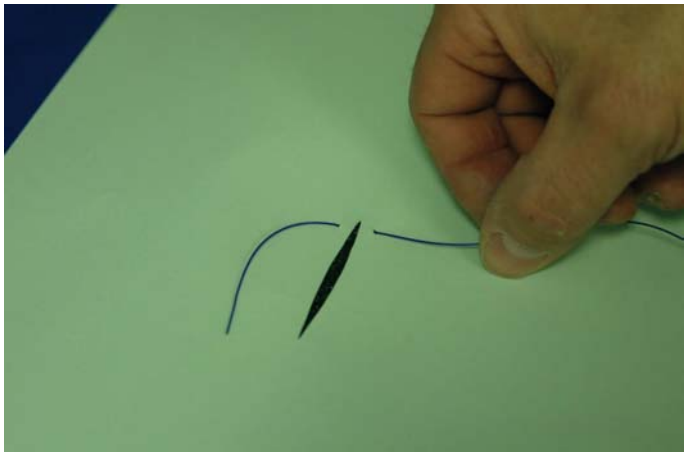
Dip surgical instruments in 70% ethyl alcohol often during surgical procedure to minimize risk of infection



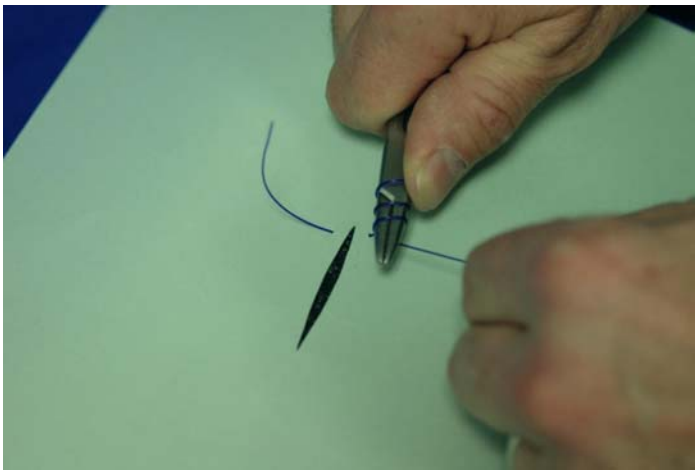
1 - Insert needle into tissue on one side of incision.



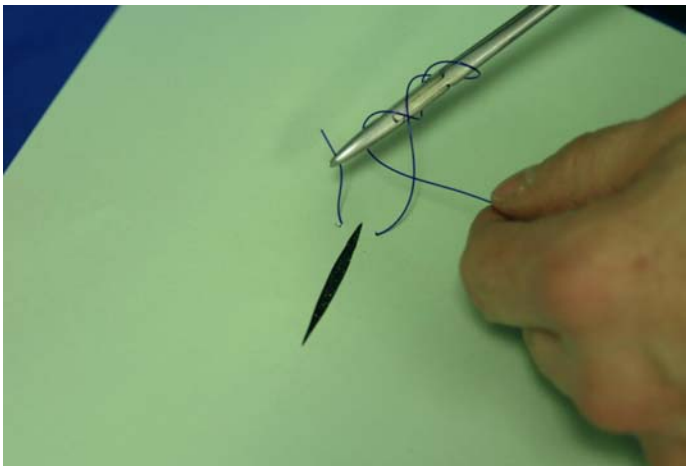
2 - Push needle up through other side of incision and pull through with forceps.



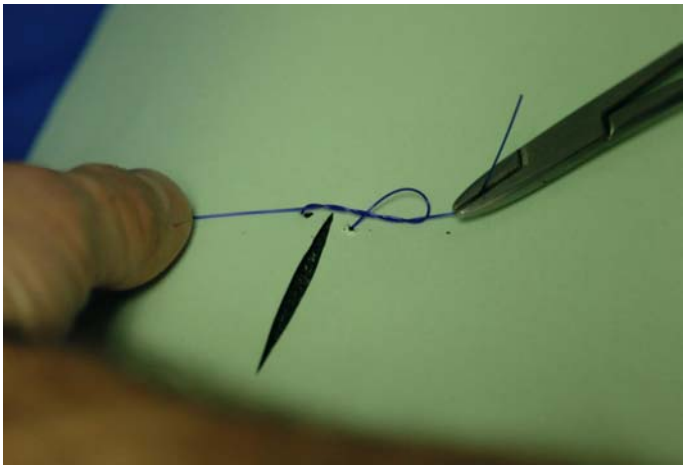
3 - Pull suture through leaving about 3-5 cm of tag.



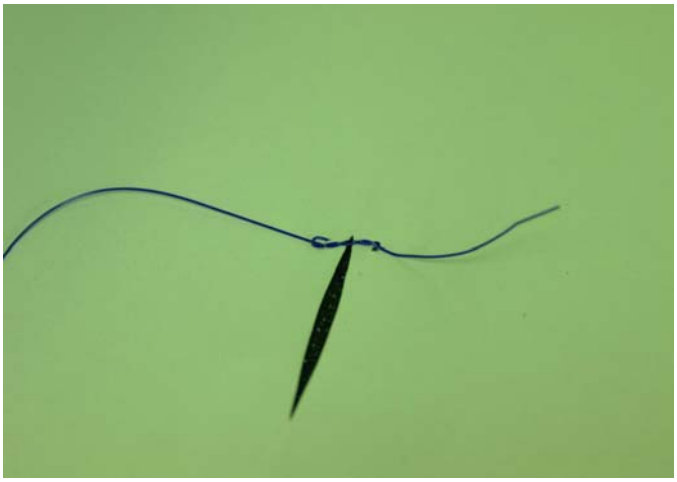
4 - Wrap suture 3 - 4 times around the tip of the needle holder.



5 - Grab the tag end of suture with the needle holder and pull tag end through the wrapped suture.

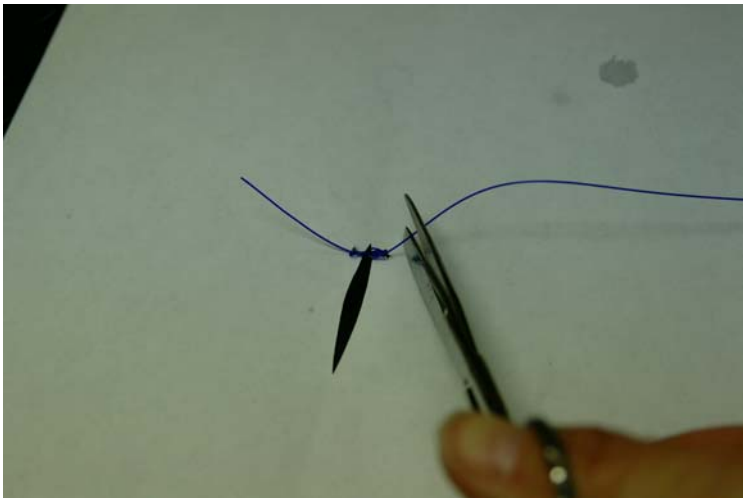


6 - Pull both ends of the suture to begin closing the incision.

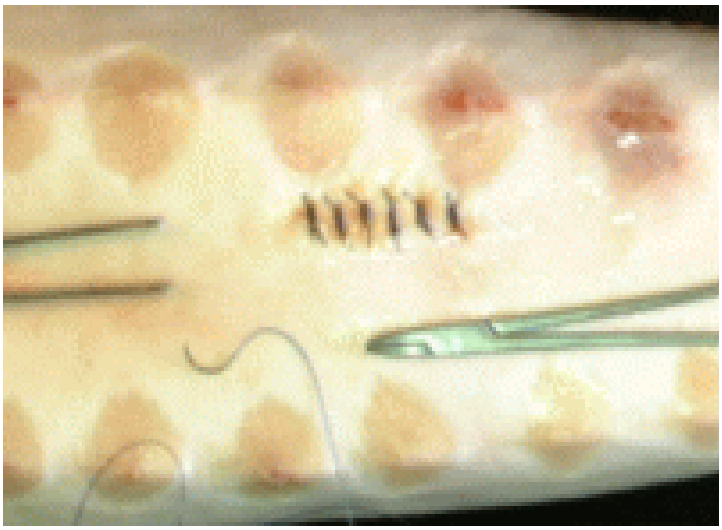


7 - Pull firmly until edges of incision are closed in the immediate area of the suture.

Repeat steps 4 through 7 to make a double knot before proceeding to the next step.



8 - Trim both ends of the suture after knot is snug. Repeat steps 1 through 8 until entire length of incision is closed



Completed set of sutures used to close a biopsy incision on an Atlantic sturgeon.

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The U.S. Fish & Wildlife Service and it's Fish Technology Centers

The U.S. Fish & Wildlife Service has a responsibility to conserve, restore, enhance, and manage the Nation's fishery resources and aquatic ecosystems for the benefit of future generations. Federal stewardship of the Nation's fishery resources has been a core responsibility of the Service for over 120 years. The National Fish Hatchery System was established in 1871 by Congress through the creation of a U.S. Commissioner of Fish and Fisheries. Today the National Fish Hatchery System is comprised of 70 Fish Hatcheries, 7 Fish Technology Centers, 9 Fish Health Centers, and 1 Historic National Fish Hatchery.

Fish Technology Centers

In 1965, the Fish & Wildlife Service established seven Fish Technology Centers nationwide to provide leadership and technology guidance to the National Fish Hatchery System and fish culture community. Fish Technology Centers originally focused on fish culture issues such as reducing costs, enhancing fish quality, and improving overall fish culture operations. Today, as fishery managers respond to increasing needs to aid in the restoration of native fish and other aquatic species and to produce healthy, genetically diverse organisms to assist in that effort, the importance of Fish Technology Centers in providing sound science and technology support is greater than ever.

The roles and responsibilities of Fish Technology Centers have correspondingly expanded to include technical support for captive propagation of imperiled aquatic species, for interjurisdictional restoration programs, for control of invasive aquatic species, and for sound genetic management of aquatic populations. Fish Technology Center activities range from developing diets and propagation techniques for rare species to developing new marking and evaluation techniques, to genetic stock identification, to providing assistance in study design.

To accomplish their work, Fish Technology Centers maintain expertise in a number of disciplines, including physiology, genetics, and biometrics. Functioning as a cohesive system, each Technology Center strengthens the others with complementary expertise, taking full advantage of various geographic differences to ensure that study results will successfully support a broad range of users and management objectives. Through their partnership role with other Service programs and federal agencies, States, Tribes, and the private sector, Fish Technology Centers provide a vital link in the Service's commitment to the conservation of our nations aquatic resources.

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