Atlantic States Marine Fisheries Commission

ATLANTIC STURGEON PROTOCOL WORKSHOP

Workshop Summary

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Annapolis, Maryland

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Goals and Objectives of the Workshop *(K. Damon-Randall)*

NOAA Fisheries Service's Northeast Regional Office contracted the Atlantic States Marine Fisheries Commission (ASFMC) to host a workshop on Atlantic sturgeon research protocols in response to the results from the Atlantic sturgeon status review and potential listing of the species. ASMFC will prepare a detailed summary of the meeting that will be used by a subgroup of the workshop participants, led by NOAA Fisheries Service, to develop a comprehensive protocol document specifically for Atlantic sturgeon.

The Status Review Team determined that Atlantic sturgeon could be grouped into five distinct population segments (DPS) in the U.S.—Gulf of Maine, New York Bight, Chesapeake Bay, Carolina, and South Atlantic—and recommended that based on the best available data three DPSs—NY Bight, Chesapeake Bay, and Carolina—warrant listing as threatened under the Endangered Species Act (ESA). NOAA will now consider the information and if listing is warranted, will publish proposed rule(s). If they are listed as threatened, then NOAA Fisheries Service will develop protective regulations (i.e., 4(d) rule) that will be published concurrent with the proposed rule.

Species listed as *endangered* automatically receive all ESA protections, including prohibition on "take" for the species. *Take* is defined under the ESA as "to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or attempt to engage in any such conduct." For species listed as *threatened*, take is not automatically prohibited. NOAA Fisheries Service must propose and publish a 4(d) rule to issue protective regulations and any limitations that it finds "necessary and advisable to provide for the

conservation of threatened species." A 4(d) rule is required by section 4(d) of the ESA. NOAA Fisheries Service must conduct a NEPA analysis on the 4(d) rule that includes an economic analysis of the impact of the provisions in the rule.

NOAA Fisheries Service determines what is "necessary and advisable to provide for the conservation of the species" by evaluating the biological status of the species and the potential impacts of various activities and programs on the species, and determining whether regulating these activities provides for the conservation of the species.

A 4(d) rule may: (1) extend some or all of the ESA Section 9 take prohibitions to all or only some activities; (2) "limit" the situations to which take prohibitions apply for threatened species; (3) identify activities representative of those to which take prohibitions do not apply; and (4) provide criteria for future activities to which take prohibitions will not apply.

Section 9 take prohibitions might not apply for the following: (1) scientific research projects that provide necessary information for the conservation and recovery of the species and follow a pre-approved research plan; (2) artificial propagation with a pre-approved hatchery and genetic broodstock management plan; and (3) rescue and salvage operations.

If, as a result of this workshop, the subgroup is capable of producing a comprehensive research protocol document for Atlantic sturgeon, a potential 4(d) rule may specify that take prohibitions do not apply to authorized research activities conducted on Atlantic sturgeon listed as threatened, provided: (1) researchers submit a research plan which adheres to the new protocols and receive written approval from NOAA Fisheries Service's Regional Administrator; (2) researchers submit annual reports of research activities; and (3) researchers notify the appropriate NOAA Fisheries Service regional contact immediately if mortalities occur. This would benefit researchers, in that an ESA scientific research permit (section 10(a)(1)(A) permit) will not be required for research activities covered under the 4(d) rule—a which may results in significant time savings.

The Northeast Regional Office is working on the listing determination now and hopes to publish a determination or proposed rule by mid-2008. A final rule must be published one year from that date. Critical habitat must be designated within one year of the final rule.

The goal of the Atlantic Sturgeon Research Protocol Workshop is to identify research activities, sampling methodologies, and other techniques that allow for information to be obtained on Atlantic sturgeon subpopulations while minimizing to the maximum extent possible the adverse impacts of the activities on the species. This information will then be incorporated by a small subgroup into a comprehensive protocol for research on Atlantic sturgeon. The Workshop objectives include: (1) identify existing and emerging research activities and sampling techniques for Atlantic sturgeon; (2) discuss the potential impacts of these activities on Atlantic sturgeon and identify ways to minimize these impacts; (3) identify uses for salvaged fish (e.g., contaminants analyses, others); (4) identify methods to resuscitate fish (e.g., how these methods could be employed by researchers and potentially trained observers or other individuals to reduce bycatch mortality); (5) determine minimum sampling requirements to confirm presence of existing subpopulations; and (6) discuss reintroduction and supplementation procedures and identify information necessary to develop hatchery and genetic broodstock management plans.

Review of Existing Sturgeon Research Protocols and Activities Permitted in ESA Section 10 Research Permits for Shortnose Sturgeon *(S. Bolden)*

Section 10 of the ESA requires a permit for *take* of a listed species. The term *take* means to "harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct" with a listed species. Currently, there are three species of sturgeon listed under the ESA: shortnose

(endangered), gulf (threatened), and green (newly listed as a threatened DPS). There are currently 18 permits issued for take of shortnose sturgeon by NMFS Headquarters; permits for take of gulf sturgeon are administered by individual states. When issuing permits for take of shortnose sturgeon, risk to the population is assessed by NMFS based on Moser *et al.*'s "A Protocol for Use of Shortnose and Atlantic Sturgeons" (2000, NOAA Tech. Memo. NMFS-OPR-18). It is important to note that a Section 6 agreement does not exempt anyone from needing a research permit.

The Moser *et al*. Protocol includes guidelines for handling, sampling, minimum sampling to confirm presence, and references. Handling includes short-term handling, identification and measurement, tagging, tissue sampling, and gastric lavage. Sampling includes the use of gillnet and trammel nets, trawls, and drift nets. For minimum sampling to confirm presence, the Protocol includes a research survey and different minimum sampling requirements for the north and south.

For ESA permitted take, researchers are encouraged to request at least one lethal take in their permit requests. The number of takes permitted varies depending on whether the take is lethal or non-lethal, the number of takes permitted annually, the life stage taken (adult, juvenile, larvae, and egg), location of the take, origin of the animal taken (hatchery v. wild), and the activity in which the take is expected to occur. If permitted to have takes, researchers must report takes annually.

Currently, the permitted activities for research on shortnose sturgeon include:

- Capture/Holding
  - Gillnets, trot lines, trawls, egg nets, buffer pads, D-traps, passive ichthyoplankton net, epibenthic sled, pump sampling
  - Weigh and measure
  - Scan for tags
  - Two hour holding with temperatures below 27°C and with a minimum DO level, a holding tank, buoyancy
  - Photographs
- Tagging
  - Internal: radio, sonic
    - Anesthesia (MS-222)
    - Antenna
    - Surgery
  - External: dart, barbel/fin clip, floy, radio, sonic, T-bar, Petersen
  - PIT
  - CART
- Tissue Sampling
  - Blood/venipuncture
  - Genetic
  - Barbel/fin clip/ray
  - Laparoscopy
  - Gastric lavage
  - Gonad biopsy
- Experience (without experience, you may be limited to)
  - Sampling in 9-15°C
  - No surgery
  - Limit net types
  - No lethal take
- Lethal take
- Dam bypass experiments
- Egg transport or removal

Long and Short-Term Holding *(B. Richardson)*

Maryland DNR hold sturgeon for short-term periods in their reward program and wild recaptures for gamete collection. Sturgeon of wild and hatchery origin sturgeon used in captive broodstock, Canadian origin sturgeon used in research, and sturgeon used for outreach and education are held for long periods.

Maryland DNR began experimental stocking of sturgeon in 1996. They received several year classes of Atlantic sturgeon from the USFWS (Lamar, PA). Following this, sturgeon were found in Maryland waters and MD DNR began a reward program for sturgeon (Sturgeon Reward Program began in 1996. This is a cooperative project with USFWS MFRO). In 2000, MD DNR initiated handling trials for wild capture sturgeon. It experienced difficulties in maintaining feeding habits of captured, wild sturgeon. In 2003, the University of Maryland AREL began successful feed training for the wild capture sturgeon. Peak collection of wild sturgeon occurred in 2004 and 2005 and in 2006, MD DNR began size-specific wild collections. MD DNR follows the FWS Culture Manual for the Atlantic Sturgeon.

Maryland DNR obtains their wild sturgeon for broodstock through a reward program that operates between Oct 1 and May 31 (watermen are provided instructions on how to care for fish). When MD DNR obtains a wild-caught sturgeon, they hold the fish in the field for no more than 24 hours. The fish is processed—scanned for a PIT tag or CWT, measured and weighed, implanted with a PIT tag (if none is present), and a sample of DNA and blood is collected. Afterward, the fish is transported to the Univ. of MD for feed training in water with DO greater than 8.0 mg/L and salinity greater than 3.0 ppt. Over the course of the Maryland Sturgeon Reward Program, MD DNR has observed sturgeon mortality of less than 0.5%. (This figure is for the reward program as a whole, not just captive broodstock. No fish brought in for captive broodstock have died as a result of the transfer. Any mortalities have occurred later, mostly due to feeding problems or hatchery system failures.)

Larvae are fed decapsulated *Artemia* and transition to a commercial larval diet. (Force-feeding was investigated as a feed training strategy early on but was found to be ineffective. All captive brood are trained by passive feeding using natural foods and transition to a gel diet before conversion to commercial diets). They progress from a diet of natural food to a gel diet to feed pellets. Large wild fish have gone off the pellets (In our experience, fish that go off feed can frequently be re-trained by initiating the gel diet again). Lamar has observed this behavior seasonally, indicating that for some sturgeon in captivity, feeding may be temperature dependent.

Maryland DNR uses intensive and extensive techniques for grow-out of cultured fish. Sturgeon are held in 20-foot, or greater, diameter tanks that are covered or fenced in. The tanks operate with water flowing through or recirculating. Water supplying these tanks comes from wells or surface water, but MD DNR regulates the salinity.

The sex ratios of hatchery origin captive broodstock in MD DNR's possession are 58% female to 42% male. The sex ratio for wild origin captive broodstock is 25% female and 75% male.

According to Bartron and King (in progress), 45% of the fish in the MD broodstock program are of Chesapeake origin and 86% are from either the Hudson River or Chesapeake Bay (Likely the James River. These two tributaries are the closest tributaries to Maryland's Chesapeake Bay that might contribute to a genetically acceptable broodstock.) This is a preliminary analysis. A final report should be ready by early 2008. This was determined from 166 samples taken from fish captured between 2003 and 2006.

Sex and sexual maturity are determined either through surgical examination or laparoscopy. MD DNR has had good results using laparoscopy methods. For laparoscopy, the fish is sedated prior to making the

incision. The body cavity is insufflated and the swim bladder is deflated, if necessary. A biopsy of the gonads is taken and the incision is sutured. Laparoscopes should be cleaned between fish. There are potential errors or problems when doing this procedure, including physical injury during sedation, the wrong dosage of sedation chemicals, too much insufflation pressure, internal organ damage (can be mitigated by using a screw cannulas), and infection or irritation (uncommon). (All of these potential problems are extremely rare if performed by trained, experienced biologists. As our proficiency has increased, mortality/morbidity is now virtually non-existent.) Vitellogenin can also be used to determine sex with a sample of blood (This is experimental and under investigation)

When spawning the Atlantic sturgeon, milt is extracted from the males. Captive female eggs are monitored for maturity throughout the spawning season to determine when the fish will respond to hormone therapy. Females are injected with hormones and their eggs are biopsied to determine when they are ready. Ripe eggs are removed for fertilization through surgical egg extraction, at which point, the fish is anesthetized with MS-222.

When keeping Atlantic sturgeon for long-term holding, there are health issues that are developmental stage-specific (i.e., affecting larvae v. juveniles v. adults). Atlantic sturgeon are classified as a food animal by FDA, therefore any fish that will potentially be released is subject to aquaculture drug label use regulations. Very few drugs are approved for use in sturgeon species since the process requires a commercial pharmaceutical sponsor and sturgeon are not a commonly cultured species (little profit incentive for the drug sponsor). Long-term holding (decades) of sturgeon at any developmental stage increases the likelihood of fish developing health issues. Common health issues for sturgeon in holding facilities include exposure to pathogens, physical injury, and inadequate nutrition.

Larvae can have low survival at CTS. They may develop health problems if they consume unhatched cysts. Larvae may also develop bacterial and fungal infections. Tank cleanliness is important for mitigating all of these. Prevention is the best course of action. Prophylactic salt treatments can reduce the chance of bacterial or fungal infections—20 ppt bath, 12-15 minutes per week. The larvae can also be fed decapsulated *Artemia* to prevent consumption of unhatched cysts; however, the use of this is limited by availability and cost.

Juvenile and adult pathogens in DNR captive stock include *Argulus sp., Mycobacterium, Aeromonas sp.* (motile aeromonad disease), pasteurellosis (*Photobacterium damselae*), *Nitzchia sturionis* (monogean flatworm), and potentially *Bacteriodes sp*. (which may cause hyperinflation of the swim bladder). *Argulus sp*. can be treated with salt, drugs, and physical removal. Therapeutants for these pathogens (and other conditions) include SLICE®, Chloramine-T®, antibiotics, spawning hormones, Calcien (SE-MARK™), potassium permanganate, and betadine. (There are dozens of other pathogens that could negatively impact captive sturgeon populations. The listed conditions are only those that we have experienced thus far.)

Juveniles and adults are also subject to physical injury, including sunburn; damage to snouts, scutes, and spines; abrasion; physical injury caused by leaping; physical injury incurred while trying to “renovate” their tanks; and other pond issues (e.g., predation, low DO). Sunburn can be prevented with a shade cloth. Other problems can be prevented or mitigated by maintaining the proper salinity (5-15 ppt) and tank cleanliness, using screens and fences, making objects in the tanks flush or using external standpipes and fixed air stones, and regularly monitoring pond conditions.

Sub-adult nutrition is also a concern in long-term holding. Feed training of Atlantic sturgeon is difficult, especially with larger wild fish. MD DNR monitors the weight of their captive wild-caught sturgeon. If their weight drops 25%, they release the fish into the wild. There needs to be a sturgeon-specific feed. MD DNR recommends research to determine appropriate broodstock diets.

There are additional sturgeon-specific considerations for long-term holding. Sturgeon have a large body size, so they require larger tanks and are difficult to move and transport. There are tagging issues (non-lethally detectable larval batch mark). Culture facilities need to be designed with large tanks, nets and cranes for lifting fish, and screens and fences to restrain leaping sturgeon. Another consideration is how to dispose of excess sturgeon. Also, sturgeon require specific equipment: PIT tag detector, laparoscope, anesthesia cart, centrifuge, cryopreservation equipment, hoop restraint, tank crane, and large animal balance.

The bottom line is that if you are holding a fish for over 20 years, you will experience every possible fish culture problem. Culture of Atlantic sturgeon captive broodstock requires continual minimization of catastrophic risk.

Atlantic Sturgeon Handling and Holding Experience *(J. Mohler)*

The Northeast Fishery Center (NEFC) in Lamar, Pennsylvania has held Atlantic sturgeon since 1991. Between 1991 and 1998, the NEFC obtained wild broodstock caught by commercial fishermen on the Hudson River spawning grounds. Juvenile fish were collected from the Delaware River near Artificial Island in 1991 and 1995 by Craig Shirey at DE DFW and given to the NEFC. In 1995, Bill Andrews (NJDFW) and commercial fishermen collected sub-adults for the NEFC out of Belmar off the coast of New Jersey.

The NEFC currently holds 6 Delaware River wild fish (all mature, 1 female), 2 Hudson River wild fish (mature males), 1 New Jersey coast wild fish (mature female), and approximately 60 hatchery fish of five year-classes (no mature females).

There is some risk associated with handling of wild spawning broodstock. Between 1991 and 2007, NEFC staff have handled at least 300 mature broodstock captured on the Hudson River spawning areas. These fish are likely at the highest stress period of their life cycle. Handling fish on the spawning grounds exemplifies the worst-case handling environment: water temperatures can reach 25°C, fish have just recently migrated from salt to fresh water, they have stored huge energy reserves in their gonads (especially for the females), fish are gillnetted from depth of 60-80 feet, fish are confined to a holding tanks, and, in handling and processing, they are probed, gouged, snipped, cut open, etc.

The NEFC uses a holding tank for handling sturgeon on the spawning grounds. The tank is eight feet log, has oxygen injection, and is filled with static water obtained from the river. Fish handled by NEFC in this way have exhibited no known mortality. NEFC protocol for safe handling of sturgeon on the river include using two thick, soft nylon ropes tied in a simple noose and looped around the sturgeon behind the pectoral fins and in front of the dorsal fins to lift the sturgeon on and off the boat. This procedure requires two people.

When transporting wild, gravid broodstock, NEFC staff use a lowboy hauling rig, which can hold eight fish. Some fish have died in transport due to stress and this is more common for females. Transporting fish works best when they are kept in cold water (use freshwater for immediate spawners) and the transport tank is equipped with oxygen injection.

The risk associated with induced spawning of wild-caught fish from the spawning grounds in season differs by sex. Induced spawning of males is low impact; the fish are restrained in a tube net for hormone injection and milt extraction. Females experience high stress and it is a huge physical insult to obtain large numbers of eggs (requires six hours of transport and a 3-4 inch incision), thus induced spawning of females has a higher risk than induced spawning of males. Only one female of 13 spawned has survived.

The best subset of wild broodstock data comes from work done by NYSDEC and the Pew Institute for Ocean Science on the Hudson River in 2006 and 2007. Eighty-two mature fish were gillnetted and handled. Sonic tags were implanted internally and anesthesia was used (medium risk category). Workshop participants suggested that anesthesia is not necessarily required and suggested that local anesthesia should be experimented with. Chris Hager (VIMS) restrains sturgeon on a stretcher and works on the fish in the water. South Carolina DNR does not use anesthesia because they think it causes more stress for the fish. Some behavioral change was observed in the Hudson River fish; fish exhibited downstream movement after handling, indicating that this activity causes medium risk. External PAT tags, sonic tags, and Carlin tags were also attached to these fish with minimal risk. Based on daily sonic tracking, no known mortalities occurred.

According to the NEFC's experience, capture and handling of wild juveniles is a low risk activity. This is based on handling of 562 fish in the Hudson River between October 2003 and November 2005. Captures occurred in the months of March, April, October and November when water temperatures ranged between <4 and >20°C. Nets set to catch juvenile sturgeon were tended every two hours, but this experimental design may be of high risk to sturgeon caught in the net. Juvenile sturgeon are held onboard the boat in the same tanks as the adults. All juveniles underwent a pelvic fin spine clip for ageing and were tagged with PIT and Carlin tags. Recaptures of these fish showed that they demonstrated good healing at the sites of the fin spine clip. NEFC staff did not observe any mortality of fish captured, handled, and tagged, although one sturgeon from the NYSDEC dataset died.

Sturgeon held at the NEFC are kept in 12' x 100' raceways or 20-foot diameter above ground tanks. The culture manual for Atlantic sturgeon published by the U.S. FWS has recommendations for the density of holding tanks for juveniles.

In its experience with holding sturgeon, the NEFC has learned things that will increase the risk of mortality for captive sturgeon including:

1. Loss of water supply through clogged inlet nozzle (can result in low DO)
2. Forgetting to turn on the water supply after working in a tank
3. Decimal error in therapeutant calculation
4. Winter kill in extensive pond culture
5. Sunburn
6. Failure of restraint fencing and netting around tanks
7. Development of anaerobic conditions in recirculation systems
8. Contraction of parasites in fish due to accumulated waste (most common for larvae)
9. *Aeromonas salmonicida* infection (systemic)
10. Hyper-inflated swim bladder (HISB) affliction that can cause starvation (potentially caused by a bacterial infection where the pneumatic duct gets blocked by an inflamed sphincter, may be able to reverse condition by inserting a cannula into the swim bladder)
11. Mortality caused by surgery, including laparoscopy (reason to use two helpers when performing this procedure)
12. Fish gorging on tad poles

In order to reduce some of the risks mentioned above, a workshop participant noted that it is possible to determine sex differentiation in fish over 9 kg without histology. Jerre was asked if he observed any negative affects when puncturing the swim bladder of fish suffering from HISB; he had not observed any, however, this treatment was not always successful in treating the condition.

Sturgeon Identification *(T. Squiers)*

According to Dadswell (**YEAR**), the following key can be used to determine if a fish is an Atlantic sturgeon:

> Mouth width inside lips usually < 55% (range 43-66%) of inter-orbital width; inter-orbital width < 29% (range 22-36%) of head length…; average TL:FL = 1.14; gill rakers 17-27 (X = 21.6); post-dorsal and pre-anal shields usually in pairs, usually 2-6 plates between anal base and lateral row of scutes…; dorsal plates generally touch or overlap; viscera pale; has fontanelle

All measurements should be made with calipers. Mouth width should be measured as the greatest distance across the transverse mouth slit with the lips excluded and mouth closed. Inter-orbital should be measured as the maximum distance across the top of the head between the bony edges of the orbit. Workshop participants advised that this information should be used in the protocol document for identification of Atlantic sturgeon as there are mistakes in the literature about using snout length to determine species because snouts exhibit allometric growth.

Sturgeon Tagging Methods and Results: Insights from a Sturgeon Study in the Penobscot River, ME *(S. Fernandes)*

PIT tags have an indefinite life and are used for individual identification (mark-recapture) experiments. They are implanted internally and have a high retention rate. In the Penobscot River, researchers used a 134.2 kHz, 12 mm, full-duplex PIT tag for sturgeon. Researchers used a hypodermic needle to implant the tag into the musculature. They closed the incision with a single non-dissolvable suture. Researchers placed the tags on the left lateral side of the fish above the row of lateral scutes and below the dorsal fin. Thirteen of fourteen recaptured fish retained their tags (i.e., tag was present and functional), a 93% retention rate. The time elapsed between when the fish were tag and when they were recaptured ranged from 23 hours to 8.8 years. For the fish that had lost the tag, the PIT tag was either non-functioning or expelled after 39 days. It was apparent from the recaptured fish that sturgeon heal well after the insertion of the PIT tag.

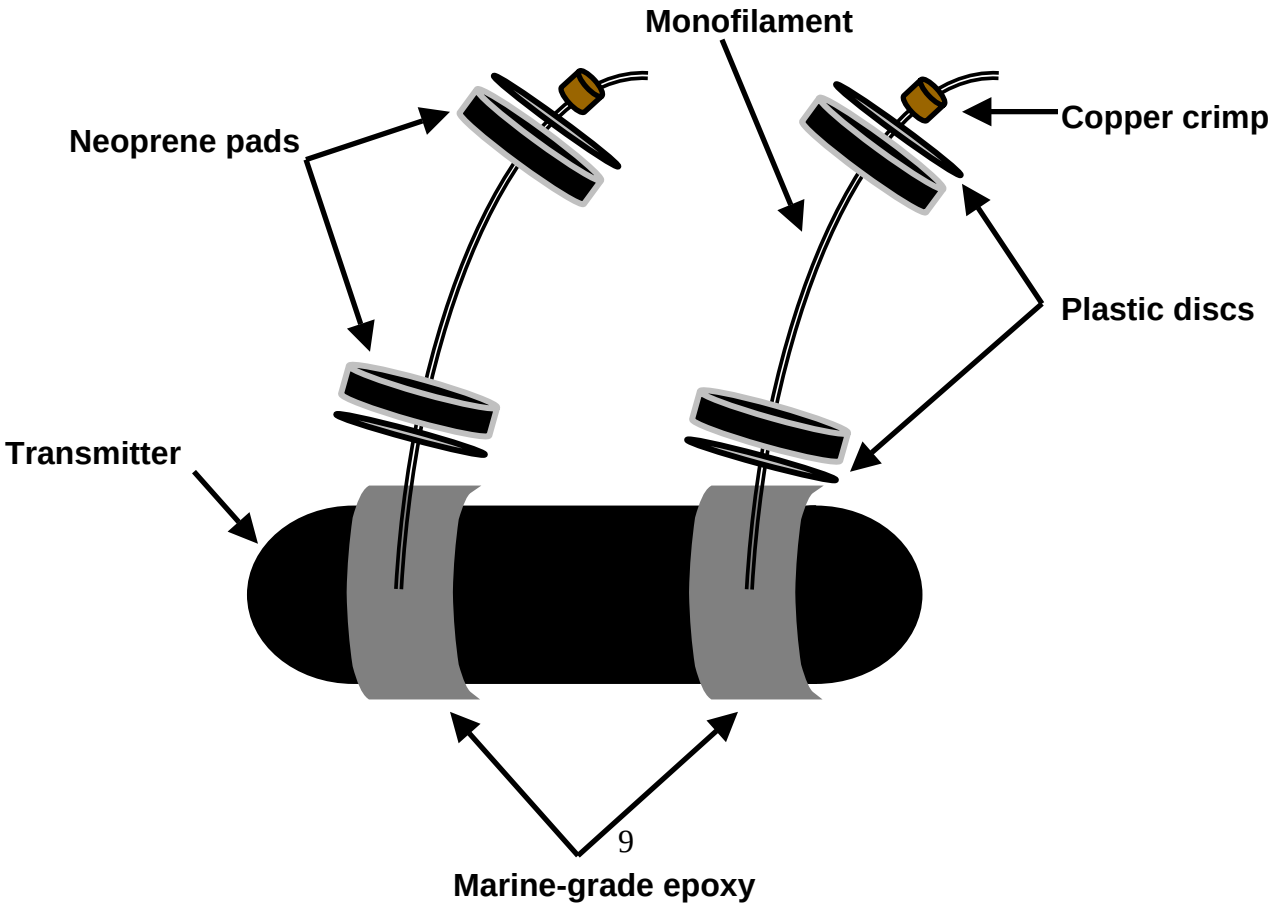
Carlin tags are external tags that are highly visible to all observers and can be used for individual identification (mark-recapture model). Researchers on the Penobscot used Carlin tags made of a plastic dangler attached to a stainless steel wire bridle. On one side of the dangler was the fish identification number and the reverse side has the toll-free phone number and notice of a reward for the recaptured fish. Initially, they attached the tags by passing one wire through the skin using a curved cutting needle and then twisted the two wires together and trimmed the excess wire. The method was modified after researchers observed that the outside connecting wire had irritated the skin of a recaptured shortnose sturgeon. The method was modified so that both wires were passed through the skin. The location of the placement of the Carlin tags was also modified—initially they were placed on the dorsal surface posterior to the dorsal fin, now they are placed on the lateral surface just below or anterior to the dorsal fin. The modification was made because some sturgeon have dense dorsal scutes posterior to the dorsal fin, which makes it difficult to attach a tag at that location. Carlin tags have had a 62% retention rate in tagged sturgeon in the Penobscot River (8 of 13 recaptured fish). The time elapsed between tagging and recapture ranges from 23 hours to 8.8 years. One fish lost the plastic disk within 23 hours, possibly when the fish was caught in the net. One fish was observed to have a Carlin tag, but the tag came loose when the fish was being untangled from the net during recapture. All fish that were recaptured more than a year after tagging had lost their Carlin tags (n=3) and the wounds where the tag had been placed were completely healed. Researchers observed that sturgeon tagged with Carlin tags had some irritation on their skin, possibly caused by the tag. Generally, the fish heal; however, placement of the tag and water temperature may affect the healing rate.

Acoustic tags track individual movements within and among systems and can last over several years. They may also transmit depth and temperature data. Researchers on the Penobscot River implanted sturgeon with two different Vemco acoustic transmitters. The V13 coded T/P tag is 13 x 45 mm and 6 grams and operates at 69.0 kHz and has a ping frequency range of 40-120 seconds. The V16 continuous

tag is 16 x 68 m and 10 grams and operates at 51, 54, 57, 60, 75, 78, and 81 kHz with a ping frequency ranging between 1000-2000 ms. The tags were implanted with a 3-4 cm surgical incision (all tags were sterilized prior to implantation). The incision was stitched using a size 2-0 reverse cutting needle. Four to five sutures were made through the skin and peritoneum using dissolvable (chromic gut) suture material and then an additional 4-5 sutures were made through the skin with non-dissolvable silk or nylon suture material. Acoustic tags were placed in the middle of the ventral body surface near the center of the abdomen. Researchers observed that after 18 days the incision had closed and that half of the dissolvable sutures were gone and all of the non-dissolvable sutures were still present; after 71 days, the incision appeared to be completely healed and all sutures were expelled. Retention rates for acoustic tags placed in Atlantic sturgeon are unknown as none were recaptured. The two recaptured shortnose sturgeon that had been implanted with acoustic tags had retained the tags. Some assumptions of acoustic tag can be made based upon tracking of the movements of the fish. Using this proxy, eight of nine Atlantic sturgeon retained their tags (90% retention). It is not known if the ninth fish expelled its tag or died. For shortnose sturgeon, 28 of 37 fish retained their tags (76% retention); one of the tagged fish was found dead.

The V13 tag was also attached to sturgeon externally (see Figure 1 for how the tag was attached). The external acoustic tags are used on pre-spawning fish in the spring or on fish captured on the spawning grounds. To attach the tag, researchers drilled two small holes through a dorsal scute and passed an 80-pound test monofilament through the holes. Neoprene pads rest against the fish's body on each side and a plastic disk provides rigidity. Small pieces of copper tubing are crimped onto the line to hold the transmitter in place. One Atlantic sturgeon was tagged using this method. The tagged fish moved 6 km upstream and into a known Atlantic sturgeon congregation area immediately after tagging. The fish then left the river two days after tagging and was detected in the Kennebec River (230 km away) 10 days later.

Figure 1. Attachment of an external acoustic tag.



Steve recommends the use and distribution of the newest PIT tags (SST ISO tags that have advanced longevity and read range). He also recommends changing to a different external tag type or implantation technique for Carlin tags because he would like to see increased retention and healing. He also sees a need for more communication between tagging studies and an organized way to share data in order to track movement between systems and potentially have an online ID code database.

In discussions following Steve's presentation, it was brought up that a sleeve around the wires of the Carlin tags might reduce the abrasions on the skin. It was noted that floy and T-bar tags have a lower retention rate in sturgeon than Carlin tags. To promote tag retention for external tags, it was suggested that the tag be attached to a scute, however, this would require that the fish be handled for a longer period of time.

On the Hudson River, they have had success with retention of Carlin tags that are attached through the cartilage at the base of the dorsal fin. In a South Carolina shortnose sturgeon tank study, the fish rubbed their bodies along rocks and detached external sonic tags within a day. The Conte Lab recommends using a suture to close the insertion site for PIT tags; not all researchers use sutures (e.g., Lamar), yet they have had high retention, instead they recommend massage the skin at the insertion site. It appears that smaller fish have greater retention problems.

USFWS Sturgeon Coastal Tagging Program & Standardization *(M. Mangold)*

The current coastal tagging database for sturgeon contains information on four types of tags (T-bar, double barb, PIT, and Carlin) used by state and federal agencies from Maine to North Carolina. The program has been in existence since 1992. There is a reward offered for captured, tagged sturgeon and captures of tagged sturgeon are reported by fishery-dependent and independent sources. The program is modeled after the striped bass reward program.

There are 18 cooperators in the tagging program that tag fish, all are fishery-independent researchers. To date, 7,381 Atlantic sturgeon have been tagged with 1,080 recaptures, and 7,027 shortnose sturgeon have been tagged with 387 recaptures.

According to the database, tags used in this program have had the following retention rates:

- Carlin-dangler tag
    - 100% retention for the first 3 years
    - No fish have been recaptured after more than 3 years from the tagging date
- T-Bar dorsal
    - 85% retention for the first year
    - 60% retention for the first 2 years
    - 53% retention for over 2 years
    - Maximum retention is 7 years
- T-Bar pectoral
    - 78% retention for the first year
    - 70% retention for the first 2 years
    - 57% retention for over 2 years
    - Maximum retention is 9 years
- Double barb
    - 84% retention for the first year
    - 29% retention for the first 2 years
    - Maximum retention is 6 years

- PIT dorsal
    - 98% retention for over 9 years

A paper by Fuller *et al*. on the performance of commercially available PIT tag systems used for fish identification and interjurisdictional fisheries management is currently in review with the North American Journal of Fisheries Management. This paper was used to determine which tags should be distributed. Tags that operate at 134.2 kHz will become the industry standard.

Standardizing tagging methods for Atlantic sturgeon on the eastern seaboard is needed. The ASMFC recommended that FWS distribute external and PIT tags and readers to cooperating organizations; FWS distributes Carlin and T-bar tags and will be distributing tag readers. FWS recommends the Destron TX1400 SST 134.2 kHz PIT tag and the AVID PT VIII, Destron FS 2001 and Destron PR EX tag readers (these readers can read multiple tags but software must be used to convert the tag ID number read by the Destron PR EX). FWS recommends researching additional external tagging techniques. The FWS/MFRO will collect data in the coastal tagging database.

When tagging a sturgeon, FWS recommends:

- Taking measurements FL, TL, and weight, if possible
- Scanning for PIT and CWT tags
- Applying a T-bar or Carlin tag to the base of the dorsal fin
- Applying PIT tags 5 cm of anterior insertion at the base of the dorsal fin, within 4 cm of the midline on the left side
- Taking a tissue sample from the caudal fin and placing it in a vial containing 95% non-denatured ethyl alcohol

The following came up in discussion following Mike's presentation:

- If you find a dead sturgeon, take a tissue sample from deep white muscle. If the fish is recently dead, you can remove a fin clip from the caudal fin and barbel.
- Sturgeon length should be measured as FL not TL
    - Other standard length measurements for sturgeon is from the tip of the snout to the last keeled scute.
    - Fishermen will often measure TL but they should be instructed to measure FL.
    - A measuring board can easily be made on the boat deck.
- Tissue samples are often taken from the caudal fin, pelvic fin or barbel. For a live sturgeon, it should not matter where the tissue sample is taken from.
- Tom Savoy will no longer collect data on tags due to a lock of cooperation among researchers.
- Workshop participants recommended collecting information on any apparent diseases the sturgeon might have when they are collecting other biological information.
- Tissues samples (1 cm^2 fin clip), they should be sent to NOS in Charleston, where they will be stored and available to other researchers. (Note: the existing protocols call for a pelvic fin clip).

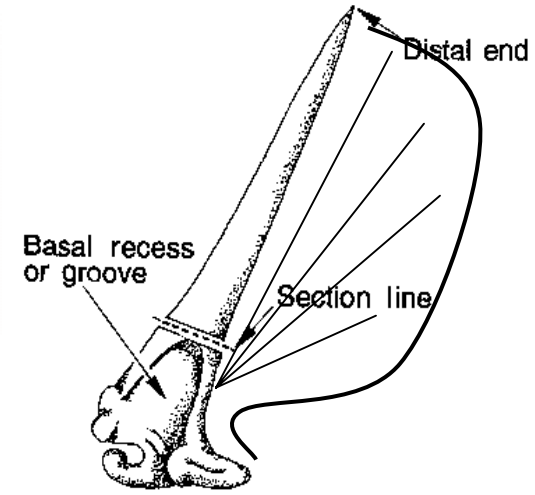
Tissue Sampling and Age Estimation of Sturgeons *(R. Woodland)*

The pectoral fin spine is the preferred ageing structure for sturgeons. Removal of a pectoral fin spine is non-deleterious, allows for mark-recapture (e.g., OTC), and there is a precedence for this technique in the literature and previous care/handling documents. There are some disadvantages to using this structure for ageing. The fin spine is composed of metabolically active material and calcium in the spine can be reabsorbed. This is also an exposed structure on the animal and is susceptible to damage. Additionally, this is method is only partially validated.

When collecting samples for ageing, researchers should minimize the amount of time that the sturgeon is out of the water. If the fish is to be held between the time of capture and tissue collection, minimize stress by using a holding pen or tank. Researchers may choose to use restraint or anesthesia (e.g., slings, stretcher, MS-222) when collecting samples, although anesthesia may cause additional stress (perhaps seasonal sampling is a good alternative).

To remove a sample, a section should be taken from the primary spine of the pectoral fin. A 1-cm sample provides sufficient material. When cutting the sample, one should minimize the distance from articulation without compromising the joint function (Figure 2). After removing the sample, disinfect the area, allow the fish to recover, and then release the fish. A variety of tools can be used to cut and remove the section, including a wire cutter, bolt cutter, hacksaw, coping saw or knife.

Figure 2. Guide to collecting a pectoral fin spine sample.



Laboratory preparation of the sample includes letting the sample dry for at least a week, mounting the sample on a cutting block (e.g., wood, spare slide), and cutting a transverse section using a low-speed diamond-blade saw. A single or double blade method can be used to cut a 0.5-1 mm section and multiple sections can be made from a single sample. The section is mounted to a microscope slide with epoxy (thermo-plastic, fingernail polish, etc.). Before viewing the slide, the sample is polished with fine grit carborundum.

The section can be viewed with reflected or transmitted light and selection of how the spine should be viewed is dependent upon the section and the reader (note: mineral oil may help provide additional contrast).

Annuli are defined as bipartite structures consisting of alternating bands of translucent and opaque concentric bands. In sturgeon fin spines, annuli often have inconsistent spacing, crowding near the edge, inclusions, banding patters, canalization and reabsorption, irregular annuli formation, opaque and indeterminate increments, multiple false annuli, and deterioration or malformation of spines, which make ageing of the section difficult.

Validation and verification exercises are a necessary component of ageing studies (Beamish and McFarlane 1983; Campana *et al.* 1995). Validation of the accuracy of age determinations can be completed through marginal increment analysis (MIA) for periodicity and timing as well as mark-recapture for known-age or time interval studies. Verification of precision of age estimation is needed to

evaluate temporal drift, inter-reader comparability, and age bias. The methodology presented above has been shown to have high precision and low bias. Periodicity and timing of annulus formation has been partially validated but known-age/interval studies are sorely needed.

Marginal incremental analysis can validate timing and frequency of increment formation. The formula for this is

$$MIR = MI/A,$$

Where *MI* is the width of the outermost opaque zone (marginal increment) and *A* is the mean width of the three opaque zones previous to the marginal increment. Increasing MIR indicates progressive increment completion. Stevenson and Secor (1999) validated annual periodicity for a mixed-age/sex sample from the Hudson River, MAB, and Delaware and Chesapeake Bays (assumed predominantly Hudson River stock, low n for older fish). It is necessary to include multiple age-classes and to avoid extrapolating results to unincluded age-classes (Campana 2001).

Accuracy of age estimates from field captures is unknown without direct validation studies, which are critical to fully validate age estimations. Hatchery-raised Atlantic sturgeon can be used to validate accuracy. Annulus formation has been verified under hatchery conditions with OTC marked fish (Secor *et al*. 1997; Stevenson and Secor 1999). Using hatchery-raised fish is a means of testing accuracy of age estimates under field conditions. Mark-recapture of wild sturgeon can also be used to validate accuracy. This is a comparative approach that uses reciprocal fin spine collection and OTC marking. Radiometric age determinations are not feasible as are elemental or isotopic cycles because they rely on metabolically available material.

To verify precision of readers, you can complete a between or among reader plot. This can assess temporal drift in interpretation of annuli, identify presence of bias between readers, and age bias. There appears to be increased variability for determining ages of older fish. Statistical tests of reproducibility can be used to calculate and compare some index of precision, such as the coefficient of variation (preferable method but is sensitive to sample size).

The following comments were generated from this presentation:

- To OTC mark a fish use 20 mg/kg of OTC.
- Ageing of sturgeon may be more difficult in the south because temperatures there may allow or promote continuous growth.
- Most pectoral fins are expected to heal if the physical structure of the fin is not damaged.

Age Estimation for Atlantic Sturgeon *(A. Rourk)*

South Carolina DNR has been researching sturgeon since the early 1980s, collecting information on population size, habitat use, fish health, diet, genetics, and age and growth. South Carolina DNR has an archive collection of 3,845 spine samples collected since 1994, primarily from the Edisto River. Sturgeon are caught in gillnets and trammel nets, tagged with external and internal tags, a pectoral fin spine sample is collected (SC DNR removes the entire spine and uses it as a ageing sample and genetic sample), and stomach contents are collected with a gastric lavage procedure.

South Carolina DNR researchers have noticed that large adults do not appear to recover well after their leading fin ray is removed (there is no grow back). South Carolina DNR will no longer be collecting samples from larger fish.

In the lab, the samples are prepped and 0.5-0.6 mm sections are cut. Analysis of age and growth has produced a smooth curve of length-at-age (figure gets bumpy for older-age fish because of small sample size). Recaptures have shown 97% agreement with the ageing (58% have agreement with exact age, 39% have agreement within one year of age).

There are issues to note with ageing of fin spines and mark-recapture:

- Left and right spines are not mirror images
- Potential difficulties in reading the spines:
    - Split annuli, false annuli
    - Secondary rays
    - Crowding of annuli
    - Defining the margin

- Adults
    - Small sample size (n=90)
    - Slow healing rate

South Carolina DNR is planning to: (1) determine annulus formation with marginal incremental analysis and month-to-month data analysis; (2) validate previously aged samples with a second reader to determine precision; and (3) finish cutting and reading the remaining spines.

South Carolina DNR researchers have not noticed a change in age structure over time. Workshop participants recommended that SC DNR look at differences in growth rates for males and females. They also recommended looking into potential errors in ageing the fish due to the month of recapture. South Carolina DNR noted that they have had the most difficulties with ageing age-0 and age-1 fish.

Gastric Lavage *(K. Damon-Randall)*

Various techniques for gastric lavage of sturgeon have been described by Haley (1998), Savoy and Benway (2004), SC DNR, Brosse *et al*. (2000), Wanner (2006), and Shuman and Peters (2007).

Haley's methodology uses a flexible, small diameter tubing (intramedic polyethylene, 1.57 mm inner diameter and 2.08 mm outer diameter). The sturgeon is lightly anesthetized using MS-222. The tube is directed past the pneumatic duct into the alimentary canal until it is felt on the ventral surface. Water is slowly injected to flush the stomach. This methodology is not recommended when water temperatures exceed 27°C. Extreme caution needs to be taken to avoid damaging the swim bladder.

The gastric lavage technique as described in the shortnose sturgeon protocol is used in one scientific research permit for a shortnose sturgeon and is modified in two other shortnose sturgeon scientific research permits. Modifications include:

- Savoy and Benway (2004): use of low-pressure hand pump to supply continuous water flow (in lieu of individual syringe). This process has been used on 246 fish with no observed ill effect.
- SCDNR: varying tube diameters based on fish size.
    - 250-350 mm FL – 1.90 mm diameter
    - 350-1250 mm FL – 4.06 mm diameter
    - >1250 mm FL – 10.15 mm diameter

The total number of shortnose sturgeon that have been permitted to be lavaged, by permit are:

- Permit 1505 – 20 (of 50) fish annually
- Permit 1447 – 100 (of 100) fish annually
- Permit 1516 – 100 (of 450) fish annually

Brosse *et al*. (2000) use a method modified from Nilo (1996) on European sturgeon. A garden sprayer equipped with a soft, 6-mm tube is used for water injection and inserted into a 12-mm outflow tube for stomach content collection. Fish are not anesthetized because of potentially harmful side effects from clove oil anesthetic in brackish water. Brosse *et al*. have not observed any mortality.

Wanner (2006) modified methods from Foster (1977) to perform gastric lavage on pallid sturgeon. A pressurized air tank is used to provide a continuous water supply. A 5.5-L, hand-pumped, pressurized garden sprayer tank is fitted with a 3.18-mm (outside diameter) polyethylene tube. Water is pulsed through the tube and the ventral surface of fish is massaged to facilitate regurgitation. No anesthesia is used. Wanner has not observed any mortalities or digestive tract damage; however, four of five fish had inflated swim bladders. By using a pressurized tank and no anesthetic, handling time was reduced and the

time required for the lavage was 2-3 minutes per fish, opposed to 20 minutes per fish in the Haley (1998) study.

Shuman and Peters (2007) used a different method for shovelnose sturgeon. A 7-L tank was fitted with aquarium tubing (6 mm outside diameter, 4 mm inside diameter) that had the end tapered and smoothed. The fish was held ventral side up at a 45° angle. Water was pulsed at and into the cardiac valve to stimulate relaxation and then the tubing was inserted until it stopped at the spiral valve. Water is then pulsed while slowly retracting the tube. When the stomach is firm, the ventral surface of the fish is gently massaged to help release water and stomach contents. Water is flushed over the gills throughout the procedure. The procedure was performed three times on each fish and not anesthetics were used. Fish in this study had 44% and 83% mortality in 2 trials and 0% mortality in 7 trials. Significant differences in temperature, DO concentration, conductivity, ammonia levels, and nitrite levels were observed among trials. Necropsy results suggested that mortalities may have been due primarily to nitrite poisoning, not the gastric lavage. Shuman and Peters recommend using a syringe for smaller fish and the Brosse technique for larger fish in order to reduce handling time. They felt that this was a good, safe technique.

South Carolina's work should be considered a study in progress, yet preliminary results suggest that in estuarine areas, Atlantic sturgeon show a polychaete specialization and shortnose sturgeon are specializing in amphipods. They have used a garden sprayer multiple times. The time required for the procedure is variable, but averages about 10 minutes per fish. South Carolina DNR does not perform any gastric lavages when water temperatures reach 28°C.

Contaminant Sampling *(K. Damon-Randall)*

Tissues from dead sturgeon (e.g., beached carcasses, ship strikes) – Whether the carcass is suitable for tissue residue analysis is a judgment call. If the carcass is not too decomposed, muscle, liver, and gonad tissues can be collected for tissue residue analyses (e.g., dioxins, furans, PCBs, organochlorine pesticides, trace elements). Depending on the analyses, tissue samples can be placed in chemical-clean jars (trace elements and organics), or wrapped in aluminum foil and placed in a zip-loc bag (organics), or wrapped in plastic wrap and placed in a zip-loc bag (trace elements). If the sample will be homogenized and split into aliquots for separate trace element and organics labs, chemical clean jars are easiest.

Tissues from fresh-killed sturgeon (e.g., scientific collection mortalities) – EROD/CYP1A can be determined from liver and gill tissue. Tissues must be stored immediately on liquid nitrogen or dry ice. Samples must be kept at -80ºC prior to analysis. Bile can be used for PAH analyses. Whole blood can also be used for contaminant analyses.

Tissues from live sturgeon – Blood plasma can be used for sex steroids (estradiol, testosterone) and vitellogenin. Similar to CYP1A samples, plasma must be stored immediately on liquid nitrogen or dry ice. Samples must be kept at minus -80ºC. Non-lethal sampling for CYP1A can be accomplished with gill tissue. Muscle plugs from live fish (not sturgeon) have been used for trace metals analyses.

The Wellfleet Atlantic sturgeon had a full screen for contaminant analysis. Samples were taken from the muscle, liver and gonad. The samples were analyzed for organochlorine pesticides (n=23 compounds), dioxins (n=7 congeners), furans (n=10 congeners), PCB congeners (n=95), polybrominated diphenyl ethers (PDBEs, n=40 congeners), and metals (n=19 elements). The cost was approximately $2,100 per sample (3 samples for one fish; total cost approximately $6,300). The time required to complete the analysis ranges between 6 months to a year (metals may take only 3 months).

A basic screen consists of a scan for organochlorines (PCB and pesticides) and trace elements (mercury and 18 others). The cost is approximately $600 per sample.

Two shortnose sturgeon collected in the Delaware River were analyzed (ERC 2002). Muscle, liver, and gonad tissue were analyzed for U.S. EPA Target Analyte List (TAL) metals, U.S. EPA TAL semi-volatile organic compounds, organochlorine pesticides and PCBs, polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and substituted isomers. Results found 16 of 23 TAL metals, 2 semi-volatile compounds, 3 organochlorine pesticides, 1 Aroclor, PCDDs, and PCFDs in one or more tissue samples. The cost for this test was approximately $1535 per sample ($4605 per fish).

The U.S. EPA list of Target Analyte Metals and Cyanide include:

- Aluminum
- Antimony
- Arsenic
- Barium
- Beryllium
- Cadmium
- Calcium
- Chromium (Total)
- Chromium (Hexavalent)
- Cobalt
- Copper
- Iron
- Lead
- Manganese
- Magnesium
- Mercury
- Nickel
- Potassium
- Selenium
- Silver
- Sodium
- Thallium
- Vanadium
- Zinc
- Cyanide (Total)
- Cyanide (Amenable

The U.S. EPA TAL Semi-volatile organic compounds include:

- Butylbenzylphthalate
- 3,3'-dicholorobenzidine
- Benzo(a)anthracene
- Chrysene
- Bis(2-ethylhexyl) phthalate
- Di-n-octylphthalate
- Benzo(b) fluoranthene
- Benzo(k) fluoranthene
- Benzo(a) pyrene
- Indeno(1,2,3,-cd) pyrene
- Dibenzo(a,h) anthracene
- Benzo(g,h,i) perylene
- 2,3,4,6-Tetrachlorophenol
- N-Nitrosodiphenylamine
- 1,2,4,5-Tetrachlorobenzene
- 4-Bromophenyl-phenylether
- Hexachlorobenzene
- Atrazine
- Pentachlorophenol
- Phenanthrene
- Anthracene
- Carbazole
- Di-n-butylphthalate
- Fluoranthene
- Pyrene

Samples from shortnose sturgeon muscle, liver, and ovary tissue were collected from fish in the Kennebec River and analyzed for inductively coupled plasma (ICP) metals, total mercury, TCL semi-volatile organic compounds, organochlorine pesticides, PCBs, PCDD/PCDF, and percent lipids. Analysis showed 14 metals, 1 semi-volatile compound, 1 Arochlor, PCDDs, and PCDFs in one or more tissue samples.

ICP metals include:

- Aluminum
- Antimony
- Arsenic
- Barium
- Beryllium
- Boron
- Cadmium
- Calcium
- Chromium
- Cobalt
- Copper
- Iron
- Lead
- Magnesium
- Manganese
- Molybdenum
- Nickel
- Potassium
- Selenium
- Silica
- Silver
- Sodium
- Thallium
- Vanadium
- Zinc

Gulf sturgeon muscle, liver, adipose, and reproductive tissues were sampled and analyzed for 9 common metals, including As, Cd, Cu, Hg, Se, Fe, Pb, Ni, and Zn. Some samples were also analyzed for Al, B, Ba, Be, Cr, Mg, Mn, Mo, Ag, Sr, Tl, and V. Other analyses were conducted to detect organochlorine pesticides, PCBs, PAHs, and aliphatic hydrocarbons. Samples were analyzed in different years and by different labs. Results indicate that concentrations of undesirable contaminants are being accumulated by Gulf sturgeon and some concentrations were at levels that have been reported to cause reproductive impairment in other fish.

Is consistency of the sampling protocol in the analytical labs used needed to ensure that the same suite of analytes is run and lab detection limits are the same? Should the research protocol be consistent in the types of analysis recommended? Who should fund this work?

Sampling for contaminant analysis may be sent to Steve Mierzykowski.

Laparoscopic Determination of Reproductive Status in Sturgeon *(B. Hickson)*

Laparoscopy was first used at Warm Springs RFC when they began research to develop a reproductively sterile shortnose sturgeon to use in life history studies. The first attempts to develop a sterile shortnose sturgeon included surgical sterilization and evaluation of triploid sturgeon. Early attempts at surgical sterilization were invasive and had mixed results. Laparoscopy proved effective at achieving program goals.

Traditionally, surgical sterilization involved a full body incision that provided full exposure and lots of room; however, this had the disadvantage of being a very large incision that had difficulties healing. Warm Springs moved on to a modified open procedure that had better healing and took less time to complete. Yet the modified open procedure was still invasive and had additional disadvantages including limited visualization, limited room to work, and required special equipment.

In contrast, the laparoscopic technique is minimally invasive and less traumatic, and because of this, the procedure minimizes public scrutiny of cruelty to animals. Because of the small incisions required for the procedure, there are no healing problems. The procedure allows for excellent visualization and magnification. While performing laparoscopy, the heart rate can be monitored with the camera and the visceral health and mullerian duct can be examined and evaluated. Tissues can be precisely selected for biopsy without injuring internal organs. There are disadvantages to laparoscopy, including the cost of equipment, the skill required, the extra time required (when compared to a biopsy without anesthesia), and the limitations of the equipment in certain temperatures.

In laparoscopy, additional equipment is required as well, including an anesthesia machine that costs $396. Anesthesia protocol begins with an initial dose (bath) of 250 mg/L MS-222 and 500 mg/L sodium bicarbonate. The fish is then given a recirculating bath of 87.5 mg/L MS-222 and 175 mg/L sodium bicarbonate. For a Gulf sturgeon weighing between 13 and 17 kg, the time to initial anesthesia is 7-10 minutes. This can be determined with a tail pinch and respiration. The surgery for gonad removal takes 45-90 minutes. Recovery after anesthesia takes 3-13 minutes.

When performing a laparoscopy, be careful to avoid the spleen and liver. A 5 mm incision is made with a scalpel just off the midline for the camera. The camera is used to guide the trocar or cannula through the fascia in order to avoid nicking the intestine. The ceolomic cavity is insufflated by applying 1-2 L/mm of ambient air to a sustained pressure of 4-8 mm Hg. Additional cannulas are used to insufflate the peritoneum and deflate the swim bladder to minimize injuries. To aspirate the swim bladder, use the camera to guide the veress pneumoperitonium through the body wall and guide the needle into the swim bladder. Suction is applied to the needle with a vacuum pump and the swim bladder is deflated. During this procedure, you can also perform an overall health exam. After the procedure is completed, each

incision is closed with a single cruciate stitch. The suture material is absorbable monofilament polydioxanone (PDS II, 3 metric, Ethicon, Inc.). Betadine ointment is spread over the stitches as a topical anti-microbial. The equipment for a laparoscopy costs approximately $7,400 and additional costs are approximately $700.

An immature sturgeon will have no gonad differentiation and histology is required to identify the sex of the fish. An immature gonad consists primarily of adipose tissue that will float when placed in freshwater. A developing immature female gonad will have deep folds in the ovary and white ova will start differentiating from germinal tissue. The developing immature female gonad is often pigmented yellow to deep orange, depending on the diet. Mature female ovaries have enlarged eggs and are white in the pre-vittelegenic phase. Eggs turn black and grow as yolk reserves are deposited. Mature testes have a smooth surface and adipose tissue on the margins that is a color ranging between cream and yellow.

An anesthesia machine can be built for field use. It requires a 12 V-120 V inverter or 12 V water pump. The boat must have a large enough deck area to move around. An additional anesthesia tank can be used in a catcher boat (100 horse trough) and the fish can recover in the catcher boat before release. An overhead cover on the boat is recommended to protect the fish.

Juvenile Atlantic Sturgeon Abundance Sampling *(M. DuFour)*

The ASMFC FMP for Atlantic sturgeon required a reliable monitoring program for juvenile abundance so NYSDEC Hudson River Fisheries Unit (HRFU) engaged in a cooperative study with the USFWS NEFC to develop a sampling program for the Hudson River juvenile population. The objectives of the study were to determine the location, season, and sampling methods, as well as the minimum amount of effort needed for effective abundance monitoring for juvenile Atlantic sturgeon in the Hudson River. The study took place between Fall 2003 and Fall 2005.

Sampling occurred in the spring (March-April) and fall (October-December) in Newburgh Bay and Haverstraw bays. Anchored gillnets were set perpendicular to the current on four different habitat types—soft/deep, hard/deep, soft/shallow, and hard/shallow.

From the study, researchers concluded the juvenile Atlantic sturgeon preferred the soft/deep bottom habitat in Haverstraw Bay. Sampling was most effective in the spring when water temperatures were above 4°C. The best gear to use were anchored gillnets with 3, 4, and 5-inch mesh (200 x 8 ft in area) set perpendicular to the current. The minimum sampling required was 100 sets per season. NYSDEC continues to employ this protocol for their sampling of juvenile abundance.

When sampling in the spring (March-April), water temperatures are well-below 27°C (high temp. = 11.2°C; average temp. = 6.5°C). During this time, DO is well-above 3 ppm (average DO = 11 ppm). DO levels below 6 ppm in Haverstraw Bay are rare. Increased stress to the fish when handling them at this time of year in Haverstraw Bay should not be a problem because water temperature and DO concentrations are of optimal levels. In the Hudson River, the salt front location is unpredictable; however, researchers neutralize handling effects by keeping the fish in an electrolyte bath (6 ppt) inside a live well and the water is changed after each net set. Handling time is also kept to a minimum; fish are processed immediately.

Anchored gillnets of 3, 4, and 5-inch mesh, 200 feet in length, and 8 feet deep are set for two-hour periods. Nets are set in cold water temperatures and when DO concentration is high. Fish may suffer abrasions from the net or their gills may be damaged and their movement may be obstructed.

When the fish are brought onboard the research vessel, physical measurements are taken—length and weight are measured with sling scales and measuring boards, and eye and mouth width are measured with

calipers. Fish are tagged prior to release. NYSDEC uses PIT tags (inserted into the musculature below the dorsal fin) and Carlin dangler tags (inserted below the dorsal fin). All tools used in tagging are sterilized with isopropyl alcohol and incisions are treated with betadine. Genetic samples are taken from the dorsal fin (tools are sterilized and wound treated with betadine) and a spine sample is removed from the left pectoral fin (<5 mm; tools are sterilized and wound treated with betadine).

Between 2003 and 2007, NYSDEC has sampled 671 fish (53 in Newburgh and 618 in Haverstraw). Researchers have only observed one mortality. The fish was likely gilled unfavorably. Soak time probably also was a factor in the fish's death; due to a large catch (55 juvenile Atlantic sturgeon), the net was in the water for 4.6 hours.

New York Adult Atlantic Sturgeon Sampling *(A. Higgs)*

New York State DEC initiated sampling of adult Atlantic sturgeon in the Hudson River to determine habitat use and located the spawning grounds and congregation areas for the fish. Researchers captured pre-spawn adult Atlantic sturgeon entering the river and tagged all unmarked fish. Fish were also equipped with sonic transmitter tags and/or pop-off archival tags (PAT).

Sampling for fish entering the river occurred in the lower river between April and June. Monofilament gillnets (40.64 cm, 45.72 cm and 50.8 cm stretched mesh) rigged with bottom line were set overnight. A total of five pre-spawn fish were captured. Although fish were in the net for an extended period of time (up to 8 hours), water temperatures were low and all fish were active and unharmed.

Captured fish were placed onboard in a holding tank filled with ambient river water that was injected with pure oxygen. The tank holding the fish was covered to keep the fish shaded and protected from sunburn. A floated pen could also be used. Holding time did not exceed 10 minutes. During processing, freshwater was pumped from the river and run over the fish's gills. Fish were kept wet and were not anesthetized (fish were originally anesthetized, but NYSDEC no longer follows that practice).

Sampling on the spawning grounds occurred between June and August. Fish were captured in monofilament and multifilament nets (30.48 cm, 35.56 cm) that were set for two-hour periods around slack tide. Water temperatures during this sampling were warmer (20-24°C). At this location and time, potential harm to the fish was higher because water temperatures were warmer and the fish were already under stress due to the freshwater and being in spawning condition.

In 2006, researchers captured 44 fish—8 released without tags, 21 released with PIT and Carlin tags, 12 released with sonic tags (4 short-term, 8 long-term), and 10 released with PAT tags (5 also had sonic tags). In 2007, researchers captured 25 fish—4 fish released with Vemco sonic tags (all male), 22 released with Lotek sonic tags (one fish double tagged), and 13 released with PAT tags (all with sonic tags; 2 confirmed females). Carlin tags were attached to the lateral side, through the musculature, just below the rear dorsal fin. PIT tags (125 kHz) were attached in the same position as the Carlin tag. Lotek sonic tags that were attached externally did not have good retention. There were 2 external sonic tags returned. The Lotek sonic tags (77 kHz) included 5-year tags (Map 32-1S), 1.5-year tags (Map 16-2; records temperature and pressure; attached with PAT 2006), and small, 110-day tags (Map 11-4; attached with PAT 2007). The Lotek tags allow you to pinpoint a fish to an exact location, which can enable researchers to determine habitat usage. The Vemco tags (V16-4H-R04K, 69 kHz) have a time delay of 20-40 seconds and a battery that lasts 440 days.

Gillnetting for Sturgeon: The Delaware Experience *(C. Shirey)*

Delaware Department of Fish and Game has a long-term (began in 1991) sampling program for sturgeon that has had successful catch rates that has observed low rates of mortality (4 fish dead in 16 years). Sampling occurs in the lower Delaware River, which can be a hostile sampling environment. In this wide portion of the river, there are heavy ship traffic, an active bluefish fishery, and swift tidal currents.

The Delaware sampling program uses multifilament braided nylon nets. Multifilament is used instead of monofilament because monofilament appears to cut the fish. The nets are in 200-foot panels, 8-10 feet deep with mesh ranging from 2-10 inches. They use the heaviest twine size available (#207, #277), which may reduce bycatch of other species. The nets are hung in a 3/2 ratio—3 units of net to 2 units of line—as was recommended by former commercial sturgeon fishermen. The nets are rigged to sink and are set in a continuous string of 4-6 panels.

Delaware DFW samples in June through November; peak catches are in the summer and fall when temperatures are high and DO concentrations are low. They set their nets during low tide when current velocity is at its minimum. This protocol reduces the sampling window. The nets are set perpendicular to the current in water ranging in depth from 20-35 feet when the start of low tide is predicted. Researchers retrieve the nets at slack tide because they are easier to pull free from the riprap bottom, which means that there is less pressure on entangled fish and less gear is lost.

Researchers remove fish from the net as they come on board. The fish are usually entangled in the net and not gilled, and they put up little struggle or resistance. Researchers cut the net if it is necessary in order to free the fish. After being freed from the net, the fish are placed in a live-car. Fish caught in this program range in length from 500-1400 mm TL. It is common for the fish to float upside down after being caught. The program has seen between 250 and 300 recaptures.

Genetic Analysis *(T. King)*

Tissue samples should be taken from fin clips and placed in 95% ethanol. All preservatives work but some make it more difficult to get a useful DNA sample. DNA samples collected from dead animals can come from the barbel, deep white muscle tissue (recommended if the animal is not freshly dead), liver and heart tissue, and the viscous fluid in the eyes. When collecting DNA samples, researchers should also collect data on Fl, sex, and location (GPS coordinate).

FTA cards can be used to collect DNA samples from live animals in the field. Tim King is willing to provide them to researchers. The FTA cards contain a cell lyse solution and DNA fix; DNA samples on the card last 10 years.

Tim's research has shown that effective population size for Atlantic sturgeon is 100 fish. Current microsatellite DNA analysis is using 12 markers, compared to the original 7. Assignment success to populations, which could be used for mixed stock analysis, increased. Delaware fish were taken out of the analysis because they could not be assigned—no confidence that they are Delaware fish. Ogeechee fish are different from their neighbors both in mtDNA and nDNA. Recently, researchers have been collecting mtDNA from sturgeon scutes found in Indian middens and from old preserved specimens. They have been successful in getting short fragments of DNA.

Breakout Session Recommendations for the Atlantic Sturgeon Research Protocol

On the second day of the workshop, the participants formed small groups to discuss the research activities that should be included in the Atlantic sturgeon research protocol. The lists below contain the activities

that were brought up in the discussion when the larger group reconvened to share the findings of the small groups.

Handling Methodologies

- Temperature relationships with handling times need to distinguish and or include some discussion of soak times as they relate to temperatures. Live wells and or surgical baths need to take into account oxygen concentration (i.e. maintain levels above 3.2 ppm?).
- Short-term holding needs to include commercial harvesters and allowances need to be made to provide for handling and short term holding of Atlantic sturgeon. It would be up to the researchers to define a generic cooperating commercial harvester. The commercial harvesters would then need to constrain their activities to conform to the definition provided by the researchers. Tethering and or net pens need to be allowed as one of the methods of holding sturgeon for short-term periods of time. Temperature needs to be considering in the timing of holding.
- Collection of biological parameters by commercial harvesters could include tagging, photography, and collection of genetic samples. The subgroup would then develop criteria (potentially including temperature) for collection of biological samples and develop standards and measures to determine the eligibility of individual harvesters and or fisheries.
- Short term holding: use flow through tanks or replace water at a certain rate
- Holding times should be determined for physiological studies, bycatch mortality, and pathological studies.
- Long term holding: there should be a statement on how fish will be treated, perhaps deferring to sturgeon culture manual
- The use of smooth rubber gloves is not necessary for general handling of Atlantis sturgeon.
- Discuss captive broodstock and collection of wild broodstock for culture. Can broodstock or already captive Atlantic sturgeon be grandfathered in or be exempt from limiting research on these fish to the protocol?

Identification and measurement

- Look to shortnose sturgeon protocols, but increase detail on ratio of mouth width to inter-orbital width.
- Standardize taking TL and FL—maximum length (pull caudal fin down as far as possible and measure TL) and then FL.
- If the researcher is inexperienced or the fish is “questionable” (i.e., fish less than 1 m in length) and in areas where both Atlantic and shortnose sturgeons are present, width measurements should be taken.

Tagging

- PIT tagging operations should follow the guidelines of the ASMFC Atlantic sturgeon technical committee including the location and type of tag. Original paragraph in the shortnose sturgeon document should suffice. Keep it general. All fish must be PIT tagged and the subgroup can also recommend an external tag, and all fish should be scanned for PIT tags
- External tags: discussion provided in shortnose sturgeon document provides a general framework.
- Scute removal needs to be considered and discussed in the document. Removal of lateral scutes is fairly easy to do and can be done “bloodless” and would allow the assignment scute removal location. On the West Coast, researchers use a scalpel to pry up a scute (posterior to anterior) and count back from the operculum for green and white sturgeon. Subgroup needs to assign river specific scutes or change scute removal location for each year-class.
- Biotelemetry needs to include satellite, PAT, archival, ultrasonic, and radio tags. Allowances should allow for internal implantation of transmitters in both juveniles and adults year-round. The need to include an upper temperature of 27°C should also be included. When temperatures exceed

17°C, the incidence of mortality is greater then long soak times are used to catch sturgeon for tagging. Not much concern for the lower temperature limits. Discussion should include tagging of fish on the spawning grounds.

- Transmitter codes should be deposited in a "clearing house" to allow for data exchange. Find funding to provide a coastwide database support to include these codes.
- Efforts should be made to move towards a standardized coastwide telemetry system. Reference AFS fish handling guidelines or use information from that document.
- Anesthesia should be buffered and should include allowances for other anesthetics including metomidate and eugenol. Levels of anesthesia to be administered should be specified for different procedures.
- Surgical methodology provided in the shortnose sturgeon document provides a good basis from which to work. Provisions should be made to allow for implantation of transmitters in small fish off the mid line. It is suggested that absorbable suture material be used during the implantation of transmitters. Other types of suture material should also be allowed.
- Tagging on the spawning grounds
- Fungal infections should be treated with salt and formalin treatment.
- When tagging on the spawning grounds, specifically using internal tags, set a maximum number or percentage of females that can be tagged based on the size of the subpopulation. It is important to tag females to track their migrations to the spawning grounds and to get information on spawning intervals.
- Standardize the placement of tags, both internal and external.
- Provide provisions for chemical marking (e.g., calcien, OTC, elastomers, electronarcosis)
- VIE tags

Tissue Sampling

- Collection and storage of tissue samples should follow the ASMFC Atlantic sturgeon Technical Committee guidelines. Take a 1 cm^2 fin clip (preferably pelvic for small fish) and store in 95% non-denatured ethanol
- Upon completion of sample analyses results should be provided back to the original investigator and the investigator should be acknowledged in the paper. (Note: Tim King is amenable to this or even co-authoring a paper).
- Fin ray removal should follow the guidelines of Collins *et al.* and those provided by Dave Secor's lab. Encourage not removing spines from larger adults. Take spines from incidental mortalities or found dead fish.
- Gill biopsies
- Gonad biopsies

Gastric Lavage

- Follow guidelines provided in day one of workshop.

Non-invasive Procedures

- Training for all procedures or qualified instruction
- X-ray, ultrasound
- MRI

Other Research Methods

- Laparoscope and related technologies (e.g., bioscope: borescope takes some practice)
- Traditional methods of assigning sex
- Cathederization
- Cryopreservation

Sampling Methodologies

- Need to include pound nets
- Trawl sampling needs to be discussed in more detail as it has been shown effective in many systems for Atlantic sturgeon. Both juvenile and YOY have been collected using trawl systems. Recommendations for trawl times based on depth or temperature/DO concentrations?
- Hydroacoutics need to be added and or discussed including but not limited to split-beam, DIDSON, multi-beam, and side scan sonar systems.
- Look to the shortnose sturgeon document for egg sampling. If eggs are captured, leave excess on egg pad and place the pad back in the water.
- Fyke nets should also be included
- Seines, traps, pots and gear listed in the shortnose protocol should be included.
- "No netting" time closures?

Minimum sampling required

- May not need to go into sampling protocol and instead go in the recovery plan.
- The divergent life histories of shortnose sturgeon and Atlantic sturgeon need to be taken into account in developing any strategy to determine extirpation from a particular system. This is a result of the wide dispersal of Atlantic sturgeon and the infrequent nature of spawning events for female Atlantics.
- Needs to take into account age-0, age-1 or spawning adults on spawning grounds.
- If a jurisdiction thinks it has an extirpated population, decision on whether enough sampling has been performed could be made by the TC (minimum sampling will be determined as jurisdiction brings evidence to TC and decided as a group).

Other

- Exposure to gear in captivity for applied research
- Determination of an LD_{50} cannot go into a 4d rule.
- Training required for certain invasive procedures (e.g., gastric lavage, laparoscope/bioscope, surgery)
- Outline the conditions under which fish can be released back into the wild after they have been used in experimentation.
- To resuscitate a non-responsive Atlantic sturgeon, flush water over gills.
- Sturgeon salvage. Any tissues for variety of analyses which may include but are not restricted to:
    - Fin spines, otoliths for microchemistry
    - White muscle for genetics
    - Samples for contaminants
    - Fish for pathological studies
    - Morphological studies

Subgroup Membership

Dewayne Fox	Jerre Mohler	Tom Savoy	Kim Damon-Randall
Albert Spells	Eric Hilton	Stephania Bolden	
Chris Hager	Brian Hickson	Erika Robbins	