

Atlantic Sturgeon Research Protocols



Picture credit: Duane Raver, Wildlife Artist

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Background

In May 2000, NOAA's National Marine Fisheries Service (NOAA Fisheries Service) published "A Protocol for Use of Shortnose and Atlantic Sturgeon" (Moser et al. 2000) (hereafter referred to as the Moser Protocol). This document provided the first guidelines for handling and sampling Atlantic Coast sturgeon and was designed to help standardize research methodologies for these unique fish. The protocol has proven to be effective and has been closely followed by many sturgeon researchers. In the period since the document was published, more emphasis has been placed on sturgeon research which has led to an increased amount of sampling. This increased sampling has provided much needed information about both shortnose and Atlantic sturgeon, and also, a great deal has been learned about methodologies to reduce injury and mortality to the fish during research activities. As a result of the ongoing status review for shortnose sturgeon, it was determined that a separate protocol document for Atlantic sturgeon was appropriate. Consequently, a group was formed to revise the protocol to incorporate new technologies as well as revise some of the existing methodologies to further reduce the potential for injury and mortality to Atlantic sturgeon.

Introduction

Atlantic sturgeon have been considered a Species of Concern since 1998 following the joint decision by NOAA Fisheries Service and the U.S. Fish and Wildlife Service (FWS) that listing the species under the Endangered Species Act (ESA) was not warranted. Due to concerns over continued declining trends in some subpopulations, in 2005, NOAA Fisheries Service initiated a second review of the status of Atlantic sturgeon. A status review team (SRT) consisting of four NOAA Fisheries Service, four FWS, and three US Geological Survey (USGS) personnel participated in the status review process. The status review was reviewed and supplemented by eight state and regional experts who provided their individual expert opinions on the scientific facts contained in the status review report and provided additional information to ensure the report provided the best available data. Lastly, the report was peer reviewed by six experts from academia and received favorable reviews. In the status review report (72 FR 15865), the SRT concluded that Atlantic sturgeon in the United States should be divided into five distinct population segments (DPSs): (1) Gulf of Maine; (2) New York Bight; (3) Chesapeake Bay; (4) Carolina; and (5) South Atlantic. The SRT also recommended that three of the five DPSs be listed as threatened (New York Bight, Chesapeake Bay, and Carolina). The SRT determined that the remaining two DPSs had a moderate risk of becoming extinct, though there were insufficient data to allow for a full assessment of these subpopulations; thus, a listing recommendation was not provided. An additional finding of the SRT was the overall lack of basic biological data for many of the Atlantic sturgeon subpopulations.

Based on the information in the status review report and other best available data, NOAA Fisheries Service is currently in the process of making a determination as to whether to list Atlantic sturgeon under the ESA, which could ultimately affect how research activities are conducted on this species. Given the current status of Atlantic sturgeon and the lack of data on many subpopulations, it is necessary to perform research activities in a manner  allows for crucial information to be obtained on Atlantic sturgeon subpopulations while minimizing the adverse impacts of the activities on the species. Should Atlantic sturgeon be listed under the ESA, researchers should refer to any relevant regulatory documents and consult with NOAA

Fisheries Service to determine if planned research (even research that follows the protocols herein) is permitted under the ESA, and whether there are special authorizations required or reporting requirements that must be satisfied.

In order to provide information on how Atlantic sturgeon protocols should be developed, a workshop sponsored by NOAA Fisheries Service and the Atlantic States Marine Fisheries Commission (ASMFC) was held in November 2007. Workshop participants were asked to identify specific activities, techniques and methodologies that should be included in an updated protocol document. Over 30 sturgeon experts from Maine to Florida attended this two day workshop, and a subgroup was formed to draft the document. Thus, these protocols have been developed by researchers who have many years of experience conducting these activities specifically on Atlantic sturgeon.

The workshop participants agreed that the Moser Protocol represented a valuable resource for conducting research activities on both species and decided that it should be used as a template to develop the revised protocols for Atlantic sturgeon. In order to maintain one comprehensive document, this report incorporates new technologies along with some of the same information from the original protocol.

As indicated in the Moser Protocol, sturgeon present some unique challenges for development of standardized methods. North American Atlantic sturgeon occur in various coastal, estuarine, and riverine habitats along the Atlantic Coast from the Saint John River in Canada to the Satilla River in Georgia (possibly as far south as the St. Johns River in Florida). The differences in habitat both within and among river systems, and latitudinal differences in temperature and sturgeon life history, have resulted in sampling methodologies that are often specific to a given region or time of year. Similar to the Moser Protocol, research methodologies for sturgeon from across their entire range of habitats and for all life stages that have been studied have been included. Specific research plans for Atlantic sturgeon should be developed by researchers based on the conditions under which research will take place and in accordance with these research protocols. Research techniques that are invasive and hold inherent risks to the well-being of Atlantic sturgeon should only be conducted by researchers with the appropriate level of training and experience. Guidance outlining appropriate means to gain sufficient experience has been provided immediately following specific discussions of each research technique in this document. Methodologies for culturing and long-term maintenance of Atlantic sturgeon in captivity have not been included as both are addressed in the Culture Manual for Atlantic Sturgeon (Mohler 2004). The authors have attempted to identify and address any new and emerging technologies in this document, but recognize that technologies change and advance over time. Thus, this should be a living document that allows for new techniques to be incorporated as they prove to be successful for Atlantic sturgeon. The protocols will be reviewed and revised as necessary every three years, with the first review beginning in January 2012. If new technologies or techniques emerge between review periods, interim revisions will be considered.

The objectives of this document are 1) provide Atlantic sturgeon researchers with particular standard protocols for conducting specific, common research activities in order to ensure that the safest and most effective protocols are followed by Atlantic sturgeon researchers; and 2) compile references to literature that provides detailed information on sturgeon biology and effective research techniques.

Identification and measurement

Two species of sturgeon are present along the East Coast of North America: Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) and shortnose sturgeon (*Acipenser brevirostrum*). The two species differ greatly in their maximum sizes (427 cm TL and 143 cm TL, respectively; Dadswell et al. 1984, Bain 1997), but juvenile Atlantic sturgeon may easily be confused with juvenile and adult shortnose sturgeon because of overall similarity in their general body form. Thus, care must be taken for correct identification, particularly among small individuals. Snout length is not a reliable character for identifying these species. Despite its common name, the rostrum of shortnose sturgeon can vary substantially in its size and shape, from truncated and rounded to moderately long and sharply pointed, even in similarly sized individuals. This matches the variation found in other aspects of the anatomy of this species (Hilton and Bemis 1999). The snout of Atlantic sturgeon generally is more sharply pointed than that of shortnose sturgeon (Dadswell et al. 1984; see also Figure 1 below), but morphological variation (including ontogenetic and other allometric variation) has not been fully described or quantified in this species.



Figure 1. Ventral view of shortnose sturgeon (left; Museum of Comparative Zoology 54265, 435 mm FL, Connecticut River, MA) and Atlantic sturgeon (right; Virginia Institute of Marine Science uncataloged, 780 mm FL, James River, VA); note short snout and wide mouth of the shortnose sturgeon. Scale bar = 2 cm. Photos: John Weinstein, Field Museum of Natural History (left); Eric Hilton, Virginia Institute of Marine Science (right).

In addition to several internal and external osteological characters that might serve to distinguish juveniles and adults of the two species (e.g., pale vs. dark viscera in Atlantic vs. shortnose sturgeon, respectively, Vladykov and Greeley 1963; the shape of rostral canal bones, Hilton 2002; shape and arrangement of frontal bones and caudal lateral line scales, Eric Hilton,

Virginia Institute of Marine Science, pers. comm.), several key external characters may be used to distinguish between the two species in the field. Juvenile Atlantic sturgeon have a solid darkly pigmented dorsum and light ventral surface, whereas juvenile shortnose sturgeon (less than c. 30 cm TL), although they have a dark dorsum and light ventral surface, also have dark, irregularly-shaped blotches along the length of their body. While Atlantic sturgeon usually have a series of bony plates in the region immediately proximal to the anal fin (i.e., between the anal fin and the series of lateral scutes), these plates have not been observed in shortnose sturgeon (Figure 2). These plates are generally larger than the irregularly shaped and randomly distributed bony elements found in the skin between the five major rows of scutes; however, they are smaller than the scutes and the bony plates found more posteriorly on the dorsal, lateral and ventral surfaces of the caudal peduncle.

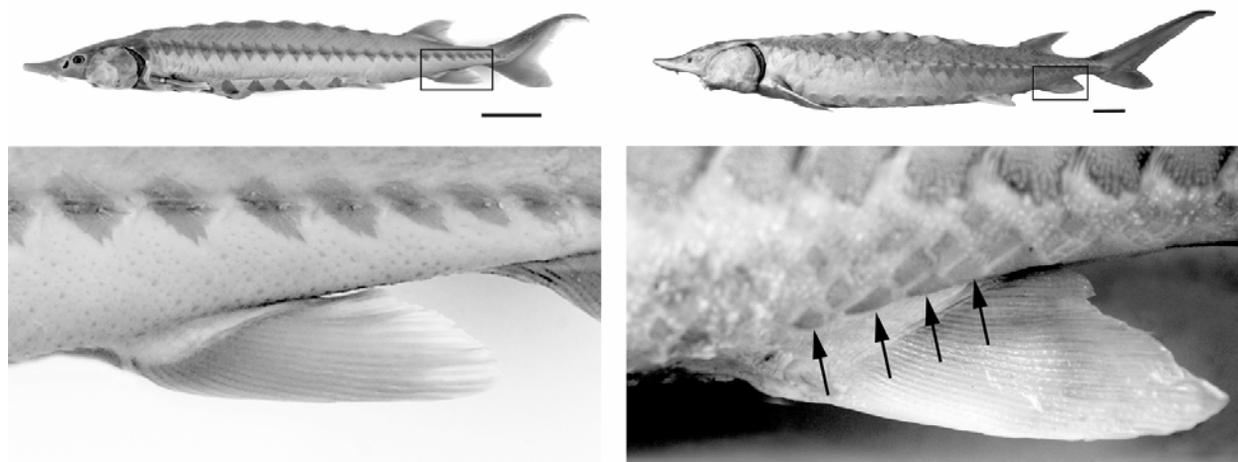


Figure 2. Lateral view of shortnose sturgeon (left) and Atlantic sturgeon (right); note the small bony plates (scutes) highlighted by the black arrows above the anal fin in the Atlantic sturgeon (same specimens as in Fig. 1). Scale bar = 5 cm. Photos: John Weinstein, Field Museum of Natural History (left); Eric Hilton, Virginia Institute of Marine Science (right).

The most widely-used and seemingly most reliable character over a broad ontogenetic range that has been used to distinguish the species is the ratio of mouth width to interorbital distance (Moser et al. 1998). Dadswell et al. (1984) reported that Atlantic sturgeon have a mouth width of less than 55% of the interorbital width (range 43-66%) and shortnose sturgeon have a mouth width of more than 62% of the interorbital width (range 63-81%). Unpublished data suggests that the range of variation may be greater for both species. Specimens from the Merrimack had the following measurements: Atlantic sturgeon, 44-70%, mean 50%, n=14; shortnose sturgeon, 59-80%, mean = 68%, n=11 (Micah Kieffer, USGS Conte Anadromous Fish Lab, unpublished data.). For fishes caught in Connecticut (Atlantic sturgeon are likely of mixed stock), the following measurements were collected: Atlantic sturgeon, 34-49%, mean 44%, n=67; shortnose sturgeon, 54-79%, mean 66%, n=80 (Tom Savoy, Connecticut DEP, unpublished data). From the Hudson River, the following measurements were taken for the two species: Atlantic sturgeon, 32-92%, mean 50%, n=442; shortnose sturgeon, 47-96%, mean = 75%, n=177 (Sweka et al. 2006) with the vast majority of Atlantic sturgeon measurements (440 of 442) between 32-76% (Jerre Mohler, U.S. Fish & Wildlife Service, pers. comm.). The level of variation within these and other morphological characters have yet to be quantified for either species, either at a species-wide or population-to-population level.

The level of variation within these and other morphological characters have yet to be quantified for either species, either at a species-wide or population-to-population level. Bath et al. (1981) described differences between larvae and small juveniles (8.4-37.0 mm TL) of the two species based on specimens from the Hudson River. They regarded the most reliable identification character to be the relative mouth width. Snyder (1988) corrected miscalculations from Bath et al. (1981) and concluded that ranges overlapped for larvae of the two species. Snyder (1988) also described differences between the larvae (post-egg resorption) of the two species that he considered more reliable than mouth width such as the presence of melanophores on the ventral surface of the abdomen of Atlantic sturgeon (absent in shortnose sturgeon) and a shorter distance between lobes of the lower lip (less than 20% mouth width in Atlantic sturgeon vs. more than 25% in shortnose). Additionally, he found a difference of pelvic fin ray number (17-22 for shortnose vs. 26-33 for Atlantic) and anal fin ray number (18-24 shortnose vs. 22-30 Atlantic) for specimens over 60 mm standard length. Scott and Crossman (1973) also reported differences in the dorsal fin ray (38-46 in Atlantic and 19-22 in shortnose) and anal fin ray (25-30 in Atlantic and 19-22 in shortnose) counts. However, Vladykov and Greeley (1963) suggested that dorsal and anal fin ray counts were too difficult to count accurately (due to the fin rays being heavily branched and embedded in a thick layer of skin) and were therefore of limited value as a distinguishing characteristic. Vladykov and Greeley (1963) found a difference in the number of gill rakers on the outside of the first gill arch of specimens greater than 20 cm (17-27, average 21.6 in Atlantics and 22-29, average 25.4 in shortnose).

Birstein et al. (1997) recommended a series of 14 body measurements and six meristic characters (scute counts) to be collected for all sturgeon species. Because there are only two species of East coast sturgeon, the authors have selected measurements that have proven to be effective at differentiating between Atlantic and shortnose sturgeon specimens. At a minimum, the five measurements specified below (Figure 3) should be taken for all individuals, particularly when there is a question of species identification. In order to ensure consistency among researchers for the various measurements, place fish on either their left or right side, and take the following measurements from that position:

total length, which is measured in a straight line along the body axis from the tip of the snout to the tip of the tail (not following the curvature of the body);

fork length, which is measured in a straight line along the body axis from the tip of the snout to the posterior edge of the fork of the tail (not following the curvature of the body);

head length, which is measured in a straight line along the body axis from the tip of the snout to the posterior edge of the bone that forms the gill cover (i.e., excluding the soft opercular flap);

interorbital width, which is measured as the distance between the lateral margins of the bony skull at the midpoint of the orbit; and

mouth width, which is measured as the distance between the left and right inside corners of the mouth (i.e., excluding the lips); this should be measured with the mouth closed.

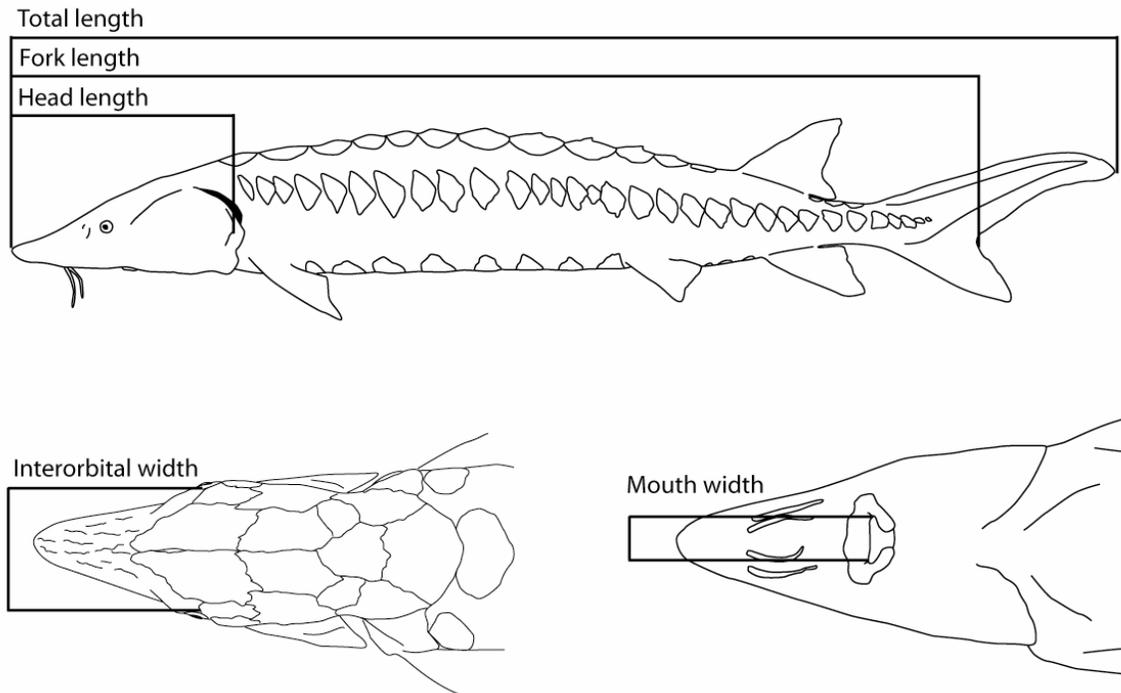


Figure 3. Depiction of the five sturgeon measurements.

Mouth width and interorbital distance should be measured with calipers. For the mouth width, several published keys, recommendations, and reports have cited this measurement as including the lips (e.g., see figure in Birstein et al. 1997; described one way in the key but illustrated the other in Musick et al. 1994); following these measurements, the ratio of mouth width to interorbital distance will be slightly higher. In order to ensure consistency of measurements for specimens throughout the range of the species, record this measurement on the inside corner of the mouth (following Vladykov and Greeley 1963 and Dadswell et al. 1984; see Figure 3) as this is the most common current practice. Measuring fork length is recommended as a reference for individual size since total length is subject to a greater level of measurement error (e.g., in “pulling” the tip of the tail ventrally, thereby over estimating the measurement) (any obvious fin erosion that impacts the measurement should be noted). If the researcher is inexperienced or the fish is “questionable” (i.e., less than 1 m in length) and in areas where both Atlantic and shortnose sturgeon occur, mouth width and interorbital distance measurements are necessary to confirm identification. Although no one diagnostic characteristic can be used to distinguish between shortnose and Atlantic sturgeon, in combination, the above characteristics are appropriate.

Training requirements

Only relatively minor training is needed for identification and measurements to ensure accuracy of identifications and consistency of measurements. Because of difficulty in distinguishing the two East Coast species, particularly at small juvenile sizes, researchers should gain sufficient experience and familiarity with identification through examination of specimens of both species in consultation with experienced researchers.

Sampling Methodologies

Choice of sampling methods for sturgeon is influenced by targeted life stage, habitat and water temperatures. Sturgeon are highly susceptible to capture in gill nets whether stationary, drifting or hung in a trammel net configuration (Buckley and Kynard 1985, Hoff et al. 1988, Dovel et al. 1992, Geoghegan et al. 1992, Kieffer and Kynard 1993, Moser and Ross 1995, Collins et al. 1996, ASSRT 2007, ASMFC 2007). Trawls can also be highly effective but are often inappropriate for estuarine or riverine use due to benthic and physical conditions (e.g., narrow passages and uneven, rocky bottoms). In regions where fyke and large hoop nets are used by commercial fishers, juvenile and sub-adult sturgeon are occasionally captured as bycatch. Pound nets due to their much larger dimensions can accommodate and hold fish of considerable size. Recently, commercial pound nets in Canada have provided large samples of adult fish to local scientists and sturgeon of lesser size are routinely captured in the Chesapeake Bay in such gear (Mike Dadswell, Acadia University, pers. comm., Chris Hager, VA Sea Grant, Marine Extension Program, pers. comm.). It is important to point out that reward programs and cooperative sampling efforts with commercial fishers are often much more cost effective than fishery independent research collection efforts and should not be overlooked as a viable alternative. Baited trot lines have proved to be an effective method for collecting white sturgeon (*A. transmontanus*) and may have potential for collecting Atlantic sturgeon (Elliott and Beamesderfer 1990).

Egg and larval nets and mats/pads

Collecting larvae and eggs requires specialized techniques and approaches. As with other fishes, eggs of several species of sturgeon have been successfully collected using egg mats (McCabe and Beckman 1990, Marchant and Shutters 1996, Sulak and Clugston 1998, Fox et al. 2000). Egg mats can be used to collect eggs as they are deposited. Egg sampling pads (e.g., floor buffing pads, approximately 2 feet in diameter (described in Fox et al. 2000)) are only effective in the immediate vicinity of spawning as sturgeon eggs become adhesive following fertilization as a result of changes in the egg membrane which cause the egg to swell and become sticky (Mohler 2004). Researchers who wish to remove fertilized eggs which are adhered to the sampling pad should avoid trying to pick them off and utilize scissors to cut the pads to prevent rupturing the egg. Individual pads should be removed from the water, quickly scanned for the presence of eggs and if present, eggs should be counted before the pad is returned to the water at the site of collection to allow successful hatching.

In other systems, eggs and larvae and early juveniles have been satisfactorily collected in cone (Kohlhorst 1976) or D-shaped nets (Taubert 1980, Kynard et al. 1999) and with the use of epibenthic sleds. Mesh sizes of 2 mm² trap sturgeon eggs and larvae while letting some debris pass through. The net is attached to a weighted and floated 1 m diameter steel ring that has been flattened to maximize contact with the substrate (D-shaped, Kynard et al. 1999). A 1 m square or 2 m x 1 m Neuston net can also be used. The net is attached to a Danforth or grapnel-type anchor via a short bridle. This arrangement allows the net to stand upright in currents up to 1.0 m s⁻¹. Depending on the current velocity and amount of debris accumulation, such gear should be fished no longer than 10 min in areas of suspected spawning. A flow meter should be positioned in the mouth of the net to allow calculation of egg or larval densities per volume of

water sieved. Such studies are best conducted with the aid of telemetry data from pre-spawning adults to identify likely spawning locations (Collins and Smith 1993, Kynard et al. 1999). D-shaped nets were used to capture eggs of Chinese sturgeon in the Yangtze River for four years. Tens of thousands of eggs were captured when the nets were set in areas occupied by telemetered fish. Eggs were reared to juvenile stages and released into the river (Wei and Kynard 1996).

Nets should be deployed beginning at the earliest time spawning would be expected. Nets should be equipped with velocity meters to allow the volume of water filtered to be estimated to develop an index of abundance and an estimate of spawning success (# ELS/volume of water sampled) (Taubert 1980). Nets should be checked routinely. Due to the relative rarity of the species, discretion by NOAA Fisheries Service will be used to determine the number of eggs collected.

Light traps have proved to be of little value for collecting Gulf or Atlantic sturgeon larvae. Small juveniles are rarely taken using traditional survey gear although some success has been achieved with modified trawls (Hrabik et al. 2007, Doyle et al. 2008). The limited success of mobile sampling gear may, in part, be due to larvae and juveniles apparent tendency to seek out crevices in rough bottom across which seines and trawls have low collection efficiencies. Due to their preference for such niche providing habitats, habitat pots which contain protective structure may be an alternative approach for sampling. These pots may work best in systems where natural protective structure is limited.

Gill nets and trammel nets

Sturgeon are highly susceptible to gill nets of various configurations. It is well established that gill nets can be selective with regard to size and species, with selectivity varying due to biotic factors including morphology, behavior and vertical and horizontal distributions (Hamley 1975, Marais 1985, Reis and Pawson 1999, Machiels et al. 1994, Dickson 1989, Purbayanto et al. 2000). Selectivity can also be affected by abiotic gear factors such as mesh size, twine material, twine diameter, hanging ratios and tie downs which influence not only species retention and size selectivity but fishing power (Hamley 1975, Machiels et al. 1994, Hovgard and Lassen 2000, Yokota et al 2001, Holst et al. 2002, Gray et al 2005, Hager 2007). 

It is easy to conceptualize why gill nets that cover more of the water column, by being longer or taller, may be more effective and result in higher catch per unit effort (CPUE) especially with regard to rarer species. What is less obvious is how construction alterations like webbing diameter, hanging ratio, vertical net height and tie downs modifications to this height alter the manner in which webbing presents and thus how the net catches and retains species. This difficulty is based on a common misconception that fish are simply gilled by gill nets. That is, they are prevented from backing out of the webbing by a mesh caught behind the gill plate. What is often overlooked is that fish and other animals are also retained by wedging - that is held by a mesh or meshes around the body. Animals with unique morphological attributes such as teeth, maxillae, scutes, snout or other projections may also be entangled in webbing without necessarily penetrating the net (Hamley 1975). Entanglement often results in struggling that subsequently wraps the fish up in additional webbing.

Sturgeon are morphologically unique with a cone shaped snout that rapidly transfers meshes over the head and along the body and thus, they are rapidly gilled and wedged. Their unique dermas covered with bony scutes also lead to rapid entanglement. When fish encounter gear that contains loss or torn webbing or if the fish is exceptionally large they may become

completely wrapped in webbing. Consequently, there can be considerable overlap between size distributions of sturgeon collected with different mesh sizes (Figure 4, Chris Hager, VA Sea Grant, Marine Extension Program, unpublished data). Due to the characteristically large size distributions of the species and varied selectivity due to gill net configurations that are not yet fully understood, caution should be taken if one is attempting to characterize size and/or age distributions of sturgeon using gill net gear.

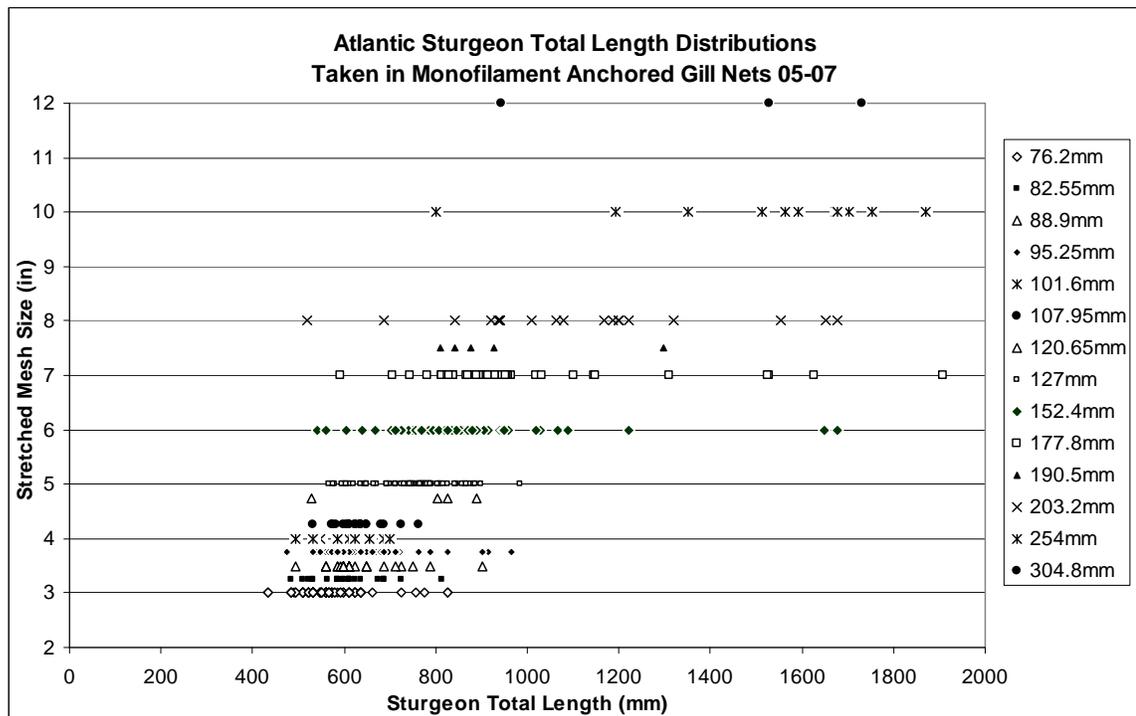


Figure 4. Atlantic sturgeon size distributions taken in anchored gill nets in Virginia are illustrated above (Chris Hager, VA Sea Grant, Marine Extension Program, unpublished data). Data supports claim by previous researchers that six inch mesh is highly effective at collecting a wide size range of fish.

Abiotic gear factors can significantly affect size distributions of fish retained and gear retention characteristics. Gill net interaction trials (Hager 2007) on captive Atlantic sturgeon, which examined the effect of twine size, hanging ratio and the use of tie downs on sturgeon retention reveal that enlarging twine (0.4-0.52 mm), augmenting hanging ratio (0.5-0.625) and removing tie downs (30" tie downs on 45" net) all significantly (Chi square, $P < 0.0002$, 0.0107, 0.0020 respectively) reduce retention rates (retained/interacted). Conversely, any gear alteration or deployment method that increases stretch in webbing, mobility of webbing, or amount of webbing per a given area increases the likelihood of gilling, wedging, entanglement and wrapping and thus, retention with respect to Atlantic sturgeon. The increased size distributions of sturgeon taken in drift gill nets (Moser et al. 2000) are evidence of this shift in selectivity. NOAA Fisheries Service (Northeast Region) observer data also supports the assertion that using tie downs, which increases webbing mobility and meshes per area, significantly increases the size distribution of sturgeon retained. When nets are tied down a series of bags is created.

NOAA Fisheries Service Northeast Region observers recorded gill nets consisting of 30.5 cm (12") stretch mesh tied down to 121.9 cm (48") retained fish from 17-250 cm in total length (ASMFC 2007, section by Chris Hager, VA Sea Grant, Marine Extension Program). The average fish was 137 cm TL.

Trammel nets are no more than numerous panels of varied sized gill net meshes hung on the same top and bottom line so that they overlap. This configuration not only provides more meshes per unit area which increase the chances of entanglement but increases the likelihood of fishes being retained because there are now two different sizes of webbing to become gilled or wedged in. Trammels not only increase the size distribution of fishes retained by increasing the likelihood of entanglement, gilling and wedging but because they consist of two overlapping walls of webbing they also entrap fish between the webbing walls that penetrate the larger outer wall but are too large to pass through the smaller inner wall. Given this construction, it is obvious why trammel nets collect a wider size distribution of fishes than other gill nets (Moser et al. 2000). Some researchers claim that the increased likelihood of entanglement that the gear affords may reduce chances of mortality but no research has been done to substantiate this claim.

Employing staked, floating gear or tie downs allows the fisher to control the depth of water fished. Staked gill nets are not anchored to the bottom using temporary benthic grabbing anchors but are attached at both top and bottom line to vertical stakes driven into the bottom. Numerous stakes are used on each net occurring every 15.2 m (50 ft) or so. In commercial fisheries, anchored gill nets of less than 91.4 m (300 feet) are rarely ever deployed and some up to 914.4 m (3000 feet) occur offshore. Shortening intervals between anchor points reduces tension on meshes due to hydrodynamic forces. This deployment methodology also helps reduce the chances of anchors becoming dislodged at either end or the top and bottom line twisting upon themselves in the currents. High rates of mortality have been observed when a net is dislodged or it becomes twisted around ensnared fish.

Both monofilament and braided mesh gill nets are effective for capturing sturgeon, though they may have different retention characteristics. Although fish are captured more efficiently with lighter twine, sturgeon can easily break free of webbing that is too light, therefore, when using larger mesh sizes, twine sizes should also be increased. In addition, lighter twine has been known to cut into the fish and cause injury (Moser et al. 2000). When targeting adults with 15 cm (6") stretched mesh, heavy multifilament nets (at least 0.52-0.57 mm) should be used. When targeting adults with larger mesh sizes of 25.4 to 35.6 cm (10-14"), twine sizes of >0.9 mm should be used to prevent loss of such large fish.

Sturgeon are benthic and generally are captured near the bottom unless they are actively migrating (McCleave et al. 1977, Moser and Ross 1995). During immigration into riverine systems, adult fish are often netted in the top half of anchored nets (Albert Spells, U.S. Fish and Wildlife Service and Chris Hager, VA Sea Grant, Marine Extension Program, pers. comm.). This location suggests that sturgeon may use the higher flow speeds in the pelagic region to aid migrations. In general, stationary nets should be heavily weighted or staked and allowed to contact the bottom. Whenever possible, nets should be set perpendicular to the current. In areas of high velocity or with heavy debris loading, this is not feasible and nets should be set in eddies, on the downstream side of islands, or parallel to the current in mid-channel (Buckley and Kynard 1985, Kieffer and Kynard 1993, Moser and Ross 1993, Kynard et al. 1999). In many southern rivers, trammel nets are set during slack tide periods only, to reduce stress on fish and debris loads.

Drift gill nets that contact the bottom can be highly effective if the bottom is relatively snag-free (O'Herron and Able 1990, McCord 1998). In addition, this method reduces debris loading because the nets drift along with the debris and do not intercept it. Generally, the short soak times, reduced pressure on webbing, and active fishing methods used in conjunction with driftnets result in less injury to captured fish (Moser et al. 2000). In upriver runs and pools very light leadline and large floats can be used. In tidal areas, buoyancy should be reduced and the net dragged along the bottom wherever possible (McCord 1998).

Entanglement in gill nets or trammel nets can result in significant sturgeon mortalities (Kieffer and Kynard 1993, Moser and Ross 1993, Collins et al. 1996, Kynard et al. 1999, Stein et al. 2004, ASSRT 2007), and mortalities are most often associated with high water temperatures and long net sets (Miller 2007). To decrease risk of mortality, precautions should be taken to reduce fish stress due to gear and environmental conditions. Collins et al. (1996) observed significant increases in gill net related mortality in water temperatures of 18° C and above in a southern shad fishery. Based on fisheries independent anchored gill net research with standardized 24 hour sets and numerous mesh sizes (5-12" stretched mesh), mortality increased from 2% (n=50, 6-17° C) to 14% (n=14, 18-24° C) in water exceeding 18° C (Chris Hager, VA Sea Grant, Marine Extension Program, unpublished data). The reasons for this are likely metabolic. Niklitschek's (2001) bioenergetic analysis of juvenile Atlantic sturgeon suggests that temperature, dissolved oxygen (DO) and salinity all significantly affect metabolism and thus, stress. At normal DO (70% saturation), instantaneous daily growth peaks at 18°C, above this temperature growth declines due to temperature stress alone. The growth curve shifts to the right slightly at higher DO (100%) with the apex moving to approximately 20°C, and at lower DO, (40%) the apex is reduced to approximately 16° C. Salinity can be an important bioenergetic limitation factor as well (Niklitschek and Secor 2005). DO and salinity are naturally augmented during summer; thus, this season becomes critical, accompanied by slow or no growth and higher mortality s from the combined effects of decreasing DO and increasing temperature and salinities.

Every effort should be made to reduce stress during removal of fish from nets. To facilitate this, eyes can be covered and the fish inverted. In some cases, net meshes should be cut to facilitate rapid removal of fish. This is especially true if the fish is gilled or meshes have entered the gill case.

Gillnet sampling and routine handling under favorable environmental conditions does not appear to cause undue harm to sturgeon. Under normal temperature conditions (not exceeding 17°C), lake sturgeon captured in gill nets (24 h net sets) and PIT tagged showed significant responses in several physiological stress indicators, but recovered nearly completely within 3 days of sampling, with no documented mortality (Baker et al. 2008). However, at elevated temperatures, sampling fish in gill nets has been shown to cause increased mortality (Murphy et al. 1995), and recovery after stress incurred from sampling can be protracted (Wilkie et al. 1996). Because Atlantic sturgeon encounter unfavorable environmental conditions in some river systems (e.g., Chesapeake Bay, Niklitschek 2001), appropriate gill net soak time guidelines based on temperature and dissolved oxygen conditions should be identified to ensure that the effects of environmental conditions on sturgeon health are not compounded unnecessarily by sampling stress. Niklitschek (2001) found that Atlantic sturgeon exhibited negative behavioral and bioenergetic responses (food consumption, routine metabolism) when water temperatures reached 28°C. Recent studies have indicated that shortnose sturgeon acclimated to higher temperatures are more tolerant of elevated temperatures (Ziegeweid et al. 2008a), and anecdotal

field observations indicate possible latitudinal variation in thermal tolerance. Atlantic sturgeon from southern river systems have been safely captured in gill nets at temperatures exceeding 30°C (Doug Peterson, University of Georgia, pers. comm.), while in some northern rivers, visible stress symptoms have been observed when Atlantic sturgeon are sampled in somewhat lower temperatures (24°C; Gayle Zydlewski, University of Maine, pers. comm.). Tolerance to elevated temperatures and low dissolved oxygen concentrations also appears to increase with age (body size) (Ziegeweid et al. 2008b, Jenkins et al. 1993). However, this research (and all research to date that has investigated effects of temperature, dissolved oxygen, and salinity) has been conducted using juvenile sturgeon, and the applicability of these results to sub-adult and adult Atlantic sturgeon is unclear. Further complicating identification of safe temperatures during which gill netting can be conducted are the additive and synergistic effects of dissolved oxygen and salinity on bioenergetic responses and survival of Atlantic sturgeon (Niklitschek 2001, Niklitschek and Secor 2009). A review of the relevant literature on the effects of hypoxic conditions on sturgeon species by Secor and Niklitschek (2001) revealed that dissolved oxygen levels below 3.3 mg/L, regardless of temperature, can cause mortality in both shortnose and Atlantic sturgeon juveniles. The authors further suggest that dissolved oxygen at 60% saturation (4.3-4.7 mg/L at 22-27°C) or higher is necessary for shortnose and Atlantic sturgeon to avoid bioenergetic responses. Additional research is required in order to understand the effects of sampling stress on sturgeon under unfavorable environmental conditions. In some cases, sampling outside of optimal conditions may be necessary. However, this is only appropriate for researchers who have extensive experience sampling in a given system, who have demonstrated ability to adapt gill net sampling to minimize stress to sturgeons, and who have not observed elevated mortality when sampling under these conditions.

When air temperatures are below 0° C, sturgeon should not be out of the water for more than a few minutes. All fish should be processed while underwater if possible. Reducing stress through this practice is especially important when tissues can freeze or when the fish is already being stressed due to other environmental extremes.

Trawls

Where benthic and hydrodynamic conditions permit the use of trawls, this gear can be effective for capturing sturgeon. Collins et al. (1996) found that 39% of all juvenile Atlantic sturgeon and 8% of the adult shortnose sturgeon tag-returns from fish tagged in the Altamaha River, Georgia, were from the commercial trawl fishery. Sampling of shortnose and Atlantic sturgeon was conducted in the tidal portion of the Hudson River from 1975-80 using 604 m and 10.7 m semi-balloon otter trawl having mesh sizes of 1.3-6.5 cm (Dovel and Berggren 1983, Dovel et al. 1992.). Fish >200-mm total length were regularly caught, with most fish around 500 mm. These trawls were fished for variable lengths of time (up to 50 min) at tow speeds of 4km/h (2.2 knots). The Connecticut Department of Environmental Protection's Long Island Sound Trawl survey has captured over 400 Atlantic sturgeon, ranging from 625 to 2135 mm FL. Sturgeon have been collected every year since the study began in 1984 from all depth strata sampled (up to 30 m). Up to 60 Atlantic sturgeon have been collected in a single tow (CT DEP unpub) with little obvious damage. The Hudson River Utilities Monitoring Program has also conducted a standardized trawling survey since 1985 using a 3 m beam trawl with 1.3- 3.8 cm mesh. This gear is towed for 5 min against the current and adult shortnose sturgeon (500-100 mm fork length) are caught regularly. This sampling indicates that even a small trawl effectively

captures sturgeon. NOAA Fisheries Service northeast observer data suggests that trawls have a significantly lower mortality incidence than gill nets. Nevertheless, gear and deployment variables (e.g., tow time, depth of fishing, retrieval speed) can and should be controlled by researchers to minimize the risk of mortality.

Electrofishing

Electrofishing has not proven to be an effective method of collecting sturgeon (Moser et al. 2000). The electric trawl developed by Aadland and Cook (1992) for the collection of benthic species in riverine environments may offer an improved electrofishing method. Studies to develop species specific electrofishing techniques should look to hatchery fish for research subjects. If efficient methodologies can be developed for sturgeon, electrofishing could possibly offer a valuable tool at least for collecting and monitoring juvenile sturgeon.

If electrofishing is to be used in habitats that may contain Atlantic sturgeon at any life history stage, the lowest effective voltages should be selected to minimize impacts on Atlantic sturgeon, whether targeted or incidentally exposed during electrofishing operations. Holliman and Reynolds (2002) found that white sturgeon (24-54 cm) were at a higher risk for hemorrhage when exposed to 60-Hz pulsed direct current (PDC) as opposed to continuous DC; thus, use of PDC is discouraged in waters containing Atlantic sturgeon. Refer to Reynolds (1996) for guidance on selecting the intensity of the electric field to be employed.

Passive Methods

Advances in non-invasive marine system sampling methodologies such as sonar, video, and highly advanced combinations of both are quickly making these methods a viable alternative to traditional, potentially mortality-causing methods. Thus, such non-invasive methods should be used if possible since none have been shown to negatively affect sturgeon behavior. Such methods can also be used in advance of traditional netting efforts to increase likelihood of success and reduce effort and potential for gear loss.

Training requirements

Safety of researchers and of the sturgeon being collected is of paramount importance. When large fish are taken, the fish itself can do considerable damage to gear and collectors if not handled correctly and quickly. In addition to these issues, the safe handling of each specimen once collected is essential. In order to safely handle large Atlantic sturgeon, the presence of experienced individuals and a crew with predefined duties is recommended to minimize risk to both collectors and fish. Due to inherent risks to both fish and human collectors during collection efforts, all researchers who wish to conduct collection efforts are strongly encouraged to participate in hands on collection efforts with an experienced researcher before conducting independent efforts. Sampling with non-invasive methods requires training only in order to effectively operate the equipment and understand the results. However, sampling with gill nets, trammel nets, and trawls should only be conducted by researchers with sufficient levels of experience, which will ideally be specific to a river system or region.

Resuscitation

At times, when Atlantic sturgeon are removed from fishing gear they appear to be non-responsive. It is often possible to resuscitate these fish by flushing water, preferably oxygen enhanced, over the gills until recovery is obvious from the fish's desire to escape. This procedure is most effective when carried out while the fish is inverted. A form of restraint may be necessary if fish size and conditions warrant caution. Resuscitation should be attempted on all non-responsive fish until such time as it is determined to be ineffective. In some cases, non-responsive fish have been tethered by the tail in local waters overnight and found in the morning to be recovered, so this process can be protracted.

Handling methodologies

Short-term holding

It is frequently necessary to hold sturgeon for short periods while fishing nets, tagging or collecting tissue samples. Various methods have been developed for holding captive sturgeon for processing depending on fish size and number caught.

Net Pens. When water quality is acceptable portable net pens are a good option for holding fish for processing. Mesh sizes should be large enough to allow for the free exchange of water but small enough to prevent entanglement of all sized fish being sampled. It is preferable to use nets with knotless webbing and have a means of covering to prevent sunburn of captive fish. It is not recommended that fish be held for extended periods of time when water temperatures are high. Thermal maximums may be different for northern and southern populations based on their acclimation to local water temperature regimes and rate of temperature change fish are exposed to during collection and holding (Ziegeweid 2006) (see discussion of thermal tolerances on page 11).

Holding Tank: Holding tanks should be designed to accommodate the size of the fish being worked on and follow guidelines provided in the Atlantic sturgeon culture manual (Mohler 2004). When fish are held on board a research vessel, they should be placed in tanks with a flow through water supply that allows for total replacement of the water volume every 15-20 min or in static bath with oxygen supplementation. Static bath water should be exchanged to maintain water temperature (Mohler 2004) and quality. A sump pump can be equipped with a long (20ft) hose to allow collection from deeper waters. While total water volume in the tanks is not critical, it should be sufficient to entirely cover the fish and allow for adequate control of temperature and oxygen levels. Oxygen saturation must not exceed 110%; this can result in oxygen receptors on the gills sending a message to the brain to slow down respiration and thereby cause build up of CO₂ (hypercapnia), which can be lethal (Molly Webb, Bozeman Fish Technology Center, pers. comm., 2003, in Golder Associates Ltd 2006, Crocker and Cech 1996). If pure oxygen cylinders are being used to augment ambient oxygen levels it is important to have a means for frequently or constantly measuring "in tank" oxygen levels to minimize environmental stress.

In static bath holding tanks osmotic stress can be relieved somewhat by the addition of 0.25 to 0.5% uniodized salt without anticaking agents (Brian Richardson, Maryland Department

of Natural Resources, pers. comm.) for non-ripe broodstock. Ripe, female broodstock should not be exposed to a salt bath due to unknown effects on egg maturation (Mohler 2004).

Tethering: Tethering should be a holding method of last resort as the possibility exists for removing the mucus and causing abrasions that can result in post handling fungal and bacterial infections (Brian Hickson, U.S. Fish and Wildlife Service, pers. comm.), and has been associated with elevated stress (Axelsen and Mauger 1993, according to a review by Dick et al. 2006). If tethering is used, it should be only as a short term method of holding and provisions should be made to protect fish from exposure by locating them in areas protected from sun and cold. When tethering in a tidal zone, care should be taken to ensure lines are long enough to allow the fish to stay on the bottom.

When multiple large fish are collected that cannot be safely held in a net pen or be moved into an onboard holding tank, then tethering may be an option that can be used (Figure 5). Golder Associates LTD (2006) describes the procedure used for tethering sub-adult white sturgeon - “This tether line (2.5 cm thick soft-lay cotton rope) is placed around the caudal peduncle of captured white sturgeon. A ‘hose noose’ (i.e., rope fed inside a garden hose to prevent rope-on-skin abrasion) can also be placed around the pectoral girdle, so that one crewmember can maneuver the fish, while another member lifts the tail and places the tether line around the caudal peduncle. The tether should be snug enough around the tail to prevent the fish from escaping, but not so tight as to cause abrasion.”

To minimize potential damage to fish, fishermen participating in reward programs should be provided with training to safely handle fish and supplied with a portable net pen or dockside holding tank to minimize handling stress if the maximum window for holding captured fish continues to be 24 hrs (Brian Richardson, Maryland Department of Natural Resources, pers. comm.).



Figure 5. Tethered white sturgeon (photo credit: Golder and Associates LTD 2006).

Stretcher: A hooded stretcher is commonly employed to handle large fish (Figure 6). Guidance for construction of a stretcher for handling broodfish can be found in Conte et al.

(1988). Generally, the fish is placed in the stretcher with the ventral surface facing up and head inserted into a hooded chamber that has a drain. The stretcher can be tethered to the side of the boat in the case of very large fish and field samples collected. Smaller fish can be lifted on board and placed in stretcher holder (See Mohler 2004). Aerated water is pumped using an aquarium style sump pump from an appropriate source through a plastic tube into the sturgeon's mouth. Flow is adjusted to a consistent outflow from both opercula. The stretcher holder should be designed with sufficient pitch to allow water to drain toward the hood. Fish should not be held in excess of 1 hour in the stretcher. 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 (Mohler 2004).



Figure 6. A hooded stretcher (photo courtesy of Jerre Mohler, U.S. Fish and Wildlife Service).

Fish are often immobilized and calmed by being placed in the recumbent position (Mohler 2004). If further anesthesia is required, a recirculating aerated anesthesia solution can be prepared in a five gallon bucket placed under the stretcher drain. Anesthesia solution should be prepared and administered according to guidance supplied in this manual, and should be appropriate for the procedures to be conducted (see section detailing laparoscopy procedure and section on anesthesia).

US Navy immobilization evacuation stretchers have been successfully modified for sturgeon handling and surgical transmitter implantation (Chris Hager, VA Sea Grant, Marine Extension Program, pers. comm.) (Figure 7). These stretchers immobilize the sturgeon by physically restraining it in a stretcher containing rigid sides. Adjustable belts and an internal jacket minimize movement and flaps allow access to the sturgeon for surgical procedures. This apparatus can also be sized with the addition of flexible foam to accommodate smaller fish.



Figure 7. Atlantic sturgeon in an immobilization stretcher (photo courtesy of Chris Hager, VA Sea Grant, Marine Extension Program).

When a captured Atlantic sturgeon has been processed, ensure that the swim bladder is deflated prior to release. The Moser protocol states that “Sturgeon are physostomous and tend to inflate their swim bladder when stressed and in air. If this occurs, efforts should be made to return the fish to neutral buoyancy prior to or during release. This can often be achieved by propelling the fish rapidly downward during release. If the fish still has air in its bladder it will float and be susceptible to sunburn or bird attacks. Often the remaining air can be released by gently applying ventral pressure in a posterior to anterior direction.” Swim bladders can also be manually deflated using techniques as described in the laparoscopy section (below).

When sampling for sturgeon downstream of dams, be aware that while the dams are generating, total gas pressure (TGP) of the water may be elevated. This condition can cause stress and in extreme cases gas bubble disease. When fish are retrieved from the depths in these conditions, mortality may result; therefore, researchers should test %TGP in locations and times when expected to exceed 100%, and should exercise caution by scheduling sampling for times when TGP is low, especially if sampling for larval sturgeon. Counihan et al. (1998) found that larval white sturgeon exposed to 118%TGP developed gas bubble disease within 15 minutes of exposure but no mortality was associated with the disease during the 10 day exposure. At 130% TGP survival at 13 days post hatch was only 50%.

Training requirements

Training should consist at a minimum of reading the relevant handling materials included in this manual and working with an experienced researcher until comfortable with fish handling techniques. Participation in an AFS sturgeon field handling technique course could substitute for working with experienced researcher. Review of the Atlantic Sturgeon culture manual is recommended.

Anesthesia

Surgical procedures requiring incision into the body cavity of sturgeon or other activities which require fish to be immobilized should be preceded by administering an appropriate sedative to the sturgeon. With regard to Atlantic sturgeon, three sedatives were evaluated by Mohler (2004): (1) Metomidate (current trade name: “Aquacalm” by Aquatic Life Sciences, Inc., Ferndale, WA); (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). Because isoeugenol, a constituent of clove oil, is thought to be carcinogenic, its use on food fish is prohibited (AADAP 2008), and clove oil should not be used on Atlantic sturgeon.

In a U.S. Fish and Wildlife Service study, the appropriate dosage for metomidate and MS-222 at water temperatures from 5-15° C was defined as the concentration necessary to sedate fish in 3-4 minutes and allow recovery in 3-4 minutes, with the exception of metomidate, which required longer recovery times regardless of concentration tested (Wade Jodun, U.S. Fish and Wildlife Service, pers. comm., in Mohler 2004). In general, sturgeon took longer to recover at colder water temperatures. Metomidate-treated fish required the lowest dosage (15 mg/L as opposed to 200 mg/L MS-222) but took the longest to recover overall (>700 seconds at either temperature), and it has been separately noted that metomidate at the same concentration often fails to induce stage 4 (surgical phase) anesthesia (Mark Matsche, Maryland Department of Natural Resources, unpublished data). Sturgeon (500-1000 g) were separately exposed for 20 minutes to the given dosages at water temperatures from 5-15° C with no mortality. For larger sturgeon (6-7 kg), the same dosages apply but recovery times can greatly exceed those for smaller individuals.

For sub-adult and juvenile sturgeon, 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 (See Figure 8). This stretcher assembly may be designed to allow the delivered anesthesia to drain back to a reservoir and be used continuously in a recirculation fashion. If recirculation of anesthesia is used, an individual not directly involved with the surgery 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 circulation system to deliver fresh water once respiration frequency slows considerably. It should be noted that under anesthesia, opercular movement can cease or be reduced to nearly imperceptible levels for Atlantic sturgeon (unlike many other fishes), and it is suggested to use other monitoring methods in combination with observing opercular movement (such as heart rate) to ensure the safety of anesthetized fish. For this reason, it is also important to follow the recommended anesthetic dosages.

Dosages of MS-222 should be administered in a manner that is appropriate to the research techniques being utilized. For surgical implantation of internal tags and procedures requiring similar incisions, sutures, and holding time, a simple anesthetic bath containing ambient water and MS-222 at 50-100 mg/L is recommended (this range of MS-222 dosage was successfully used on Gulf sturgeon in Fox et al. 2000). A small amount of electrolyte treatment

(approximately .25 ml of electrolyte treatment for every liter of river water, Gayle Zydlewski, University of Maine, pers. comm.) can be useful to preserve the protective slime coat on sturgeon. The concentration of MS-222 suggested for surgical implantation of transmitters should be sufficient to anesthetize the fish and eliminate any observable response during the procedure while allowing for rapid post-operative recovery; thereby, minimizing holding time.

For more invasive procedures (e.g., laparoscopy) that require prolonged surgical phase anesthesia, the anesthetic protocol detailed in the laparoscopy section of this document may be appropriate. When it is necessary to place sturgeon under prolonged, deep anesthesia, it is recommended that procedures be conducted in a controlled laboratory setting. Physiological stress from prolonged periods of restraint required for some procedures should be avoided if possible through the use of anesthesia (AFS 2004).



Figure 8. Sturgeon being anesthetized (Photo courtesy of Jerre Mohler, U.S. Fish and Wildlife Service).

Although other anesthetics have been tested on sturgeon (Mohler 2004), only MS-222 is currently approved for use in the field. In low pH conditions, it is advisable to check the pH of the anesthesia bath and adjust as necessary with sodium bicarbonate since MS-222 is a hydrochloride, and can acidify water.

Laparoscopy and related technologies, non-invasive procedures, and traditional methods of assigning sex

Laparoscopy

Laparoscopy is a valuable surgical technique for examining the internal anatomy in sturgeon and has gained popularity in recent years since it is less invasive and provides a superior view of internal anatomy than more traditional biopsy techniques which often require large abdominal incisions. For Atlantic sturgeon, laparoscopic procedures are mostly used to determine sex and degree of sexual maturity, but can also be used to observe other anatomical features for reasons of fish health. Laparoscopy requires specialized surgical equipment and

should only be performed by individuals who are experienced or have had adequate training in laparoscopic techniques and anesthesia application. An experienced individual can determine sex and degree of sexual maturity in as little as 15 minutes per fish if no unforeseen complications arise. A minimum of two people are necessary to perform laparoscopy on sturgeon. Mature broodstock are often 2 meters in length and often require more than two people to be safely handled.

Laparoscopic equipment is of medical quality, requires a source of 110 Volt power, and is most suitable for use in controlled conditions with protection from the elements. The basic components of the system include:

- a) A stretcher or surgery table equipped with an anesthesia delivery system large enough to accommodate the fish being examined
- b) A light source with a flexible fiber-optic cable
- c) A 6mm stainless steel, rigid telescope which attaches to the fiber-optic cable
- d) A hollow cannula which is just large enough in diameter to permit insertion of the telescope. The cannula is equipped with a cutting tip and exterior threads which allow it to be screwed through the abdominal body wall of the sturgeon.
- e) A small video camera attached to the eyepiece of the telescope
- f) An LCD monitor upon which the internal anatomy is displayed
- g) A small air pressure/vacuum pump with flexible air lines
- h) A Verres insufflation needle
- i) Surgical supplies to make and eventually close the small incision (scalpel, suture material, needle holder, forceps, scissors, betadine antiseptic). PDS monofilament sutures have been recommended for sturgeon (Mohler 2004, Matsche and Bakal 2008).

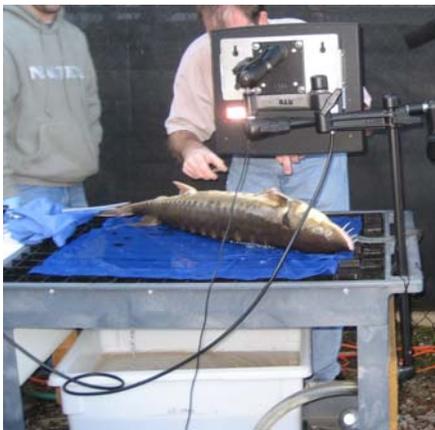


Figure 9. Laparoscopy

The standard operating procedures and details for performing laparoscopy on Atlantic sturgeon found have been described (Mohler 2004, Matsche and Bakal 2008); both citations recommend the following:

- 1) Prepare the area where the procedure will be done, and assemble the laparoscopy equipment.
- 2) Prepare anesthesia. There are 2 options for anesthetizing the sturgeon in preparation for

laparoscopy: (a) use of a tub or other container large enough to immerse the entire fish to be anesthetized or (b) use of a stretcher assembly outfitted with a pump to recirculate anesthesia across the gills of the subject fish. For large, mature fish, a stretcher assembly with re-circulating anesthesia system is sometimes more desirable since a much smaller total volume of anesthesia is required. Regardless of which option is used, prepare an “induction” dose of MS-222 anesthesia at 250 mg/L which is buffered with baking soda at 500 mg/L. *Note** Do not dry-mix the MS-222 and baking soda for any length of time before its use as the effectiveness of the MS-222 may be diminished.* Also prepare a separate “maintenance” dose of MS-222 at 87mg/L buffered with 175 mg/L baking soda in preparation for the laparoscopy procedure.

- 3) Administer the induction dose of anesthesia. Once the sturgeon has been immobilized (refer to Matsche and Bakal 2008 for appropriate measures of response), the fish should be placed on the operating platform/stretcher with the maintenance dose of anesthesia re-circulated across the gills to keep the fish immobile during the procedure. Always make sure that the dissolved oxygen levels are maintained between 8 – 15 mg/L in the anesthesia solutions. One assistant should be designated to monitor the vital signs (i.e., opercular movement and heart activity). Opercular movement will slow considerably under anesthesia but if all movement ceases, the fish should be quickly prepared for removal from the operating table/stretcher and resuscitated by gently moving the fish back and forth in an upright position in a tank of untreated water to flush the gills until rhythmic respiration is resumed.
- 4) Make a small incision about 6-7 mm in length into the abdominal wall (Matsche and Bakal 2008). For sex determination, the fish should be ventral side up with the body tilted somewhat so that the gonad will fall away from the body wall slightly to facilitate the sex determination. Look for a location on the fish’s right side between the 3 and 5th scute anterior of the pelvic fins and offset and from the abdominal mid-line to make the incision. **Tip** -Look for a favorable surface location which has fewer visible inclusions of dermal bone so that it will be easier to make and close the incision. The incision does not have to be completely through the body wall but should be large and deep enough so the tip of the threaded cannula will begin screwing itself through the body wall with its cutting tip as it is inserted in the incision and twisted.
- 5) Screw in the cannula. **Tip** -Once the cannula begins to screw in, a slight upward pressure on the cannula while twisting may prevent damage to the intestines or other organs as it breaks through the abdominal wall and into the body cavity. Experience will allow you to determine when the cannula has finished cutting through the body wall and enters the cavity as you will feel less resistance when the tip breaks through. Alternatively, the telescope can be inserted into the cannula during entry to visualize the progress (Matsche and Bakal 2008).
- 6) Insert the telescope. As you slide the telescope down through the cannula you should begin to see some internal anatomy. If the view is obstructed, you may not have screwed the cannula in far enough to break through the body wall. If you can see some internal anatomy but the view is cloudy, the lens of the telescope may be smudged and should be wiped off before proceeding. If the view is still obstructed, you may need to insufflate the body cavity

and/or deflate the swim bladder to create more open space.

- 7) Insufflation of the body cavity can be achieved by attaching a low pressure air line to the air fitting on the telescope and introducing atmospheric air at no more than 1 L/min. via a low pressure pump. Air pressure should be less than 14 mmHg to prevent embolism or gas bubbles in the vascular system, which will lead to rapid death of the sturgeon. Because of this possibility, *insufflation should be used sparingly*.



Figure 10. Insertion of cannula and insufflation technique.

- 8) Determination of sex. Gonads lie adjacent to each side of the body wall and are examined for condition and texture for sex assignment (Bruch et al. 2001, Mohler 2004).
- 9) Closure of incision. Normally, a single suture can be used for closure of laparoscopy incisions or punctures made with the Veress needle. Necessary instruments and other details concerning incisions closure are found in Matsche and Bakal (2008) and Mohler (2004).



Figure 11. Suturing the incision.

- 10) Post-surgery recovery. Move the sturgeon to its culture tank or back into the natural water supply and orient the fish upright using a gentle back and forth motion to flush water across

the gills. Once the fish is able to maintain itself in an upright position, it can be released. Normal recovery time is around 20 minutes but will likely be longer at cooler temperatures (Mohler 2004).

Traditional Methods of Assigning Sex of Atlantic Sturgeon

In circumstances where laparoscopy equipment is not available yet sex must be determined, additional options may include: (1) traditional surgical biopsy (coeliotomy); (2) expression of sexual products; (3) blood analysis for sex-specific hormones; (4) ultra-sound imaging; (5) use of meristic indices; and, (6) shape of the urogenital opening. Each procedure is briefly explained below:

- 1) Coeliotomy- This technique requires an incision though the ventral body wall large enough to view internal organs with the naked eye. This procedure is very invasive and should only be performed by individuals whom have had adequate training in both surgery and sex identification. This procedure is limited to larger fish (10 kg or larger) with visually differentiated gonads. Insert a blunt probe into the exposed coelom for manipulation of the gonad into a position where the germinal tissue can be viewed (Mohler 2004). This can be a difficult procedure because the germinal portion of the gonad lies against the lateral body wall. For fish large enough to be sexually mature (about 130 to 150 cm in Atlantic sturgeon) it is relatively easy to identify sex with this technique because of advanced gonad development. Nonetheless, it remains highly invasive with increased risk of fish mortality when performed by untrained individuals. This technique will not provide an immediate analysis in young fish (< 9 kg) as they do not possess visually differentiated gonads (Joel Van Eenennaam, University of California, Davis, pers. comm.). For these small fish, instead prepare a tissue sample of the gonad for histological determination.



Figure 12. Immature male – solid, smooth white strip of spermatic tissue visible surrounded by orange colored adipose tissue.



Figure 13. Immature female. Pinkish, grooved ovarian tissue visible surrounded by orange colored adipose tissue.



Figure 14. Dissected section of immature male (top) and female (bottom) gonad.

- 2) Expression of sexual products- If mature, wild individuals are captured during spawning migration, sexual products may be expressed from the genital opening, especially in males (Mohler 2004). For wild females it is rare, but possible, to obtain ovulated eggs from the genital opening. In order for this to be the case, the female would likely have been captured in the process of egg deposition. In the hatchery, suspected mature individuals can be injected with spawning hormones as per Mohler (2004). Mature males will spermiate if injections are performed at the proper time. Individuals expressing milt can be positively identified as males, but injected individuals which did not express milt cannot necessarily be assigned female sex due to the fact they may still be immature males.
- 3) Blood analysis for sex-specific hormones- This procedure requires that a blood sample be taken from the caudal vein and sent to a laboratory for analysis of the blood plasma

hormones estradiol and testosterone via a procedure known as radio-immuno assay (Webb et al. 2002). Obviously, this procedure does not give immediate results for sex assignment but can be valuable for determining sex and level of maturity in hatchery fish or analysis of the reproductive condition of wild populations. Blood samples must be collected, processed, and taken for analysis the same day. A centrifuge and means of keeping samples chilled are also needed along with specific techniques to obtain and ship the samples (Matsche and Bakal 2008).

- 4) Ultrasound imaging – Advancements in technology have made it possible to use portable ultrasound imaging equipment for non-invasive sexing of sturgeon. Ultrasound imaging has been shown to be effective for determining the sex of the stellate sturgeon *Acipenser stellatus* (Moghim et al. 2002). In a study with shovelnose sturgeon *Scaphirhynchus platorynchus*, Colombo et al. (2004) concluded that ultrasound imaging was 86% accurate and could be applied to other species of Acipenseriformes. However, ultrasound equipment costs are relatively expensive at this time.
- 5) Meristic indices – Currently there are no meristic indices which can be used to determine sex specifically for Atlantic sturgeon, but Mal'tsev and Merkulov (2006) report a biometric measurement scheme using the head of the Russian sturgeon *A. guldenstaedtii* to permit sex determination in live sturgeons. As such, a discriminate equation is used to indicate the sex of individuals in a wide size-age range, irrespective of the maturity stage of the gonads. Given a sufficient number of measurements, it is likely that such an index could be developed for Atlantic sturgeon via discriminant function analysis software.
- 6) Shape of urogenital opening – Vecsei et al. (2003) reported 82% accuracy in determining sex of live white, green, Atlantic and shortnose sturgeon using the shape of the urogenital opening. Males were found to have a Y shaped opening whereas the openings in females were in the shape of the letter O.

Training requirements

Mortality of research fish is less likely if individuals who wish to perform techniques such as laparoscopy or coeliotomy receive hands-on training with an experienced instructor using live fish. Expression of sexual products requires only a short amount of instruction from an experienced individual but since live, ripe fish are difficult to have available for training, preserved or dead specimens could be substituted. Taking blood samples for the purpose of possible sex determination also requires hands-on training with live fish but mostly for success in locating the proper area and technique for extraction of the sample rather than prevention of fish mortality.

Ultrasound imaging and meristic evaluations only require training in the use of the equipment and techniques but for proper sex determination, some training would be required from an experienced individual present. It would likely be possible to use fresh-dead or preserved specimens of known sex for this training.

Gastric lavage

A variety of techniques for non-lethal sampling of stomach contents have been developed, but gastric lavage is recommended. Gastric lavage is relatively cost effective, non-labor intensive and is reasonably safe and effective (Seaburg 1957, Foster 1977, Meehan and Miller 1978, Light et al. 1983, Haley 1998, Savoy and Benway 2004, Wanner 2006, Savoy 2007, Shuman and Peters 2007). Only two papers have noted negative effects. Brosse et al. (2002) reported a statistically significant higher weight loss of lavaged sturgeon (7.97%) over a control group (5.84%), but all of the fish held lost weight over the 60 day holding period, indicating the presence of an additional stressor and confounding the results of that study. While Sprague et al. (1993) reported that 4 of 12 fish pumped died within 1 week of lavage from apparent water pressure damage, they provided few details on the technique, their experiment or holding process.

Anesthesia: Haley (1998) and Savoy and Benway (2004) reported success while utilizing anesthesia (MS-222) during gastric lavage, as it is thought to relax muscles in the sturgeon and aid insertion of the lavage apparatus. Although this automatically extends handling time, use of anesthesia is encouraged to minimize the risk of injury during the procedure.

Required use of anesthetics for lavage is still a question for debate. Anesthetics are only discussed in this section as they pertain to the lavage process. Additional information on anesthetics and their uses/limitations can be found in the Anesthesia section. The Moser Protocol and some sturgeon researchers utilize anesthetics to calm fish and ostensibly to aid in insertion of the lavage apparatus into the pharynx. Use of chemical anesthetics automatically extends handling time to allow for fish to succumb and to recover from the anesthesia and requires additional space in which to anesthetize and recover fish. Additional concerns of the utilization of chemical narcotics involve the specific water chemistry where work is being conducted, and interactions with the desired chemical agent. Working with salinities greater than approximately two parts per thousand or elements/solids/chemicals in solution may render some anesthetics ineffective, and MS-222 may become toxic to fish when in solution with seawater and exposed to sunlight (Bell 1987). Lastly, researchers must take care to follow proper handling and disposal of treated water. Some researchers have noted that rolling sturgeon (and other fish) onto their backs calms them down enough to allow handling. It must be noted that this is not an absolute and some fish may react strongly. In addition, v-boards or props should be used to keep fish inverted and well supported all along the fish's length. Given the size and strength of Atlantic sturgeon, restraints are advised for fish over 1 m TL to prevent injury to both fish and researcher. Ultimately, it must be noted that current information available about use of anesthetics, topically applied or otherwise is equivocal as to whether they aid in carrying out the lavage procedure.

Tube diameter and flexibility: Relatively flexible small diameter tubing is an essential part of this procedure. A small diameter tube (on the order of 2.0 mm outside diameter) is to be used for the average to mid-sized sturgeon (75.0 to 150.0 cm FL) to be lavaged, and smaller diameter tubes for the correspondingly smaller, immature fish. The flexibility of the tubing is an aid to prevent forcing the tubing through the walls of the alimentary canal. Aquarium tubing and the like should not be used owing to their stiffness. Haley (1998) specifically recommended intramedic type tubing over others due to its ductile nature and small diameter. The leading edge of the tubing should have all sharp edges blunted through heating or other manual means. While the flexibility of intramedic tubing seems to protect sturgeon from injury, it can take several attempts to get the tubing into the esophagus instead of curling around and exiting the mouth or through the gills. While unproven, forcing water out of the tubing while inserting the tubing into

the alimentary canal is intuitively effective and should assist in allowing the tubing to enter the canal and prevent puncturing the walls of the canal by the tube. This may be an alternative to the anesthetic, which Haley (1998) noted might be required to aid in relaxing the muscular gizzard region of the alimentary canal of sturgeon. Researchers must take all care to prevent forcing the tubing into the fish and thus, causing damage. Gently moving the tube in and out while pumping seems to enhance the effectiveness of regurgitation. Some researchers have utilized a large diameter tube to aid in inserting the flexible small diameter tube down the esophagus; using it as a sleeve to assist in getting the highly flexible small diameter tube past the oral cavity. This technique works well for some researchers and better for some species. Atlantic sturgeon have relatively narrow mouth widths for their size so it is unclear if the two tube technique is applicable.



Figure 15. Gastric lavage (South Carolina Department of Natural Resources).

Water delivery device: A variety of water receptacles and means of forcing water into the stomachs have been employed: syringes, garden sprayers (approx 2.5 gallon), hand operated and electric pumps. Regardless of the water delivery device utilized, it should allow researchers the ability to limit the amount of pressure in forcing water into the fish given the fragile nature of internal organs. If high volume or pressure pumps are used, a flow/pressure restricting device is imperative, although it can not be stated what the upper pressure/volume limit is at this time. Positive results have been noted for both continuous water flow and pulsed or interrupted flow. No specific requirements can be made at this time without further directed study, particular to Atlantic sturgeon. Additional studies also need to be made on the effects of internal water chemistry, i.e. changing the osmotic balance of fish after introducing volumes of water to internal organs where fluid/chemical uptake is possible. Only Shuman and Peters (2007) have examined water chemistry after exposing shovelnose sturgeon to pulsed gastric lavage, and they report no negative effects. Additional research on Atlantic sturgeon specifically, is needed.

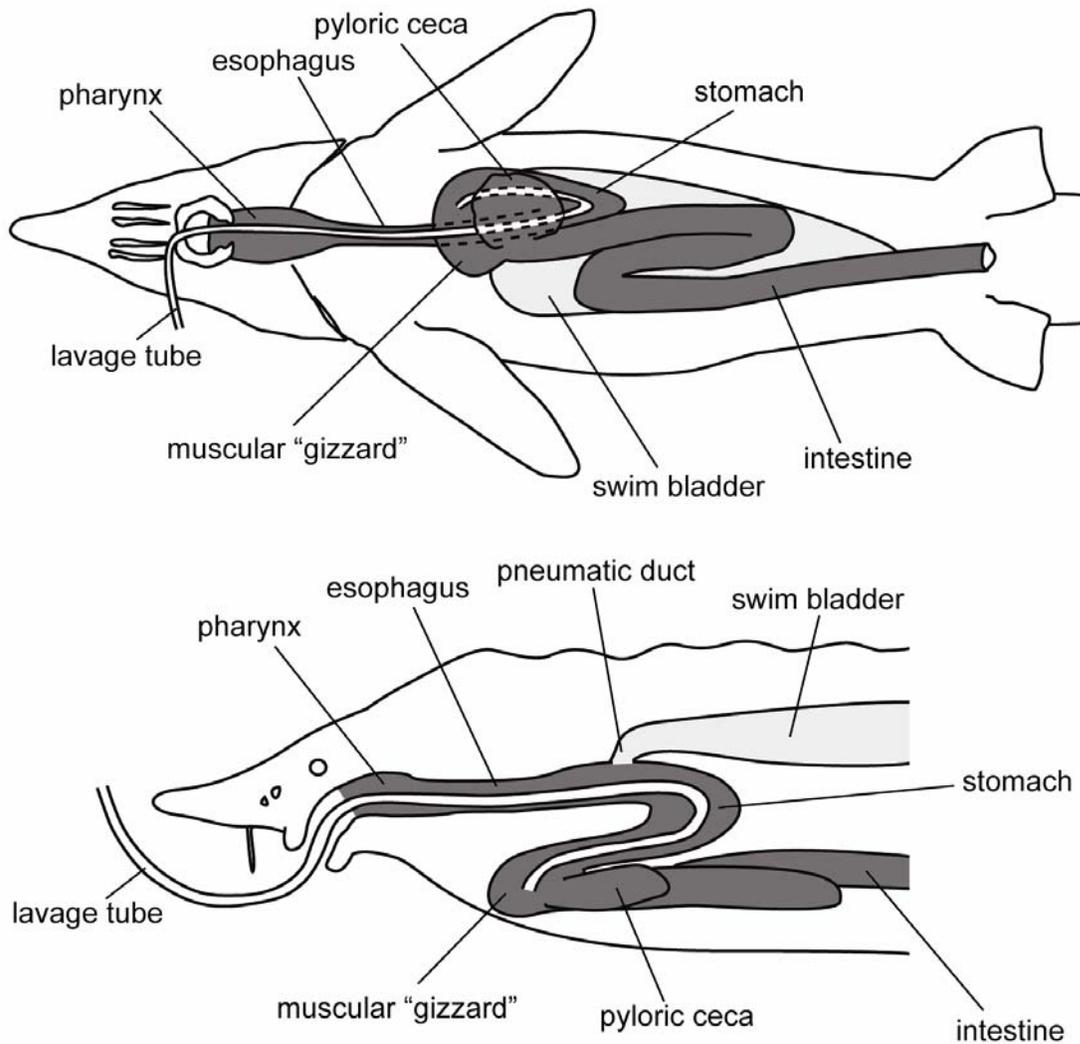


Figure 16. Generalized depiction of the gastric lavage technique for Atlantic sturgeon.

Temperature restrictions: Few specific temperature restrictions have been noted in published reports, but this may be a function of the small volume of work performed to date and general lack of work at temperature extremes. Shuman and Peters (2007) suggest water temperatures above 30°C may be problematic. Conducting lavage under freezing weather conditions may present unique dangers to the fish. General guidelines discourage exposure of fish to air temperatures below freezing for more than a couple of minutes rendering lavage ineffective as it would be difficult to collect stomach contents while keeping a sturgeon submerged in water. Highly stressed individuals (from temperature change/capture stress or other means) should not be subjected to lavage techniques.

Training requirements

Gastric lavage is a relatively simple but moderately invasive technique, and at a minimum, observation of an experienced individual and a review of Haley (1998) should be completed prior to performing gastric lavage on live Atlantic sturgeon. Brosse et al. (2002) and

Buddington and Christofferson (1985) can be consulted for rough anatomy of the digestive tract of sturgeon as it pertains to lavage. Gastric lavage is likely to only remove those prey items in the esophagus and stomach or material located anterior to the gizzard. Although, digestion rates of sturgeon are generally unknown (Buddington and Christofferson 1985), the prey contents flushed from the beginning of the alimentary canal should probably be considered as recently eaten. Some polychaetes retrieved from shortnose sturgeon were noted to still be alive upon examination in the lab, several hours after the lavage (Savoy and Benway 2004).

Tagging

PIT Tagging

A number of Atlantic sturgeon population studies use passive integrated transponder (PIT) tags to provide long term marks. These tags are injected into the musculature below the base of the dorsal fin and above the row of lateral scutes on the left side of the fish (Eyler et al. 2009), where sturgeon experience the least new muscle growth. Additional safeguards to ensure long term retention include using a single suture or adhesive to close the PIT tag insertion wound, and insertion of PIT tags in an anterior to posterior direction is thought by some researchers to reduce the likelihood of tag loss. It is recommended that the needles and PIT tags be disinfected in isopropyl alcohol or equivalent rapid acting disinfectant. Tags should be inserted antennae first in the injection needle after being checked for operation with a PIT tag reader. Fish should be examined on the dorsal surface posterior to the desired PIT tag site to identify a location free of dermal scutes at the injection site. The needle should be pushed through the skin and into the dorsal musculature at approximately a 60 degree angle. When in the musculature adjust angle of needle to close to parallel and push through to the target PIT tag site, then inject the tag. After withdrawing the needle the tag should be scanned to check operation again and tag number recorded. Some researchers check tags in advance and place them in individual 1.5 ml microcentrifuge tubes with the PIT number labeled to save time in the field. Due to the previous lack of standardization in placement of PIT tags, we recommend that the entire dorsal surface of each fish be scanned with a PIT tag reader to insure detection of fish tagged in other studies. Due to the long life span and large size attained, Atlantic sturgeon may grow around the PIT tag, making it difficult to get close enough to read the tag in later years. For this reason, full length (highest power) PIT tags should be used. PIT tags far out perform external tags which are known to experience high shedding rates. However, laboratory studies indicate that sturgeon smaller than 200 mm TL shed PIT tags at a rate of over 50%, due to the less developed nature of the musculature at this size (Moser et al. 2000). Recent studies at the Bears Bluff National Fish Hatchery on shortnose sturgeon found that fish with a good condition factor could be tagged by a skilled operator with short (11.5mm) and long tags (14mm) at a minimum size of 300 mm TL with good survival (97%) and tag retention (98%) at 180 days post implant. There were no significant differences between cranial and dorsal implant of tags on weight gain or condition factor. Fish smaller than 250 mm were tagged only in the cranial location by necessity; retention rates were high (97%) but survival lower (70%) (James Henne, U.S. Fish and Wildlife Service, unpublished data). Due to these high observed mortality rates and desire for consistency in implant location, sturgeon should not be tagged in the cranial location, and until safe dorsal PIT tagging techniques are developed for sturgeon <300 mm, only

fish >300 mm should receive PIT tags. Sutures were not used to close the PIT tag insertion point during this study (James Henne, U.S. Fish and Wildlife Service, pers. comm.).

A paper by Fuller et al. (2008) on the performance of commercially available PIT tag systems provides guidance on the quality of currently available tags and readers and provides recommendations on the most flexible systems that can be integrated into existing research efforts while providing a platform for standardizing PIT tagging programs for Atlantic sturgeon on the East Coast. The results of this study were consulted to assess which PIT tags/readers should be recommended for distribution. To increase compatibility across the range of these species, the authors currently recommend the Destron TX1411 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). The FWS/MFRO will collect data in the coastal tagging database and provide approved tags for distribution to researchers.

Golder Associates (2006) reported on preliminary trials with a remote, underwater PIT tag reader equipped with an external antenna that successfully energized and recorded PIT tag numbers at a location baited to attract juvenile white sturgeon. When refined this tool may prove valuable for studying seasonal habitat use and movement.

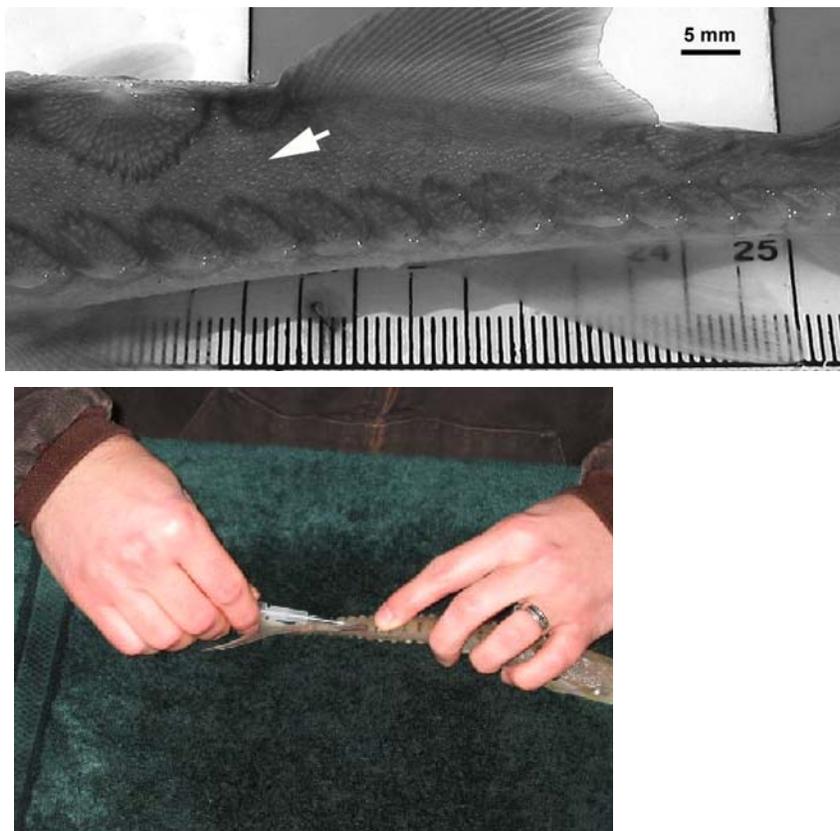


Figure 17. Illustration of PIT tag location (indicated by white arrow; top), and photo of a juvenile Atlantic sturgeon being injected with a PIT tag (bottom). Photos courtesy of James Henne, U.S. Fish and Wildlife Service.

Scute Marking

Scute removal is a technique used by west coast sturgeon researchers as a secondary permanent mark to document fish that have been PIT tagged and injected with oxytetracycline (OTC) for staining hard parts in ageing studies. Rien et al. (1994) validated the technique with white sturgeon in the Columbia River Basin. In a two year study 99% of fish marked by scute removal were clearly distinguishable upon recapture. Trained observers correctly read the mark with 93% accuracy where lay individuals read the mark correctly with 74% accuracy. In sturgeon smaller than 2 ft the scute can be removed using a number twelve curved scalpel blade to cut out the scute. The removal of a lateral scute should be accomplished by cutting in anterior direction starting at the posterior margin and continuing anteriorly. The blade is angled slightly upward so the cutting surface lies against the lower scute surface. When the scalpel reaches the front of the scute the thumb is placed on the posterior face and the scalpel is pulled up to complete the scute removal.



Figure 18. Scute removal (left), and marking and insertion of coded wire tags (right) in lake sturgeon.

The scalpel should be disinfected and allowed to dry between fish. For sturgeon >500 mm, a sharp filet knife can be used for scute removal. If skin is cut to the sub dermis layer, antiseptic ointment should be available to apply to the wound. Fish should be anesthetized with a light dosage of anesthesia i.e. 75-90 mg/L MS-222 to immobilize the fish for this procedure. Fish larger than 300mm can safely be maintained at this dosage for 20 minutes. Care needs to be taken to ensure adequate oxygenation and temperature control in both the anesthesia and recovery chambers. During white sturgeon juvenile relocation into John Day reservoir fish are intramuscularly or intraperitoneally injected with Liquamycin-LP at a rate of 0.2 ml/kg to impart a mark on the pectoral spine for age validation studies (Apperson and Anders 1991, R.L. & L. 1996, Golder Associates Ltd. 2006). To prevent the chemical breakdown of OTC keep the bottles in a cool area, protect them from direct light, and note the expiration date. A secondary benefit of this activity is providing an antibiotic treatment to minimize possible bacterial

infections resulting from fish handling including scute removal. In addition to OTC, US FWS has been granted an Investigative New Animal Drug permit exemption allowing the use of calcein “SE-MARK®” to mark hard parts of fish including sturgeon¹.

The southeastern lake sturgeon reintroduction program has been using scute marking to aid in the identification of year classes of hatchery reared fish. The fish are marked as sub-yearlings (152-350 mm). Some fishery managers recapturing these fish have observed that as the fish grow there is a tendency for migration and joining of scutes adjacent the site of scute removal, making interpretation difficult. Marking sub-yearlings may require removal of multiple scutes to obtain a clear mark or switching from the left to right lateral scute on a rotating cycle. We recommend that a standardized procedure for assigning marks be developed for the eastern populations if scute marking is implemented for coastal populations. Until this is done, scute marking should be used on a case by case basis with mark location being coordinated among researchers. As scutes are thought to play an important role as an anti-predation device, especially in small sturgeon, we recommend that long-term studies designed to assess the impact of scute removal on mortality rates be conducted.

Coded Wire Tags

Coded wire tags (CWT) have been tested in several species of sturgeon with mixed results. Isely and Fontenot (2000) took a novel approach to use of CWT to mark shortnose sturgeon by injecting sequentially numbered tags along the pectoral spine to allow for multiple recoveries during age validation studies. Retention rates were 78% for this placement. In the same study, placement of tags in the dorsal fin base and pectoral fin base were 98% and 96% respectively. Collins et al. (1994) found highly variable retention rates for shallow placement (1-2 mm) of CWT in the snout cartilage of shortnose sturgeon (70-100% over the course of 400 days). Bordner et al. (1990) working with white sturgeon juveniles (150-260g) had 100% retention for CWT placement below the dorsal scute and deep injection (3-4 mm) into the snout cartilage. Shallow placement (1-2mm) resulted in a 60% retention rate over 6 months. MS-222 was used for anesthesia at a dosage of 90mg/L with no adverse affect on growth or survival. CWT injections were conducted without the use of a head mold. Mohler (1994) had 100% retention of CWT when injected underneath the first dorsal scute. As a result of insufficient data regarding effects of inserting CWT in the snout region, and due to the presence of multiple sensory systems, we recommend that CWT be injected underneath the first dorsal scute. The standardization of one location will help researchers in the conduction of broad-scale studies. Peterson et al. (2000) used coded wire tags to study population dynamics and wild juvenile shortnose sturgeon recruitment in the Hudson River system. CWT provided an inexpensive mark

¹ Calcein is currently available for use on potential food fish such as sturgeon under an Investigative New Animal Drug (INAD) permit exemption administered by the U.S. Fish & Wildlife Service. Researchers desiring to use calcein for fish marking must register with the Aquatic Animal Drug Approval (AADAP) office in Bozeman, MT www.fws.gov/fisheries/aadap/calcein.htm to obtain a study number and an understanding of requirements for experimental use of calcein. Requirements for use of calcein on Atlantic sturgeon may differ depending upon Federal listing status of the Distinct Population Segment of interest.

to mass mark hatchery fish for use in mark–recapture studies to provide evidence of decreased recruitment of juvenile wild fish to the system. Coded wire tags have limited utility for marking wild fish in the field due to the expense and complexity of the equipment but may be a valuable tool for specialized studies using juvenile hatchery reared sturgeon that are too small to mark with PIT tags. Field researchers working in regions where these studies are being conducted should have access to a wand to detect CWT's during routine surveys. Because CWT equipment is expensive and has not been widely employed by sturgeon researchers, its use is not recommended for routine studies.

External Tags – (non telemetry)

The use of an internal tag (e.g., PIT) is preferable for long term mark-recapture studies due to higher retention rates (Isley and Fontenot 2000). However, there are occasions when having an external tag allows for identification of Atlantic sturgeon that are unexpectedly encountered by harvesters, the general public, and fisheries professionals. A variety of external tag types (e.g., dart, Carlin, and T-bar) have been used in a numerous locations (base of dorsal fin, scute drilling, and pectoral/pelvic fins) with mixed levels of success (Huff 1975, Carr et al. 1996, Collins et al. 1996). Researchers are encouraged to standardize the placement of external tags as much as possible. If an external tag is desired, researchers should contact the USFWS Maryland Fisheries Resources Office. These T-bar tags should be implanted in a standardized location – based on experience of the authors, it is recommended that T-bar tags be placed at the base of the left dorsal fin musculature. If an existing tag is found in an Atlantic sturgeon at the time of collection, the researcher should pull lightly on the existing tag to estimate the level of attachment. If replacement is warranted, a new tag should be affixed (at the base of the left dorsal fin musculature). All data from both PIT tag and T-bar tagged individuals should be provided to the USFWS Atlantic sturgeon tagging database at the conclusion of the sampling season for inclusion into the coast-wide tagging database.

Biotelemetry

As a result of high cost and difficulty in collecting Atlantic sturgeon, researchers conducting telemetry studies are often concerned with long term attachment/implantation of transmitters. Recent advances in battery/transmitter design have lead to longevities exceeding 10 years. This increase in the longevity of transmitters when coupled with the highly migratory nature of Atlantic sturgeon makes the issues of system compatibility and tag code collision/redundancy an important issue that researchers must consider when designing and implementing telemetry studies. Researchers interested in conducting multi-year studies are encouraged to utilize internal implantation of transmitters (Dovell  Berggren 1983, Moser and Ross 1995, Zehfuss et al. 1999, Fox et al. 2000) to increase the likelihood of transmitter retention/attachment. Both anecdotal and published results (e.g., Smith et al. 1990, Savoy 1991, Zehfuss et al. 1999, and Fox et al. 2000) have shown that attachment rates for external transmitters are lower than internal transmitters. Advances in casting materials used for transmitters have reduced rejection rates, but researchers who are concerned with maximizing retention rates may use a biologically inert compound (e.g., Silastic® Dow Corning) which is thought to reduce tissue reactivity and expulsion rates (Boyd Kynard, USGS Conte Anadromous

Fish Lab, pers. comm.). Another low cost solution may be found in the use of bee's wax, although reactivity rates for it are not well understood. 

Although retention rates of externally attached radio and acoustic transmitters have been shown to be lower (Zehfuss et al. 1999), there are occasions where external attachment may be warranted. Where planned research activities are scheduled for less than 4 months, researchers should consider external attachment, as it requires a less invasive procedure. Additionally, the use of pop-off satellite transmitters and archival tags requires external attachment for proper functioning. A number of techniques have been used to attach external tags including attachment at the base of the dorsal fin (Erickson and Hightower 2007, Erickson et al. in review) and by drilling holes through a dorsal scute (Edwards et al. 2007). In both the dorsal fin and scute attachment methods, the use of a backing plate coupled with a sheath around wires/cables should be used to minimize irritation and maximize retention times.

Fisheries scientists using radio or hybrid (radio/acoustic) transmitters, which necessitate an antenna for signal transmission, should consider antenna placement. Signal transmission values are maximized by trailing antennas, but the rate of infection and irritation via the opening in the body wall are considered greater than with internal antenna (coiled and trailing). Researchers are urged to consider the need for signal transmission values when considering either external or trailing antennas. Trailing antennas should only be used in cases where signal transmission range must be maximized (i.e. deep rivers, aerial surveys, dam passage studies). When a trailing antenna is required, researchers should consider the placement of the transmitter and the angle of the antenna at the exit point in the body wall. Researchers should utilize a shallow/more oblique angle of antenna exit as opposed to the antenna exiting at a perpendicular angle. The use of the shallow/oblique antenna is thought to decrease irritation and subsequent infection and promote healing rates.

Surgical implantation of internal transmitters should only be conducted on Atlantic sturgeon that are in excellent condition at times when they are not stressed due to temperature/dissolved oxygen extremes (see discussion on tolerance to high temperatures and low dissolved oxygen conditions; page 11). Anesthesia should be administered according to guidance provided in the anesthesia section of these protocols (beginning on page 17). All reasonable means should be used to check fish for existing biotelemetry tags prior to surgery. If it is determined that a fish has received an internal tag in the past, implantation of a new tag is not recommended. Possible tag detection methods include hydrophones, coded wire tag wands, and metal detectors. Surgical implantation of transmitters should be conducted using sterilized transmitters and equipment to help minimize post operative infection rates (Mohler 2004). Surgery protocols should follow published guidelines for the location, size, and closure of incisions as well as application of betadine ointment (Conte et al. 1988, Fox et al. 2000, and Mohler 2004). The intent of the betadine ointment is to coat the sutures until the fish's mucous has a chance to coat them (Robert Bakal, U.S. Fish and Wildlife Service, pers. comm.). The betadine has antibacterial properties but also antifungal properties, which may be even more important (Robert Bakal, U.S. Fish and Wildlife Service, pers. comm.). Upon completion of transmitter insertion, telemetered Atlantic sturgeon should be released immediately after regaining equilibrium at or near the location of capture. In cases where fixed collection gear remains in the area, the researcher should release Atlantic sturgeon in a location so as to minimize the potential of recapturing recently tagged individuals.

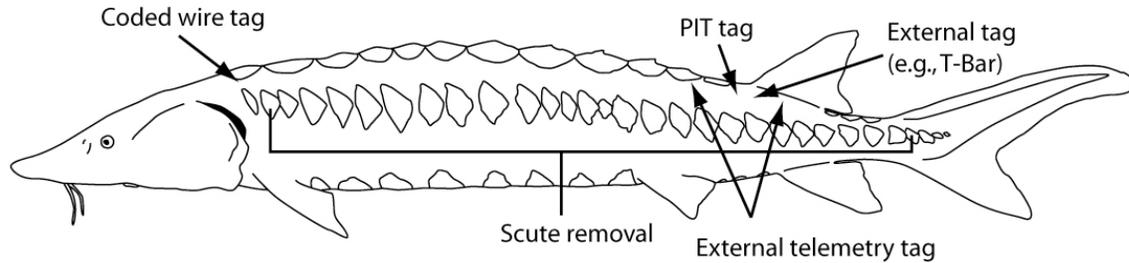


Figure 19. External tag attachment locations for Atlantic sturgeon (line drawing adapted from Figure 3).

Training requirements

Due to inherent needs of sturgeon handling, all researchers who wish to conduct biotelemetry studies should participate in either hands on training from an existing or approved sturgeon researcher or an approved course on the surgical/handling procedures for Atlantic sturgeon.

Tissue sampling

Tissue samples for genetic analysis should be collected during sampling from all Atlantic sturgeon which are confirmed to be first time captures and not recaptures (confirmed while in the field through the presence of external tags or PIT tags). Collection of tissue samples for chemical analyses or age and growth studies is also strongly encouraged. Descriptions of tissue sampling techniques are provided below. Full use should be made of mortalities and salvaged specimens in order to maximize the information derived.

Biopsy

Collection of samples of internal organ tissues (e.g., for biological contaminant analysis) often requires sacrifice of a fish, salvaged specimens, or incidental mortalities, and may be obtained through dissection during a necropsy. However, biopsies can be taken of some organ tissues (e.g., gills, gonads, muscle) through surgeries with minimal impact on a fish. Muscle biopsies can be taken from the thickest portion of the dorsal (epaxial) musculature, as described in the Moser Protocol. After making a small incision, a biopsy can be used to take the tissue sample, and the wound closed with two sutures.

Gonad biopsies in particular have been used successfully to identify sex for several species of sturgeon and are commonly employed, particularly in aquaculture facilities. As Chapman and Park (2005) noted, “Although invasive, gonad biopsies in sturgeon cause minor trauma and remain the most reliable method of identifying their sex and stage of sexual maturity, especially at an early age.” Procedures for gonad biopsies are described in detail in several publications (e.g., Chapman et al. 1996, Fox et al. 2000, and Webb and Erickson 2007). In short, a 40-mm incision is made on the ventral surface of the anesthetized fish using a sterile scalpel.

Once the gonad is located, a 1 cm³ sample can be taken using a pair of forceps and a scalpel or a sample can be taken with an Eppendorfer biopsy punch or similar biopsy tool. The gonad sample must contain germinal tissue in order to be meaningful. The germinal portion of the gonad is oriented facing the body wall making a proper sample difficult to obtain in some cases. This is especially true when working with immature fish where a non-lethal sample is desired. In immature fish the gonad is comprised largely of yellowish-orange adipose tissue, and if the biopsy sample contains only adipose tissue it will not reveal sex. Once the proper sample has been taken, the incision is then closed with a suture as described below. Also see section: (Laparoscopy and related technologies, non-invasive procedures, and traditional methods of assigning sex).

Chapman and Park (2005) favored using Vicryl suture for closing the wound following biopsies because it did not require removal and did not irritate the skin, as opposed to some other suture material. A more quantitative approach was taken in a comparative study which examined the tissue reactivity in a teleost fish. The findings of Hurty et al. (2001) suggest that Vicryl or cat gut materials, while cheaper, were found to cause more tissue reaction in comparison to absorptive materials (e.g., Maxon and PDS). Samples should be preserved in buffered 10% formalin for histological analysis or frozen for contaminant analysis. An alternative technique for gonad biopsy via laparoscopic techniques is also described in Matsche and Bakal (2008).

Blood sampling and caudal venous puncture

AFS (2004) cites three methods suitable for extracting blood from fishes: heart puncture, venous puncture, and caudal bleeding. Because of their morphology, heart puncture is impractical for sturgeon (i.e., the heart is well protected by the bony shields of the pectoral girdle). The preferred method for bleeding is through caudal venous puncture (venipuncture). Collection of blood can be achieved by puncturing the caudal vein (located ventral to vertebral column in the midline of the caudal peduncle) with a hypodermic needle (Figure 20). Unlike typical bony fishes, sturgeons do not have bony haemal spines protecting the caudal vein. Instead, the caudal vein runs in a canal formed by short, block-like cartilaginous vertebral elements; these elements are thickest on their lateral sides and are thinner ventrally. In caudal venous puncture, the needle is inserted at a 45° angle until the cartilage of the vertebral column is contacted (Stoskopf 1993); in large fish the vessels will be completely surrounded by cartilage and the needle will need to be pushed through the thin ventral cartilage to puncture the vessel; care should be taken to not push too deep and puncture the notochord. The needle should then be backed off slightly and blood can be drawn (Stoskopf 1993). Negative pressure should be created in the syringe by pulling up on the plunger while inserting the needle so that blood will be drawn when the vein is punctured. While the needle should be inserted at an angle, this may not be possible or effective for juvenile fish, in which the needle may need to enter perpendicular to the vein for blood to be extracted (e.g., see Figure 20). Because of the anatomy of the vertebral column and the caudal peduncle of sturgeon, a ventral approach for caudal venous puncture is most commonly applied. However, it is possible to collect blood with an insertion of the needle on the lateral surface of the caudal peduncle, ventral to the lateral scutes (a lateral insertion is most commonly applied for other types of fishes; Stoskopf 1993); this lateral technique has been used successfully for blood collections from Gulf Sturgeon (Brian Hickson, U.S. Fish and Wildlife Service, pers. comm.). Alam et al. (2000) used 10cc disposable syringes with 20 gauge needles to draw blood from juvenile Gulf sturgeon; smaller or larger gauge

needles may be appropriate depending on the size of the individuals being sampled. Glass syringes should not be used for collection of blood due to increased chances for premature coagulation (Stoskopf 1993). In the field, blood samples should be stored on ice. Blood samples should be transferred to sterile containers (e.g., heparinized vacutainers) and need to be stored in a freezer (Alam et al. 2000 stored samples at 0° C until sent out for analysis). For long term storage, samples should be maintained at -20 to -80° C (Molly Web, Bozeman Fish Technology Center, pers. comm.). In their study of bioaccumulation of metals and organochlorine compounds in Kootenai River white sturgeon, Kruse and Scarnecchia (2002) centrifuged samples to separate the plasma from the blood cells, and froze the plasma until shipped to labs for analysis. Collection of plasma samples requires collection of blood using heparinized syringes or immediate transfer to heparinized vacutainers before centrifugation.



Figure 20. Caudal venous puncture (performed on a small, two year old fish).

Genetic tissue samples

For genetic analyses, a 1 cm² fin clip from one of the pelvic fins from living sturgeon should be taken and placed in a labeled vial with an o-ring cap containing 95% non-denatured ethyl alcohol (ETOH) for genetic analyses (the pelvic fin is regarded as least intrusive, particularly for small individuals). It is recommended that the tissue be stored in a refrigerator for the first 24-48 hours (Tim King, USGS, pers. comm.). If long-term storage is necessary, the tissue should be placed in a refrigerator or freezer after the initial steeping and stored at 4° C to -20° C (Tim King, USGS, pers. comm.). This will aid in preventing evaporation of the ethanol. There may be some utility for collection of disease status information from pectoral fin clips. This should be considered on an as needed basis, with the standard sampling location for genetic samples being the pelvic fin. Fin clips provide sufficient DNA for extraction and analysis, and they are viewed as minimally invasive to the animal (e.g., AFS 2004). Tissue samples collected from dead sturgeon can include fin clips, the barbel, deep white muscle tissue (recommended if the animal is not freshly dead), liver, heart, or the viscous fluid in the eyes. Note that it is not recommended to clip barbels from living sturgeon for tissue samples.

A relatively new tissue collection method for genetic samples is the use of FTA™ cards (Whatman Inc., Clifton, NJ). Sample collection involves simple contact of a tissue to a specially coated paper which lyses the cells, stabilizes the DNA, and allows for long term sample storage at room temperature. The use of FTA cards is widespread and has been used for forensics, bacteriology, and plant and animal genetics (e.g., Purvis et al. 2006, Mbogori et al. 2006). Livia et al. (2006) found FTA-collected genetic samples to be reliable for microsatellite and RFLP

analysis derived from samples of mucus and buccal cells of northern pike and brown trout. Borisenko et al. (2008) found the use of FTA cards to be less effective in a mammal survey than alcohol or cryopreserved tissue samples. They concluded that the observed DNA degradation was due to high humidity (e.g., tropical conditions), tissue type that was sampled (e.g., liver, because of high enzymatic activity, degrades quickly; Hanner et al. 2005) or oversampling (e.g., too much tissue was blotted on the paper and the DNA did not fix properly). However, because of ease of use (including tissue collection from live specimens, including buccal swabs, mucous samples, as well as tissues sampled through biopsies; Livia et al. 2006), transport (e.g., they can be sent through the mail with no need for special shipping with dry ice or alcohol), and archiving of samples, FTA cards may be suitable for many applications involving tissue collection for genetic studies of sturgeon.

When collecting tissues, researchers should also perform the 5 measurements described on page 8, as well as collect information such as sex (if known), date of collection, and location (i.e., a GPS coordinate) of each fish sampled. The tissue sample (or a subsample, if the tissue is to be used by the researcher) and the corresponding collection data and any tag numbers available should be delivered to the NOS archive in South Carolina (attention Julie Carter, NOS Marine Forensics Branch, 219 Fort Johnson Road, Charleston, SC 29412). Because genetic information (e.g., natal rivers, etc.) is important information for a broad range of aspects of sturgeon biology and management, results will be provided to the investigator who originally submitted the tissue; the investigator shall also be acknowledged for their efforts in any resulting publications or reports.

Fin spines

The pectoral fin spine is the preferred calcified structure for ageing sturgeon (Brennan and Cailliet 1989). Removal or partial removal of a pectoral fin spine is non-deleterious (cf. otoliths, for which sturgeon must be sacrificed), allows for mark-recapture (e.g., OTC or calcein (see footnote on page 32)), and there is a precedence for this technique in the literature and previous care/handling documents (e.g., Cuerrier 1951, Rossiter et al. 1995; for Atlantic sturgeon specifically by Stevenson and Secor 1999, see also Moser et al. 2000). There are some disadvantages to using this structure for ageing, including that the fin spine is composed of metabolically active material and calcium in the spine can be reabsorbed. The fin spine is an exposed structure and is susceptible to damage. This method for ageing has only been partially validated; see Rien and Beamesderfer (1994), Rossiter et al. (1995), Stevenson and Secor (1999), Whiteman et al. (2004), and Secor and Woodland (2005) for discussion of pros and cons, and information on precision and validation of annuli counts for sturgeon fin spines generally. Split annuli, false annuli, inclusions of additional rays in the spine, crowding of annuli in older fishes, and difficulty defining the margin are concerns in using fin spines for ageing (e.g., see Whiteman et al. 2004). Paragramian and Beamesderfer (2003) suggested that age estimates based on fin spines may underestimate true ages in their study of Kootenai white sturgeon. Also, for mark-recapture studies using OTC or calcein, it needs to be noted that left and right fin spines are not mirror images (note: spines marked with OTC or calcein must be stored in a dark location to prevent mark degradation). However, even with these disadvantages and caveats, fin spines remain the most practical structure for collecting age estimates for sturgeon.

Fin spine removal should follow the protocol outlined by Collins and Smith (1996), in which the spine is cut near its base with a minihacksaw (or similar saw or cutting instrument,

e.g., bolt cutters, wire cutter, hacksaw, coping saw or knife) and the more distal part of the spine is carefully separated from the fin rays with a scalpel. A recommended less invasive alternative technique involves taking a 1 cm section of the fin spine (e.g., Rien and Beamesderfer 1994, and Secor and Woodland 2005). In this procedure, two cuts are made on either side of the section to be removed. In either procedure, when cutting the sample one should minimize the distance from the articulation without compromising the joint function or cutting into the basal recess of the spine, which houses a portion of the internal skeletal supports for the fin (Figure 21). After removing the sample, be sure to disinfect the wound and allow the fish to recover before releasing the fish.

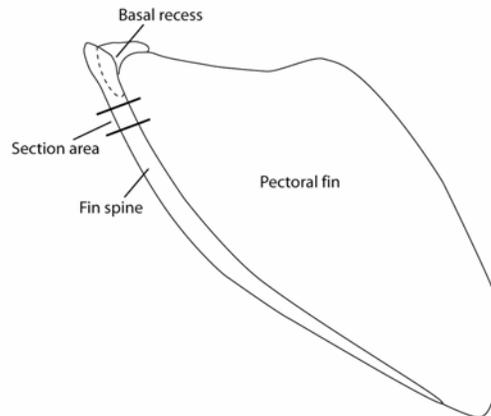


Figure 21. Depiction of a pectoral fin spine.

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, particularly during the spawning season.

Most pectoral fins that have had the fin spines removed for ageing are expected to heal if the physical structure of the fin is not damaged (Collins and Smith 1996). However, there is evidence that large adults do not recover well after their leading fin ray is removed (Mark Collins, South Carolina Department of Natural Resources, pers. comm.), and it is encouraged that spines are not removed from larger adults due to potential deleterious effects. Full spines (i.e., including the base of the spine) may be removed from dead specimens.

Salvage specimens

Dead or salvaged specimens can be invaluable for a number of basic and applied aspects of sturgeon biology and conservation. In the effort to maximize the information and data that is able to be derived from dead sturgeon, it is recommended that, depending on the condition of specimen (i.e., level of decomposition), tissues be taken as soon as practical, including fin clips for genetic analyses, muscle tissue for contaminant analyses, and fin spines for age and growth analyses. Deep muscles (i.e., close to the vertebral column) can be usefully sampled from even partially decomposed fish, and even badly decomposed specimens may prove valuable for osteological and other morphological and comparative analyses.

It is important to maintain salvaged specimens and their derivative tissues so that they are available for future researchers (e.g., for morphological analyses, as voucher specimens for

genetic studies, etc.). Natural history collections have great intrinsic value as a resource for future generations of researchers, students, fisheries agency officials and resource managers. However, collection data for museum holdings of Atlantic sturgeon specimens are generally incomplete, particularly for large individuals (Eric Hilton, Virginia Institute of Marine Science, pers. comm.). These specimens are, therefore, of decreased value for many studies. Minimum standard data that should be kept with specimens include date of collection or recovery, locality, and the names of the collectors.

Contaminants sampling

Several tissues, including (but not limited to) gonads, muscle, liver, gills, and blood, may be sampled for tissue residue analyses (e.g., dioxins, furans, PCBs, organochlorine pesticides, trace elements) to gauge effects of bioaccumulation and environmental contaminants (see review of contaminants in Gulf Sturgeon by Berg 2006). Depending on the analyses to be employed, tissue samples can be placed in chemical-clean jars (trace elements and organics), 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. Specimens should be stored frozen until analysis. MacDonald et al. (1997) and Agusa et al. (2004) stored their frozen samples at -20°C whereas Alam et al. (2000) stored specimens at 0°C. For some analyses (e.g., EROD/CYP1A analysis based on liver and gill tissue or that of sex steroids - estradiol, testosterone - and vitellogenin from blood plasma), tissues must be stored immediately on liquid nitrogen or dry ice and samples must be kept at -80°C prior to analysis. Brundage (2003) reported on analysis of ICP metals, mercury, TCL semivolatile organic compounds, organochlorines, PCBs, PCDD/PCDF and substituted isomers, and percent lipids from a shortnose sturgeon killed during dredging; the specimen was frozen after recovery. The specimen was partially thawed to allow for dissection of samples, which were kept on dry ice for transportation to the lab for analysis.

Although tissues generally degrade after death, with some analyses requiring fresh specimens that are immediately fixed or frozen, many usable tissues may also be taken from salvaged sturgeons; 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; muscle samples should be taken as deep as possible.

As an example, an Atlantic sturgeon carcass salvaged from Wellfleet, MA in 2007 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 (PBDEs, 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).

Training requirements

The precise degree to which researchers should be trained for taking tissue samples should be matched to the level of invasiveness of the protocol. Because tissue sampling often involves a variety of specialized techniques and protocols, particularly for invasive aspects of

sampling (e.g., biopsies, fin spine sampling, blood sampling, etc.), a high level of training or experience should be acquired by researchers for most tissue sampling activities. For taking fin clips for genetic sampling, a moderate amount of experience should be achieved so as to not take an excessively large portion of the fin. Tissue sampling from salvaged specimens requires minimal training, but experience and knowledge of particular protocols for sample preservation should be attained prior to sampling.

Cryopreservation

Cryopreservation of gametes may play an important role in sturgeon aquaculture and conservation biology. Most effort has been made for cryopreservation of spermatozoa, although recent advances on the cryopreservation of eggs of other fishes have been made (e.g., salmonids, Kobayashi et al. 2007); these and other protocols may be extended to sturgeon in the future. For collecting gametes, the researcher is referred to Mohler (2004).

Sperm stored undiluted in refrigerators (on ice and given oxygen daily) has been shown to retain its fertilizing potential for up to 14 days for paddlefishes, but only 5-7 days for *Acipenser brevirostrum* and *A. oxyrinchus desotoi* (Park and Chapman 2005) and with significant decrease in sperm motility. For short term storage of sperm, Bill Wayman (USFWS Warm Springs Fish Technology Center, pers. comm.) recommended keeping fresh sperm viable by keeping it in oxygen filled sealable plastic bags or centrifuge tubes on ice (see DiLauro et al. 1994). Containers should only be filled half way with sperm in order to provide enough volume for sufficient oxygenation, and multiple samples should be taken per fish in order to ensure that some are viable (Curry Woods, University of Maryland, pers. comm.). Samples should be resuspended daily by gentle inversion, and oxygen should be replenished daily. Various extenders have been used for cryopreservation of sturgeon sperm, including buffered sodium chloride (NaCl) or potassium chloride (KCl) and saccharose solutions diluted one to one sperm to extender (e.g., see Billard et al. 2004). Bill Wayman (USFWS Warm Springs Fish Technology Center, pers. comm.) used HBSS-S at 100 mOsm/kg as an extender at a ratio of 1 ml of sperm to 4 ml of extender. Park and Chapman (2005: table 2) provide a protocol for mixing an extender for short-term storage of sturgeon sperm (up to 28 days at 4°C). Sperm can be cryopreserved using dimethyl-sulfoxide (DMSO), dimethyl acetate (DMA), methanol, or ethylene glycol; different cryoprotectants show different results depending on the species of sturgeon involved. No equilibration time may be needed for sperm that is frozen immediately (Jähnichen et al. 1999), but generally is no more than 10-15 minutes. Sperm can be frozen by directly placing pellets on dry ice, in vials or straws placed in programmable freezers, or by suspending straws or vials in a rack 3-5 cm above liquid nitrogen (Billard et al. 2004).

Sperm can be thawed quickly in warm water (e.g., for 9 sec in a 40°C water bath; Bill Wayman, USFWS Warm Springs Fish Technology Center, pers. comm., see also Jähnichen et al. 1999 and Billard et al. 2004). Activators may also include sodium, calcium, or saccharose, which may improve sperm motility or fertilizing capacity (Billard et al. 2004). Changes in both motility and acrosome structure of cryopreserved sperm affect the fertilization potential of the sperm, but these may be offset by use of excess sperm during fertilization (Jähnichen et al. 1999, Billard et al. 2004). Bill Wayman (USFWS Warm Springs Fish Technology Center, pers. comm.) reported 10-20% motility of cryopreserved Atlantic sturgeon sperm when thawed.

Training requirements

Because cryopreservation involves specialized protocols and invasive techniques, a high level of training or experience should be acquired by researchers prior to initiation of activities.

References

- AADAP (Aquatic Animal Drug Approval Partnership Program). 2008. AQUI-S use on food fish under IDAD 10-541 strictly prohibited. AADAP Newsletter 4-2:1.
- ASMFC 2007. Estimation of Atlantic Sturgeon Bycatch in the Coastal Atlantic Commercial Fisheries of New England and the Mid-Atlantic. Atlantic States Marine Fisheries Commission Washington D.C.
- Aadland, L.P., and C. Cook. 1992. An electric trawl for sampling bottom dwelling fishes in deep turbid streams. *North America Journal of Fisheries Management* 12: 62-656.
- Agusa, T., T. Kunito, S. Tanabe, M. Pourkazemi, and D. G. Aubrey. 2004. Concentrations of trace elements in muscle of sturgeon in the Caspian Sea. *Marine Pollution Bulletin* 49: 789-800.
- Alam, S. K., M. S. Brim, G. A. Carmody, and F.M. Parauka. 2000. Concentrations of heavy and trace metals in muscle and blood of juvenile Gulf sturgeon (*Acipenser oxyrinchus desotoi*) from the Suwannee River, Florida. *Journal of Environmental Science and Health* A35:645-660.
- American Fisheries Society (AFS). 2004. **Guidelines for the Use of Fishes in Research; American Fisheries Society**, American Institute of Fishery Research Biologists, American Society of Ichthyologists and Herpetologists. Use of Fishes in Research Committee Members; J. G. Nickum, Chair, H. L. Bart, Jr., P. R. Bowser, I. E. Greer, C. Hubbs, J. A. Jenkins, J.R. MacMillan, J. W. Rachlin, J. D. Rose, P. W. Sorensen, and J.R. Tomasso. Website address: http://www.fisheries.org/afs/docs/policy_guidelines2004.pdf
- Apperson, K.A., and P.J. Anders. 1991. Kootenai River white sturgeon investigations and experimental culture. Annual Progress Report to Bonneville Power Administration, Portland Or., FY 1990: 67 p.
- Atlantic Sturgeon Status Review Team (ASSRT). 2007. Status Review of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*). Report to National Marine Fisheries Service, Northeast Regional Office. February 23, 2007. 174 pp. Web address: [http://www.NOAA Fisheries Service.noaa.gov/pr/pdfs/statusreviews/atlanticsturgeon2007.pdf](http://www.NOAA.Fisheries.Service.noaa.gov/pr/pdfs/statusreviews/atlanticsturgeon2007.pdf)
- Bain, M. B. 1997. Atlantic and shortnose sturgeon of the Hudson River: common and divergent life history attributes. *Environmental Biology of Fishes* 48: 347-358.

- Bath, D. W., J. M. O'Connor, J. B. Alber, and L. G. Arivdson. 1981. Development and identification of larval Atlantic sturgeon (*Acipenser oxyrinchus*) and shortnose sturgeon (*A. brevirostrum*) from the Hudson River estuary, New York. *Copeia* 1981: 711-717.
- Bell, G.R. 1987. An outline of anesthetics and anesthesia for salmonids, a guide for fish culturists in British Columbia. *Can. Tech. Rep. Fish. Aquat Sci.* 1534: 16p.
- Berg, J. 2006. A review of contaminant impacts on the Gulf of Mexico sturgeon, *Acipenser oxyrinchus desotoi*. Report to United States Fish and Wildlife Service, Panama City, Florida.
- Billard, R., J. Cosson, S. B. Noveiri, and M. Porkazemi. 2004. Cryopreservation and short-term storage of sturgeon sperm, a review. *Aquaculture* 236:1-9.
- Birstein, V. J., W. E. Bemis, and J. R. Waldman. 1997. The threatened status of acipenseriform species: a summary. *Environmental Biology of Fishes* 48: 427-435.
- Bordner, C.E., S.I. Doroshov, D.E. Hinton, R.E. Pipkin, R.B. Fridley, and F. Haw. 1990. Evaluation of marking techniques for juvenile and adult White Sturgeon reared in captivity. *American Fisheries Society* 7: 293-303.
- Borisenko, A. V., B. K. Lim, N. V. Ivanova, R. H. Hanner, and P. D. N. Hebert. 2008. DNA barcoding in surveys of small mammal communities: a field study in Suriname. *Molecular Ecology Resources* 8(3): 471-479.
- Brennan, J. S., and G. M. Cailliet. 1989. Comparative age-determination techniques for white sturgeon in California. *Transactions of the American Fisheries Society* 118: 296-310.
- Brosse, L., P. Dumont, M. Lepage, and E. Rochard. 2002. Evaluation of a gastric lavage method for sturgeon. *North American Journal of Fisheries Management* 22: 955-960.
- Bruch, R.M., T.A. Dick, and A. Choudhury. 2001. A field Guide for the identification of stages of gonad development in Lake sturgeon, (*Acipenser fulvescens* Rafinesque), with notes on Lake sturgeon reproductive biology and management implications. Wisconsin Department of Natural Resources, Oshkosh, WI, 40 p.
- Brundage, H. M. 2003. Contaminant analysis of tissues from a shortnose sturgeon (*Acipenser brevirostrum*) from the Kennebec River, Maine. Report to National Marine Fisheries Service, Protected Resource Division, Gloucester MA.
- Buckley, J., and B. Kynard. 1985. Yearly movements of shortnose sturgeon in the Connecticut River. *Transactions of the American Fisheries Society* 114: 813-820.
- Buddington, R.K., and J.P. Christofferson. 1985. Digestive and feeding characteristics of the chondrosteans. *Environmental Biology of Fishes* 14:31-41.

- Carr, S. H., F. Tatman, and F. A. Chapman. 1996. Observations on the natural history of the Gulf of Mexico sturgeon (*Acipenser oxyrinchus desotoi* Vladykov 1955). *Ecology of Freshwater Fish* 5: 169-174.
- Chapman, F.A., J.P. Van Eenennaam, and S.I. Doroshov. 1996. The reproductive condition of white sturgeon (*Acipenser transmontanus*), in San Francisco Bay, California. *Fishery Bulletin* 94: 628-634.
- Chapman, F.A. and C. Park. 2005. Comparison of sutures used for wound closure in sturgeon following a gonad biopsy. *North American Journal of Aquaculture* 67: 98-101.
- Collins, M.R., and T.I.J. Smith. 1993. Characteristics of the Adult Segment of the Savannah River Population of Shortnose Sturgeon. *Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies* 47: 485-491.
- Collins, M.R., T.I.J. Smith, and L.D. Heyward. 1994. Effectiveness of six methods for marking juvenile shortnose sturgeon. *Progressive Fish-Culturist* 56: 250-254.
- Collins, M.R. and T.I.J. Smith. 1996. Sturgeon fin ray removal is nondeleterious. *North American Journal of Fisheries Management* 16: 939-941.
- Collins, M.R., S.G. Rogers, and T.I.J. Smith. 1996. Bycatch of sturgeons along the Southern Atlantic coast of the USA. *North American Journal of Fisheries Management* 16: 24-29.
- Colombo, R.E., P.S. Wills, and J.E. Garvey. 2004. Use of ultrasound imaging to determine sex of shovelnose sturgeon. *North American Journal of Fisheries Management* 24: 322-326.
- Conte, F.S., S.I. Doroshov, P.B. Lutes, E.M. Strange. 1988. Hatchery manual for the white sturgeon (*Acipenser transmontanus* Richardson) with application to other north American Acipenseridae. University of California Cooperative Extension Service Publication 3322, Oakland, California, 104 pp.
- Counihan, T.D., A.I. Miller, M.G. Mesa, and M.J. Parsley. 1998. The effects of dissolved gas supersaturation on white sturgeon larvae. *Transactions of the American Fisheries Society* 127: 316-322.
- Crocker, C.E., Cech, J.J. Jr. 1996. The effects of hypercapnia on the growth of juvenile white sturgeon, *Acipenser transmontanus*. *Aquaculture* 147: 293-299.
- Cuerrier, J. P. 1951. The use of pectoral fin rays for determining age of sturgeon and other species of fish. *Canadian Fish Culturist* 11: 10-18.
- Dadswell, M.J., B.D. Taubert, T.S. Squiers, D. Marchette, and J. Buckley. 1984. Synopsis of biological data on shortnose sturgeon, *Acipenser brevirostrum* LeSueur 1818. NOAA Technical Report NOAA Fisheries Service 14.

- Dick, T.A., S.R. Jarvis, C.D. Sawatzky, and D.B. Stewart. 2006. The Lake Sturgeon, *Acipenser fulvescens* (Chondrostei: Acipenseridae): An Annotated Bibliography. Canadian Technical Report of Fisheries and Aquatic Sciences 2671.
- Dickson, W. 1989. Cod gill net simulation model. Fisheries Research 7:149-174.
- DiLauro, M.N., W.F. Krise, M.A. Hendrix, and S.E. Baker. 1994. Short-term storage of Atlantic sturgeon sperm. The Progressive Fish-Culturist 56: 143-144.
- Dovel, W.L., and T.J. Berggren. 1983. Atlantic sturgeon of the Hudson Estuary, New York. New York Fish and Game Journal 30: 140-172
- Dovel, W.L., A.W. Pekovitch, and T.J. Berggren. 1992. Biology of the shortnose sturgeon (*Acipenser brevirostrum* Lesueur, 1818) in the Hudson River estuary, New York. Pages 187-216 in C.L. Smith, editor, Estuarine Research in the 1980s. State University of New York Press, Albany, New York.
- Doyle, W. C. Paukert, A. Starostka, T. Hill. 2008. A comparison of four types of sampling gear used to collect shovelnose sturgeon in the Lower Missouri River. Journal of Applied Ichthyology. 24: 637-642.
- Edwards, R.E., F.M. Parauka, and K.J. Sulak. 2007. New Insights into Marine Migration and Winter Habitat of Gulf Sturgeon. Pages 183-196 in Anadromous Sturgeons: Habitats, Threats, and Management. J. Munro, D. Hatin, J.E. Hightower, K. McKown, K.J. Sulak, A.W. Kahnle, and F. Caron, editors. American Fisheries Society, Bethesda, MD.
- Elliott, J., and R. Beamesderfer. 1990. Comparison of efficiency and selectivity of gears used to sample white sturgeon in a Columbia River reservoir. California Fish and Game 76: 174-180.
- Erickson, D. L., and J. E. Hightower. 2007. Oceanic Distribution and Behavior of Green Sturgeon. American Fisheries Society Symposium 56: 197-211.
- Eyler, S., M. Mangold, and S. Minkinen. 2009. Atlantic Coast Sturgeon Tagging Database. Summary Report, February, 2009. U.S. Fish & Wildlife Service, Annapolis, MD. 34pp.
- Foster, J.R. 1977. Pulsed gastric lavage: An efficient method of removing the stomach contents of live fish. Progressive Fish-Culturist 39:166-169.
- Fox, D.A., J.E. Hightower, F.M. Parauka. 2000. Gulf Sturgeon spawning migration and habitat in the Choctawhatchee River system, Alabama-Florida. Transactions of the American Fisheries Society 129:811-826.
- Fuller, S.A., J. Henne, J. Seals, and V. Mudrak. 2008. Performance of commercially available Passive Integrated Transponder (PIT) tag systems used for fish identification and

- interjurisdictional fisheries management. *North American Journal of Fisheries Management* 28: 386-393.
- Geoghegan, P., M.T. Mattson, and R.G. Keppel. 1992. Distribution of the shortnose sturgeon in the Hudson River estuary, 1984-1988. Pages 217-277 in C.L. Smith (editor). *Estuarine research in the 1980s*. State University of New York Press, Albany, New York.
- Golder Associates Ltd. 2006. Columbia River Adult White Sturgeon Capture, Transport and Handling Manual, May 2006 Version. Prepared for Upper Columbia White Sturgeon Recovery Initiative. Golder Associates Ltd.
- Golder Associates Ltd. 2006. Upper Columbia River juvenile white sturgeon monitoring: Phase 4 investigations, 2005 – 2006. Report prepared for BC Hydro, Castlegar, B.C. Golder Report No. 05-1480-058F: 70 p. + 6 app.
- Gray, C.A., D.D. Johnson, M.K. Broadhurst, and D.J. Young. 2005. Seasonal, spatial, and gear-related influences on relationships between retained and discarded catches in multi-species gill net fishery. *Fisheries Research* 75: 56-72.
- Hager C. 2007. Sink Gillnet Fisheries and Description of Factors that can Contribute to Higher or Lower Interaction and Retention Rates in Estimation of Atlantic Sturgeon Bycatch in Coastal Atlantic Commercial Fisheries of New England and the Mid-Atlantic, A Special Report to ASMFC Atlantic Sturgeon Technical Committee.
- Haley, N.J. 1998. A gatric lavage technique for characterizing diets of sturgeon. *North American Journal of Fisheries Management* 18: 978-981.
- Hamley, J.M. 1975. Review of gill net selectivity. *J. Fish. Res. Board Can.* 32: 1943-1969.
- Hanner, R., A. Corthals, and H.C. Dessauer. 2005. Salvage of genetically valuable tissues following a freezer failure. *Molecular Phylogenetics and Evolution* 34: 425-455.
- Hilton, E. J., and W. E. Bemis. 1999. Skeletal variation in shortnose sturgeon (*Acipenser brevirostrum*) from the Connecticut River: implications for comparative osteological studies of fossil and living fishes. Pages 69–94 in *Mesozoic Fishes 2 – Systematics and Fossil Record*. G. Arratia and H.-P. Schultze (eds.). Verlag Dr. Friedrich Pfeil, München, Germany.
- Hilton, E.J. 2002. Observations on the rostral canal bones of two species of *Acipenser* (Actinopterygii, Acipenseriformes). *Copeia* 2002: 213–219.
- Hoff, T.B., R.J. Klauda, and J.R. Young. 1988. Contributions to the biology of shortnose sturgeon in the Hudson River Estuary. Pages 171-189 in C.L. Smith (editor) *Fisheries research in the Hudson River*, State University of New York Press, Albany, New York.

- Holst, R., D. Wileman, and N. Madsen. 2002. The effect of twine thickness on the size selectivity and fishing power of Baltic cod gill nets. *Fisheries Research* 56: 303–312.
- Hovgard, H., and H. Lassen. 2000. Manual on estimation of selectivity for gill net and longline gears in abundance surveys. *FAO Fisheries Technology, Paper*, p. 397
- Hrabik, R. A., D. P. Herzog, D. E. Ostendorf, M. D. Petersen. 2007. Larvae provide first evidence of successful reproduction by pallid sturgeon, *Scaphirhynchus albus*, in the Mississippi River. *Journal of Applied Ichthyology*. 23: 436-443.
- Huff, J.A. 1975. Life history of Gulf of Mexico sturgeon, *Acipenser oxyrinchus desotoi*, in Suwannee River, Florida. No. 16, Florida Department of Natural Resources, Marine Research Laboratory.
- Hurty, C.A., D.E. Diaz, J.L. Campbell, and G.A. Lewbart. 2001. Chemical analysis of six commercial adult iguana, *Iguana iguana*, diets. *Journal of Herpetological Medicine and Surgery* 11(3): 23-26.
- Isely, J.J., and Q.C. Fontenot. 2000. Retention of coded wire tags in juvenile shortnose sturgeon. *North American Journal of Fisheries Management* 20: 1040-1043.
- Jähnichen, H., D. Warnecke, E. Trölsch, K. Kohlmann, H. Bergler, and H. J. Pluta. 1999. Motility and fertilizing capability of cryopreserved *Acipenser ruthenus* L. sperm. *Journal of Applied Ichthyology* 15: 204-206.
- Jenkins, W.E., T. Smith, L. Heyward, and D.M. Knott. 1993. Tolerance of shortnose sturgeon, *Acipenser brevirostrum*, juveniles to different salinity and dissolved oxygen concentrations. *Proceedings of the Southeast Association of Fish and Wildlife Agencies* 47: 476–484.
- Kieffer, M., and B. Kynard. 1993. Annual movements of shortnose and Atlantic sturgeons in the Merrimack River, Massachusetts. *Transactions of the American Fisheries Society* 122: 1088-1103.
- Kobayashi, T., Y. Takeuchi, T. Takeuchi, G. Yoshizaki, and G. Yoshizaki. 2007. Generation of viable fish from cryopreserved primordial germ cells. *Molecular Reproduction and Development* 74: 207-213.
- Kohlhorst, D. W. 1976. Sturgeon spawning in the Sacramento River in 1973 as determined by distribution of larvae. *California Fish and Game*. 62: 32-40.
- Kruse, G.O., and D.L. Scarnecchia. 2002. Assessment of bioaccumulated metal and organochlorine compounds in relation to physiological biomarkers in Kootenai River white sturgeon. *Journal of Applied Ichthyology* 18: 430-438.

- Kynard, B., M. Kieffer, M. Burlingame, and M. Hogan. 1999. Studies on shortnose sturgeon. Final Report to Northeast Utilities Service Co., Berlin, Connecticut.
- Light, R.W., P.H. Adler, and D.E. Arnold. 1983. Evaluation of gastric lavage for stomach analyses. *North American Journal of Fisheries Management* 3: 81-85.
- Livia, L., P. Antonella, L. Hovirag, N. Mauro, and F. Panara. 2006. A nondestructive, rapid, reliable and inexpensive method to sample, store and extract high-quality DNA from fish body mucus and buccal cells. *Molecular Ecology Notes* 6: 257—260.
- MacDonald, D.D., M.G. Ikonomou, A.L. Rantalaine, I.H. Rogers, D. Southerland, and J. Van Oostdam. 1997. Contaminants in white sturgeon (*Acipenser transmontanus*) from the Upper Fraser River, British Columbia, Canada. *Environmental Toxicology and Chemistry* 16: 479-499.
- Machiels, M.A.M., M. Klinge, R. Lanters, and W.L.T. van Densen. 1994. Effect of snood length and hanging ratio efficiency and selectivity of bottom-set gill nets for pikeperch *Stizostedion lucioperca* L. and bream *Abramis brama*. *Fisheries Research* 9: 231-239.
- Mal'tsev, A.V. and Y.G. Merkulov. 2006. A biometric method for determining the sex of Acipenserids, including the Russian sturgeon *A. guldenstaedtii* (Acipenseridae) of the Azov population. *Journal of Ichthyology* 46:460-464.
- Marais, JFK. 1985. Some factors influencing the size of fishes caught in gill nets in eastern cape estuaries. *Fisheries Research* 3:251-261.
- Matsche, M., and R. Bakal. 2008. General and reproductive health assessments of shortnose sturgeon with application to Atlantic sturgeon: anesthesia, phlebotomy, and laparoscopy. Maryland Department of Natural Resources. Oxford, Maryland. 27 p.
- Mbogori, M.N., M. Kimani, A. Kuria, M. Lagat, and J.W. Danson. 2006. Optimization of FTA technology for large scale plant DNA isolation for use in marker assisted selection. *African Journal of Biotechnology* 5: 693-696.
- McCabe, G.T. and L. G. Beckman. 1990. Use of an artificial substrate to collect white sturgeon eggs. *California Fish and Game* 76: 248-250.
- McCleave, J.D., S.M. Fried and A.K. Towt. 1977. Daily movements of shortnose sturgeon, *Acipenser brevirostrum*, in a Marine estuary. *Copeia* 1977: 149-157
- McCord, J.W. 1998. Investigation of fisheries parameters for anadromous fisheries in South Carolina. Completion report to National Marine Fisheries Service (AFC-53).
- Meehan, W.R, and R.A. Miller. 1978. Stomach flushing: effectiveness and influence on survival and condition of juvenile salmonids. *Journal of the Fisheries Research Board of Canada* 35: 1359-1363.

- Miller, T.J. 2007. Factors Associated with Mortality of Incidentally Caught Sturgeon in the Northwest Atlantic Ocean in Estimation of Atlantic Sturgeon Bycatch in Coastal Atlantic Commercial Fisheries of New England and the Mid-Atlantic. A Special Report to ASMFC Atlantic Sturgeon Technical Committee.
- Moghim, M., A.R. Vajhi, A. Veshkini, and M. Masoudifard. 2002. Determination of sex and maturity in *Acipenser stellatus* by using ultrasonography. *Journal of Applied Ichthyology* 18: 325-328.
- Mohler, J.W. 1994. Evaluation of tagging procedures and associated tag retention in hatchery-produced yearling of Atlantic sturgeon (*Acipenser oxyrinchus*). USFWS Northeast Fishery Center Fiscal Year 1994 Annual Report. Study number L-94-03.
- Mohler, J.W. 2004. Culture Manual for the Atlantic Sturgeon, *Acipenser oxyrinchus oxyrinchus*. U. S. Fish and Wildlife Service Region 5, Hadley MA. Web address: <http://www.fws.gov/northeast/fisherycenter/pdfs/ASTCultureManual.pdf>
- Moser, M.L., and S. W. Ross. 1993. Distribution and movements of shortnose sturgeon (*Acipenser brevirostrum*) and other anadromous fishes of the lower Cape Fear River, North Carolina. Final Report to the U.S. Army Corps of Engineers, Wilmington, North Carolina.
- Moser, M.L., and S.W. Ross. 1995. Habitat use and movements of shortnose and Atlantic sturgeons in the lower Cape Fear River, North Carolina. *Transactions of the American Fisheries Society* 124: 225-234.
- Moser, M.L., J.B. Bichy, and S.B. Roberts. 1998. Sturgeon distribution in North Carolina. Final report to the U.S. Army Corps of Engineers, Wilmington District, Wilimington, North Carolina.
- Moser, M.L., M. Bain, M.R. Collins, N. Haley, B. Kynard, J.C. O'Herron II, G. Rogers, and T. S. Squiers. 2000. A protocol for use of shortnose and Atlantic sturgeon. NOAA Technical Memorandum NOAA Fisheries Service-OPR-18.
- Murphy, M.D., R.F. Heagey, V.H. Neugebauer, M.D. Gordon, and J.L. Hintz. 1995. Mortality of spotted seatrout released from gill-net or hook-and-line gear in Florida. *North American Journal of Fisheries Management* 15: 748-753.
- Musick, J. A., R.E. Jenkins, and N.B. Burkhead. 1994. Sturgeons, Family Acipenseridae. Pp. 183-190 In: Jenkins, R.E., and N.B. Burkhead, *Freshwater Fishes of Virginia*. American Fisheries Society, Bethesda MD.
- Niklitschek, E.J. 2001. Bioenergetics modeling and assessment of suitable habitat for juvenile Atlantic and shortnose sturgeons (*Acipenser oxyrinchus* and *A. brevirostrum*) in the Chesapeake Bay. Ph.D. dissertation. University of Maryland, College Park.

- Niklitschek, E.J., and D.H. Secor. 2005. Modeling spatial and temporal variation of suitable nursery habitats for Atlantic sturgeon in the Chesapeake Bay. *Estuarine, Coastal and Shelf Science* 64: 135–148.
- Niklitschek, E.J., and D.H. Secor. 2009. Dissolved oxygen, temperature, and effects on the ecophysiology and survival of juvenile Atlantic sturgeon in estuarine waters: I. Laboratory results. *Journal of Experimental Marine Biology and Ecology*, In Press.
- O'Herron, J.C., and K.W. Able. 1990. A study of the endangered shortnose sturgeon (*Acipenser brevirostrum*) in the Delaware River. Final Performance Report to U.S. Fish and Wildlife Service and New Jersey Fish, Game, and Wildlife, Department of Environmental Protection, New Brunswick, NJ. Project AFS-10-R.
- Paragramian, V.L. and R.C.P. Beamesderfer. 2003. Growth estimates from tagged white sturgeon suggest that ages from fin rays underestimate true ages in the Kootenai River, USA and Canada. *Transactions of the American Fisheries Society* 132: 895-903.
- Park, C., and F.A. Chapman. 2005. An extender solution for the short-term storage of sturgeon semen. *North American Journal of Aquaculture* 67: 52-57.
- Peterson, DL, M.B. Bain, and N. Haley. 2000. Evidence of declining recruitment of Atlantic sturgeon in the Hudson River. *North American Journal of Fisheries Management* 20: 231-238.
- Purbayanto, A., A. Tsunoda, S. Akiyama, T. Arimoto, T. Tokai. 2000. Survival of Japanese whiting *Sillago japonica* and by-catch species captured by a sweeping trammel net. *Fisheries Science* 67: 21–29.
- Purvis, L.B., P. Villegas, and F. Perozo. 2006. Evaluation of FTA[®] paper and phenol for storage, extraction and molecular characterization of infectious bursal disease virus. *Journal of Virological Methods* 138: 66-69.
- Rien, T.A. and R.C. Beamesderfer. 1994. Accuracy and precision of white sturgeon age estimates from pectoral fin rays. *Transactions of the American Fisheries Society* 123: 255-265.
- Rien, T.A., R. C. P. Beamesderfer, and C.A. Foster. 1994. Retention, recognition, and effects on survival of several tags and marks for white sturgeon. *California fish and Game* 80(4): 161-170.
- Reis, E.G. and M.G. Pawson. 1999. Fish morphology and estimating selectivity by gill nets. *Fisheries Research* 39: 263-273.
- Reynolds, J. B. 1996. Electrofishing. Pages 221-253 in B. R. Murphy, and Willis, D.W., editor. *Fisheries Techniques*, 2nd edition. American Fisheries Society, Bethesda.

- R.L. & L. Environmental Services Ltd. 1996. Columbia River white sturgeon investigations. 1995 study results. Report Prepared for B.C. Hydro, Kootenay Generation, Vancouver, B.C., and BC Ministry of Environment, Lands and Parks, Nelson Region. R.L. & L. Report No. 96-377F: 94 p. + 6 app.
- Rossiter, A., D.L. Noakes, and F.W.H. Beamish. 1995. Validation of age estimation for the Lake Sturgeon. *Transactions of the American Fisheries Society* 124: 777-781.
- Savoy, T. 1991. Sturgeon Status in Connecticut waters. Connecticut Department of Environmental Protection, Completion Report AFC-18-3, Waterford. CT.
- Savoy, T. 2007. Prey eaten by Atlantic sturgeon in Connecticut waters. *American Fisheries Society Symposium* 56:157-165.
- Savoy, T., and J. Benway. 2004. Food habits of shortnose sturgeon collected in the Lower Connecticut River from 2000 through 2002. Pages 353-360 in P. M. Jacobson, D. A. Dixon, W. C. Leggett, B. C. Marcy, Jr., and R. R. Massengill, editors. *The Connecticut River Ecological Study (1965-1973) revisited: ecology of the lower Connecticut River 1973-2003*. American Fisheries Society, Monograph 9, Bethesda, Maryland.
- Scott, W. B. and E. J. Crossman. 1973. *Freshwater Fishes of Canada*. Fisheries Research Board of Canada, Bulletin 184: 1-966.
- Seaburg, K.G. 1957. A stomach sampler for live fish. *Progressive Fish-Culturist* 19:137-139.
- Secor, D.H., and E.J. Niklitschek. 2001. Hypoxia and sturgeons: Report to the Chesapeake Bay Program dissolved oxygen criteria team. Technical Report Series No. TS-314-01-CBL.
- Secor, D.H., and R. Woodland. 2005. Recovery and status of shortnose sturgeon in the Hudson River. Report to Hudson River Foundation for Science and Environmental Research, Inc. HRF 011/02A, UMCES-CBL 07-4-35255.
- Shuman, D.A., and E.J. Peters. 2007. Evaluation of pulsed gastric lavage on the survival of captive shovelnose sturgeon. *Journal of Applied Ichthyology* 23: 521-524.
- Smith, T.I.J., S.D. Lamprecht, and J.W. Hall. 1990. Evaluation of tagging techniques for shortnose sturgeon, *Acipenser brevirostrum*, and Atlantic sturgeon *A. oxyrinchus*. *American Fisheries Society Symposium* 7: 134-141.
- Snyder, D.E. 1988. Description and identification of shortnose and Atlantic sturgeon larvae. *American Fisheries Society Symposium* 5: 7-30.
- Sprague, C.R., L.G. Beckman, and S.D. Duke. 1993. Prey selection by juvenile white

- sturgeon in reservoirs of the Columbia River. Pages 229-243 in R.C. Beamesderfer and A.A. Nigro, editors. Status and habitat requirements of the white sturgeon populations in the Columbia River downstream from McNary Dam, vol. 2. Oregon Dept. Fish and Wildlife to Bonneville Power Administration, Final report, Portland, Oregon.
- Stein, A.B., K.D. Friedland, and M. Sutherland. 2004. Atlantic sturgeon marine bycatch mortality on the continental shelf of the northeast United States. *North American Journal of Fisheries Management* 24(1): 171-183.
- Stevenson, J.T. and D.H. Secor. 1999. Age determination and growth of Hudson River Atlantic sturgeon, *Acipenser oxyrinchus*. *Fishery Bulletin* 97: 153-166.
- Stoskopf, M. K. 1993. Clinical Physiology. Pages 48-57 in *Fish Medicine* (Stoskopf, M. K., ed.). Philadelphia, PA: W. B. Saunders Company.
- Sulak, K.J. and J.P. Clugston. 1998 Early life history stages of gulf sturgeon in the Suwannee River, Florida. *Transactions of the American Fisheries Society*. 127:758-771.
- Sweka, J.A., J. W. Mohler, and M.J. Millard. 2006. Relative Abundance Sampling of Juvenile Atlantic Sturgeon in the Hudson River. Final Study Report to the New York Department of Environmental Conservation, Hudson River Fisheries Unit. U.S. Fish & Wildlife Service, Lamar, PA. 46pp.
- Taubert, B.D. 1980. Reproduction of shortnose sturgeon (*Acipenser brevirostrum*) in Holyoke Pool, Connecticut River, Massachusetts. *Copeia* 1980:114-117.
- Vecsei, P., M.K. Litvak, D.G. Noakes, T. Rien, and M. Hochleithner. 2003. A Noninvasive Technique for Determining Sex of Live Adult North American Sturgeons. *Environmental Biology of Fishes* 68: 333-338.
- Vladykov, V.D., and J.R. Greeley. 1963. Order Acipenseroidei. Pages 24-60 in Bigelow, H. B., C. M. Breder, D. M. Cohen, G. W. Mead, D. Merriman, Y. H. Olsen, W. C. Schroeder, L. P. Schultz, and J. Tee-Van, editors. *Fishes of the Western North Atlantic*, Memoir 1, Part Three, of the Sears Foundation for Marine Research. Yale University, New Haven.
- Wanner, G.A. 2006. Evaluation of a gastric lavage method on juvenile pallid sturgeon. *North American Journal of Fisheries Management* 26: 587-591.
- Webb, M.A.H., G.W. Feist, E.P. Foster, C.B. Schreck, and M.S. Fitzpatrick. 2002. Potential classification of sex and stage of gonadal maturity of wild white sturgeon using blood plasma indicators. *Transactions of the American Fisheries Society* 131: 132-142.
- Webb, M.A.H. and D.L. Erickson. 2007. Reproductive structure of the adult green sturgeon, *Acipenser medirostris*, population in the Rogue River, Oregon. *Environmental Biology of Fishes* 79: 305-314.

- Wei, Q., and B. Kynard. 1996. Study of Chinese Sturgeon spawning in the Yangtze River. Yangtze River Fishery Research Institute, Progress Report.
- Whiteman, K.W., V.H. Travnicek, M.L. Wildhaber, A. DeLonay, D. Papoulias, and D. Tillett. 2004. Age estimates for shovelnose sturgeon: A cautionary note based on annulus formation in pectoral fin rays. *North American Journal of Fisheries Management* 24: 731-734.
- Wilkie, M.P., K. Davidson, M.A. Brobbel, J.D. Kieffer, R.K. Booth, A.T. Bielak, and B.L. Tufts. 1996. Physiology and survival of wild Atlantic salmon following angling in warm summer waters. *Transactions of the American Fisheries Society* 125: 572-580.
- Yokota, K., Y. Fujimori, D. Shiode, and T. Tokai. 2001. Effect of thin twine on gill net size-selectivity analyzed with the direct estimation method. *Fisheries Science* 67: 851-856.
- Zehfuss, K.P., E. Hightower, and K.H. Pollock. 1999. Abundance of Gulf sturgeon in the Apalachicola River, Florida. *Transactions of the American Fisheries Society* 120: 130-143.
- Ziegeweid, J.R. 2006. Ontogenetic changes in salinity and temperature tolerances of young-of-the-year shortnose sturgeon, *Acipenser brevirostrum*. Master's Thesis, University of Georgia, Athens.
- Ziegeweid, J.R., C.A. Jennings, D.L. Peterson. 2008a. Thermal maxima of juvenile shortnose sturgeon acclimated to different temperatures. *Environmental Biology of Fishes* 82: 299-307.
- Ziegeweid, J.R., C.A. Jennings, D.L. Peterson, M.C. Black. 2008b. Effects of salinity, temperature, and weight on the survival of young-of-year shortnose sturgeon. *Environmental Biology of Fishes* 137: 1490-1499.