NOAA Technical Memorandum NMFS-NE-215

Atlantic Sturgeon Research Techniques

US DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Science Center
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Atlantic Sturgeon Research Techniques

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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, 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 Moser 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 (*Acipenser brevirostrum*) and Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*), 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. If Atlantic sturgeon are listed under the Endangered Species Act (ESA), it is possible that NOAA Fisheries Service may use the techniques described in this document as a baseline for development of consistent sampling protocols.

INTRODUCTION

Atlantic sturgeon have been considered a Species of Concern since 1998 following the joint decision by NOAA Fisheries Service and the US Fish and Wildlife Service (FWS) that listing the species under the ESA was not warranted. Because of 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 examined and supplemented by eight state and regional experts who provided both their individual insights and 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 determining whether to list Atlantic sturgeon under the ESA, which could ultimately affect how and what type of 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 that allows for

crucial information to be obtained on Atlantic sturgeon subpopulations while minimizing potential adverse impacts on the species. The consistent methods and approaches across subpopulations as elucidated in the summaries of this document would benefit future assessments and ranking of comparative population health. 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. 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 Moser 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 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. These techniques should be reviewed and revised as necessary, approximately every three years. If new technologies or techniques emerge between review periods, interim revisions should be considered.

The objectives of this document are (1) provide Atlantic sturgeon researchers with guidelines for conducting research to ensure that the safest, most recent, and effective techniques are used; and (2) compile the most recent literature review to support the use of these techniques.

IDENTIFICATION AND MEASUREMENT

Two species of sturgeon are present along the east coast of North America: Atlantic sturgeon and shortnose sturgeon. The two species differ greatly in their maximum total length (TL:427 cm and TL:143 cm 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 variation matches that found in other aspects of the anatomy of this species (Hilton and Bemis 1999). There is significant variation in the shape and length of the snout of Atlantic sturgeon at different life history stages (e.g., juvenile vs. adult), although this has yet to be quantified (Bain 1997; Eric Hilton, Virginia Institute of Marine Science, pers. comm.). The snout of Atlantic sturgeon generally is more sharply pointed than that of shortnose sturgeon (Dadswell et al. 1984; Figure 1), but again, the morphological variation (including ontogenetic and other allometric variation) has not been fully described or quantified in this species.

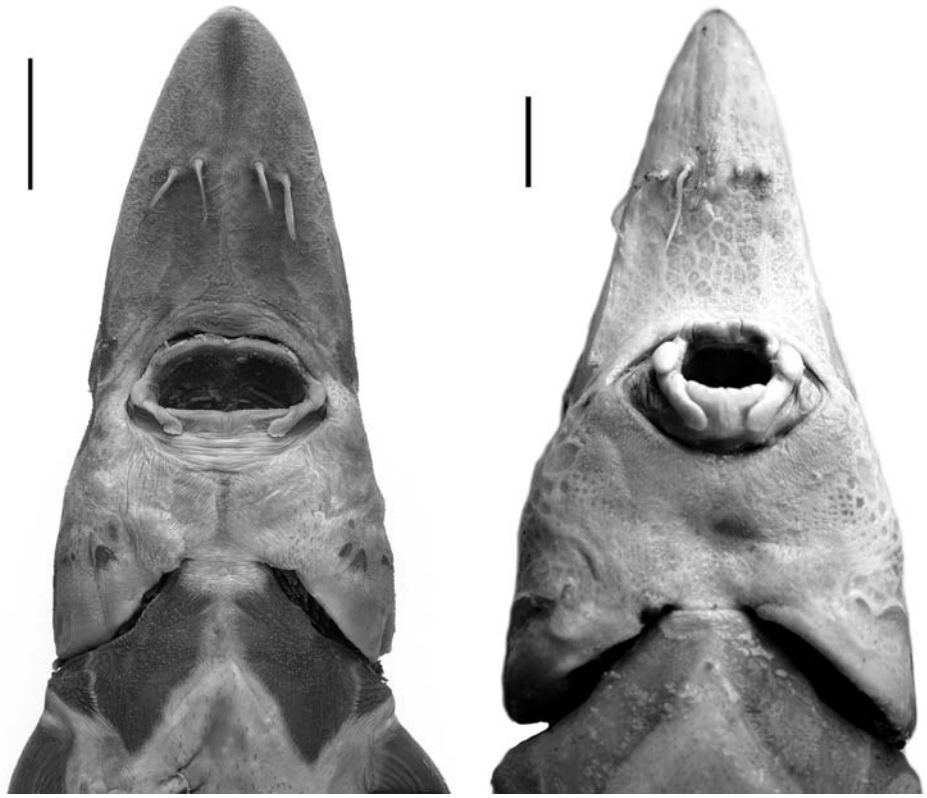

Figure 1. Ventral view of shortnose sturgeon (*Acipenser brevirostrum*; left; Museum of Comparative Zoology 54265, 435 mm FL, Connecticut River, MA) and Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*; right; Virginia Institute of Marine Science uncataloged, 780 mm FL, James River, VA); note short 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.

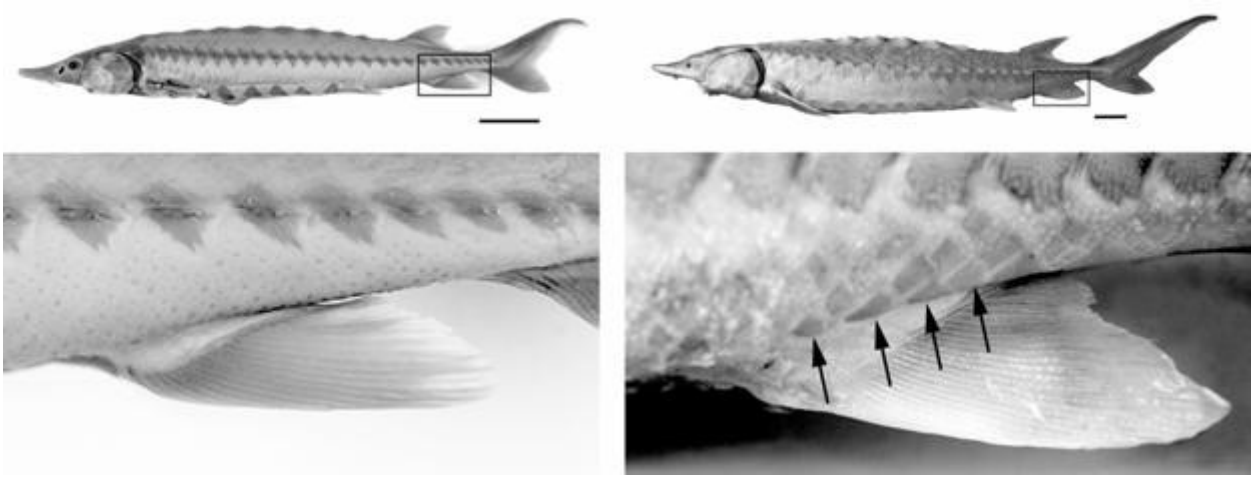

Figure 2. Lateral view of shortnose sturgeon (*Acipenser brevirostrum*; left) and Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*; right); note the small bony plates (scutes) highlighted by the black arrows above the anal fin in the Atlantic sturgeon (same specimens as in Fig. 1). Scale bar = 5 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 between 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 suggest that the range of variation may be greater for both species. Specimens from the Merrimack River (NH, MA) 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, US FWS, 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.

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 (NY). 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 rays were too difficult to count accurately (because the fin rays are 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 Atlantic sturgeon and 22-29, average 25.4 in shortnose sturgeon).

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: 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: 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: 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: distance between the lateral margins of the bony skull at the midpoint of the orbit

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

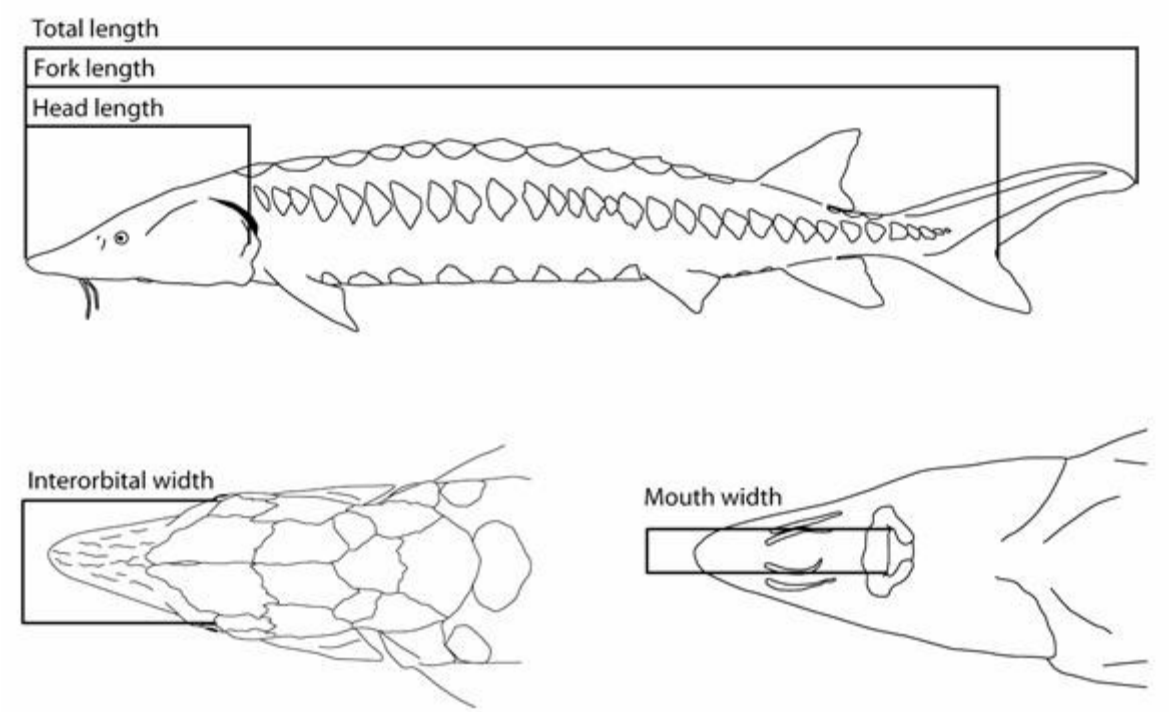

Figure 3. Depiction of the five sturgeon measurements (drawing courtesy of Dr. Eric Hilton, Virginia Institute of Marine Science).

Mouth width and interorbital distance should be measured with calipers. Several published keys, recommendations, and reports have cited mouth width 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.

Summary

For proper species identification of juvenile fish, the following measurements should be taken on all individuals sampled:

- Mouth width
- Interorbital width

In addition, for general information and consistency between studies, we recommend that the following measurements also be taken:

- Total length
- Fork length
- Head length

Training requirements

Only relatively minor training is needed for identification and measurements to ensure accuracy of identification 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 because of 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 subadult sturgeon are occasionally captured as bycatch. Because of their much larger dimensions, pound nets 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 can be more cost effective than fishery independent research collection efforts and should be considered as a means to collect data. Baited trot lines have proved to be an effective method for collecting white sturgeon (*Acipenser 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 with 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 ft in diameter [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 instead cut the pads with scissors 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 mm^2 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 prespawning 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 (*Acipenser sinensis*) 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. Because of 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 (*Acipenser oxyrinchus desotoi*) or Atlantic sturgeon larvae. Small juveniles are rarely taken with 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 the apparent tendency of larvae and juveniles to seek out crevices in rough bottom across which seines and trawls have low collection efficiencies. Because of 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; however, it is well established that gill net selectivity with regard to size and even species is a function of biotic and abiotic factors. 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) and abiotic gear factors such as mesh size, twine material, twine diameter, hanging ratios, and tie downs influence species retention, size selectivity, and 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). The historic commercial fishery for Gulf sturgeon provided evidence of age specific differences in gear vulnerability with larger and smaller sturgeon escaping preferentially (Huff 1975).

It is easy to conceptualize why gill nets that cover more of the water column, by being longer or taller, may increase interaction rates and result in higher catch per unit effort (CPUE). Other gear construction alterations also significantly affect the species specific retention rates of the gear. The assumption that fish are simply gilled (i.e., are prevented from backing out of the webbing by a mesh caught behind the gill plates) by gill nets is false. Fish and other animals are retained in the nets in numerous ways, and the degree to which a species is retained is influenced by its body size and configuration of the gear it encounters. Organisms can be wedged (i.e., held by a mesh or meshes around the body) or become entangled by unique morphological attributes such as teeth, maxillae, scutes, snout, or other projections (Hamley 1975). Entanglement often results in struggling that subsequently wraps the animal in additional webbing. Gill nets can also be constructed so that they contain pockets or funnels of loose mesh that simply entrap and hold species until harvest. Tie downs create such consecutive bags of webbing and are used to increase likelihood of entanglement and entrapment between mesh walls. Finally, a net can be fished in a specific manner that promotes retention. Shad fishermen using anchored gill nets traditionally fish on slack tide to promote retention. The species has reduced/weak opercle plates and thus, typically does not gill well. Net retrieval in slack water allows fishers to literally scoop the fish up in the mesh and prevent them from falling free. This confinement of fish between webbing walls also promotes sturgeon retention upon gear retrieval as the weight of the fish is supported across numerous meshes instead of being concentrated on only a few.

Sturgeon are morphologically unique. Their cone shaped snout rapidly transfers meshes over the head and along the body and thus, they are rapidly gilled or wedged. Their unique dermis covered with bony scutes increases the likelihood of entanglement and wedging, as meshes are rapidly caught as the fish attempts to pass through or around webbing. Larger fish may subsequently become wrapped in webbing once entangled while struggling, and smaller fish may be held by a single monofilament strand hung around a scute. Considerable overlap between sturgeon size distributions retained in varied mesh sizes results from these highly varied forms of retention (Figure 4, Chris Hager, VA Sea Grant, Marine Extension Program, unpublished data).

Abiotic factors also affect how a fish interacts with the gear, and thus, retention characteristics. Interaction trials 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 reduce retention rates (retained/interacted) (Hager 2007). This finding indicates that gear alterations or deployment methods that increase stretch in webbing, mobility of webbing, or amount of webbing for a given area will increase the likelihood of retaining Atlantic sturgeon. Increased sturgeon size distributions have also been noted in drift gill nets (Moser et al. 2000). Such nets are not only characteristically loosely hung, but the lack of anchors or lead line results in fish or other animals easily becoming wrapped in the net; thereby, increasing the likelihood of retention. NOAA Fisheries Service’s (Northeast Region) observer data indicate an increase in the size distribution of sturgeon retained when tie downs are used in gill nets. 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) with the average fish being 137 cm TL. This difference in size selectivity is likely due to the fact that tie downs increase webbing mobility and meshes per unit area and create bags of webbing that entrap fish. Such alterations likely increase retention rates of sturgeon given that they are easily entangled.

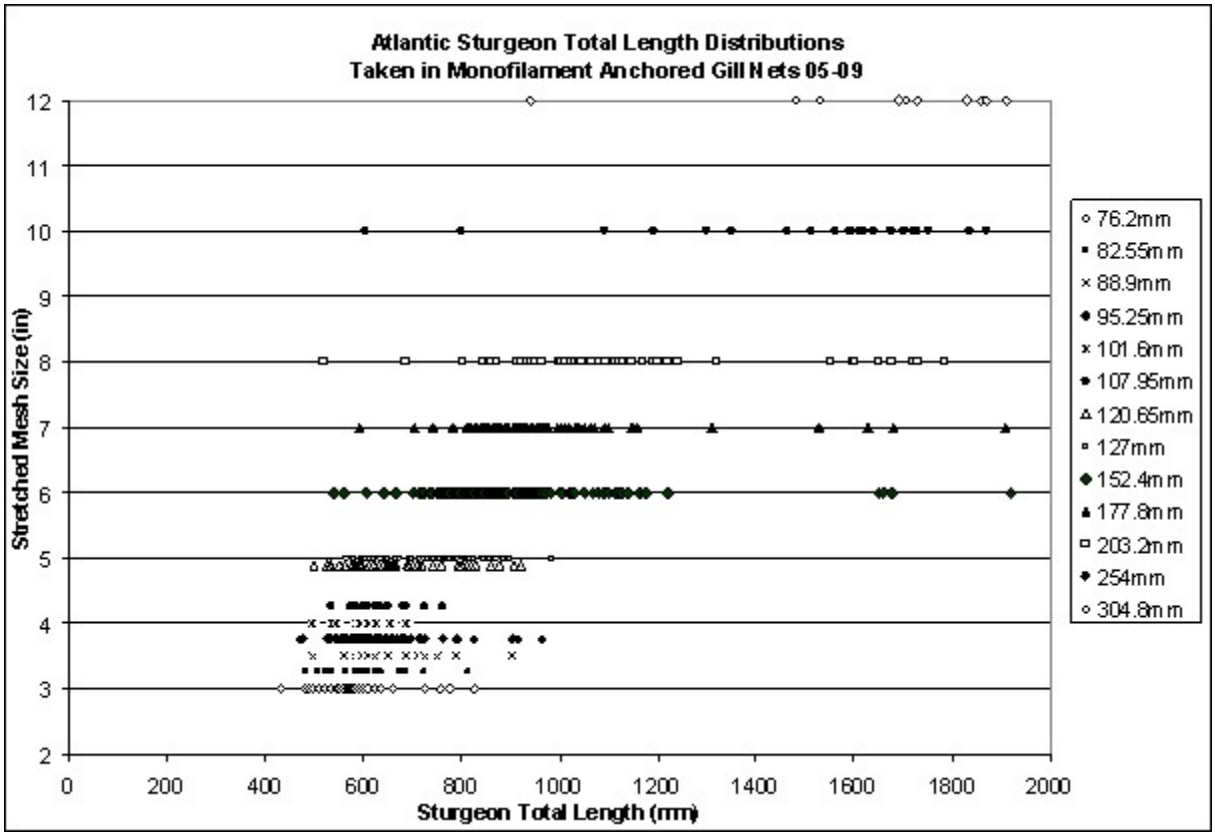

Figure 4. Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) size distributions taken in anchored gill nets in Virginia are illustrated above (Chris Hager, VA Sea Grant, Marine Extension Program, unpublished data). Data support claims by previous researchers that 6 in mesh is highly effective at collecting a wide size range of fish. It also suggests that larger mesh sizes result in greater size disparity.

Both monofilament and braided mesh gill nets have been found to be effective for capturing sturgeon, although they may have different retention characteristics. While fish are captured more efficiently with smaller diameter twine, sturgeon can easily break free of webbing that is weak. Lighter twine has also been known to cut into sturgeon and cause injury (Moser et al. 2000); therefore, when using larger mesh sizes, twine sizes should also be increased. When targeting adults with 15 cm (6") stretched mesh, multifilament nets of at least 0.52-0.57 mm should be used; with larger mesh sizes of 25.4-35.6 cm (10-14"), twine sizes of at least 0.9 mm are preferred to prevent loss and/or injury.

Trammel nets consist of overlapping gill net panels (usually three) of varied mesh sizes hung on a single top and bottom line. This configuration not only provides more meshes per unit area but increases the likelihood of gilling, wedging, and entanglement through the congruent application of varied mesh sizes. Trammels also entangle and entrap fish between their overlapping walls of webbing. Fish may penetrate the larger webbing of the outer wall but subsequently fail 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.

Sturgeon are benthic feeders and are generally captured near the benthos 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, US FWS and Chris Hager, VA Sea Grant, Marine Extension Program, pers. comm.). This capture location within the nets 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 midchannel (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. This deployment method also maximizes the gear's inherent entanglement/entrapment methodology.

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 but are attached at both the top and bottom line to a series of vertical stakes driven into the substrate. Stakes are placed every 15.2 m (50 ft) or so. In commercial fisheries, anchored gill nets of less than 91.4 m (300 ft) are rare, and offshore lengths can reach 914.4 m (3000 ft). Shortening intervals between anchor points reduces tension on meshes caused by hydrodynamic forces. This deployment methodology also helps reduce the chances of anchors becoming dislodged at either end or of the top and bottom line twisting upon themselves in the currents. High rates of mortality have been observed when a net is dislodged or becomes twisted around retained fish.

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). Drifting reduces debris loading because the nets move with the debris and thus, intercept less of it. Generally, the short soak times, reduced pressure on the webbing, and active fishing methods used in conjunction with driftnets also 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).

Gill net sampling and routine handling under favorable conditions do not appear to cause undue harm to sturgeon. In temperatures not exceeding 17°C, lake sturgeon (*Acipenser fulvescens*) captured in gill nets (24 h net sets) and Passive Integrated Transponder (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). In an effort to reduce stress during removal from meshes, the fish should be inverted and their eyes covered. In some cases, net meshes should be cut for rapid and safe removal; in particular, if the fish is gilled and/or meshes have entered the gill case. Every effort should be made to revive nonresponsive fish, as in many cases fish that appear to be dead have recovered.

In some cases, significant sturgeon mortalities have been documented in gill nets and trammel nets (Kieffer and Kynard 1993; Moser and Ross 1993; Collins et al. 1996; Kynard et al. 1999; Stein et al. 2004; ASSRT 2007). Mortalities were often associated with elevated water temperature, extended soak times (Miller 2007), and net interactions that prevented normal respiration (Albert Spells, US FWS and Chris Hager, VA Sea Grant, Marine Extension Program, pers. comm.) To decrease mortality risk, precautions should be taken to reduce fish stress from collection methodology and environmental and biological conditions. When air temperatures are below 0°C, sturgeon should not be out of the water for more than a few minutes. Sampling fish

in gill nets in elevated temperatures has also been shown to increase mortality (Murphy et al. 1995) and protract recovery (Wilkie et al. 1996). All fish should be processed while underwater if possible, as tissues can freeze.

Collins et al. (1996) observed significant increases in gill net related mortality in the commercial drift gill net fishery for southern American shad (*Alosa sapidissima*) when water temperatures exceeded 18°C. Based on a 5 year fisheries independent anchored gill net survey with standard 24 hour sets and numerous mesh sizes (4.88-12" stretched mesh, n=430, 6-24°C), mortality likelihood did not increase homogenously across mesh sizes with rising water temperature; at some mesh sizes, it was negatively correlated. Interestingly, predicted mortality significantly increased across mesh sizes with fish size, with larger adults experiencing higher mortality than smaller, more resident individuals. It is worth noting that adult fish were recent immigrants to the system and only common in elevated water temperatures (>18°C). These findings may suggest that osmoregulation alterations upon freshwater immigration and long migrations to spawning grounds are significant stressors for adults. Conversely, higher adult death rates may simply be a reflection of the fact that most of these adults were retained in large mesh nets after having been gilled in a manner that restricted their respiration (Chris Hager, VA Sea Grant, Marine Extension Program, unpublished data).

Niklitchek'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 for small juveniles of Hudson origin peaks at ~18°C. Above this temperature, growth declines from temperature stress alone. The growth curve shifts to the right (21°C) at higher DO (100%) and to the left (16°C) at lower DO (40%). Salinity alone can be an important bioenergetic limitation factor (Niklitschek and Secor 2005). DO and salinity are naturally augmented during summer in Atlantic estuaries; thus, this season may be critical with regard to juvenile habitat limitation. If habitat is being limited by rising water temperatures, physiological tolerances and thermal sanctuaries may become increasingly important to species recovery, as systems are likely to become more bioenergetically challenging as climate continues to warm.

Atlantic sturgeon may encounter unfavorable environmental conditions in many estuarine systems (Niklitschek 2001). Niklitschek (2001) found that juvenile 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).

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 juvenile 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 Niklitshcek (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.

It is important to note that sturgeon physiological research has only been conducted on juvenile sturgeon of Hudson origin. Applicability of results to subadult and adult Atlantic sturgeon and/or fish from different DPSs is unclear. Adult anadromous fish often have very different metabolisms than do juveniles for obvious evolutionary reasons. In some species, latitudinal differences are so extreme that spawning adults die instead of converting their metabolic systems to handle the osmotic challenges associated with freshwater habitation. Genetic variation presumably imparts inherent advantages to a given population uniquely suited for the physical characteristics of its native habitat. Atlantic sturgeon DPSs are unique at least in part because of this assumed adaptation to each system of origin. Clearly, more research needs to be done to derive applicable physiological limitation models that sufficiently describe the diverse physiological tolerances within Atlantic sturgeon populations. Once appropriate research has been conducted, 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. 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 the ability to adapt gill net sampling to minimize stress to sturgeons, and who have not observed elevated mortality when sampling under these conditions

Though we are only beginning to understand how and to what degree retention can be manipulated through various gear configurations, it is increasingly obvious that gill nets vary with regard to their selectivity. Because of the characteristically large size variation of the species and varied selectivity from gill net configurations, caution should be taken if one is attempting to characterize size or age distributions of sturgeon with fishery-dependent gill net data or independent data when gear is not uniform in construction. Temporal and spatial aspects of deployment may be equally important (WE Pine and S Martell, unpublished data). Researchers have even suggested that fish avoid the gear once educated (Pine et al. 2006), though numerous repeat captures of a single Atlantic sturgeon on consecutive days and fish that have been repeatedly captured in the same region for several months would cast doubt on this theory as would controlled interaction experiments (Chris Hager, VA Sea Grant, Marine Extension Program, unpublished data).

Differences in sampling methods between programs and even within programs must be taken into account even when comparing CPUE. For example, despite recognition of a relationship between area fished and a gear's efficiency, CPUE is commonly expressed as catch per linear length which ignores the vertical component of the gear. Differences in data collection and analysis can easily influence model outputs to the degree that they are misguiding or severely limit their usefulness. The structure of the data derived in each program is driven by the sampling program's collection methodology and biology of the species, which may be unique within a given system and is likely varied between DPSs. Power to detect alterations in populations is affected by the presence of larger populations, larger changes in population, a higher probability of capture, and longer sampling periods (Zehfuss 2000). To be able to estimate and time dependent capture probabilities, capture rates must be consistently relatively high for each age class during each sampling period in order to separate whether an animal was present and not caught or was not present (WE Pine and S Martell, unpublished data). Gear

retention is obviously influenced by presence or absence of target species, but it is also influenced significantly by biotic and abiotic factors unique to each study. Inconsistency in sampling biases predicted capture probabilities and thus, mortality estimates. Though biotic differences will never be eliminated between systems, they can be minimized. Abiotic factors can be effectively addressed through standardization.

Standardizing equipment and developing temporal and spatial consistency between monitoring programs would greatly benefit future population analysis and allow for comparative studies (WE Pine and S Martell, unpublished data). Such standardization between studies or the establishment of surveys within each DPS will be required to understand population alterations within a DPS, exchanges between DPSs, and to be able to establish research priorities under a DPS structure.

Trawls

Where benthic and hydrodynamic conditions allow 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, GA, were from the commercial trawl fishery. Sampling of shortnose and Atlantic sturgeon was conducted in the tidal portion of the Hudson River, NY 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, unpublished data) with little obvious external damage. The Hudson River Utilities Monitoring Program has also conducted a standardized trawling survey since 1985 by 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 suggest that trawls have a significantly lower mortality incidence than gill nets (ASMFC 2007). 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 noninvasive marine system sampling methodologies such as sonar, video, and combinations of both are quickly making these methods a viable alternative to traditional, potentially mortality-causing methods. Thus, such noninvasive methods should be used when 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.

Summary

We recommend that demographic assessments through surveys using gill nets be established and standardized with regard to biotic and abiotic factors known to influence catch composition. Establishment and support for such surveys should follow the ASSRT (2007) suggestions in that populations that are highly reduced in abundance should be viewed as high priorities. Standardization of typical sampling gear containing numerous meshes is not enough. Gear should also be standardized with regard to temporal and spatial deployment and other abiotic factors that significantly affect retention rates.

Training requirements

Safety of researchers and of the sturgeon being collected is of paramount importance. When large fish are taken, the fish can do considerable damage to itself, gear, and collectors if not handled correctly and quickly (AFS 2004). In order to safely handle large Atlantic sturgeon, experienced individuals and a crew with predefined duties are recommended to minimize risk. Because of inherent risks to both fish and humans, all researchers who wish to conduct collection efforts are strongly encouraged to actively participate in collection efforts with an experienced researcher prior to conducting independent efforts. Sampling with noninvasive 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, Atlantic sturgeon removed from fishing gear appear to be nonresponsive. 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. The most effective way to resuscitate fish is through the mouth, as if the fish were swimming forward. Dragging the fish back and pushing it forward is not ideal, but if there is no other option, then the drag back should very gentle so that the gill filaments are not damaged (Joel Van Eenennaam, University of California, pers. comm.). Also, the amount of oxygen exchange provided with this retrograde flow while pulling back is less than that provided when moving the fish forward (Joel Van Eenennaam, University of California, pers. comm.). The forward motion should be a faster

motion than pulling back. The best method is to have a pump and hose of freshwater directed into the mouth, and if the end of the hose is actually placed into the mouth, a soft piece of sponge should be used to keep the metal or hard plastic from injuring the inside of the fish's mouth (Joel Van Eenennaam, University of California, pers. comm.). Holding fish in large floating pens or in onshore raceways has been proven to be a successful resuscitation technique for both Atlantic and shortnose sturgeon in the Hudson River, NY (David Secor, University of Maryland, pers. comm.). 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 nonresponsive fish until such time as it is determined to be ineffective. In some cases, nonresponsive 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 often necessary to hold sturgeon for short periods while fishing with 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 captive fish to prevent sunburn. 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 the rate of temperature change fish are exposed to during collection and holding (Ziegeweid 2006) (see discussion of thermal tolerances on page 12).

Holding Tank: Holding tanks should be designed to accommodate the size of the fish being worked on and should 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 CO_2 (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 0.25-0.5% uniodized salt without anticaking agents (Brian Richardson, Maryland Department of Natural Resources, pers. comm.) for nonripe broodstock. Ripe, female Atlantic sturgeon

broodstock should not be exposed to a salt bath because of unknown effects on egg maturation (Mohler 2004); however, other species such as white and green sturgeon (*Acipenser medirostris*) have shown no adverse effects (Joel Van Eenennaam, University of California, pers. comm).

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, US FWS, pers. comm.) and has been associated with elevated stress (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. Tethered fish should never be pulled out of the water and hung by the tether for weighing or other purposes as this will result in tearing the mesenteries of large fish (David Secor, University of Maryland, pers. comm.).

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 to hold fish for later processing. Golder Associates LTD (2006a) describes the procedure used for tethering subadult 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.).

Stretcher: A hooded stretcher is commonly employed to handle large fish (Figure 5). 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. For very large fish, the stretcher can be tethered to the side of the boat for collection of field samples. Smaller fish can be lifted on board and placed in a stretcher holder (Mohler 2004). Aerated water is pumped with 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).

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).

Figure 5. A hooded stretcher (photo courtesy of Jerre Mohler, US FWS).

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 6). 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 6. Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) in an immobilization stretcher (photo courtesy of Chris Hager, VA Sea Grant, Marine Extension Program).

Prior to releasing a captured sturgeon, ensure that the swim bladder is deflated. 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 with the techniques 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 percentage 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%.

Summary

For short term holding, we recommend maintaining fish in floating net pens or holding tanks with flow through water to maintain proper temperature and dissolved oxygen concentrations. Field researchers should always carry instrumentation for monitoring water quality when handling sturgeon and make efforts to acclimate fish when water quality conditions are sufficiently different between bottom and surface waters. Tethering fish should be the method of last resort for short term holding.

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

Reasons for anesthetizing fish include: minimizing handling stress resulting from physical restraint, increasing safety for the fish and handlers, and reducing fish movement to facilitate performing a research-related procedure, thus providing for the greatest welfare during handling. However, whether fish feel pain is an ongoing debate, and the distinction between an anesthetic agent with analgesic properties and immobilizing drug is not always clear (Neiffer and Stamper 2009). We suggest at this time there is no one agent for fish which provides both analgesic and anesthetic properties which would prevent detection of noxious stimuli as well as block the physiological stress response to restraint. For procedures on Atlantic sturgeon which may require small incisions and can be performed quickly (<10 min) by experienced researchers,

use of anesthesia may not be the best course of action to obtain the desired results. If used, anesthesia should be administered to provide the lowest stage and plane of anesthesia as possible to safely conduct the specific procedure, and for the shortest exposure time. For most procedures, this would be defined as the point at which there is loss of equilibrium and failure to respond to tactile stimuli.

Some additional guidance is provided by the American Fisheries Society whose policy statement indicates that prolonged stressful restraint should be avoided, but in some cases, utilization of general anesthesia for restraint may be advisable. However, the benefits of anesthesia and potential effects on data derived from anesthetized fish should be weighed against results obtained from fishes that have not been anesthetized. The full range of potential effects on the subject fish, not just the anesthetic qualities, must be considered. Physiological stress from prolonged periods of restraint required for some procedures should be avoided if possible through the use of anesthesia (AFS 2004).

With regard to Atlantic sturgeon, three sedatives were evaluated at 5°C and 15°C in 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, WA); 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), therefore clove oil as an anesthetic for Atlantic sturgeon is not recommended. In the above evaluation, the appropriate dosage for metomidate and MS-222 was defined as the concentration necessary to sedate fish in 3-4 minutes (i.e., loss of equilibrium and lack of response to tactile stimuli) and to allow recovery in 3-4 minutes, with the exception of metomidate, which required longer recovery times regardless of concentration tested (Wade Jodun, US FWS, pers. comm. in Mohler 2004). In general, Atlantic 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 (>700 seconds at either temperature). Atlantic sturgeon (0.5-1 kg) were separately exposed for 20 minutes to the given dosages at water temperatures with no mortality. For larger Atlantic sturgeon (6-7 kg), the same dosages apply, but recovery times can greatly exceed those for smaller individuals.

For subadult and juvenile Atlantic 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 Atlantic sturgeon onto a stretcher assembly and delivering anesthetic solution to the fish via an electric pump and tubing (Figure 7). 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 overexposure does not occur. With any sedative, risk of lethal overexposure 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.

Figure 7. Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) being anesthetized (Photo courtesy of Jerre Mohler, US FWS).

MS-222- 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 postoperative recovery; thereby, minimizing holding time. 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 (Harms and Bakal 1994; and used on Gulf sturgeon (*Acipenser oxyrinchus desotoi*) by Fox et al. 2000). 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 when used in freshwater settings.

For invasive procedures (e.g., laparoscopy) that require prolonged surgical phase anesthesia, the anesthetic protocol for MS-222 detailed in the laparoscopy section of this document is more appropriate. When it is necessary to place Atlantic sturgeon under prolonged anesthesia, it is recommended that procedures be conducted in a controlled laboratory setting.

Metomidate- If the intent for anesthesia is to minimize the stress response (suppression of the hypothalamo-pituitary-interrenal (HPI) axis and related cortisol release), metomidate is the only commonly used drug that performs this function. However, it is a hypnotic and does not induce general anesthesia as is evident in muscle fasciculations (i.e., twitching of small nerve fiber bundles) generally observed at the site of an incision and is probably a poor analgesic

(Neiffer and Stamper 2009). A concentration of 15 mg/L is recommended for Atlantic sturgeon with sedation occurring in about 8 min (Jerre Mohler, US. FWS, pers. comm.). As previously stated, recovery times for fish treated with metomidate are much longer than for those with MS-222. If metomidate or any other nonapproved drug is to be used on Atlantic sturgeon released back into the wild, the national Investigative New Animal Drug (INAD) coordinator at the US FWS's Bozeman Fish Technology Center must be contacted for coordination purposes. See (http://www.fws.gov/fisheries/aadap/contactstaff.htm).

Other investigative anesthesia drugs- At this time, there are two INADs for experimental anesthesia which can be used by registering with the Aquatic Animal Drug Approval Partnership (AADAP) office (for contact information see web address above). These additional drugs are known as AQUI-SE® and BENZOAK®. Currently, there is no withdrawal period as long as fish are not susceptible to harvest within 72 hours of administration (http://www.fws.gov/fisheries/aadap). Their current use is investigative, and they have not been evaluated for use with Atlantic sturgeon.

Electro-anesthesia- Low intensity electrical currents have been used as a means of physical anesthetization in fish and provide an alternative to chemical anesthetics. Researchers have used alternating current (AC), straight direct current (DC), and pulsed direct current (PDC) to administer electronarcosis, and much of it is based on the same principles applied during electrofishing (Hartley 1967; Walker et al. 1994; Henyey et al. 2002; Barton and Dwyer 1997). The recommended method of applying electronarcosis to sturgeon is described by Henyey et al. (2002) using DC. Benefits of electronarcosis include shorter induction and recovery time, low risk of mortality, no visible sublethal effects (swimming and feeding behavior appear normal, no grossly apparent burns; Henyey et al. (2002)), and currents needed are imperceptible to researchers handling the fish and water containing current. Given these benefits, the utility of electronarcosis for even invasive techniques might prove to exceed that of chemical anesthetics such as MS-222. At this time, however, uncertainty exists over how electornarcosis works, and whether it is a true anesthetic or simply immobilizes and relaxes fish during research procedures (Hartley 1967; Henyey et al. 2002).

Summary

Because the administration of some anesthetics results in additional stress to fish, some researchers contend that it is an unnecessary and possibly risky procedure. However, others assert that for invasive procedures, it is more humane for the fish and safer for the researcher to administer anesthetics. We recommend that whenever it is practical for the researcher to administer anesthetics at the proper dosage, anesthetics be used both to reduce the risk to the researcher conducting the activity and to ensure humane treatment of Atlantic sturgeon.

LAPAROSCOPY AND RELATED TECHNOLOGIES, NON-INVASIVE PROCEDURES, AND TRADITIONAL METHODS OF DETERMINING SEX AND STAGE OF GONADAL DEVELOPMENT

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 do more traditional biopsy techniques which often require large abdominal incisions. For Atlantic sturgeon, laparoscopic procedures are mostly used to determine sex and stage of gonadal development, but they 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 stage of gonadal development in as little as 15 min per fish (including anesthesia but not recovery) if no unforeseen complications arise. A minimum of two people are necessary to perform laparoscopy on sturgeon. Mature broodstock are often 2 m 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 (Figure 8). 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 8. 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 steps:

1. Prepare the area where the procedure will be done, and assemble the laparoscopy equipment.

2. Prepare anesthesia. There are two 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 recirculating 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 is sufficiently anesthetized (see anesthesia section), the fish should be placed on the operating platform/stretcher with the maintenance dose of anesthesia recirculated 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 or 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 third and fifth scute anterior of the pelvic fins and offset and from the abdominal midline 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 (Figure 9). 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*.

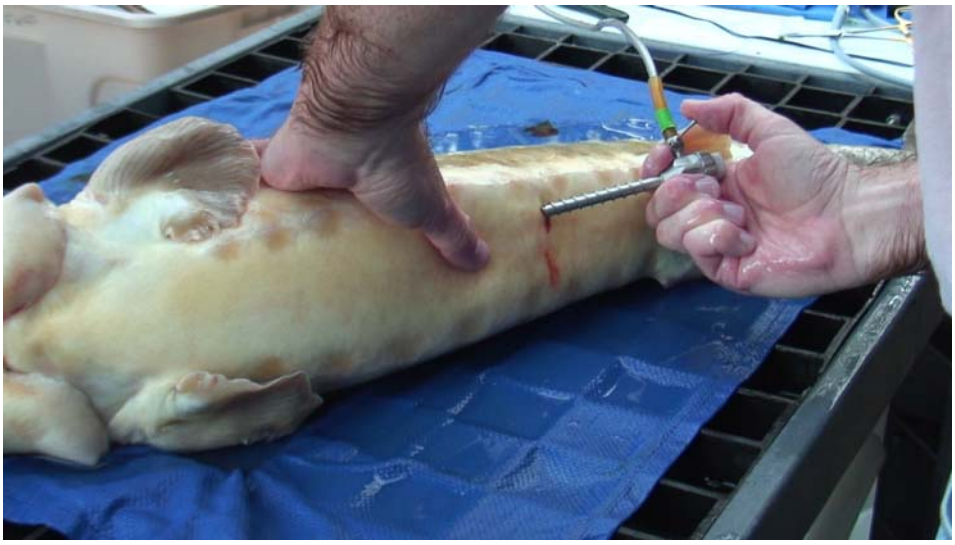

Figure 9. Insertion of cannula and insufflation technique.

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 (Figure 10). 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).

10. Post-surgery recovery. Move the sturgeon to its culture tank or back into the natural water supply, and orient the fish upright with 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).

Figure 10. Suturing the incision made during laparoscopy (Photo courtesy of Jerre Mohler, US FWS).

Traditional Methods of Determining Sex and Stage of Gonadal Development 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 plasma analyses of steroid levels; (4) ultrasound imaging; (5) other techniques such as the use of a borescope, meristics, and the shape of the urogenital opening. Each procedure is briefly explained below:

1. Coeliotomy- This technique requires an incision though the ventral abdominal wall large enough to view internal organs with the naked eye. This procedure is very invasive and should only be performed by individuals who have had adequate training in both surgery and sex identification (Figure 11). 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 abdominal wall. For fish large enough to be sexually mature (about 130-150 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. Sex can be identified after differentiation of the ovary and testis (the age and size of when this occurs is approximately 9 kg; Jerre Mohler, US FWS, pers. comm.). To determine the stage of gonadal development of small fish, a tissue sample of the gonad can be preserved in 10% buffered formalin, sectioned, embedded in paraffin, and stained for histological evaluation (Van Eenennaam and Doroshov 1998).

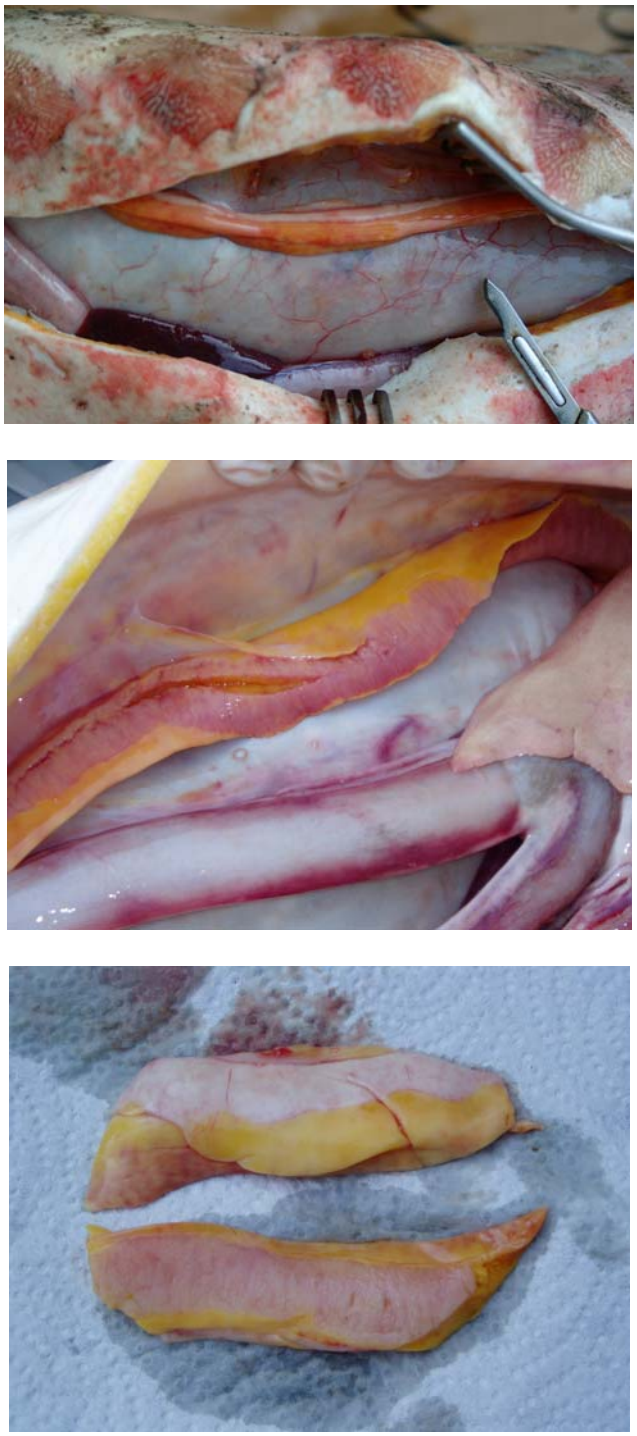

Figure 11. Gonadal tissue of immature sturgeons (Photos courtesy of Jerre Mohler, US FWS). (Top photo) Immature male. Turgid, smooth white strip of testicular tissue surrounded by orange colored adipose tissue. (Middle photo) Immature female. Pinkish, grooved ovarian tissue surrounded by orange colored adipose tissue. (Bottom photo) Dissected section of immature male (top) and female (bottom) gonad.

2. Expression of sexual products- If mature, sexual products may be expressed from the genital opening of wild individuals captured during spawning migration, especially in males (Mohler 2004). It is extremely rare, but possible, to obtain ovulated eggs from the genital opening of wild females. In order for this to be the case, the female would have been captured in the process of egg deposition.

3. Blood plasma analyses of steroid levels- 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 11-ketotestosterone via a procedure known as radio-immuno assay (Webb et al. 2002; Fiest et al. 2004; Wildhaber et al. 2006). 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). (See blood sampling and caudal venous puncture section below for more detail). In a recent study with 12 hatchery Atlantic sturgeon ranging from 9–17 kg, 42% of individuals were correctly classified as to gender by using radio-immuno assay as verified by histology while 75% were correctly classified using laparoscopy (Jerre Mohler, US Fish &Wildlife Service, pers. comm.).

4. Ultrasound imaging – Advancements in technology have made it possible to use portable ultrasound imaging equipment for identifying the sex of sturgeon, as well as determining other aspects of gonad development. Ultrasound offers a quick (10 seconds to 2 minutes per individual after significant experience), noninvasive method for sex identification (Chebanov and Galich 2009). In this procedure, the transducer is held against the posteroventral portion of the abdominal region of the sturgeon. Specific characteristics of male and female gonads allow the reader to determine which gonad is being examined (testis or ovary) and at what stage the gonad has developed. Ultrasound imaging has been shown to be effective for determining the sex of the stellate sturgeon (*Acipenser stellatus*) (Moghim et al. 2002) and numerous other Eurasian species of sturgeon (Mikhail Chebanov, pers. comm.; Chebanov and Galich, 2009). 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. In another study of shovelnose sturgeon (Wildhaber at al. 2005), however, ultrasound was shown to be less effective than use of an endoscope and resulted in correctly determining gender only 68-70% of the time, while use of an endoscope through an abdominal incision resulted in correct sex determinations 92% of the time. Bryan et al. (2007) also used ultrasound to study aspects of the reproductive condition of shovelnose sturgeon and pallid sturgeon (*Scaphirhynchus albus*), such as egg diameter, fecundity, and gonad volume. Although it was found that there was significant measurement error that required correction (e.g., egg diameter was underestimated by 52%), it was determined that ultrasound "accurately measure[d] the gonad volume, but needed a correction to accurately measure egg diameter and fecundity" (Bryan et al. 2007: 418). Because of variation in the anatomy of different species (e.g., thickness of body wall because of overall size of individuals; relative thickness and density of bony plates in the skin between the scutes), it is likely that there are species-specific aspects to interpretation of

ultrasound data. At this time, no ultrasound studies have been performed with Atlantic sturgeon. The quality and ease of interpretation of ultrasound images will be dependent on the condition of the individual being evaluated. For instance, it has been well established that adipose tissue disrupts the ultrasound and prevents a clear image from being obtained. It is therefore suggested that ultrasound is most effective for sex determination after overwintering and before feeding is initiated (or in hatchery conditions, after a period of starvation) and large amounts of fat tissue are laid down and the gonads can be clearly seen in the ultrasound image (Chebanov and Galich 2009). Although ultrasound equipment costs are relatively expensive at this time, it is a promising method for quickly determining the sex of an individual by means of a noninvasive method given sufficient training in interpretation of ultrasound images.

5. Other – A borescope was used by Kynard and Kieffer (2002) to determine gender in shortnose sturgeon by viewing the gonads through the urogenital duct, and they were able to determine the oocyte maturation stage by gonad color. However, they found no characteristic of a male testis that enabled them to conclusively distinguish between males and immature females. Bryan et al. (2007) used a flexible endoscope with female shovelnose sturgeon to determine egg diameter and fecundity by viewing the gonads through the urogenital opening as well as a small incision through the abdominal wall. A number of other techniques, such as meristics and the shape of the urogenital opening, have been tried with other species but have not been sufficiently tested on Atlantic sturgeon for reliable use at this time.

Summary

There is no single gender determination technique which can be recommended for all occasions. Numerous factors relative to an individual researcher's particular study must be taken into account before selecting one or more of the above-described techniques. Important factors to consider should include but are not limited to: (1) suspected level of sexual maturity likely to be encountered; (2) skill and experience of the researchers; (3) speed with which results are desired; (4) availability of equipment; and (5) number of fish expected to require analysis.

Analysis of gonadal tissue samples via histology is the most accurate form of sex determination since specific structures associated with either spermatogenesis or oogenesis can be verified microscopically (Van Eenennaam and Doroshov 1998). For a more immediate sex determination of Atlantic sturgeon, laparoscopy gives the best view of internal organs in live fish and allows the most accurate determination of sex in individual fish having visually-differentiated gonads (usually fish >10 kg). Use of laparoscopy is best suited to a hatchery or laboratory setting, but with the proper power supply requirements and overhead protection from sun glare and adverse weather, laparoscopy equipment can be set up on a vessel or shore-based operation near the capture location. Use of coeliotomy to determine gender is not recommended for most occasions since the size of the incision must be large enough (> 40 mm) to spread open the body cavity using a surgical retractor. Unless the fish is a reproductively mature individual, the target tissue that must be viewed lies obscured against the abdominal wall and is difficult to manipulate for a satisfactory view without some magnification or other technologic aid. Improvements in the above techniques and technologies will likely continue over time.

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 nonlethal sampling of stomach contents have been developed, but gastric lavage is recommended (Figure 12). Gastric lavage is relatively cost effective, nonlabor intensive, and 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.

Figure 12. Gastric lavage (South Carolina Department of Natural Resources).

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 (see anesthesia section above for full discussion). 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 midsized sturgeon (75.0-150.0 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 because of 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 (Figure 13). 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. Large diameter tubes have been utilized by researchers to aid in inserting the flexible small diameter tube down the esophagus; they have been used 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.

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 cannot 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 specifically on Atlantic sturgeon specifically is needed.

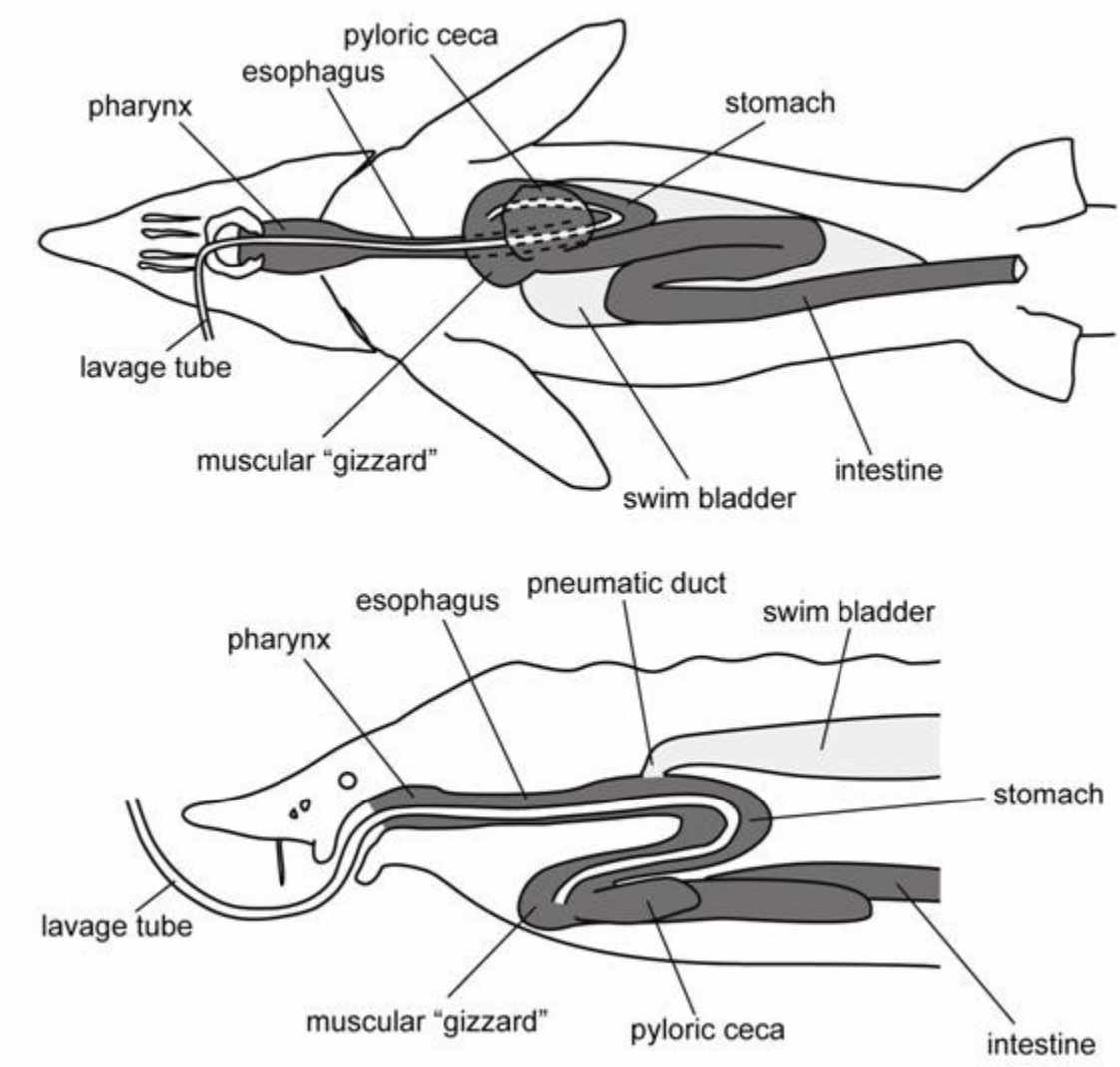

Figure 13. Generalized depiction of the gastric lavage technique for Atlantic sturgeon (drawing courtesy of Eric Hilton, Virginia Institute of Marine Science).

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 30ºC 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.

Summary

Gastric lavage is the least injurious, nonlethal technique available for examination of Atlantic sturgeon stomach contents. However, lavage is considered an invasive procedure, and full care and attention are necessary to minimize the potential for negative effects resulting from the procedure. As such, the smallest size water tubing feasible should be used with additional trade-offs between flexibility and ability to insert the tubing beyond the oral cavity. It is generally desired to hold fish in an inverted position, with the head end lower. Tubing must be inserted gently while expressing water. Tubing should never be forced if resistance is noted. It may take several tries to successfully get the tube into and beyond the pharynx. Volume of water and water pressure to be flushed through the fish also needs to be monitored and regulated. Continuous monitoring of the fish is required, and if water/discharge from the anus is noted, the tubing should be extracted somewhat and/or water pressure or volume reduced. Additional study of both short and long term effects, specific to Atlantic sturgeon are recommended.

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 AND MARKING

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 Atlantic sturgeon (Eyler et al. 2009), where sturgeon are believed to experience the least new muscle growth. It is

recommended that the needles and PIT tags be disinfected in isopropyl alcohol or equivalent rapid acting disinfectant. After any alcohol sterilization, we recommend that the instruments be air dried or rinsed in a sterile saline solution, as alcohol can irritate and dehydrate tissue (Joel Van Eenennam, University of California, pers. comm.). Tags should be inserted antennae first in the injection needle after being checked for operation with a PIT tag reader. Atlantic sturgeon 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 (Figure 14). After insertion into the musculature, the needle angle should be adjusted to close to parallel and pushed though to the target PIT tag site while injecting 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. Because of 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 ensure detection of fish tagged in other studies. Because of 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.

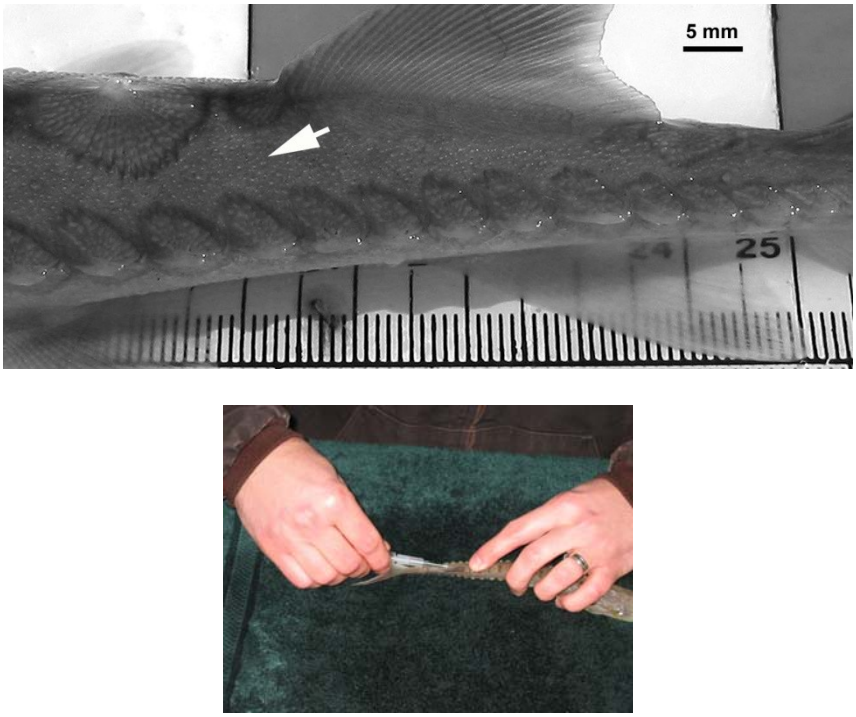

Figure 14. Illustration of PIT tag location (indicated by white arrow; top), and photo of a juvenile Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) being injected with a PIT tag (bottom). Photos courtesy of James Henne, US FWS.

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%, because of 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, US FWS, unpublished data). Because of 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 smaller than 300 mm, only Atlantic sturgeon larger than 300 mm should receive PIT tags. Sutures were not used to close the PIT tag insertion point during this study (James Henne, US FWS, pers. comm.).

Fuller et al. (2008) provide guidance on the quality of currently available PIT tags and readers and offer 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/Maryland Fishery Resources Office (MFRO) will collect data in the coastal tagging database and provide approved tags for distribution to researchers.

Golder Associates, Ltd. (2006b) 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.

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 (Figure 15). 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 while lay individuals read the mark correctly with 74% accuracy. In sturgeon smaller than 2 ft, the scute can be removed with a number twelve curved scalpel blade. The lateral scute should be removed by cutting from 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.

Scalpels should be disinfected and allowed to dry between fish. For sturgeon larger than 500 mm, a sharp filet knife can be used for scute removal. If skin is cut to the subdermis layer, antiseptic ointment should be available to apply to the wound. Fish can be anesthetized with a light dosage of anesthesia (75-90 mg/L MS-222) to immobilize the fish for this procedure (see anesthesia section for further detail). 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 (OTC) 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. 2006b). 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 that the antibiotic treatment minimizes possible bacterial infections resulting from fish handling, including scute removal. In addition to OTC, US FWS has been granted an INAD permit exemption allowing the use of calcein "SE-MARK®" to mark hard parts of fish, including sturgeon[1].

Figure 15. Scute removal (left), and marking and insertion of coded wire tags (right) in lake sturgeon (*Acipenser fulvescens*).

The lake sturgeon reintroduction program occurring in the southeastern United States has been using scute marking to aid in the identification of year classes of hatchery reared fish. The fish are marked as subyearlings (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 subyearlings 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 Atlantic sturgeon if scute marking is implemented. 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 antipredation device, especially in small Atlantic sturgeon, we recommend that long-term studies designed to assess the impact of scute removal on mortality rates be conducted.

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[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 US Fish & Wildlife Service. Researchers desiring to use calcein for fish marking must register with the Aquatic Animal Drug Approval Partnership (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.

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 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 because of the presence of multiple sensory systems, we recommend that CWT be injected underneath the first dorsal scute for Atlantic sturgeon. The standardization of one location will help researchers in the conduction of broad-scale studies. Peterson et al. (2000) used CWTs to study population dynamics and wild juvenile shortnose sturgeon recruitment in the Hudson River system. CWTs provide an inexpensive mark to mass mark hatchery fish for use in mark–recapture studies to provide evidence of decreased recruitment of juvenile wild fish to the system. A CWT was detected in a captured 15-yr-old Atlantic sturgeon released into the Hudson River as a fingerling but even with the proper detector, numerous attempts were made before a positive detection was obtained. The presence of a visual secondary mark (i.e., pelvic fin removal) indicated the fish had received a CWT prior to its release, causing the researchers to perform repeat attempts at tag detection (Jerre Mohler, US FWS, pers. comm.). CWTs have limited utility for marking wild fish in the field because of the expense and complexity of the equipment but may be valuable tools 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 CWTs during routine surveys. Because CWT equipment is expensive and has not been widely employed by Atlantic sturgeon researchers, its use is not recommended for routine studies.

External Tags – (nontelemetry)

The use of an internal tag (e.g., PIT) is preferable for long term mark-recapture studies because of 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 US FWS Maryland Fisheries Resources Office (MFRO). 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 (Figure 16).

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 multiyear studies are encouraged to utilize internal implantation of transmitters (Dovel and 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 (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 for 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 beeswax, although reactivity rates for it are not well understood (Helm and Tyus 1992).

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, 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) (Knights and Lasee 1996; Bauer et al. 2005). 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 (Bauer et al 2005). 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 to 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 from temperature/dissolved oxygen extremes (see discussion on tolerance to high temperatures and low dissolved oxygen conditions; 12). Anesthesia should be administered according to guidance provided in the anesthesia section of these protocols (beginning on page 19). All reasonable means should be used to check fish for existing biotelemetry transmitters prior to surgery. If it is determined that a fish has a currently functioning internal transmitter, implantation of a new transmitter is not recommended. Possible transmitter detection methods include hydrophones, coded wire tag wands, and metal detectors. Surgical implantation of transmitters should be conducted with 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, US FWS, pers. comm.). The betadine has antibacterial properties but also antifungal properties, which may be even more important (Robert Bakal, US FWS, 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 selected to minimize the potential of recapturing recently tagged individuals.

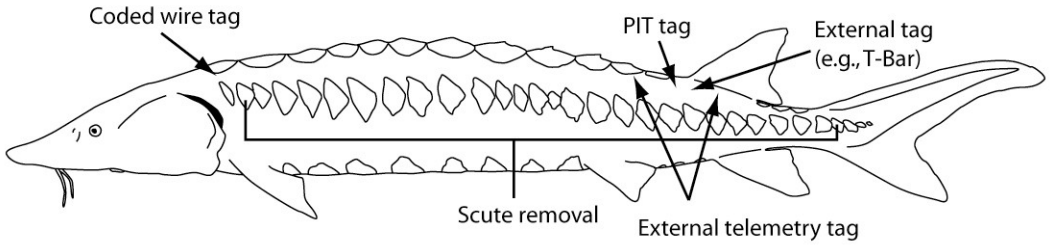

Figure 16. External tag attachment locations for Atlantic sturgeon (line drawing adapted from Figure 3; courtesy of Eric Hilton, Virginia Institute of Marine Science).

Summary

All Atlantic sturgeon greater than 300 mm should be tagged with PIT tags. Approved PIT tags and external t-bar tags should be acquired from the US FWS/MFRO office and data should be housed in their coastal tagging database. Because of the long life span and wandering nature of Atlantic sturgeon, researchers should consider both the long term and short term implication of any tagging studies. Researchers likely to encounter Atlantic sturgeon in other sampling activities should be provided the means to both scan captured Atlantic sturgeon and implant PIT tags in individuals not previously tagged. Scute removal as a means of externally marking Atlantic sturgeon should not be undertaken until more detailed studies on the long term effects on the health of Atlantic sturgeon are conducted. If an external tag is needed, researchers should contact the US FWS/MFRO to receive standard t-bar tags to be placed at the base of the

left dorsal fin musculature. Given the high costs associated with telemetry studies, we recommend that researchers give consideration to the issues of system compatibility and code collision when initiating a telemetry study on Atlantic sturgeon. Short term telemetry studies on Atlantic sturgeon should consider the use of external transmitters while multiyear studies should utilize internal implantation techniques to minimize transmitter loss.

Training requirements

Because of 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 and stage of gonadal development 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 (Chapman et al. 1996; Fox et al. 2000; Webb and Erickson 2007). In short, as small as an incision as possible (25-40 mm) is made on the ventral surface of the anesthetized fish with a sterile scalpel. Once the gonad is located, a 1 cm^3 sample can be taken with 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 nonlethal sample is desired. In immature fish the gonad is composed 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, noninvasive procedures, and traditional methods of determining sex and stage of gonadal development).

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 much as 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 comparion 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). Blood can be collected by puncturing the caudal vein (located ventral to vertebral column in the midline of the caudal peduncle) with a hypodermic needle or vacutainer (Figure 17). 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 (Figure 17). 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. 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 because of 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 with heparinized syringes or immediate transfer to heparinized vacutainers before centrifugation.

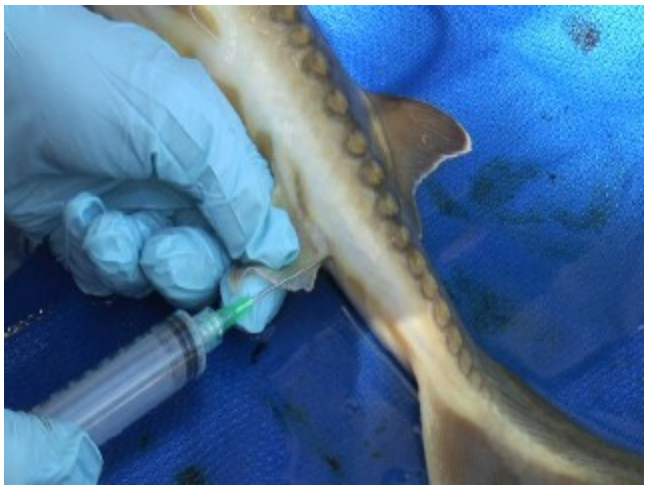

Figure 17. Caudal venous puncture (performed on a small, two year old fish).

Genetic tissue samples

For genetic analyses, a 1 cm^2 fin clip from one of the pelvic fins from living sturgeon should to be taken and placed in a labeled vial with an o-ring cap containing 95% nondenatured 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 (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 they have been used for forensics, bacteriology, and plant and animal genetics (Purvis et al. 2006; Mbogori et al. 2006). Livia et al. (2006) found FTA-collected genetic samples to be reliable for microsatellite and Restriction Fragment Length Polymorphism (RFLP) analysis derived from samples of mucus and buccal cells of northern pike (*Esox lucius*) and brown trout (*Salmo trutta*). 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 caused by 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, such as 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 five measurements described on page 5, 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), the corresponding collection data, and any tag numbers available should be delivered to the National Ocean Service (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 for a broad range of aspects of sturgeon biology and management, results will be provided to the investigator who originally submitted the tissue; the investigators will 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 nondeleterious (cf. otoliths, for which sturgeon must be sacrificed), allows for mark-recapture (e.g., OTC or calcein [see footnote on page 36]), and there is a precedence for this technique in the literature and previous care/handling documents (Cuerrier 1951; Rossiter et al. 1995; for Atlantic sturgeon specifically Stevenson and Secor 1999; 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 (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 River 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 (Rien and Beamesderfer 1994; 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 18). After removing the sample, be sure to disinfect the wound and allow the fish to recover before releasing the fish.

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 because of potential deleterious effects. Full spines (i.e., including the base of the spine) may be removed from dead specimens.

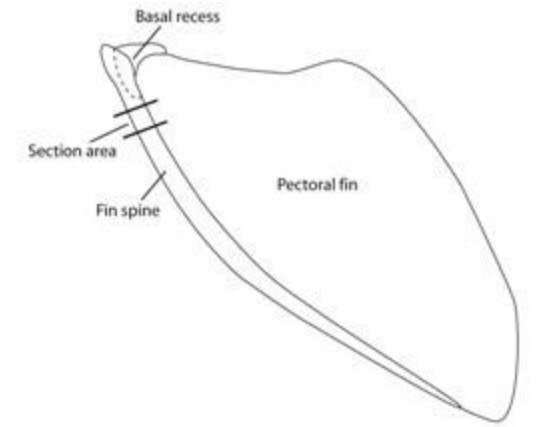

Figure 18. Depiction of a pectoral fin spine (drawing courtesy of Eric Hilton, Virginia Institute of Marine Science).

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 can 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). 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 -80ºC prior to analysis. Brundage (2003) reported on analysis of ICP metals, mercury, Target Compound List (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 (three 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).

Summary

- Tissue samples collected from live or dead specimens can provide a variety of useful information to researchers. Biopsies of internal organ tissues are best suited for salvage specimens. When conducting muscle biopsies on live fish, tissue collected should be from the thickest portion of the dorsal musculature.
- Gonad biopsies of 1 cm^3 to determine sex and stage of gonadal development can be taken with a 25-40 mm incision; to be effective samples must include germinal tissue, which can be difficult to obtain, especially in immature fish.
- It is recommended that blood samples be taken by ventral venipuncture of the caudal peduncle; the needle should be inserted at a 45° angle, but in small fish this may not be

possible, and an insertion perpendicular to the vein may be most effective. Blood samples collected in the field should be stored on ice; if long-term storage is desired, samples should be kept in sterilized containers at -20 to -80°C. Blood should not be collected in glass syringes unless specifically requested in the protocol. Syringes and vacutainers can be used interchangeably as long as the type of vacutainer meets the blood collection objective (i.e. serum, vs. plasma).

- For genetic analysis, we recommend that 1 cm^2 tissue samples be taken from the pelvic fins of all fish, refrigerated or placed on ice for the first 24 hours if possible, and placed in 95% nondenatured EtOH; if long-term storage is desired, samples can be stored after initial steeping at 4 to -20°C. Collecting genetic samples requires the use of clean, sterile instruments for each fish. A specific field protocol for sterilizing instruments would involve scrubbing flesh off the instrument and rinsing in fresh water then placing instruments in a 10% Clorox bath to degrade residual DNA. Instruments can then be rinsed in distilled water and dried before use. Ratio of ethanol to tissue should be approximately 10:1 for storing the tissue. A subsample of the genetic tissue sample and the corresponding collection data should be delivered to the NOS archive in South Carolina (attention Julie Carter, NOS Marine Forensics Branch, 219 Fort Johnson Road, Charleston, SC 29412).
- A 1 cm section can be taken from the pectoral fin spine for ageing (see Rien and Beamesderfer 1994, and Secor and Woodland 2005 for technique details) but should not be taken from larger adults because of evidence that these individuals do not recover well from the procedure.
- All savage specimens should be reported to NOAA Fisheries Service's salvage network so that specimens can be obtained and sampled if desired; sampling of salvaged fish should be conducted to maximize the information gained.

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), 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

Collection and storage 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, see Mohler (2004).

Short term storage

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 shortnose sturgeon and Gulf sturgeon (Park and Chapman 2005) and with significant decrease in sperm motility. Dorsey (2009) found that wild caught Atlantic sturgeon had higher quality sperm than captive individuals, perhaps because of environmental or diet stressors on captive individuals (captive samples did not survive after 21 days). For short term storage of sperm, Bill Wayman (US FWS 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 (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. Dorsey (2009) found that sperm stored with oxygen was of higher quality (viability, motility, curvilinear velocity, and cell ATP level) than sperm stored in an oxygen free environment. Various extenders have been used for storage and 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., Billard et al. 2004). Horvath et al. (2005) used a modified Tsvetkova's extender in a 1:1 ratio with sperm to cryopreserve sperm of shortnose sturgeon. 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 4ºC). Dorsey (2009) tested two extenders and found that the one described by Park and Chapman (2005) in the presence of oxygen was most effective and recommended dilution immediately upon collection to avoid contamination issues.

Cryopreservation

Sperm can be cryopreserved with 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^{o}C water bath; Bill Wayman, USFWS Warm Springs Fish Technology Center, pers. comm.; see also Jähnichen et al. 1999; 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.

Summary

To maximize the success and value of collected sperm, we recommend:

- Diluting sperm immediately after collection with an appropriate extender (as described by Park and Chapman (2005)
- Store sperm in the presence of oxygen, and resuspend the sperm dailyTraining requirements

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

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APPENDIX – FIELD DATA SHEET

ATLANTIC STURGEON FIELD DATA COLLECTION FORM

For use in documenting Atlantic sturgeon capture and tagging in the wild

COLLECTOR'S CONTACT INFORMATION
Pre-filled with Researcher Info
Name: First _____ Last _____
Agency Affiliation _____ Email _____
Address _____
_____
Area code/Phone number _____

UNIQUE IDENTIFIER (Assigned by NMFS)
AOX000000(STATE)(DATE)

DATE CAPTURED:
Month ☐☐ Day ☐☐ Year 20☐☐

DATE RELEASED:
Month ☐☐ Day ☐☐ Year 20☐☐

LOCATION FOUND: ☐Offshore (Atlantic or Gulf beach) ☐Inshore (bay, river, sound, inlet, etc)
River/Body of Water_____ City_____ State ____
Descriptive location (be specific)_____
_____
_____
Latitude _____N (Dec. Degrees) Longitude _____ W (Dec. Degrees)

WATER CONDITION at time of capture:
Water Depth _____ Ft
Water Temp _____ F°/C°
Salinity _____
Dissolved Oxygen_____ mg/L

SEX:
☐ Undetermined
☐ Female ☐ Male
How was sex determined?
☐ Necropsy
☐ Eggs/milt present when pressed
☐ Borescope
☐ Other

MEASUREMENTS: circle unit
Length ☐ actual ☐ estimate
Fork length _____ cm / in
Total length _____ cm / in
Mouth width (inside lips, see reverse side) _____ cm / in
Interorbital width (see reverse side) _____ cm / in
Head length _____ cm / in
Weight ☐ actual ☐ estimate _____ kg / lb

TAGS PRESENT? ☐ Yes ☐ No **(If Yes, please fill in tag #, type, and location)**

Tag #	Tag Type	Location of tag
_____	_____	_____
_____	_____	_____
_____	_____	_____

NEW TAGS PLACED? ☐ Yes ☐ No **(If Yes, please fill in tag #, type, and location)**

Tag #	Tag Type	Location of tag
_____	_____	_____
_____	_____	_____
_____	_____	_____

GEAR INFORMATION:
Capture Gear_____
_____
Soak Time _____hrs
Mesh Stretch_____in

LOCATION RELEASED:
☐Same as Above ☐Offshore (Atlantic or Gulf beach) ☐Inshore (bay, river, sound, inlet, etc)
River/Body of Water_____ City_____ State ____
Descriptive location (be specific)_____
_____
_____
Latitude _____N (Dec. Degrees) Longitude _____ W (Dec. Degrees)

TISSUE SAMPLES COLLECTED? ☐ Yes ☐ No

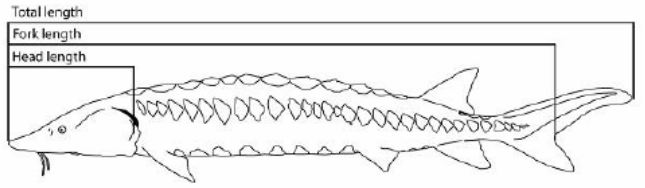
Sample	How preserved	Disposition (person, affiliation, use)
_____	_____	_____
_____	_____	_____
_____	_____	_____

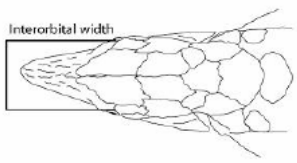
SEE BACK ➡

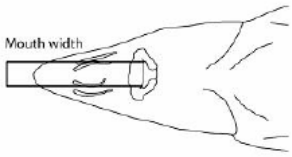
Distinguishing Characteristics of Atlantic Sturgeon

Characteristic	Atlantic Sturgeon, *Acipenser oxyrinchus*
Maximum length	> 9 feet/ 274 cm
Mouth	Football shaped and small. Width inside lips < 55% of bony interorbital width
*Pre-anal plates	Paired plates posterior to the rectum & anterior to the anal fin.
Plates along the anal fin	Rhombic, bony plates found along the lateral base of the anal fin (see diagram below)
Habitat/Range	Anadromous; spawn in freshwater but primarily lead a marine existence

* From Vecsei and Peterson, 2004

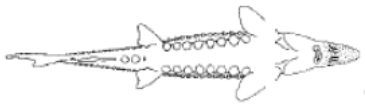






Circle any areas where wounds/abnormalities were found:

Ventral



Describe any wounds / abnormalities (note tar or oil, gear or debris entanglement, propeller damage, etc.). **Please note if no wounds / abnormalities are found.**

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