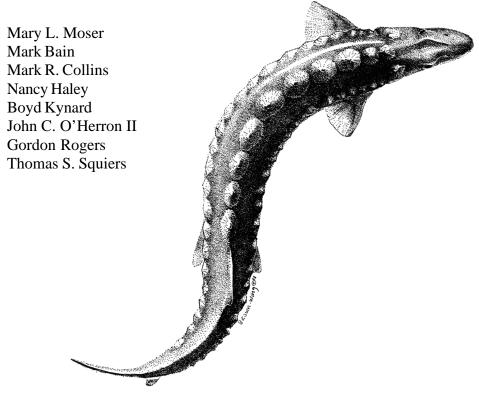
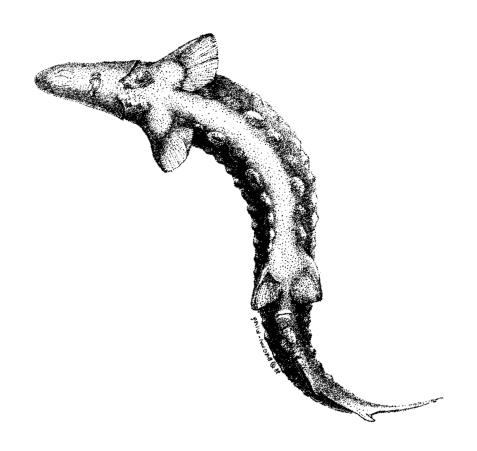
# A Protocol for Use of Shortnose and Atlantic Sturgeons





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#### **Abstract**

Guidelines for handling and sampling of Atlantic coast sturgeons are needed to protect these fishes and to facilitate standardization of methodologies used by sturgeon researchers. The shortnose sturgeon, *Acipenser brevirostrum*, is a federally listed endangered species and the Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus*, is considered a species of special concern. Consequently, special techniques have been developed to reduce stress and mortality resulting from sampling and handling these species. In this document we review the most acceptable methods for short-term holding, identification and measurement, tagging, tissue sampling, gastric lavage, and collection using a variety of gear types. In addition, we provide a protocol for sampling to establish whether shortnose sturgeon are present in systems where their status is unknown.

#### Introduction

In recent years, a need has developed for standardization of sampling and handling methods for Atlantic coast sturgeons: shortnose (*Acipenser brevirostrum*) and Atlantic (*A. oxyrinchus oxyrinchus*). The shortnose sturgeon has been federally-listed as an endangered species since the Endangered Species Act of 1973. In the past few years the Atlantic sturgeon has been petitioned for listing and has been designated as a candidate species. Because the shortnose sturgeon has been listed for so long, it has been the subject of a relatively large number of research projects; however, this research has been conducted by only a handful of individuals. The Shortnose Sturgeon Recovery Plan (National Marine Fisheries Service 1998) specified the need for a sampling and handling protocol because of: 1) the likely increases in research on sturgeon in future years by a larger number of scientists and the concomitant need for standardization of methods, 2) the need for guidance in permitting research activities that may harm sturgeon, and 3) the need for minimum sampling requirements to determine that sturgeon are extant in a given system.

Sturgeon present some unique challenges for development of standardized methods. Both shortnose and Atlantic sturgeon may occur in a variety of habitats in Atlantic drainages from southern Canada (Saint John River) to northern Florida (St. Johns River). The differences in habitat both within and among river systems, and latitudinal differences in temperature and sturgeon life history, have resulted in sampling methods that are often specific to a given region or time of year. To make this document as comprehensive as possible, we have incorporated methodologies from research conducted across the entire range of habitats where these sturgeons occur and for the all sturgeon life stages that have been studied in the wild. We make no attempt here to suggest methodology for culture or long-term maintenance of sturgeon. In reviewing the literature and incorporating our own experiences in this protocol, we noted that innovations in research occur rapidly. Consequently, we emphasize that this protocol should be a living document that incorporates new techniques as they are

developed and perfected. This protocol represents many years of collective experience in sampling and handling sturgeons and should provide useful guidelines for future research. Our intent is not to discourage development of new techniques or to limit or restrict sturgeon research.

## **Handling Methodologies**

Both shortnose and Atlantic sturgeons are very hardy species. The ability of sturgeon to survive under extremely stressful conditions is well established and was exploited during early fisheries for their flesh and roe. The sturgeon's hardy nature also permits the use of research practices that stress these fish, potentially resulting in negative, but sub-lethal, impacts. For example, excessive handling of pre-spawning adults during their migration can result in interruption or even abandonment of upstream migration (Moser and Ross 1995). Moreover, sturgeon are very sensitive to handling during periods of high water temperature or low dissolved oxygen, and sturgeon can be lethally stressed in a short time if handled improperly during these conditions. The following handling protocol therefore includes guidelines for a variety of conditions.

## Short-term holding

It is frequently necessary to hold sturgeon for short periods while fishing nets, tagging or collecting tissue samples. If possible, sturgeon should be held in floating net pens or live cars during processing. When fish are held on board the research vessel, they should be placed in flow-through tanks that allow total replacement of the water volume every 15 - 20 min. While total water volume in the tanks is not critical, adequate control of temperature and oxygen levels is absolutely essential. Fish should not be held on board for longer than 2 h when water temperatures are equal to or less than 27°C. If water temperature exceeds 27°C, sturgeon should never be held on board for longer than 30 min. Dissolved oxygen levels below 3 ppm are also stressful to sturgeon (Jenkins et al. 1993). Therefore, oxygenation of the water in holding tanks may be necessary during periods of high temperature or low dissolved oxygen and handling should be minimized. The use of an electrolyte bath (such as Stress Coat, marketed by aquaculture suppliers) can also help to reduce stress and restore the slime coat when fish are collected in fresh water. Sturgeon are very sensitive to chlorine; so, very thorough flushing is required if holding tanks are sterilized with bleach between sampling periods.

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.

## *Identification and measurement*

Identification of sturgeon to species, sex and reproductive condition may involve use of both external and internal morphology. Juvenile Atlantic sturgeon and juvenile or adult shortnose sturgeon are easily confused and care should be taken in use of morphological characters for identification. The most consistently accurate external character is the ratio of bony inter-orbital width to mouth width (Moser et al. 1998). Use of other characters such as snout length and scute patterns can be misleading. For weight measurements, sturgeon should be supported using a sling or net and handling should be minimized throughout processing. Use of smooth rubber gloves is recommended to reduce abrasion of skin and removal of mucus.

Neither sturgeon species can be sexed on the basis of external morphology. A close magnifier at the end of a light beam (Bioscope) can be used to distinguish sexes and even to stage eggs without surgery. This instrument is gently inserted through the genital opening and rotated to view the gonads internally. This technique is quick, far less intrusive than surgical procedures, and with experience its use will allow differentiation of females that will spawn during the next spawning period from immature and post-spawned females. However, it cannot provide maturity stage data for males, nor differentiate between males and immature females.

## Tagging

The life history, morphology, behavior, and physiology of sturgeons present a plethora of challenges for tagging studies. Sturgeon are long-lived; so, for many studies it is essential that tags be retained for extended periods. In addition, they exhibit very rapid juvenile growth rates and, in the case of Atlantic sturgeon, can achieve very large sizes (> 3 m). Therefore, tags must be retained even as the tag placement area changes size and shape. Moreover, sturgeon are adept at rubbing off external tags and can actually extrude internal tags through the body wall to rid themselves of tags placed in the body cavity (Kynard and Kieffer 1994). Our collective experiences with a variety of tagging methods and materials, in addition to laboratory studies of tag retention, were drawn upon to provide the following recommendations for tagging.

External tags generally have lower retention rates than internal tags, but are often needed in studies that require participation of people other than the researcher (such as tagrecapture studies that rely on tag returns from fishermen). A variety of external tag designs and placement sites have been used on both Atlantic and shortnose sturgeon. The first laboratory studies of tag retention by shortnose sturgeon indicated that Carlin tags placed just below the dorsal fin and internal anchor tags inserted laterally into the abdomen had the highest retention rates of the tags tested (Smith et al. 1990). More than 50 shortnose sturgeon marked with Carlin tags in the Hudson River from 1979-80 were recovered in recent

research, indicating that these tags can have long retention times. About half of the tag disks were clearly legible and provided valuable data on fish at large for over 15 years. However, Carlin tag retention in both the Connecticut River and Delaware River has been poor when compared to passive integrated transponding (PIT) and anchor tags, respectively. Anchor tags placed at the base of the dorsal fin in 1981-87 are now being recovered in the Delaware River over a decade later. Collins et al. (1994) tested a variety of external tag designs in the laboratory and found that a T-anchor tag inserted into the lateral abdominal wall provided the greatest retention. However, it was noted that healing of the insertion wound was slow (or did not occur) for all tags that protruded through the skin. While external tags clearly have lower retention than internal tags, anchor tags in the dorsal musculature show the most promise for greatest longevity with least impact to the fish.

A number of sturgeon studies use PIT tags in addition to an external tag. These tags are injected just below the skin along the dorsal mid-line anywhere from the posterior edge of the fourth dorsal scute to the posterior edge of the dorsal fin. Due to the lack of standardization in placement of PIT tags, we recommend that the entire dorsal surface of each fish be scanned with a waterproof PIT tag reader to insure detection of fish tagged in other studies. We note that juvenile 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, the largest (highest power) PIT tags should be used for both sturgeon species, and tags should be placed posterior to the dorsal fin, where tissue growth is least. PIT tags far out perform external tags. However, laboratory studies indicate that sturgeon smaller than 200 mm TL shed PIT tags at a rate of over 50%, due to the lack of musculature at this size. The likelihood of high PIT tag loss should therefore be considered when marking sub-yearling sturgeon.

A variety of methods have been used to outfit sturgeon with sonic or radio transmitters. Due to their large body size, sturgeon can carry large transmitters having extended battery life. Consequently, it is important that these tags be retained for as long as possible. External attachment of the transmitters is the least intrusive method; however, a number of field studies have indicated that both sonic and radio tags are shed at rates of 15 - 60% within the first 4 – 6 mo. of external attachment (Smith 1988, Moser and Ross 1993, Kieffer and Kynard 1993, Rogers and Weber 1995). In a tank study using cultured shortnose sturgeon, externally-attached transmitter loss began on day 2, and 100% were lost by day 60. It was obvious that the sturgeon actively rubbed the transmitters on any available surface.

In spite of the problems with tag loss, only external attachment of transmitters should be used for pre-spawning fish in spring or those on the spawning ground. In addition, surgical implants should not be attempted when water temperature exceeds 27°C (to reduce handling stress) or is less than 7°C (incisions do not heal rapidly in low temperatures). External transmitters are retained longest when they are as small as possible and are attached through the dorsal fin using monofilament line or stainless steel leader and a PVC backing

plate (Rogers and Weber 1995). The addition of a neoprene pad between the fish's body and the transmitter or backing plate helps to protect the fish.

Internal implantation of radio or sonic transmitters provides greater retention than external attachment. Radio range is maximized with a trailing antenna, however, there is less chance of infection if the antenna is also implanted internally. In a recent tank study, radio transmitters were surgically implanted in cultured shortnose sturgeon, but the antennas were externally trailing. After 90 days, all of the fish had openings around the antenna exit area and were still bleeding or obviously infected. In some cases the antenna had cut large wounds through the abdominal wall and the transmitter and internal organs were visible. Field trials using this method of attachment indicated less significant impacts to wild shortnose sturgeon in the upper Connecticut River. Eight fish tagged internally with transmitters having a trailing radio antenna were recaptured after 12 months at large. While the tissue at the antenna exit area was darkened, there was no sign of infection or of abrasion to the fins on any of these fish (Kynard et al. 1999). We conclude that radio transmitter antennas should be internally implanted whenever possible to minimize injury to the fish. However, when it is absolutely necessary to obtain maximal signal range (aerial surveys, passage studies around dams, etc.), trailing antennas may be used with caution. This method should not be used when tagging a significant percentage of a given population.

Surgery to implant transmitters should only be attempted when fish are in excellent condition. Methods of Summerfelt and Smith (1990) should be used as general guidelines for sturgeon anesthesia using tricaine methane sulfonate (MS-222); however, the dose should be reduced to only that needed to immobilize the fish during surgery, if at all. Placing fish upside down in a cradle or trough during surgery is often sufficient to immobilize them. Also, sturgeon may be safely immobilized using galvanonarcosis (low voltage DC). The transmitters and internally implanted coiled antennas can be coated with an inert elastomer (Silastic MDX4.4210) to reduce tissue irritation and subsequent tag rejection. However, some transmitter coatings are quite inert and do not need this treatment, and some transmitter models coated with Silastic have been expelled by cultured shortnose sturgeon in tank studies. Also, transmitters with externally trailing antennas should not be coated to allow sturgeon tissue to adhere to the tag and hold it in place in the body cavity (Kynard et al. 1999).

The transmitter and all surgical instruments should be sterilized immediately prior to use. A lateral incision approximately 30 mm long should be made 40 - 60 mm anterior to the pelvic fin and about 10 - 20 mm above the ventral row of scutes (although the specific location will vary with fish size). This location reduces abrasion of the transmitter on the incision. However, lateral muscle tissue in large adults may be quite thick, so a ventral incision is recommended for them. The incision should be closed with either absorbable or non-absorbable suture material (absorbable material is superior for tying knots but there has

been no documented differences in healing of wounds with either suture type) and a large cutting needle. Individual sutures should be closed with separate, double, square knots so that the muscle tissue firmly touches but is not drawn tightly. After surgery the fish should be released as soon as it recovers from the anesthesia.

## Tissue sampling

Tissue sampling is required for genetic evaluation, studies of contaminant loading, assays of physiological condition, and ageing. A 1 cm² pelvic fin clip is recommended for genetic analysis. Muscle samples for contaminant analysis or energetic evaluation should be taken from the thickest dorsal musculature using a mammalian tissue punch. First, a v-shaped flap of skin should be peeled back using a sterilized scalpel. The punch is then used to cut a small core of tissue, which may be removed with cutting pliers. The flap of skin should then be replaced and two sutures used to close the wound. Blood samples may be taken from the ventral caudal peduncle. Egg samples may also be removed using a large gauge hypodermic needle (as used for PIT tag insertion). The needle is inserted through a small ventral incision in the abdomen and a small number of eggs drawn out, if the female has ovulated (i.e., eggs are loose in the abdomen). A gonad biopsy for histological analysis can be obtained from either sex at any point in the reproductive cycle by making a small incision and inserting an Eppendorfer biopsy punch. These techniques should not be used in systems having small populations and should be limited to only a few individuals.

The removal of pectoral fin rays for ageing studies is controversial. Concerns raised include potential impacts to fish swimming performance in high current velocity areas and the equivocal data that may be obtained from these structures. In tank tests, ray regeneration was rapid and sturgeon swimming performance was unaffected (Collins and Smith 1996). Continued study of the impacts of ray removal on sturgeon performance, validation of annuli, and investigations into alternative methods of ageing are sorely needed.

#### Gastric lavage

A safe and effective technique for flushing food items from the stomach of live sturgeons has recently been developed (Haley 1998). Due to the morphology of the gut tract and the physostomous swim bladder, gastric lavage of sturgeons was previously considered a risky procedure. Consequently, diet information was only available from fish that had been killed. The new lavage method requires the careful use of a flexible, small diameter tubing (intramedic polyethylene, 1.57-mm inner diameter and 2.08 mm outer diameter). The fish is lightly anesthetized using MS-222 and the tube is directed past the pneumatic duct and into the alimentary canal until it can be felt on the ventral surface of the fish. Water is slowly injected into the tubing to flush the stomach. After lavage the fish are allowed to recover and are immediately released. This method is not recommended when water temperature

exceeds 27°C and extreme caution should be taken to avoid damage to the swim bladder, which can result in mortality.

## **Sampling Methodologies**

Preferred sampling methods for sturgeon are dictated by the habitat where they occur, season of capture, and life stage. In general, large juvenile and adult sturgeon are efficiently captured in stationary or drifting gillnets or trammel nets (Buckley and Kynard 1985, Hoff et al. 1988, Dovel et al. 1992, Geoghegan 1992, Kieffer and Kynard 1993, Moser and Ross 1995, Collins et al. 1996). Trawl sampling is also an effective means of capturing sturgeon, but much of the time this gear is not feasible for use, due to the rapid current conditions and excessive amount of bottom structure in riverine or estuarine sturgeon habitat. Sturgeon are also susceptible to pound nets, but this gear has not been used for research purposes, other than to assess commercial capture rates. Similarly, sturgeon are occasionally captured on hook and line (usually baited trotlines or via snagging); however, this gear has not been employed for research sampling. Baited trotlines are a safe and effective method for capturing white sturgeon (*A. transmontanus*), and this method probably has potential for shortnose and Atlantic sturgeon research as well (Elliott and Beamesderfer 1990).

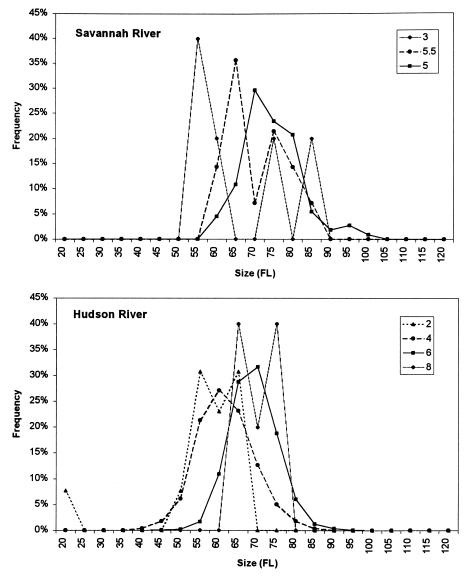
Very small juveniles (larvae and young-of-the-year) are rarely captured in traditional survey sampling. Young sturgeon seek cover in gravel crevices and amongst structure for about 9 d after hatching and then the larvae move downstream. Sturgeon eggs and/or larvae have successfully been collected in some rivers using D-shaped drift nets (Kynard et al. 1999), epibenthic sleds, and textured pads to which the eggs adhere. Recent studies have been conducted to confirm that light traps are not effective for capture of sturgeon larvae.

Electrofishing has not proven to be an effective method for capture of sturgeon in most systems because the fish tend to sink immediately upon being stunned. This is unfortunate, because many resource agencies conduct regular survey sampling with this gear. In very shallow areas with clear water it may be possible to retrieve stunned sturgeon from the bottom with a long handled dipnet. The more widespread use of sophisticated electrofishing equipment that allows control of amperage, voltage, and waveform may result in development of electrofishing methods that are specific to sturgeon (such as those for specific collection of catfish). Moreover, Aadland and Cook (1992) have developed an electric trawl for use in sampling benthic river fishes that may be very useful for collecting sturgeon. Studies to examine the efficacy of electrofishing gear should be undertaken using hatchery fish.

#### Gillnets and trammel nets

Both shortnose and Atlantic sturgeon are very susceptible to gillness and trammel nets as adults or large juveniles. These gears (especially gillness) are size selective and

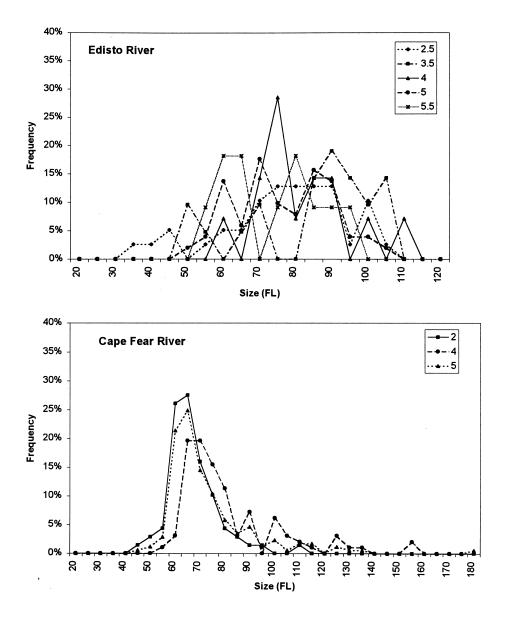
therefore should be used with caution when determining sturgeon size or age distributions. However, length frequencies from studies using gillnets having different mesh sizes indicate that there is considerable overlap between size distributions of sturgeon collected with different mesh sizes (Figure 1). Sub-yearling sturgeon (200 –300 mm FL) have been captured using 5 cm (2") stretched mesh nets in the Hudson, Cape Fear, Edisto and Savannah rivers but in all cases the catch rates were low. This was probably due to low abundance of



**Figure 1.** (Above and facing page) Size frequencies (in cm fork length) of shortnose and Atlantic sturgeon captured using various gillnet mesh sizes (in inches stretched mesh): 2 (5.1 cm), 2.5 (6.4 cm), 3 (7.6 cm), 3.5 (8.9 cm), 4 (10.2 cm), 5 (12.7 cm), 5.5 (14.0 cm), 6 (15.2 cm), and 8 (20.3 cm). Data from the Savannah River, S.C. and the Hudson River, N.Y. are for shortnose sturgeon captured in stationary gillnets. Data from the Edisto River, S.C. (J. McCord, S.C. Department of Natural Resources, unpubl. data) are for shortnose sturgeon caught in drifting gillnets. Data from the Cape Fear River, N.C. are for Atlantic sturgeon caught in stationary gillnets.

small size classes in these rivers, rather than gear selectivity. For post-yearlings, all mesh sizes greater than 6.4 cm (2.5") stretched mesh result in similar length frequencies (Figure 1). Trammel nets collect a wider size distribution than gillnets and are often less stressful than gillnets because the fish are frequently entangled rather than gilled.

Both monofilament and braided nylon mesh are effective for capture of sturgeon; however, twine size should be increased if large fish are targeted. Although fish are captured more effectively with light twine, sturgeon can easily break through webbing that is too light. Also, light twine is more likely to cut into the fish and cause injury. When targeting adults, heavy multifilament nylon (size 208 - 233) with 15 cm (6") stretched mesh can be used to reduce sturgeon injury.



Sturgeon are benthivores and generally are captured near the bottom unless they are actively migrating (McCleave et al. 1977, Moser and Ross 1995). Therefore stationary gillnets or trammel nets should be heavily weighted and allowed to contact the bottom. In low velocity areas, nets should be set perpendicular to the current. However, in areas of high velocity or having heavy debris loading, this is not feasible. In this case, nets should be set in back 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.

Drifting gillnets can be used very effectively to capture sturgeon by drifting through relatively snag-free areas while dragging near or on the bottom (O'Herron and Able 1990, McCord 1998). Often this method results in lower debris loading because the nets drift along with the debris and do not intercept it. Generally, the short soak times and reduced pressure on driftnets also result in less injury to captured fish. This method can be used through upriver runs and pools without large entanglements by using very light leadline (just enough to take the net to the bottom). The net should be buoyed at the ends with large floats (8-15 L displacement) to facilitate operating the net and to avoid snags. In tidal areas, buoyancy should be reduced and the net dragged along the bottom wherever possible (McCord 1998).

Entanglement in gillnets or trammel nets can result in sturgeon mortalities (Kieffer and Kynard 1993, Moser and Ross 1993, Collins et al. 1996, Kynard et al. 1999). To reduce the risk of mortality, precautions should be taken to reduce stress to fish during netting. Gillnets and trammel net soak times should never exceed 2 hrs in water temperatures > 27°C. During lower water temperatures, soak times up to 24 h are acceptable, but soak times should be reduced as much as possible as temperature rises. Sturgeon should also not be exposed to air temperatures below 0°C for more than a few minutes. In these conditions, fish should be processed while held underwater to reduce the risk of freezing tissue. Every effort should be made to reduce stress during removal of fish from nets and net meshes should be cut to facilitate rapid removal of fish.

#### **Trawls**

Where conditions permit the use of trawls, this gear can be effective for the capture of sturgeon. Collins et al. (1996) found that 39% of all juvenile Atlantic sturgeon and 8% of the adult shortnose sturgeon tag returns from fish tagged in the Altamaha River, Georgia were from the commercial trawl fishery. Sampling of shortnose and Atlantic sturgeon was conducted in the tidal portion of the Hudson River from 1975 - 80 using a 6.4 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 4-km h<sup>-1</sup> (2.2 knots). The Hudson River Utilities Monitoring Program has also conducted a standardized trawling survey since 1985 using a 3 m beam trawl with 1.3 - 3.8 cm mesh. This gear is towed for 5 min against the current and adult shortnose sturgeon (500 - 1000 mm fork length) are caught regularly. This sampling indicates that even a small trawl effectively captures sturgeon.

## Drift nets

D-shaped or rectangular drift nets have been used effectively to catch shortnose sturgeon eggs and larvae in both northern (Kynard et al. 1999) and southern (Smith et al. 1993) rivers. Mesh sizes of 2 mm<sup>2</sup> 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 1m square or 2 m 1m 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 of up to 1.0 m s<sup>-1</sup>. Depending on the current velocity and amount of debris accumulation, such gear should be fished for 10 min – 1 h in areas of suspected spawning. A flow meter should be positioned in the mouth of the net to allow calculation of egg or larval densities per volume of water sieved. Such studies are best conducted with the aid of telemetry data from pre-spawning adults to identify likely spawning locations (Collins and Smith 1993, Kynard et al. 1999). Little to no mortality occurs with this gear type if the samples are processed in the field. The D-shaped nets have been used to capture eggs of Chinese sturgeon in the Yangtze River for four years. Tens of thousands of eggs have been captured when the nets have been set in areas occupied by telemetered fish. These eggs are reared to juvenile stages and released into the river (Wei and Kynard 1996). Egg samples can also be collected using artificial substrates to which they adhere (anchored buffer pads, Moser et al. 1998).

## Minimum Sampling Required to Confirm Presence of Shortnose Sturgeon

Guidelines for minimum sampling necessary to confirm that shortnose sturgeon still exist in a system are desperately needed for management of this species. Shortnose sturgeon are no longer extant in many rivers where they historically occurred (Dadswell et al. 1984). However, the Shortnose Sturgeon Recovery Plan (NMFS 1998) stipulates that restoration efforts (stocking of cultured fish) should not be undertaken until it is confirmed that wild fish have been extirpated. In addition, sampling for the presence of shortnose sturgeon is often required when activities that jeopardize the existence of this fish are proposed in an area where their status is unknown. Consequently, the National Marine Fisheries Service and

other regulatory agencies require guidelines for sampling efforts that are adequate to address such questions.

Unfortunately, it is impossible to absolutely confirm that shortnose sturgeon no longer exist in a given system due to their life history and problems associated with sampling them. Shortnose sturgeon are long-lived (over 30 yrs) and do not spawn every year (Dadswell et al. 1984). Therefore, sampling over multiple years is needed to insure that a strong year class has not been missed. Moreover, sturgeon are rarely captured using traditional survey sampling, so specialized sampling methods in specific habitats are needed, particularly in systems where sturgeon are very rare. Even studies specifically designed to capture sturgeon can only confirm their presence, as negative data does not necessarily indicate that the fish are extirpated. However, given adequate sampling, an acceptable degree of confidence that the fish are extirpated (or functionally extirpated) can be gained. Based on the types and amounts of effort conducted in other systems to date, we developed the following sampling guidelines as the best available approach to assessing shortnose sturgeon presence in areas where they historically occurred.

## Research Survey

The first step in any system is to conduct a literature survey and to contact people who currently or historically fished in the area using gear that captures sturgeon. Often museum records, archeological remains (scutes in middens), or patterns in historical collections can provide vital clues to appropriate areas and times to sample for shortnose sturgeon. Personal contact with local fishers is also essential. They can provide detailed information on exact sampling locations that were historically productive, tricks to effective use of gear, and observations on the timing of sturgeon movements. In addition, people currently fishing in the system may have recently captured shortnose sturgeon as bycatch and be willing to provide anecdotal information on these captures or actual specimens (Collins and Smith 1993, Moser and Ross 1993, Collins et al. 1996, Moser et al. 1998). The U.S. Fish and Wildlife Service has successfully obtained shortnose sturgeon specimens by offering monetary rewards for live fish in Chesapeake Bay (J. Skejeveland, Maryland Fisheries Resources, personal communication). While this technique may put more fish at risk or result in targeted fishing for sturgeon, the ability to enlist the help of commercial fishers greatly increases the chances of documenting the presence of fish in areas where they are thought to be extirpated.

Finally, prior to any fieldwork, literature from neighboring systems should be reviewed. Patterns of sturgeon habitat use and movements are similar over small spatial scales (Dadswell et al. 1984). By mapping suspected aggregation areas (spawning grounds, wintering areas, summering sites) from adjoining systems, sites to sample in the study area can be more accurately identified. Any available maps of water quality or bottom substrate in the study area should be collected to help identify likely spawning sites and aggregation areas.

Patterns of habitat use and movements of shortnose sturgeon vary latitudinally. Therefore, our recommendations for minimum sampling are divided into two main groups: 1) northern rivers where < 7°C water temperature regularly occurs in winter and temperatures occasionally reach >27°C in summer (Chesapeake drainages north), and 2) southern rivers where >27°C occurs regularly in summer and temperatures seldom drop below 7°C in winter (south of Chesapeake drainages).

## Minimum Sampling Requirements in Northern Rivers

Northern rivers having sturgeon habitat can be subdivided into two groups: northerly (systems in Maine and Canada), and north central (Chesapeake drainages to Massachusetts). It is necessary to subdivide the northern region because sturgeon in the most northerly rivers exhibit a greater degree of anadromy, venturing into high salinity regions. Shortnose sturgeon in north central rivers spend more time in freshwater and make only short forays into relatively low salinity areas to feed (Dadswell et al. 1984, Kynard 1997).

Sampling in northerly rivers (Maine and Canada) should be conducted for a minimum of two years. Attempts should first be made to capture pre-spawning adult shortnose sturgeon at the base of the first dam or falls that they would encounter. This sampling should be conducted weekly for 8 - 10 weeks during early spring when water temperatures range from 8 – 18°C. Four to six, 100 m, 15.2 cm (6") stretched mesh, stationary sinking gillnets should be set as recommended in the sampling protocol for at least two days each week and checked at least every 24 h (minimum sampling effort = 128, 100 m net days). In the event that no fish are captured in the first spring, sampling should be conducted in the estuary (1 – 12 ppt) along marsh edges and in tidal creeks that summer and the following summer. This sampling should occur weekly with four to six, 100 m, 15.2 cm (6") stretched mesh sinking gillnets (2 - 3 day/week) in June – August (8 - 10 weeks) when water temperatures range from 20 – 25°C (minimum sampling effort = 128, 100 m net days). Telemetry studies are recommended so that any fish captured in the estuary can be tracked to their river of origin.

Sampling in north central rivers (Chesapeake drainages to Merrimack River) should initially concentrate on capture of pre-spawning adults with gillnets at the base of the first dam or falls (protocol as described for northerly rivers) for two years (minimum sampling effort = 128, 100 m net days). If no fish are collected in the first spring, sampling efforts should be directed to likely aggregation areas that summer. Areas targeted should be between the saltwater/freshwater interface and the first dam or falls. Habitats sampled should include the deepest part of the water body in every curve and around each island (Kynard et al. in press). Sampling should continue weekly through two summers (June – October) using four to six, 100 m, 15.2 cm (6") stretched mesh sinking gillnets set for at least 3 days each week (soak times should be 24 h unless water temperature exceeds 27°C, see previous section on gillnet methodology).

Minimum Sampling Requirements in Southern Rivers

Adult and juvenile shortnose sturgeon in southern rivers aggregate in deep areas near the saltwater/freshwater interface in summer (Hall et al. 1990, Weber 1996, Moser and Ross 1995, Collins et al. in press). Sampling for shortnose sturgeon should initially be focused in these summer aggregation areas, but extreme caution must be exercised to avoid killing any fish captured during high water temperatures. Sampling should begin in summer when temperature exceeds 27°C (July in most southern rivers) and continue until the temperature drops below 27°C (October in most southern rivers).

Three sinking gillnets of 13-14 cm stretched mesh (5-5.5 in) or trammel nets with 5-8 cm (2-3 in) stretched mesh inner panels and 35 cm (14 in) stretched mesh outer panels should be set as specified in this sampling protocol. Nets should be 100 m long, or else shorter nets with the equivalent combined length of 300 m should be used (e.g., six, 50 m nets). All nets should be set for 2 h during the slack tide (neap tides are preferred) in the deepest part of the water body near the upper extent of the salt wedge (0-3 ppt) or up to 2 km above the saltwater-freshwater interface. In deltaic systems there may be more than one area that fits this definition. In this case all candidate sites should be sampled in random order during the summer. Sampling should be conducted 3 times per week for 8-10 weeks (minimum sampling effort = 288 net hours).

If no shortnose sturgeon are collected in the first summer of sampling at the saltwater/freshwater interface, sampling for pre-spawning adults should be initiated at the base of the first dam or falls in January – April. Some rivers on the coastal plain do not present such obstacles to migration and possible aggregation areas are unknown. In such cases, likely spawning habitats based on research in other southern rivers (as identified in Hall et al. 1993) should be identified and sampled. Three, 100 m sinking gillnets of 13-14 cm stretched mesh (5-5.5 cm) or 100 m trammel nets with 5-8 cm (2-3"") stretched mesh inner panels and 35 cm (14"") stretched mesh outer panels should be set bi-weekly as specified in the sampling protocol. In many upriver areas it may be necessary to use shorter nets, in which case their total length should equal 100 m. Sampling should be conducted for at least 8 weeks in two years, with three days of effort per week (24 h sets) from January until the water temperature exceeds  $18^{\circ}\text{C}$  (minimum sampling effort = 144, 100 m net days).

#### Conclusion

Sampling and handling procedures for Atlantic coast sturgeons have evolved over the past 30 years and differ among systems and sampling situations. Minimum sampling requirements also vary across systems. While we have addressed latitudinal differences in developing sampling guidelines, inter-system differences in sturgeon abundance can also affect minimum sampling requirements. The amount of effort required to document sturgeon presence is negatively correlated with sturgeon abundance (Figure 2). Therefore, we have

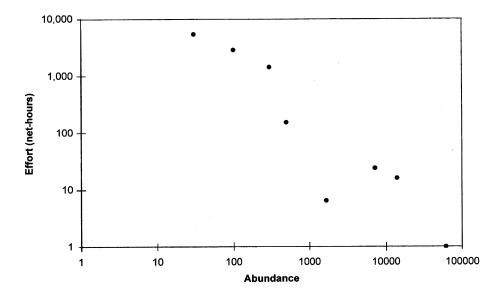
attempted to provide conservative estimates of effort required so that sturgeon presence may be detected in systems where these fish are rare.

The minimum sampling protocols will certainly be affected by the availability of reliable anecdotal/historical information on sturgeon occurrence. With this information, sampling can be directed to specific sites within the protocol framework. We emphasize that obtaining this information is critically important. Sturgeon fishing has become an activity of the past, and sturgeon fishers are aging. When they die, a wealth of information about historical occurrences of sturgeon, movement patterns, and capture methods will be lost.

New sampling and handling methodologies may be developed on the basis of information from fishers or via research innovations and experimentation. We reiterate that this protocol is to serve as a current set of guidelines for use with Atlantic Coast sturgeons, and should in no way restrict testing of new techniques. However, we recommend that cultured sturgeon be used first when testing new and potentially harmful methods.

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**Figure 2.** Effort expended (100 m gillnet set for 1 hour) to capture the first shortnose sturgeon in each of eight different systems vs. estimated shortnose sturgeon abundance in each system.

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