A Protocol for Use of Shortnose, Atlantic, Gulf, and Green Sturgeons

Jason Kahn and Malcolm Mohead

U.S. Department of Commerce
National Oceanic and Atmospheric Administration
National Marine Fisheries Service

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Atlantic sturgeon (Robert Michelson, Photography by Michelson, Inc.)

Gulf sturgeon (Oscar Sosa, *New York Times*)

Green sturgeon (Thomas Dunklin)

Cover: shortnose sturgeon (credit: Robert Michelson)

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Introduction

The goal of the National Marine Fisheries Service (NMFS) protocols for the use of sturgeon is standardization of research practices to benefit the recovery of Gulf of Mexico (Gulf), green, Atlantic, and shortnose sturgeon while also minimizing potentially negative impacts of research. As with *A Protocol for the Use of Shortnose and Atlantic Sturgeon* (Moser *et al.* 2000a), these protocols provide guidelines for consistent and safe sampling methods when conducting research on sturgeon. They were developed from a comprehensive review of the best available scientific information at the time of publication, including peer reviewed journals, technical memorandums, species status reviews, interviews with researchers, and empirical evidence provided by researchers. Currently, some state agencies have been delegated authority for issuing research permits for Gulf and green sturgeon. However, due to previous lack of protocols established for these species, they were incorporated into this document.

The majority of research conducted on sturgeon falls into several categories: capturing, handling, holding, standard research, anesthetization, tagging, gastric lavage, sex identification and stage of maturation, and age estimation. First, sturgeon must be captured, which may also require consideration of the waterway sampled to mitigate impacts on other federally listed threatened or endangered species. NMFS has determined that measuring, passive integrated transponder (PIT) tagging, and genetic sampling are essential procedures to provide NMFS with the most basic information on each fish and therefore those procedures are strongly recommended. After those procedures are completed, other discretionary research might include telemetry tagging, gastric lavage, sex identification, and age estimation. These discretionary procedures should use either chemical or physical anesthesia, potentially increasing risks to sturgeon.

These protocols were developed to allow for safe, non-lethal research on sturgeon, balancing the necessary negative impacts of research while still allowing researchers to gather information vital to the recovery of listed species under the Endangered Species Act (ESA). These protocols are based on a thorough and comprehensive review of the best available scientific information on current research methods and the subsequent risk to these species. When researchers or managers have reason to exceed recommendations in this document using less known or riskier techniques, NMFS recommends first using surrogate Acipenserids or hatchery-reared sturgeon. When researchers or managers feel non-recommended methods must be conducted on wild listed or candidate species, the researchers should consult with the appropriate permitting agency in order to justify why their methodology is necessary to provide information for the recovery of these species.

Non-Targeted Species Concerns in the Research Area

When sampling shortnose, Atlantic, Gulf, and green sturgeon, the potential exists for researchers to encounter other ESA or Marine Mammal Protection Act (MMPA) listed species, in addition to other locally or state protected species. These circumstances will vary with location and NMFS encourages consultation with the appropriate management authority in all cases.

When other ESA protected species are potentially present in an action area, the researcher must contact NMFS or the US Fish and Wildlife Service (USFWS) for clarification on the likelihood to adversely impact any listed species, or destroy or adversely modify any critical habitat for that species. The presence of listed species may require researchers to alter sampling plans to avoid taking listed fish, such as Pacific or Atlantic salmonids, or mammals, such as Stellar sea lions or manatees.

In many other locations, marine mammals, protected under the MMPA but not the ESA, may be present. The MMPA places a moratorium, with certain exceptions, on the taking and importing of marine mammals and marine mammal products. In 1981, Congress amended the MMPA to allow the incidental, but not intentional, taking of small numbers of marine mammals by U.S. citizens who engage in a specified activity (other than commercial fishing) within a specified geographical region. If marine mammals, including non-ESA listed pinnipeds or cetaceans, have the potential to be taken incidental to scientific research activities on sturgeon (e.g., there is a chance of entanglement), the researcher should consult with NMFS under section 101(a)(5) of the MMPA to determine if an incidental take authorization is warranted. Contact: Office of Protected Resources, Silver Spring, Maryland (301-713-2289).

In other instances, predators may frequent sampling areas posing threats to listed sturgeon species. In such cases, nets must be monitored at all times and pulled if predators are evidenced. Pinnipeds have been seen feeding on listed sturgeon by researchers (Fernandez 2008, Marty Gingras, California Department of Fish and Game, pers. comm.), potentially other predatory species such as odontocetes and sharks could take sturgeon while trapped in gillnets or trammel nets. If there are reasons to believe sturgeon could be harmed by predators while captured in gillnets or trammel nets, those nets should be continuously monitored.

Capture

Researchers most often capture Gulf, Atlantic, green, and shortnose sturgeon using a variety of gears including gillnets (drift and anchored), trammel nets, seine nets, trawls, trot lines, pound nets, and electrofishing. Nets of varying length and mesh size are chosen to target different life stages of sturgeon (Mason and Clugston 1993, DeVries 2006).

Generally, sturgeon are hardy, allowing some research methods lethal to other fish. These methods can still be stressful to sturgeon, occasionally resulting in lethal and, more often, sub-lethal effects. For example, during pre-spawning activities, capture and handling is thought to have resulted in immediate downstream migration or aborted spawning runs (Moser and Ross 1995, Kynard *et al.* 2007, Gail Wippelhauser, Maine Department of Marine Resources, pers. comm.). Also, during periods of warm water or low dissolved oxygen (DO), fish have been lethally stressed (Hastings *et al.* 1987, Secor and Gunderson 1998). NMFS recommends capturing adult sturgeon while they are still in their winter staging areas, but does not recommend targeting sturgeon during their upstream spawning migration due to the risks of aborted spawning runs. However, when the purpose of the research is to document the size of the spawning run, managers must determine whether the information to be gained is worth the risk posed by the research.

Dissolved Oxygen, Temperature, and Salinity

For all sturgeon species, research has revealed that survival is affected by a relationship between temperature, DO, and salinity and this vulnerability may be increased by the research-related stress of capture, holding, and handling. The following environmental information is considered relevant for establishing recommendations for directed sampling on early life stages to adult life stages of sturgeon.

Jenkins *et al.* (1993), Secor and Gunderson (1998), Niklitschek (2001), Secor and Niklitschek (2001 and 2002), and Niklitshek and Secor (2009a and 2009b) demonstrated shortnose and Atlantic sturgeon survival in a laboratory setting was affected by reduced DO, increased temperature, or increased salinity. Other researchers have demonstrated similar relationships between temperature, DO, and salinity in green sturgeon (Van Eenennaam *et al.* 2005, Allen *et al.* 2006, Allen and Cech 2007). Likewise, Altinok *et al.* (1998), Sulak and Clugston (1998), Sulak and Clugston (1999), and Waldman *et al.* (2002) reported high temperatures, low DO, and high salinities result in lower survival of Gulf sturgeon.

Though there may be differences between populations in different geographical regions, optimal growth for both Atlantic and shortnose sturgeon has been shown to occur at 70% oxygen saturation with a temperature of approximately 20°C (Niklitschek 2001). Shortnose sturgeon have also been shown to experience significant reductions in food consumption when temperatures exceed 25.8°C (Niklitschek 2001). Green sturgeon require cooler temperatures, growing optimally between 15° and 19°C, and experiencing reduced growth rates between 20° and 24°C (Mayfield and Cech 2004). However, larval green sturgeon grow more optimally at 24°C compared to 19°C (Allen *et al.* 2006). Gulf

sturgeon also appear dependent on temperature for optimal growth, fasting during hot summer months and feasting during winter when water temperatures and DO in the Gulf of Mexico and tributaries are more optimal (Sulak and Randall 2002).

Considerable work has been conducted on temperature tolerances of sturgeon (Wang *et al.* 1985, Wehrly 1995, Kynard 1997, Campbell and Goodman 2004, Cech and Doroshov 2004, Van Eenennaam *et al.* 2005, Ziegeweid *et al.* 2007, Sardella *et al.* 2008). In recent work on critical thermal maximum, Ziegeweid *et al.* (2007) demonstrated hatchery-raised young of year shortnose sturgeon can tolerate between 28° and 30°C, while the maximum safe temperature limits for adults ranges between 28° and 31°C. Kynard (1997) also notes empirical temperatures of 28° to 30°C in summer months creates unsuitable shortnose sturgeon habitat. Atlantic sturgeon experience lower survival when water temperatures exceed 28°C (Niklitshek and Secor 2005). Mayfield and Cech (2004) estimated the lethal water temperature for green sturgeon in the wild at 27°C. Sardella *et al.* (2008) found green sturgeon lethal limits in a laboratory is approximately 33°C, in freshwater and sea water, although the maximum respiratory response evidenced is 26° to 28°C. Although Gulf sturgeon reside in freshwater during summer months where water temperatures range from 28° to 32°C, there have been no studies estimating lethal temperature limits for Gulf sturgeon. It is worth noting, however, the healthiest population of Gulf sturgeon occurs in the Suwannee River, where temperatures are generally maintained at 28°C by springs in parts of the river.

There is no clear evidence to suggest minimum water temperatures negatively affect sturgeon when captured beyond the early life stages. Therefore, this document identifies only upper water temperature restrictions to establish safe sampling limits for threatened or endangered sturgeon. However, when air temperatures are below freezing, handling procedures should be limited to less than two minutes to prevent exposure of a sturgeon's skin to freezing temperatures.

Because warm water can hold less DO, percent oxygen saturation is a measurement that accounts for water temperatures and DO concentrations, providing a general index of how much DO is available to sturgeon under various environmental conditions. All three measures are used in this document to highlight risks to sturgeon survival (Table 1). The 24 hour LC50 (concentration lethal to 50% of the test fish) of DO for shortnose sturgeon is documented between 2.2 and 3.1 mg/L at temperatures ranging from 22°C to 29°C (Campbell and Goodman 2004). Secor and Niklitschek (2002) reported the critical DO concentration for Eurasian sturgeons to be 4.5 mg/L at 24°C, but also found 3.6 mg/L DO critical at 20°C. Following a similar pattern, critical concentrations of DO between 4.3 and 4.7 mg/L were found for shortnose and Atlantic sturgeon at temperatures ranging from 22° and 27°C respectively. Further, acute lethal effects to shortnose and Atlantic sturgeon were observed when DO was 3.3 mg/L at temperatures between 22° and 27°C (Secor and Niklitschek 2002). Survival of Atlantic sturgeon was observed to be 100% in water temperatures of 26°C with 7 mg/L DO; however, 12% survival was observed in waters with 3 mg/L DO at the same temperature (Secor and Gunderson 1998). Even when water temperatures were only 19°C and DO was 3 mg/L, 25% of the Atlantic sturgeon died. Similar to reduced growth rates experienced by shortnose sturgeon when

temperatures are above 25°C, both shortnose and Atlantic sturgeon growth is impaired when DO is less than 4.7 mg/L (Secor and Niklitschek 2002). Jenkins *et al.* (1993) confirmed 12% mortality for 339 mm juvenile sturgeon when held at 2.5 mg/L DO and 22.5°C, while no sturgeon died when DO was above 4 mg/L at any temperature. Likewise, Secor and Gunderson (1998) found the DO level required avoiding mortality was 5 mg/L. Specific DO tolerance levels have not been established for green or Gulf sturgeon, although hypoxia for many *Acipenser* species has been documented to begin at 4 mg/L (Cech *et al.* 1984, Jenkins *et al.* 1993, Secor and Gunderson 1998). Similarly, Cech and Crocker (2002) identified hypoxia for sturgeon as 58% oxygen saturation.

Table 1. Water temperature, dissolved oxygen, percent oxygen saturation of the water, and survival rates of sturgeon tested.

Authors	Species	Temp (°C)	DO (mg/L)	% Saturation	Effects
Jenkins *et al.* 1993	Shortnose	22.5	2.5	29%	88% survival
Campbell and Goodman 2004	Shortnose	22 – 29	2.2 – 3.1	25 - 41%	50% survival
Secor and Niklitschek 2002	Atlantic and shortnose	22 – 27	3.3	38 – 42%	Acute lethal effects
Secor and Gunderson 1998	Atlantic	26	3	37%	12% survival
Secor and Gunderson 1998	Atlantic	19	3	33%	75% survival
Secor and Niklitshek 2002	Eurasian	24	4.5	54%	Critical DO concentration, onset of sub-lethal effects
		20	3.6	40%	
Secor and Niklitshek 2002	Atlantic and shortnose	22 – 27	4.3 – 4.7	50 – 60%	Critical DO concentration, onset of sub-lethal effects

NMFS recognizes the synergistic effects of water temperature and DO present difficulties when establishing finite levels for safe sturgeon sampling (Table 1). It is clear from reported empirical catch data and scientific literature, higher temperatures and lower DOs stress sturgeon even if the percent oxygen saturation remains constant or increases. Water temperature and DO can be responsible for mortality events. Each individual sturgeon will react differently to changes in environmental conditions such as water quality, salinity, and stress associated with capture and handling, which compounds the difficulty of conducting a risk assessment.

Using data reported from capture of shortnose and Atlantic sturgeon from the 1970s to present and the critical thresholds and LC50s reported in the scientific literature as reference points, NMFS established safe environmental limits for capturing and handling sturgeon species. NMFS recommends not capturing or handling Gulf, Atlantic and shortnose sturgeon when DO concentrations are below 4.5 mg/L. Green sturgeon should not be captured or handled when DO concentrations are below 5 mg/L. Additionally, NMFS recommends not sampling for Gulf, shortnose, or Atlantic sturgeon when temperatures exceed 28°C and green sturgeon should not be captured when water temperatures exceed 25°C. When establishing these recommendations, NMFS also considered the percent oxygen saturation of water and recommends not sampling for Gulf, Atlantic, or shortnose sturgeon when oxygen saturation is below 55% or green sturgeon when oxygen saturation is below 58%. Sampling at higher temperatures or lower DO levels may be possible if the percent oxygen saturation in water is maintained at these levels.

Gillnets and Trammel Nets

Researchers typically use gillnets and trammel nets to capture sturgeon. These netting techniques, while potentially lethal for many species of fish, are somewhat safer for sturgeon. However, given the implications of water temperature, DO, and percent oxygen saturation, both soak times and mesh size are important factors considered for safely capturing and handling sturgeon. Mesh size that is too small for the targeted life stage is more likely to constrict gills resulting in mortality via suffocation. The mesh size chosen for gill netting sturgeon, therefore, should be carefully considered and appropriate for the species and life stage targeted. Experimental nets with multiple mesh sizes may be appropriate for researchers to discover the safest and most effective mesh size. For example, due to disproportionately high reports of mortality using ten inch stretch mesh with Atlantic sturgeon (Balazik *et al.* 2009), this size mesh should not be used to sample adult Atlantic or Gulf sturgeon.

Safe net soak times are influenced by water temperature, DO, and, to a lesser extent, salinity. While there are no publications documenting the effects of soak times on mortality rates of sturgeon, there is consensus amongst sturgeon researchers that shorter soak times are safer than longer soak times (Mark Collins, South Carolina Department of Natural Resources; Matt Fisher, Delaware Division of Fish and Wildlife; Dewayne Fox, Delaware State University; Chris Hager, Virginia Institute of Marine Science; Doug Peterson, University of Georgia; William Post, South Carolina Department of Natural Resources; Mike Randall, United States Geological Survey (USGS); and Ken Sulak, USGS, pers. comm.). By monitoring signs of stress such as excessive redness, mucous production, or lethargy, experienced researchers will often shorten net deployment regardless of measured environmental conditions (Kathryn Hattala, New York State Department of Environmental Conservation; Tom Savoy, Connecticut Department of Environmental Protection; and Doug Peterson, University of Georgia, pers. comm.).

When using anchored gillnets while targeting Atlantic and shortnose sturgeon, soak times of 14 hours are safe when water temperatures at the sampling depth are under 15°C. However, soak times should not exceed four hours in waters up to 20°C, two hours in

waters up to 25°C, and one hour in waters up to 28°C at the sampling depth (Table 2). Similar effects were alluded to in Moser *et al.* (2000a), but were not clearly defined. Gulf sturgeon net set durations should not exceed four hours under any conditions. Mortalities have been documented in the empirical records of researchers while fishing above 20°C at net set durations ranging from 45 minutes to 24 hours. However, mortalities have been extremely rare when fishing nets less than two hours and at temperatures between 20° and 25°C. The one hour soak time at water temperatures between 25° and 28°C (Table 2) accommodates standard research practices of netting at slack tides (i.e., the occurrence of relatively still water at the turn of the low tide). There have been only two recorded sturgeon mortalities documented when fishing in this manner.

Table 2. Appropriate fishing protocols for Gulf, Atlantic, and shortnose sturgeon.

Net set duration (hours)	Temperature at sampling depth	Minimum DO at sampling depth	% oxygen saturation at sampling depth
14†	Up to 15°C	4.5 mg/L	55%
4	15° to 20°C	4.5 mg/L	55%
2	20° to 25°C	4.5 mg/L	55%
1	25° to 28°C	4.5 mg/L	55%
No sampling	Over 28°C	4.5 mg/L	55%

† Net set duration for Gulf sturgeon should not exceed four hours for all temperatures up to 20°C.

When fishing for green sturgeon, NMFS recommends that gill net fishing not be conducted in the Sacramento River, California all year to prevent interactions with listed salmonids and to also protect green sturgeon during their upstream migrations. NMFS also recommends that no gillnetting or trammel netting occur in the Feather River between October 31st and March 1st of each year to protect spawning salmonids. When fishing for green sturgeon in other locations, the risk of interactions between gillnets or trammel nets and listed salmonids or pinnipeds requires the nets to be manned at all times. Additionally, pinnipeds are protected by the MMPA and the presence of gillnets in the water could pose an entanglement risk and require an Incidental Take Authorization (Section 101(a)(5) of the MMPA). NMFS recommends net soak times should not exceed four hours in water temperature up to 19°C, two hours between 19° and 23°C, and one hour for water temperature between 23° and 25°C (Table 3).

Table 3. Appropriate fishing protocols for green sturgeon.

Net soak times (hours)	Temperature at sampling depth	Minimum DO at sampling depth	% oxygen saturation at sampling depth
4	Up to 19°C	5 mg/l	58%
2	19° to 23°C	5 mg/l	58%
1	23° to 25°C	5 mg/l	58%
No netting	Over 25°C	5 mg/l	58%

When following the protocols in Table 2 between 2005 and 2009, East Coast sturgeon researchers recorded over 3,800 captures of shortnose sturgeon resulting in no mortality.

However, while fishing outside of these recommended criteria, the same researchers experienced a 0.6% mortality rate of captured shortnose sturgeon. This is the same mortality rate documented for shortnose sturgeon captured between 2000 and 2004 when researchers followed the Moser *et al.* (2000a) protocols.

When drift gillnetting, nets are allowed to drift on the rising tide or in slack tide until just after high tide for approximately thirty minutes to several hours, depending on the location and swiftness of the tide. Water quality conditions and net soak times for drift gill nets are the same as for anchored gillnets. However, drift nets must be tended because of the risk of gear entanglement or loss of gear resulting in ghost nets. For drift gillnet fishing, gear should be pulled immediately if it is obvious a sturgeon has been captured.

Electrofishing

Electrofishing gear poses documented risks and potentially lethal effects to all sturgeon species (Moser *et al.* 2000b, Holliman and Reynolds 2002). Sturgeon have exceptional electro-sensory abilities and actively avoid electrofishing gear (Moser *et al*. 2000b). If sturgeon are likely present in areas where agencies are using electrofishing gear to target other species, only low voltage direct current should be used if no alternative sampling method is available. While electrofishing likely reduces feeding and alters spawning behavior (Moser *et al.* 2000b), such sub-lethal effects may not be significantly different than effects caused by other capture methods. However, due to more effective and safer methods of capture, NMFS prohibits electrofishing to capture Gulf, green, Atlantic, or shortnose sturgeon.

Other Non-Lethal Sampling Gear

While fyke, hoop, and pound nets are not commonly used by researchers to capture sturgeon, they occasionally capture sturgeon as bycatch in several fisheries. Usually sturgeon captured as bycatch in these gear types are found in relatively good condition. Large numbers of sturgeon captured in fyke, hoop, and pound nets have been used by researchers in cooperation with these commercial fisheries in Canada. Because these nets are less stressful to sturgeon, they are an acceptable alternative to gillnets.

Set lines have also been used to effectively sample white, pallid, shovelnose, and lake sturgeon and are approved options for sampling Gulf, green and Atlantic sturgeon as well. Shortnose sturgeon are less likely to be taken on a set line because of their diets. The two concerns with set lines are predation and hooking mortality. If there are predators such as pinnipeds in the area, the set line should be monitored constantly and pulled if any predators are seen surfacing. The hooks can be swallowed, damaging organs such as the gills and stomach, if the hook sizes are too large or small for the targeted sturgeon life stage. Every effort should be made to limit and monitor adverse effects, including not using set lines in some locations if they cannot be fished without mortality.

Trawling
While gillnets and trammel nets are most commonly used for targeting adult and sub-adult sturgeon, they are not as effective as trawls at capturing young of the year juvenile sturgeon. In larger river systems such as the Mississippi and Missouri River, and more recently in Atlantic coastal rivers, researchers have successfully employed a modified "Missouri trawl" (Herzog *et al.* 2005) — a two-seam (i.e., standard) slingshot balloon trawl (Gutreuter *et al.* 1995) completely covered with heavy, delta-style mesh.

Trawls in general are limited by shallow water (less than 20 inches) and benthic obstacles. The location of trawling should be monitored using a sounding device and global positioning system to avoid snags and limit repeated disturbance of the same location. The tow rope should be quickly released from the boat if any debris is caught and the trawl unengaged to minimize damage to the substrate or catch. Ideally, a chase boat is recommended to assist with recovery of the cod end or assisting with snags, but if that is not possible, a buoy should be attached to a single 70 to 100 foot rope line fastened to the cod end of the trawl to assist retrieval if the trawl becomes snagged.

The footrope of a trawl should maintain contact with the substrate during conditions of heavy current, fast tow speeds, or undulating bottom surfaces (e.g., sand waves). The trawl should be operated attached to the boat with 100 to 200 foot towlines, the length dependent on water depth (i.e., deeper water required longer towlines as reported in Brabant and Nedelec 1979). The trawl should be manually deployed and retrieved by powering the boat in reverse (bow upstream) with continued movement downstream. A standard haul should be approximately 300 to 500 feet, lasting approximately 10 minutes, and towed at a range of three to five knots (Gutreuter *et al.* 1995).

Areas successful for trawling are characterized by a variety of habitat substrate including fine and course sands with mobile bedforms (sand dunes) and mudflats. Particularly productive areas are located at the mouths of tributaries entering a larger river. However, any large, straight river segment, devoid of benthic material that may entangle nets, can be successfully trawled.

D-Nets
When targeting eggs and early life stage (ELS) sturgeon, the two commonly used sampling methods are D-nets and artificial substrates. Both techniques can be non-lethal, but due to the risk of mortality, no more eggs and ELS sturgeon should be captured than are absolutely necessary. While not mandatory, in rivers with unknown spawning populations, adults can be tagged and tracked to document possible spawning runs and spawning areas prior to sampling for eggs (Kieffer and Kynard 1996). Otherwise, D-nets should be deployed well before the earliest time spawning would be expected. Due to the risks associated with capturing and impinging ELS sturgeon in the D-Nets, however, they should be checked at least every three hours to minimize incidental mortality (Boyd Kynard, USGS, pers. comm.). D-nets should also be equipped with flow meters to calculate filtered water volume when developing an index of abundance and spawning success (# ELS/ volume of water sampled) (Taubert 1980). If the purpose of the research is to verify the occurrence of spawning, nets should be checked every hour. As soon as

ELS are captured, sampling should be discontinued. If the purpose of the research is to verify duration of the spawning period, then additional samples may need to be taken, but the acceptable number of ELS fish to be captured would depend on the status of the sturgeon populations in the river.

Egg Mats

Artificial substrates consist of floor buffing pads or similar materials, approximately two feet in diameter (described in Fox *et al.* 2000) for the purpose of collecting eggs as they are deposited in the water column. These pads should be anchored to the river bottom in suspected spawning areas. No more pads should be fished than is necessary. If the researcher is unsure of the number of pads required to identify spawning areas and success, no more than 100 to 150 pads should be fished at once across several sites. Pads should be checked at least twice a week or more frequently if circumstances allow. The artificial substrates should be examined in the field for sturgeon eggs and only returned to the river if more samples are needed. If it is not necessary to remove the eggs from the mat, the mat can be returned to the river bottom allowing the eggs to incubate and hatch before being removed. For every artificial substrate that collects an egg, environmental conditions such as latitude, longitude, velocity, substrate type, depth, dissolved oxygen, etc. should be collected.

Other Methods of Egg Collection

There are other methods of sampling eggs and ELS, such as epibethic sleds, ichthyoplankton nets, and pump sampling. These methods are not considered as effective as the other described methods, though they are acceptable sampling methods.

Recommendations

General

- NMFS recommends capturing adult sturgeon while they are still in their winter staging areas, but does not recommend targeting sturgeon during their upstream spawning migration due to the risks of aborted spawning runs.

Water Temperature, Dissolved Oxygen, and Salinity

- When air temperatures are below freezing, handling procedures should be limited to less than two minutes to prevent exposure of a sturgeon's skin to freezing temperatures.
- NMFS recommends Gulf, Atlantic, and shortnose sturgeon are not captured or handled when DO concentrations are below 4.5 mg/L. Green sturgeon should not be captured or handled when DO concentrations are below 5 mg/L.
- NMFS recommends not sampling for Gulf, shortnose, or Atlantic sturgeon occur when temperatures exceed 28°C; while sampling for green sturgeon should not occur when temperatures exceed 25°C.
- NMFS recommends not sampling for Gulf, Atlantic, or shortnose sturgeon when the oxygen saturation is below 55% and not sampling green sturgeon when the oxygen saturation is below 58%.

Gillnets and Trammel Nets

- Due to disproportionately high reports of mortality using ten inch stretch mesh with Atlantic sturgeon, this size mesh should not be used to sample adult Atlantic or Gulf sturgeon.
- NMFS recommends no gill net fishing be conducted in the Sacramento River, California all year round to prevent interactions with listed salmonids and also to protect green sturgeon during their upstream migrations.
- NMFS also recommends that no gillnetting or trammel netting take place in the Feather River, California between October 31st and March 1st of each year to protect spawning salmonids.
- NMFS recommends net soak times should not exceed four hours in water temperature up to 19°C, should not exceed two hours between 19° and 23°C, and one hour for water temperature between 23° and 25°C.
- Gillnets should be used sparingly and carefully in waters where other listed species may be encountered. The researcher must contact NMFS or the USFWS when other listed species may be incidentally affected.

Electrofishing

- NMFS prohibits electrofishing to capture Gulf, green, Atlantic, or shortnose sturgeon.

Other Non-Lethal Sampling Gear

- Fyke, hoop, and pound nets are an acceptable alternative to gillnets for Gulf, green, Atlantic, and shortnose sturgeon.
- Set lines are approved options for sampling Gulf, green, and Atlantic sturgeon.

Trawling

- NMFS recommends trawling as safe, efficient sampling gear to target small juvenile Gulf, Atlantic, shortnose, and green sturgeon; however, small mesh gillnets and trammel nets are also acceptable.

D-Nets

- NMFS recommends D-nets and egg mats to sample rivers for eggs or ELS of Gulf, Atlantic, shortnose, or green sturgeon.
- Due to risks associated with capturing and impinging ELS sturgeon in D-Nets, they should be checked at least every three hours to minimize incidental mortality.

Egg Mats

- No more egg mats should be fished than is necessary. If the researcher is unsure of the number of pads required to identify spawning areas and success, no more than 100 to 150 pads should be fished at once.

Handling and Holding

Handling of sturgeon refers to the time period actual research activities are conducted on live fish and does not refer to the time a fish is held in live cars before and after research activities. Holding is the period of time a sturgeon is in possession but kept in live cars either waiting to be handled or recovered from handling prior to being released.

Proper Handling of Sturgeon

Improper handling can result in lethal or sub-lethal impacts to sturgeon. In some cases, sturgeon may display altered behavior after being released, for example, swimming towards the ocean rather than remaining in the river, or, in some instances, aborting spawning runs completely (Moser and Ross 1995, Schaffter 1997, Kelly *et al.* 2007, Benson *et al.* 2007, Moser and Lindley 2007). There are no other alternatives to handling sturgeon during research; however, the researcher's primary focus should be the well-being of the sturgeon.

NMFS strongly recommends standard handling procedures performed on all sturgeon captured including measuring, weighing, PIT tagging, and tissue sampling. The total time required to complete routine research procedures should not exceed 15 minutes. Additional procedures such as internal tagging, lavage, boroscoping, etc. will take more time for handling and recovery. However, only one additional discretionary procedure to the standard handling procedures should be performed on each sturgeon, thus minimizing handling time prior to release. For example, if a sturgeon is fitted with a telemetry tag, it should not also undergo gastric lavage. And when water temperatures are above 23°C for green sturgeon or 25°C for Gulf, shortnose, or Atlantic sturgeon, the extent of research should be limited to the standard handling procedures of measuring, weighing, PIT tagging, and tissue sampling.

Fish should be handled rapidly, but with care and kept in water to the maximum extent possible during handling. During handling procedures, each fish should be immersed in a continuous stream of ambient water passing over the sturgeon's gills. Many sturgeon researchers provide sturgeon with supplemental compressed oxygen, thereby reducing stress and ensuring DO does not fall below acceptable saturation levels.

Researchers should also attempt to support larger sturgeon in slings preventing struggle during transfer. Sturgeon should be weighed using hand held sling scales or a platform scale for larger sturgeon. Also, because sturgeon are sensitive to direct sunlight, they should be covered and kept moist.

Short-Term Holding

All captured sturgeon should be removed from the capture gear and immediately transferred to short-term holding. When multiple fish are captured, those not processed immediately should be held in a net pen or live car while waiting to be transferred by hand or sling to a processing station on board. Net pens measuring three feet wide, six feet long, and three feet deep can safely hold about 20 adult shortnose sturgeon or comparably sized juvenile Atlantic, Gulf or green sturgeon when temperatures are below

15°C (Doug Peterson, University of Georgia, pers. comm.). Larger net pens (8 feet long) are required for holding adult Atlantic, green, and Gulf sturgeon or they should be processed as quickly as possible (or scheduled first) instead of subjected to confined holding conditions. When water temperature is between 15° and 25°C, fewer fish should be held in the same enclosure because overcrowding animals amplifies short term stress, particularly at higher temperatures (Safi *et al.* 2006). If the fish are being held on-board a vessel in a holding tank, compressed oxygen should be added to increase DO in the water. If the researcher observes a visually stressed sturgeon, efforts should be made to revive the fish and release it in a healthy condition. In some cases, recovery can be achieved by allowing a sturgeon to rest in an appropriately sized net pen for several hours prior to release.

Sturgeon should never be held in gillnets if there isn't enough room to safely hold them in net pens. In some rivers with large populations of sturgeon, catches can exceed the number of fish that can possibly be held safely in live cars or net pens. In such cases, researchers should have multiple holding bins at their disposal. If more fish are captured than can be processed and released within two hours, those excess fish may need to be released to minimize stress or lethal injury.

When sturgeon are held on-board research vessels, they should be placed in flow through tanks where the total volume of water is replaced every 15 to 20 minutes. Traditionally, some species of sturgeon have been held for research purposes by tethering with ropes looped around tails to the sides of research vessels until they can be handled. In a study of lake sturgeon (Axelsen and Mauger 1993 cited in Dick *et al.* 2006), tethered fish experienced greater stress and higher mortality than sturgeon kept in uncrowded cages. Therefore, NMFS recommends only using on-board holding tanks or net pens large enough to hold a large sturgeon. NMFS does not recommend holding any sturgeon by tethering its caudal peduncle to the research vessel. However, while a rope should never be tied around the caudal peduncle, it may be necessary to use a rope placed under the sturgeon immediately posterior to the pectoral fins when moving large sturgeon from net pens onto the boat.

Following handling procedures, fish should be returned to the net pen for observation and to ensure full recovery prior to release. Total holding time in the net pens would be variable depending on water temperature and the condition of each fish, however, the maximum amount of time a fish should be held after removal from capture gear is approximately two hours, unless more time is needed to recover from the effects of an anesthetic or because prolonged holding would benefit a sturgeon. When water temperature is above 25°C for Gulf, shortnose, and Atlantic sturgeon, or 23°C for green sturgeon, they should be held for as little time as possible. Holding time includes the time to remove any other captured sturgeon, time to process other fish, and time necessary for recovery ensuring the safety of the fish.

Prior to release, sturgeon should be examined and, if necessary, recovered by holding fish upright and immersed in river water, gently moving the fish front to back, aiding freshwater passage over the gills to stimulate it. The fish should be released when

showing signs of vigor and able to swim away under its own power. A spotter should watch the fish, making sure it stays submerged and does not need additional recovery.

Recommendations

Proper Handling of Sturgeon

- NMFS strongly recommends standard handling procedures performed on all sturgeon captured including measuring, weighing, PIT tagging, and tissue sampling.
- Only one additional discretionary procedure to the standard handling procedures should be performed on each sturgeon, thus minimizing handling time prior to release.
- When water temperatures are above 23°C for green sturgeon or 25°C for Gulf, shortnose, or Atlantic sturgeon, the extent of research should be limited to the standard handling procedures of measuring, weighing, PIT tagging, and tissue sampling.
- During handling procedures, each fish should be immersed in a continuous stream of ambient water passing over the sturgeon's gills.
- Researchers should attempt to support larger sturgeon in slings preventing struggle during transfer.
- If the researcher observes a severely stressed sturgeon, efforts should be made to revive the fish and release it in a healthy condition.

Short-Term Holding

- Sturgeon should never be held in gillnets while waiting to be handled, but should instead be transferred to a net pen for holding.
- NMFS recommends only using on-board holding tanks or net pens large enough to hold a large sturgeon. NMFS does not recommend tethering sturgeon to the boat by its caudal peduncle.
- The maximum amount of time a fish should be held after removal from capture gear is approximately two hours, unless more time is needed to recover from the effects of an anesthetic or because prolonged holding would benefit a sturgeon.
- Adult Atlantic, green, and Gulf sturgeon over six feet in length should be processed as quickly as possible (or scheduled first) instead of subjected to confined holding conditions.

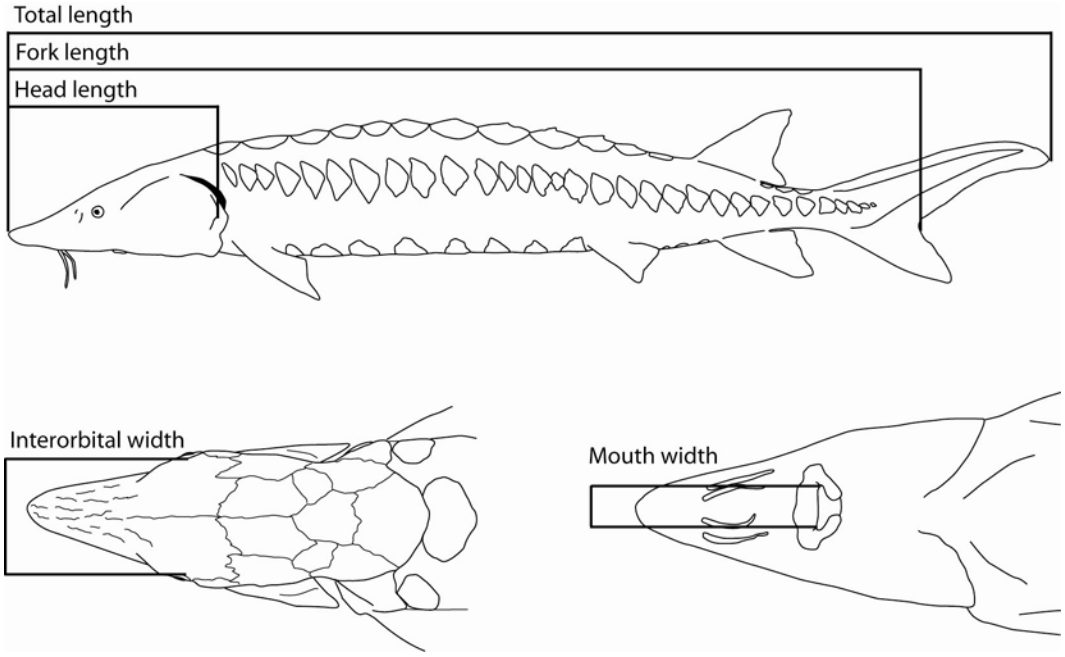
Standard Research Methods

Upon capturing a green, Gulf, shortnose, or Atlantic sturgeon, there are several research procedures strongly recommended on all sturgeon. First, the captured fish is to be measured. The sturgeon should also be weighed if possible. It can also be photographed, if possible. Then, their entire bodies should be scanned for previously inserted PIT tags; and, if none are found, one should be properly inserted. Finally, a small sample of the soft tissue of the pelvic fin should be removed for genetic identification.

Measuring

Standardized length measurements for all sturgeon should be taken from the snout to the fork in the tail (i.e., fork length – FL). The measuring device should be a solid ruler or board, so the measurement does not measure the curvature of the body. Additional length measurements should be taken at the researcher's discretion for total length (TL) or head length (Figure 1). While the heterocercal tail of larger fish may be damaged or shortened, the total length can still be obtained by pressing down the tail at the caudal peduncle and measuring to the tip of the tail. Girth measurements should also be taken at the widest part of the body. While not mandatory, measurements of the ratio of mouth width to interorbital width can also be obtained to differentiate between shortnose and Atlantic sturgeon (Dadswell *et al.* 1984). Interorbital width is measured as the distance between the lateral margins of the bony skull at the midpoint of the orbit and mouth width is measured as the distance between the left and right inside corners of the closed mouth (i.e., excluding the lips) (Figure 1).

Figure 1. Diagram of different types of measurements for sturgeons. Drawings by Eric Hilton, Virginia Institute of Marine Science.



Weighing
All captured sturgeon should be weighed if possible. Weights allow a better understanding of the conditioning of captured sturgeon during various seasons of the year or life span of the fish. For weighing sturgeon, animals should be supported with a sling or net and handling should be minimized throughout the procedure.

Boats used for researching green, Gulf, and Atlantic sturgeon should accommodate larger fish with scales available to safely weigh a 200 pound fish. When targeting shortnose sturgeon (or juvenile green, Gulf, or Atlantic sturgeon), hand-held sling scales are acceptable. When using a bench scale or platform scale to weigh large sturgeon, a five to six foot flat platform will be necessary to support the fish.

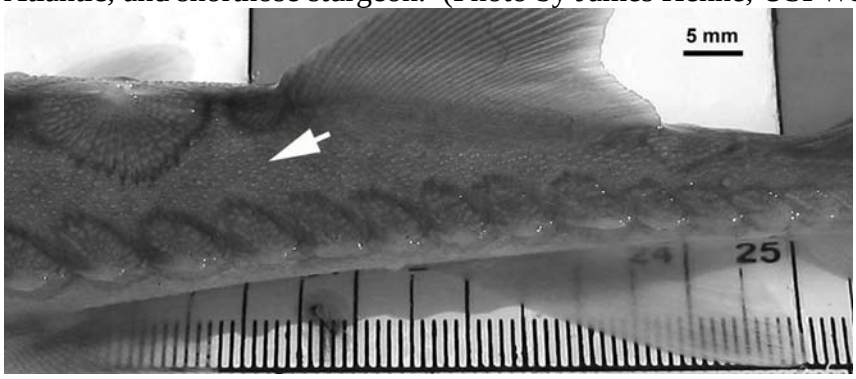
Photographing
When handling sturgeon, optional photography is often used to document the health of fish, research methods, and any identifying marks on the sturgeon potentially useful in the future. Although it is recommended to take as many pictures as needed, researchers should do so without interfering with other research activities.

PIT Tags
Every sturgeon should be scanned for PIT tags along its entire body surface ensuring it has not been previously tagged. Untagged sturgeon should then be appropriately PIT tagged (Figure 2) and the identifying number recorded. Each PIT tag consists of integrated circuitry and an antenna encapsulated in glass. PIT tags are "passive" because they contain no batteries; their internal code is activated and transmitted to the receiver when exposed to the transceiver's electromagnetic signal. The newest PIT tags, and those recommended by NMFS, use a frequency of 134.2 kHz.

Standardized PIT tag placement for Gulf, green, Atlantic, and shortnose sturgeon would enable subsequent researchers to locate prior PIT tags quickly and consistently. Sturgeon, are large fish growing a considerable amount from the time they're first PIT-tagged until they reach their adult size. If muscles grow over the PIT tag as they mature, the tag can become increasingly more difficult to read.

For this reason, NMFS strongly recommends PIT tag placement in all four sturgeon species to be located to the left of the spine, immediately anterior to the dorsal fin, and posterior to the dorsal scutes (Figure 2). This positioning would optimize PIT tag readability over the animal's lifetime as sturgeon experience the least new muscle growth in this location during their lifetimes (Berg 2004, Simpson and Fox 2006). After the tag is inserted, it should be scanned to ensure it is readable before the fish is released. If necessary, to ensure tag retention and prevent harm or mortality to small juvenile sturgeon of all species, the PIT tag can also be inserted at the widest dorsal position just to the left of the 4^{th} dorsal scute.

Figure 2. Standardized location for PIT tagging all green, Gulf, Atlantic, and shortnose sturgeon. (Photo by James Henne, USFWS)



PIT tags have the highest reported retention rate of all identification tags, though they are not visible to the researcher or fisherman upon capture. Clugston (1996) found PIT tags implanted in gulf sturgeon have approximately a 90% retention rate. Musick and Hager (2007) tagging 445 Atlantic sturgeon reported a 99% retention rate of PIT tags after 96 hours. Smith *et al.* (1990) noted 100% retention after 60 days in wild shortnose sturgeon. In the Penobscot River, retention rates for PIT tags in Atlantic sturgeon were 93% after as much as 8.8 years (Gayle Zydlewski, University of Maine, pers. comm.). Nelson *et al.* (2007) report approximately 100% retention of PIT tags in recaptured white sturgeon.

Other researchers have had different results. Researchers with EDI Environmental Dynamics (2006) reported recapturing three white sturgeon, with 66% retention of PIT tags. DeHaan *et al.* (2008) recorded 51 to 95% retention when PIT-tagging juvenile pallid sturgeon, which is similar to rates observed by Henne *et al.* (unpublished).

As with all research procedures, there is a risk of injury or mortality either directly or indirectly related to PIT tagging. When PIT tags are inserted into animals having large body sizes relative to tag size, empirical studies generally conclude they have no adverse effect on the growth, survival, reproductive success, or behavior of individual animals (Brännäs *et al.* 1994, Elbin and Burger 1994, Keck 1994, Jemison *et al.* 1995, Clugston 1996, Skalski *et al.* 1998, Hockersmith *et al.* 2003). However, smaller sturgeon may experience mortality within the first 24 hours, usually as a result of inserting the tags too deeply or from pathogenic infection. When analyzing mortality of small sturgeon caused by PIT tags, Henne *et al.* (2008) found 11 and 14 mm tags inserted into shortnose sturgeon longer than 300 mm was safe. In this study, they found that when fish are under 300 mm, factors other than length, such as weight or condition, most influence the likelihood of mortality. Therefore, NMFS recommends only sturgeon over 300 mm should receive PIT tags.

A negative aspect of using PIT tags in sturgeon research is the difficulty for NOAA observers or non-researchers to detect tags in recaptured sturgeon without the benefit of a PIT tag reader. Rien *et al.* (1994) and Nelson *et al.* (2004) recommend removal of the second left lateral scute indicating the presence of a PIT tag in white sturgeon. This methodology has been subsequently used for green sturgeon as well. While removal of

scutes rarely results in bleeding, and is not considered deleterious, there are other, safer means for externally marking sturgeon. NMFS believes a standardized PIT tag location is less stressful to animals and is easily located. If an external mark is necessary, NMFS recommends using other external tags identified in this document. Those external tags are not only obvious to other researchers, but also to the general public for identifying recaptured animals to alert researchers of their recapture. NMFS therefore recommends using external tags to identify the presence of a PIT tag, if necessary, but researchers should not remove scutes from sturgeon for any reason.

Genetic Tissue Sampling

Tissue sampling is a common practice in fisheries science characterizing the genetic "uniqueness" and quantifying the level of genetic diversity within a population. NMFS strongly recommends genetic tissue samples be taken from every sturgeon captured unless, due to marks or tags, the researcher knows a genetic sample has already been obtained. Tissue samples should be a small (1.0 cm^2) fin-clip collected from soft pelvic fin tissues using a pair of sharp scissors. Tissue samples should be preserved in individually labeled vials containing 95% ethanol. There is no evidence that this procedure harms any species of sturgeon.

Recommendations

Strongly Recommended

- Researchers should measure all captured green, Gulf, Atlantic, and shortnose sturgeon. The sturgeon should also be weighed, if possible.
- Researchers should scan captured sturgeon for previously inserted PIT tags; and, if none are found, one should be properly inserted.
- Researchers should remove a small tissue sample by clipping the soft tissue of the pelvic fin.

Measuring

- Standardized length measurements for all sturgeon should be taken from the snout to the fork in the tail.
- NMFS recommends measuring the ratio of mouth width to interorbital width to differentiate shortnose and Atlantic sturgeon.

PIT Tags

- NMFS recommends PIT tag placement in all four sturgeon species to be located to the left of the spine, immediately anterior to the dorsal fin, and posterior to the dorsal scutes.
- NMFS recommends using 134.2 kHz PIT tags.
- If necessary, to ensure tag retention and prevent harm or mortality to small juvenile sturgeon of all species, the PIT tag can also be inserted at the widest dorsal position just to the left of the 4^{th} dorsal scute.
- NMFS recommends only sturgeon over 300 mm should receive PIT tags.
- NMFS recommends using external tags to identify the presence of a PIT tag, if necessary, but researchers should not remove scutes from sturgeon for any reason.

Genetic Tissue Sampling

- NMFS strongly recommends genetic tissue samples be taken from every sturgeon captured unless, due to marks or tags, the researcher knows a genetic sample has already been obtained.
- Tissue samples from Gulf, green, Atlantic, and shortnose sturgeon should be archived at the NOAA/NOS Tissue Archive in Charleston, South Carolina. Proper certification, identity, and chain of custody of samples should be maintained during transfer of tissue samples.

Anesthetization

Anesthetics are physical or chemical agents preventing the initiation and conduction of nerve impulses (Summerfelt and Smith 1990). Therefore, the primary functions of anesthetics on ESA listed sturgeon are to immobilize the animal allowing precise, autorized procedures to be performed while blocking nerve impulses which might otherwise adversely affect the fish. This section, therefore, attempts to balance the risk of stress from invasive procedures with the risk posed by using an anesthetic, while also considering the risk of an unanesthetized sturgeon moving suddenly during a procedure resulting in trauma or hemorrhaging.

Invasive research activities can be stressful to fish, even if immobilized. The use of an anesthetic reduces the potential for short term stress response and risk of mortality during those procedures (Iwama *et al.* 1989, Small 2003, Wagner *et al.* 2003, Coyle *et al.* 2004, Roubach *et al.* 2005, Wanner *et al.* 2007). However, the use of some anesthetics have also proven to be stressors to fish (Iwama *et al.* 1989) as evidenced by the buildup of the cortisol hormone. NMFS recommends that noticeably stressed sturgeon should not be anesthetized.

Documented lethal or sub-lethal effects caused by improper dosage or exposure of anesthetics (Iwama *et al.* 1989, Summerfelt and Smith 1990) raises concerns whether it is acceptable to use anesthetic when handling listed Gulf, green, shortnose, or Atlantic sturgeon. In tests where anesthetics were not used during invasive procedures, cortisol levels were found significantly higher than when fish were anesthetized with tricaine methanesulfonate (MS-222) or clove oil (Wagner *et al.* 2003). Conversely, Wagner *et al.* (2003) found unanesthetized fish had lower cortisol levels than either of two anesthetized groups after one hour, demonstrating recovery of fish is more rapid without anesthetization. Nevertheless, in controlled studies when prolonged handling took place (30 minutes or more), Strange and Schreck (1978) documented fish had a higher survival rate when anesthetized.

Summerfelt and Smith (1990) and Bowser (2001) note a normal condition and six stages of anesthesia: light sedation, deep sedation, partial loss of equilibrium, total loss of equilibrium, loss of reflex reactivity, and asphyxia (Table 4). Light sedation occurs when there is a slight loss of reactivity, while deep sedation occurs when only the strongest external stimuli will elicit a response, but in both cases, the fish is able to maintain equilibrium. Partial loss of equilibrium is also characterized by partial loss of muscle tone and an increase in opercular movement, while total loss of equilibrium is characterized by total loss of muscle tone, the loss of spinal reflexes, and slow and steady opercular rate. The loss of reflex reactivity is when the fish losses all reflex response, but also when the heart rate becomes very slow and the opercular movements become slow and irregular. The final stage of anesthesia is a complete medullary collapse, when opercular movement ceases. Death is typically caused by an overdose or overexposure leading to eventual mortality.

Table 4. Stages of anesthesia (Summerfelt and Smith 1990).

Stage	Descriptor	Behavioral Response of Fish
0	Normal	Reactive to external stimuli; opercular rate and muscle tone normal
I	Light sedation	Slight loss of reactivity to external stimuli; opercular rate slightly decreased; equilibrium normal
II	Deep sedation	Total loss of reactivity to all but strong external stimuli; slight decrease in opercular rate; equilibrium normal
III	Partial loss of equilibrium	Partial loss of muscle tone; swimming erratic; increased opercular rate; reactivity only to strong tactile and vibration stimuli
IV	Total loss of equilibrium	Total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes
V	Loss of reflex reactivity	Total loss of reactivity; opercular movements slow and irregular; heart rate very slow; loss of all reflexes
VI	Medullary collapse (asphyxia)	Opercular movements cease; cardiac arrest usually follows quickly

The primary risks associated with anesthetizing sturgeon are overexposure and overdosing. Overexposure can occur when sturgeon are left in an anesthetic bath longer than necessary to achieve narcosis. Fish often have difficulty recovering with normal response time when overexposed, and sometimes will not respond for extended periods requiring continuous respiration to revive them. Overdosing can take place when the concentration of anesthetic is higher or more toxic than fish can tolerate. Both conditions often result in immediate or delayed mortality. As an anesthetic is applied, the sturgeon's opercular movement should be monitored closely. It should not be allowed to stop as this condition could result in blood hypoxia and high stress response, or even mortality of the anesthetized animal (Iwama *et al.* 1989).

There are various research activities commonly performed on sturgeon that present enough risk to the fish that they should only be done using anesthesia (Table 5). However, the same level of narcosis is not needed for each activity and therefore the researcher would not use the same concentrations of anesthetic. Physical restraint is not an appropriate substitute for anesthetization.

The rate at which anesthesia is induced in a fish is also important at minimizing stress. Prolonged induction generally leads to increased stress responses (e.g. prolonged thrashing during excited phase), while excessively rapid induction times (<1 minute) risks taking the fish beyond the surgical anesthesia plane because animals may skip typical behavioral signs characterizing stages of anesthesia. NMFS recommends initiating anesthesia gradually to reduce the risks of overdosing. NMFS also recommends monitoring the sturgeon during induction to avoid overexposure. If the desired stage of narcosis cannot be reached within 15 minutes (Summerfelt and Smith 1990), the sturgeon should be placed in freshwater to recover before being released.

Table 5: Procedures and stages of anesthesia.

Procedure	Stage of Anesthesia (see Table 4)
Internal tagging	III
Biopsy	III
Laparoscopy	IV
Gastric lavage	I
Boroscope	0 or I
Fin ray sectioning	II
Genetic fin clip	0
Blood sample	0
PIT tag	0
External tagging	0 but I is acceptable if necessary

Cold water species respond more rapidly and at lower doses to chemical anesthetics than do warm water species (Bowman *et al.* 2003, Coyle *et al.* 2004). Currently, it has not been demonstrated if shortnose, Atlantic, Gulf, or green sturgeon exhibit variable inter- or intra-species responses to chemical anesthetics with respect to temperature. As identified previously, however, larger green sturgeon grow more optimally at cooler temperatures than do shortnose or Atlantic sturgeon. This suggests green sturgeon are better adapted to cooler waters, may also be more likely to respond to lower levels of anesthetic than shortnose or Atlantic sturgeon. Correspondingly, Gulf sturgeon may need higher doses than the other species at cooler temperatures. Likewise, northern populations of shortnose and Atlantic sturgeon may be better adapted to cooler waters and respond differently to anesthesia.

Chemical Anesthetic

MS-222

A wide variety of chemical compounds have been utilized to anesthetize fish in fisheries research. However, tricaine methanesulfonate (MS-222) is the only anesthetic with a label for use with fish granted by the Food and Drug Administration (FDA) and as such, is the only chemical anesthetic recommended by NMFS for use on green, Gulf, Atlantic, and shortnose sturgeon.

MS-222 is absorbed rapidly through the gills and it prevents the generation and conduction of nerve impulses, with direct actions on the central nervous system and cardiovascular system. MS-222 is excreted in fish urine within 24 hours and tissue levels decline to near zero in the same amount of time (Coyle *et al.* 2004).

Proper dosing depends on the degree of anesthetization desired, the species and size of fish, water temperature and water hardness. In general, levels of MS-222 recommended do not typically exceed 100mg/L for salmonids or 250 mg/L for warm water fish (Coyle *et al.* 2004). To euthanize fish using MS-222, the recommended dosage varies from 150 to 500 mg/L for one minute or more depending on the species (DeTolla *et al.* 1995, Cho and Heath 2000, Callahan and Noga 2002, Borski and Hodson 2003).

There are two methods commonly used by sturgeon researchers to anesthetize sturgeon. The first method incorporates a "knockout" initiation dose of MS-222 followed by a safer maintenance concentration (DeTolla *et al.* 1995, Callahan and Noga 2002, Thorsteinsson 2002, Borski and Hodson 2003). Alternately, researchers anesthetize sturgeon using the lowest possible dose of MS-222, raising it to achieve the desired stage of narcosis based on the procedure (Table 5). Neither method, when performed correctly, is safer than the other. However, more risk is associated with overdosing fish exposed to higher induction rates.

For most procedures, sturgeon should initially be lightly anesthetized with MS-222, and if needed, more should be added only to the level considered necessary to perform the appropriate procedures. MS-222 solutions are highly acidic, therefore the pH of the solution should be buffered to a neutral pH with equal amounts of sodium bicarbonate prior to use. In cooler water temperatures, either higher doses or longer exposure times may be necessary to achieve the proper narcosis because the absorption rate is lower at lower temperatures (Coyle *et al.* 2004). Additionally, because MS-222 is a hypoxic agent, the anesthetic container should be vigorously aerated to maintain DO levels equivalent to ambient river water.

Total loss of equilibrium (Stage IV) is the deepest level of narcosis acceptable for anesthetizing listed sturgeon. It may not be possible to reach this stage of narcosis by gradually increasing the dosage and instead, the researcher would need to begin with a high induction dose and then drop back to a maintenance dose. Because of the risks associated with this type of anesthetization, NMFS recommends inexperienced researchers first conduct this type of anesthesia in a laboratory using a heart rate monitor to prevent overdose. Only once a researcher has demonstrated the ability to consistently perform this type of anesthetization safely should they do this in the field.

When immersed in MS-222, sturgeon will initially experience rapid gill movement followed by marked reduced gill movement as the agent begins to have an effect. As gill movement slows, sturgeon will lose equilibrium and eventually turn upside down or float to the surface. At this stage, sturgeon should be watched closely to confirm continuous involuntary gill movement. If the procedure is brief, once the desired stage of anesthesia has been reached, sturgeon may be placed on a surgical cradle and the gills irrigated with fresh water to ensure respiration and to begin recovery as the procedure is quickly completed. After completing the procedure, the fish should be placed in a clean, anesthetic free recovery tank and observed until fully recovered. Once recovered, the sturgeon can be released.

Following is a review of the various concentrations and induction methods of MS-222 when anesthetizing Gulf, Atlantic, shortnose, and green sturgeon. Fleming *et al.* (2003a) suggested concentrations of MS-222 of up to 400 mg/L failed to adequately anesthetize Gulf sturgeon. These researchers concluded the anesthetic was potentially dangerous to the sturgeon. However, Hernandez-Divers *et al.* (2004) successfully anesthetized Gulf sturgeon submerging them in an initiating dose of 250 mg/L followed by a maintenance bath of 87.5 mg/L. Harris *et al.* (2005) anesthetized Gulf sturgeon using 160 mg/L MS-

222. Parkyn *et al.* (2006) anesthetized Gulf sturgeon using a single phase induction of 150 mg/L MS-222. Lankford *et al.* (2005) anesthetized green sturgeon placing them in concentrated baths of 350 mg/L of MS-222 followed by less concentrated doses of 150 mg/L. Kaufman *et al.* (2007) anesthetized green sturgeon using 350 mg/L removing them from the solution when anesthetized. However, Serge Doroshov, (University of California Davis, pers. comm.) regularly uses 100 mg/L when working on green sturgeon. Joe Cech (University of California Davis, pers. comm.) starts green sturgeon anesthesia in baths of 150 mg/L and then when respiration stops, places them in a second, less concentrated bath of 75 mg/L. The majority of shortnose and Atlantic sturgeon researchers interviewed for this document reported concentrations of MS-222 from 50 to 100 mg/L were sufficient to induce anesthesia for most invasive procedures (Boyd Kynard Permit #1549, Mark Collins Permit #1447, Michael Kennison Permit #1595, Doug Peterson Permit #10037, Haley 1998, Oakley and Hightower 2007, Savoy 2007). The USFWS' Biological Procedures and Protocols for Researchers and Managers Handling Pallid Sturgeon recommends using MS-222 at doses between 50 and 150 mg/L (USFWS 2008).

Induction and recovery times for chemical anesthetics vary based on the dosage level and duration the fish is under anesthesia. For rainbow trout in MS-222, Wagner *et al.* (2003) found induction takes two to three minutes at 60 mg/L with recovery taking 5 to 6 minutes. For Gulf sturgeon in MS-222, Hernandez-Divers *et al.* (2004), when initiating anesthesia at 250 mg/L, induction took 5 to 11 minutes before lowering the dosage to 87.5 mg/L, after which recovery took 3 to 13 minutes. For green sturgeon at 50 to 100 mg/L MS-222, induction and recovery both required 10 to 15 minutes at 18° to 21°C, but at cooler temperatures it took longer (Joel Van Eenannaam, University of California Davis, pers. comm.).

Sturgeon face several risks posed by MS-222, such as overdose, increased stress, or being released prior to recovering. Weakened fish are more susceptible to anesthetic shock and thus are more likely to be accidentally overdosed (Coyle *et al.* 2004). Even when anesthetized with MS-222, fish still experience elevated levels of plasma cortisol, indicating they are stressed either by handling or by additive stress of MS-222 (Coyle *et al.* 2004). After being handled under anesthesia, plasma cortisol levels increased 8 times over base in channel catfish (Small 2003) and nine times over base in rainbow trout (Wagner *et al.* 2003). Studies by Pirhonen and Schreck (2003) found fish anesthetized with MS-222 ate significantly less (15-20%) than control fish. If the dose of MS-222 is too high or the exposure is too long, recovery is longer if it occurs at all. Therefore, NMFS recommends monitoring sturgeon closely during recovery and taking protective measures if fish appear stressed and not recovering normally (e.g., providing supplementary DO and moving water across the gills until fully recovered).

Recovery is also influenced by the size and sexual condition of fish. Because MS-222 is fat soluble (Coyle *et al.* 2004) longer recovery times are experienced by larger sturgeon and gravid females. Holcomb *et al.* (2004) showed doses of 225 mg/L MS-222 had no effect to eggs or sperm of white sturgeon and could be used to harvest gametes. However, doses of 2,250 mg/L resulted in lower hatching success and doses of 22,500

mg/L resulted in complete loss of fertility. At the dosages typically used by researchers to anesthetize sturgeon, however, no impact to their eggs is expected.

Although the FDA permits the use of MS-222, it also requires a 21 day withdrawal period before an anesthetized fish can be consumed. This poses concerns for humans when non listed fish are released into the wild where they may be consumed. However, a 21 day withdrawal is not a consideration for threatened or endangered sturgeon, as taking or possessing them is prohibited by the ESA. Therefore, no external marks or tags are required for Gulf, green, Atlantic, or shortnose sturgeon following anesthetization with MS-222.

Clove Oil

Clove oil is approximately 90 to 95% eugenol with smaller portions of methyleugenol and isoeugenol and was initially experimented with as a substitute for MS-222 (Bowman *et al.* 2003). Showing promise as an anesthetic, it was marketed as AQUI-S (isoeugenol , 2-methoxy-4-propenylphenol) in an attempt to gain FDA approval. However, in 2007, the National Toxicology Program concluded exposure of male mice to isoeugenol resulted in clear evidence of cancer. As a result of its concern that isoeugenol's carcinogenic properties could be transmitted through the food web, the FDA's Center for Veterinary Medicine officially rescinded authorization for the "investigational food use" of AQUI-S under INAD 10-541 (AADAP 2008). Consequently, both NMFS and the FDA (2007) are concerned isoeugenol could have direct adverse effects to threatened and endangered aquatic species. NMFS does not authorize the use of clove oil or AQUI-S on Atlantic, green, Gulf, or shortnose sturgeon.

Physical Anesthetic

Electronarcosis

Electronarcosis (also referred to as electroanesthesia and galvanonarcosis) is a non-chemical method of anesthetization and, as such, does not require FDA approval. Researchers investigating the use of electricity to immobilize fish have used various methods and species of fishes. Alternating current (AC), rectified AC, constant direct current (CDC), and pulsed direct current (PDC) have all been tested (Hartley 1967, Walker *et al.* 1994, Barton and Dwyer 1997, Henyey *et al.* 2002). Some researchers leave the electricity on for the entire time the fish is immobilized (Gunstrom and Bethers 1985) while others apply a short burst of relatively high voltage resulting in immobization of the fish for several minutes after the electric current is discontinued (Sterritt *et al*. 1994). Much of what has been learned about electronarcosis is based on the same principles applied during electrofishing.

Fish exposed to electric current may show electrotaxis (forced swimming), electrotetanus (muscle contractions), or electronarcosis (muscle relaxation). AC causes tetanus (Henyey *et al.* 2002) and at higher voltages pulsed direct current causes tetanus, whereas constant direct current causes narcosis first, and then will eventually cause tetanus as the voltage is increased (Summerfelt and Smith, 1990). Typically, when researchers have studied electronarcosis, the electricity used was either AC or PDC, or was CDC of a sufficiently

high voltage that the fish were immobilized by electrotetanus. Further, most studies using AC and PDC reported adverse effects including some bruising, burning, hemorrhaging, and mortality (Tipping and Gilhuly 1996, Redman *et al*. 1998, Holliman and Reynolds 2002). Consequently, NMFS does not recommend using AC or PDC currents for inducing anesthesia in listed sturgeon. When using CDC, the risks to sturgeon are over-applying the direct current resulting in either tetany or cessation of opercular movement. These adverse affects can be avoided by monitoring the sturgeon and reducing the voltage depending on the fish's behavior.

Henyey *et al.* (2002) describe using low voltage CDC to induce electronarcosis (muscle relaxation) in shortnose sturgeon without any changes in swimming or feeding behavior, burns, bruising, or mortality after monitoring the fish for six weeks (Boyd Kynard, USGS, pers. comm.). All evidence indicates electronarcosis induced by the method described is similar to the condition induced by chemical anesthetics; nevertheless, more research is needed on the physiological mechanisms by which it works. NMFS recommends low voltage direct current electronarcosis as described by Henyey *et al.* (2002) as a viable alternative to chemical anesthesia.

Electronarcosis has been used successfully by Boyd Kynard (USGS, pers. comm.) to anesthtize shortnose sturgeon since the 1980s. Since 2004, USFWS researchers in Maryland have also followed the Henyey *et al.* (2002) protocol to anesthetize Atlantic and shortnose sturgeon on the Potomac River and Chesapeake Bay with no adverse affects reported (Mike Mangold, USFWS, pers. comm.). Researchers in South America have also followed these methods reporting similar success (Alves *et al.* 2007).

As described in Henyey *et al.* (2002), a tank is prepared by positioning positive cathode and negative anode plates at opposite ends. With the sturgeon oriented head towards the cathode, a CDC is applied quickly so the fish loses equilibrium and then the voltage is adjusted downward until the fish is relaxed and exhibiting strong opercula movement. In practice, when inducing electronarcosis, if gill ventilation becomes irregular or stops, the electric current should be decreased and the fish will recover steady ventilation immediately (Boyd Kynard, USGS, pers. comm.). The amperes should be set to the minimal level (0.01A). Depending on the individual sturgeon and water chemistry, about 0.3 to 0.5 volts per centimeter is recommended to immobilize sturgeon. Typically, sturgeon should be supported by a net so only half of the body either dorsal or ventral depending on the work being conducted, is out of the water. Under these conditions, the researcher will feel nothing while working in the water (Hartley 1967, Boyd Kynard, USGS, pers. comm.) but researchers with sensitive skin or hand abrasions are also encouraged to wear rubber gloves during the procedure.

Induction and recovery from electronarcosis both require less than 10 seconds because as soon as fish are placed in or removed from the electrical current, it is no longer anesthetized (Gunstrom and Bethers 1985, Summerfelt and Smith 1990, Henyey *et al.* 2002). Henyey *et al.* (2002) state electronarcosis is ideal for non-invasive research. The methods in Henyey *et al.* (2002) elicited narcosis, not tetany; and Boyd Kynard (USGS, pers. comm.) states narcosis is induced by blocking nerve impulses at the medulla

oblongata. Kynard and Lonsdale (1975) demonstrated electronarcosis and MS-222 yielded similar states of muscle relaxation and immobility.

Recommendations

General

- NMFS recommends that noticeably stressed sturgeon should not be anesthetized.
- Physical restraint is not an appropriate substitute for anesthetization in procedures requiring anesthesia.
- NMFS recommends initiating both chemical and physical anesthesia gradually to reduce the risks of overdosing.

Chemical Anesthetic

- Because of the risks associated with high initial induction doses followed by a lower maintenance dose of MS-222, NMFS recommends using this technique in a controlled environment such as a laboratory and also using a heart rate monitor to prevent overdosing.
- NMFS also recommends monitoring the sturgeon during induction to avoid overexposure and if the desired stage of narcosis cannot be reached within 15 minutes, the sturgeon should be placed in freshwater to recover before being released.
- Tricaine methanesulfonate (MS-222) is the only chemical anesthetic with a label for use on fish granted by the FDA and as such, is the only chemical anesthetic recommended by NMFS for use on green, Gulf, Atlantic, and shortnose sturgeon. Dosages of MS-222 should be between 50 and 150 mg/L as identified in the pallid sturgeon protocols (USFWS 2008) and by green, Gulf, Atlantic, and shortnose sturgeon researchers.
- NMFS recommends monitoring sturgeon closely during recovery and taking protective measures if fish appear stressed and not recovering normally (e.g., providing supplementary DO and moving water across the gills until fully recovered).
- A 21 day withdrawal, normally associated with the use of MS-222 on food fish, is not a consideration for threatened or endangered sturgeon, as taking or possessing them is prohibited by the ESA.
- NMFS does not authorize the use of clove oil and AQUI-S on Atlantic, green Gulf, or shortnose sturgeon.

Physical Anesthetic

- NMFS recommends low voltage direct current electronarcosis as described by Henyey *et al.* (2002) as a viable alternative to chemical anesthesia but does not recommend using AC or PDC currents for inducing anesthesia in listed sturgeon.

Tagging

Tagging is an essential function of sturgeon research, serving to identify unique information about a captured or recaptured animal. PIT tags, as discussed earlier, should be inserted in all Gulf, green, shortnose, and Atlantic sturgeon without a PIT tag. Determining the life history, morphology, behavior, movement, and physiology of sturgeons are all highly dependent on proper tagging methods. Because sturgeon can live for decades, it is essential tags be retained for extended periods. In addition, because sturgeon exhibit very rapid juvenile growth rates and can achieve very large sizes, tags must be retained even as the tag placement area changes size and shape. Moreover, sturgeon are adept at shedding external tags and can also extrude internal tags through the body wall (Kieffer and Kynard 1993). Consequently, sturgeon researchers should keep well informed on the effectiveness of tagging methods and the technology best suited for local conditions.

Tagging varies based on tag function, location, method, technology, retention rates, and size. Internal tags (acoustic or radio) are surgically implanted in sturgeon for tracking movements, whereas externally mounted tags can be used for tracking or identification. Despite lower retention rates for some external tags, there are situations where external tags are the only option, such as tracking pre-spawning females. External archival tags and satellite tags can also passively record water quality information or geographic position without arrays. Other types of external-identifier tags are useful when non-researchers are involved in research activities, such as studies relying on fishermen to return data from tags on marked fish.

Telemetry Tags

Acoustic tags outperform radio tags in deeper water (or saline water) where sturgeon spend a majority of their lives; however, acoustic tags have disadvantages associated with limited range and ineffectiveness in turbulent or turbid waters. Acoustic signals can be monitored by field crews using either mobile hydrophones or, more commonly, stationary hydrophone arrays. Because the stationary arrays are designed to passively capture the location of transmitted signals from near-by fish, many researchers are converting to acoustic tag technology, collecting data over a longer period of time and downloading it at later intervals (Reine 2005).

Radio transponders emit radio signals from transmitter antennae to the atmosphere where they can then be monitored by researchers with a receiving antenna. For highly migratory species such as sturgeon, researchers can locate and track fish at distances up to three kilometers via airplane. Radio signals are also effective in environments having more physical disruptions such as turbidity (Thorsteinsson 2002). Combined acoustic and radio transmitter (CART) tags provide the researcher the advantages of each transmitter type.

Implanting internal telemetry tags is stressful to sturgeon and should be done using anesthesia. To gain access to the abdominal cavity, a two to four centimeter incision is made between the 3rd and 4th ventral scute between the anal and pelvic fin slightly left or

right of the mid-ventral line. Internal tags should be coated with a biologically inert substance, soaked in alcohol and allowed to dry, and then pushed deeply into the abdominal cavity to prevent tags from rubbing against the incision (Kynard and Kieffer 1991). In studies by Kynard and Kieffer (1997) no tags were rejected from shortnose sturgeon when they were coated in biologically inert material but when uncoated tags were used, they were rejected 33% of the time. Of those rejected, sonic tags were expelled within two weeks, while the radio tags were rejected within 14 weeks (Kynard and Kieffer 1997). Collins *et al.* (2002) recorded no mortality using completely internal tags during a three month study on tagging methods. Due to slower recovery time at lower temperatures, internal tags should not be implanted when water temperatures are below 8°C (Moser *et al.* 2000a, Ream *et al.* 2003, Kieffer and Kynard *in press*). Also, due to increased stress at higher temperatures, incisions should not be made in sturgeon when water temperatures exceed 27°C (Moser *et al.* 2000a, Kieffer and Kynard *in press*).

Some researchers have experimented with an internal tag having external trailing antennae threaded through a permanent hole in the lateral wall of sturgeon. These tags, allowing for better transmission of radio frequencies, are known as Internal/External tags (I/E tag). However, depending on the surgical procedure used to anchor the trailing antennae at the exit point, certain harmful effects resulted from the chaffing and cutting of the trailing antenna. In one lake sturgeon I/E tagging study, Peterson and Bezold (2008) tagged both wild and hatchery raised fish, allowing them to recover for 14 to 21 days prior to release. In this study, wild fish experienced 9% mortality but hatchery-reared sturgeon experienced 90% mortality. In an I/E tagging study by Collins *et al.* (2002), laboratory sturgeon tagged in this manner endured large exit wounds resulting from the trailing antenna and eventually suffered 100% mortality. In the same study, internal telemetry tagging techniques and two methods of external tagging resulted in only one mortality.

More recent results documented by Kieffer and Kynard (*in press*) found trailing antennae did not appear deleterious to the health of shortnose sturgeon when designed to exit the body wall through holes drilled in lateral scutes. Five wild fish tagged in the Connecticut River with I/E tags exiting through the scute were tracked for a year. All fish were recaptured, but the exit holes in all scutes had become larger. Until these techniques are better documented, NMFS recommends I/E tagging should not be done on green, Gulf, shortnose, or Atlantic sturgeons.

Historically, external tags were easily shed. Collins *et al.* (2002) showed hatchery shortnose sturgeon were able to shed 100% of their external transmitters (9 cm long, 1.7 cm diameter) when attached with a wire through the dorsal fin. However, the same researcher reported no external transmitter tags lost when attached to a dart tag using heat shrunk plastic wrap. Counihan and Frost (1999) found no external tags were shed by juvenile white sturgeon after one to three weeks. Sutton and Benson (2003) reported a 14.4% shedding rate for external tags (2.1 – 4.0 cm), with 27% of the larger tags (3.4 - 4.0 cm) shed.

More recently, researchers have documented higher retention rates with the advent of newer, smaller external tags and better methods of attachment (Figure 3). These external tags range in size between 18 and 46 mm long and only 7 to 9 mm in diameter. Using 70 to 100 lb test monofilament line, Mike Randall and Ken Sulak (USGS, pers. comm.) described a method for attaching such tags bound externally to the dorsal fin using lightweight heat shrink electrical splice tubing and five minute, two-part epoxy. These researchers documented over 96% retention rates on Gulf sturgeon during 2005 to 2008 using the following method. Their method (Mike Randall and Ken Sulak, USGS, pers. comm.) is described as:

> About 25 cm of monofilament is centered in approximately 20 mm length of heat shrink. A small quantity of epoxy is added to the tag which is then seated into the heat shrink tubing. The tubing is then shrunk with a heat gun until snug. This also warms up the mono line enough to make right angle bends at the ends of the heat shrink tubing. A small amount of epoxy should extrude from each end of the heat shrink tubing making a smooth union. Once the attachment is cooled and the epoxy hardened, the tag should be re-checked and the tag's magnet affixed to the tag. A tape label with the identifying tag number is also wrapped around the monofilament. A hole is then made through the base of the sturgeon's dorsal fin with a PIT tag needle which is also used as a guide to thread the mono line through the dorsal fin. Similarly another hole is made through the dorsal fin anterior to the first hole and the aft monofilament line is passed through. As the transmitter tag is pulled snugly to fit within the crease at the base of the dorsal fin and the body, the two monofilaments ends are joined on the opposite side of the dorsal fin by a short length of steel leader. The external tag is then secured by threading the monofilament through crimps pre-fastened on the ends of the steel leader. As the monofilament lines are pulled with opposite pressure, the leader line crimps are compressed. Finally any trailing ends of the monofilament or leader are cut. The leader will eventually corrode freeing the external tag from the fish.

Figure 3: Location of external telemetry tag (USGS Southeast Ecological Science Center).

NMFS recommends acoustic telemetry tags for tracking the movements of Gulf, green, Atlantic, and shortnose sturgeon. NMFS would suggest tagging sturgeon externally, though both methods are acceptable.

External Identifier Tags

NMFS has authorized a variety of external-identifier tag designs and placement sites on shortnose sturgeon over the past 10 years. Some examples of external-identifier tags are: Carlin (Peterson) tags, coded wire tags, dart tags, disk anchors, double barb tag, elastomer, and Floy T-bar tags. Minimal research has been conducted on the effects of these types of tags on sturgeon species.

The need for researchers to identify sturgeon with external-identifier tags has been called into question by Bergman *et al.* (1992) as sturgeon can be uniquely recognized by PIT tags. Additionally, the effectiveness and retention of these external-identifier tags is uncertain (Bergman *et al.* 1992). However, using external identifier tags can be helpful for identifying wide ranging sturgeon, like the Gulf, Atlantic, and green sturgeons that can be captured in distant locations by other researchers or commercial fishermen. Shortnose sturgeon are less likely to travel great distances through the ocean and into different rivers; therefore, external identifier tags are not as beneficial for them. Consequently, NMFS recommends the use of external tags to assist with the identification of migratory sturgeon when that information will contribute to the species' recovery.

Smith *et al.* (1990) compared the effectiveness of dart tags with nylon T-bars, anchor tags, and Carlin tags in shortnose and Atlantic sturgeon. Carlin tags applied to scutes had low retention rates as did dart tags; however, they also noted the dart tags caused some tissue damage. Carlin tags applied at the dorsal fin and anchor tags inserted in the abdomen showed the best retention. Although anchor tags resulted in lesions and eventual breakdown of the body wall if fish entered brackish water prior to their wounds healing, Collins *et al.* (1994) found no significant difference in healing rates between fish tagged in freshwater or brackish water. Clugston (1996) also looked at T-bar anchor tags placed at the base of the pectoral fins, finding beyond two years, retention rates were about 60%. Collins *et al.* (1994) compared T-bar tags inserted near the dorsal fin, T-anchor tags implanted abdominally, dart tags attached near the dorsal fin, and disk anchor tags implanted abdominally. He found that T-anchor tags were most effective long-term (92%), but also noted that all of the insertion points healed slowly or not at all and, in many cases, lesions developed. Collins *et al.* (1994) also inserted coded wire tags into the sturgeons' snout and found a 100% retention after 62 days, but only 74% after two years, though the tags may not have been inserted deeply enough. Bordner *et al.* (1990) inserted coded wire tags deeply into the snouts of white sturgeon and found 100% retention after 180 days; and Isely and Fontenot (2000) also found that coded wire tags inserted near the dorsal fin have a 98% retention rate after 120 days.

Winter (1983) suggested the appropriate tag weight to body weight ratio for fish was 2% for the tag weight in air and 1.25% for the tag weight in water. Generally, heavier tags

reduce growth or affect the swimming ability of tagged fish. But, as noted by Brown *et al.* (1999), different species of fish are better able to respond to tag weight, handling higher ratios of tag weight to body weight. In a tag to body weight ratio study conducted on lake sturgeon, Sutton and Benson (2003) recommended tag weight in air not to exceed 1.25% of body weight. In a separate study by Counihan and Frost (1999), using the ratio of wet tag weight to sturgeon weight of less than 1.25%, they found the swimming performance of white sturgeon was affected. However, this effect was more attributed to the tag placement rather than the weight itself as external tags attached to the rear dorsal fin resulted in increased drag and unbalanced weight. Currently, NMFS is sponsoring directed research on a variety of sturgeon species to determine the appropriate tag to body weight ratio. However, until resolved, NMFS recommends not exceeding a tag to body weight ratio of 1.25% in water and 2% weight in air for all tags cumulatively.

Recommendations

General

- PIT tags are strongly recommended to be inserted in all Gulf, green, shortnose, and Atlantic sturgeon without a PIT tag.
- NMFS recommends not exceeding a tag to body weight ratio of 1.25% in water and 2% weight in air.

Telemetry Tags

- NMFS recommends I/E tagging should not be done on green, Gulf, shortnose, or Atlantic sturgeons.
- NMFS recommends acoustic telemetry tags for tracking the movements of Gulf, green, Atlantic, and shortnose sturgeon. NMFS would suggest tagging sturgeon externally, though both methods are acceptable.

External Identifier Tags

- When appropriate, NMFS recommends the use of external tags to assist with the identification of migratory sturgeon.

Gastric Lavage

The pulsed gastric lavage technique, demonstrated by Foster (1977) to sample diets of pickerel and largemouth bass, has not worked well for sturgeon species. This is largely due to the difficulty in navigating the lavage tube past the U-shaped bend of the alimentary canal in sturgeon, which begins after the pneumatic duct of the swim bladder joins the anterior end of the stomach (Figure 4, also see Haley 1998 and Brosse *et al.* 2002). Serious injury and mortality has occurred when lavaging sturgeon. Sprague *et al.* (1993), showed gastric lavage tubes positioned prior to the pneumatic duct filled and burst the swim bladder and when passed beyond the bend of the alimentary canal, those tubes were capable of puncturing the canal and stomach lining of an unrelaxed gut.

Haley (1998) modified the Foster (1977) protocols for gastric lavage to create a lavage technique appropriately safe and effective for use on sturgeon (Figure 4). Haley's (1998) technique has been modified a few times with different methods created for water delivery into the stomach through intramedic tubing. Murie and Parkyn (2000), Savoy and Benway (2004), and Collins *et al.* (2008) each used slight variations of the water delivery system, but essentially used the procedures described in Haley (1998) to safely lavage sturgeon. NMFS recommends researchers follow these methods, as described below, when conducting gastric lavage of Gulf, green Atlantic, or shortnose sturgeon.

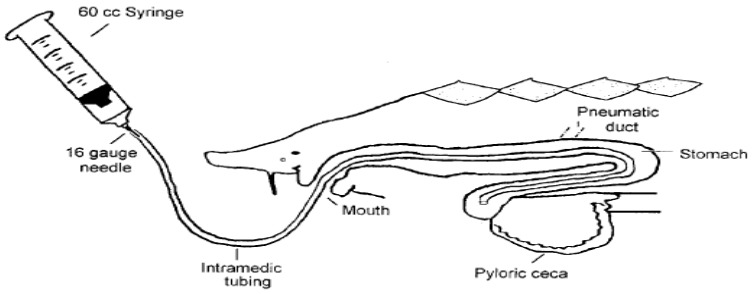


Figure 4: Depiction of the gastric lavage technique used by Haley *et al.* (1998).

First the sturgeon is anesthetized to the appropriate stage (Table 5, Stage I) causing the sturgeon's esophageal and gastric muscles to relax. The sturgeon is then placed ventrally head down on a stretcher or sling with an irrigation tube in its mouth to irrigate the gills during the procedure to ensure respiration. With water running over the gills, a fine mesh strainer is positioned under the sturgeon's mouth to capture the regurgitated contents of the stomach as it is lavaged. With the sturgeon correctly positioned, a soft, flexible intramedic tubing (typically polyethylene) is inserted into the mouth of the sturgeon and carefully directed down the alimentary canal past the pneumatic duct into the stomach region. At the point of resistance reached at the U-shaped bend of the stomach, the flexible tube is twisted ventrally and gently pushed further down the alimentary canal until the tube can be felt on the ventral surface of the fish. If the researcher is more conservative, the lavage procedure can begin once the tube reaches the point of resistance at the U-shaped bend in the stomach, as this method has been shown to be equally effective.

Once the tube is correctly positioned, the stomach contents are evacuated with injected pulses of water. Haley used a syringe to inject water into the stomach, flushing the contents into a strainer. Variations of Haley's technique have been used by other researchers to inject water using a garden sprayer holding a larger reservoir of water to administer the flushing, either timed (Savoy and Benway 2004) or manually (Collins *et al.* 2008). The contents are collected into an appropriately small meshed sieve, preserved in an alcohol filled container and the contents later identified in the laboratory.

In order to conduct gastric lavage procedures, researchers should have the following items:

- Garden sprayer or another appropriately sized water delivery device
- Intramedic tubing
- Means of anesthetization
- 500 micrometer sieve
- A sling or stretcher for holding the fish in the head down position
- Jars filled with alcohol for preserving gut content samples

Kamler and Pope (2001) and Shuman and Peters (2007) report Haley's (1998) protocols are more effective for smaller fish because the syringe can only deliver a small volume of water. Brosse *et al.* (2002), Nilo *et al.* (2006), Savoy (2007), and Collins *et al.* (2008) developed their methods to deliver larger volumes of water to effectively lavage larger Atlantic and shortnose sturgeon. These researchers used varying diameter tubes and depending on the size of the fish, flushing slightly less than a gallon of water into the sturgeon's stomach to completely evacuate its contents.

When gastric lavage was first used with sturgeon, there were serious perceived risks to the individual fish. Sprague *et al.* (1993) reported 33% mortality (4 of 12) of white sturgeon they attempted to lavage. Farr *et al.* (2001) practiced their technique on three dead green sturgeon but were unable to maneuver the tubing around the bend of the alimentary canal. In both methods, the swim bladder filled with water resulting in damage to the alimentary canal and stomach. Both of these studies however used a less flexible aquarium tubing, a factor which potentially prevented the tubing from bending with the stomach and reaching the ventral portion of the stomach near the pyloric caeca. To avoid adverse affects in future research, NMFS recommends practicing on non-listed or hatchery-reared sturgeon before attempting the procedure in the wild.

Several sturgeon researchers have also expressed concerns that delayed mortality and other risks associated with gastric lavage remains unknown and may not be worth the risks of data collection. The only way to adequately measure adverse affects is conducting gastric lavage on sturgeon in a laboratory setting and subsequently monitoring post-lavage survival, growth, and behavior. Brosse *et al.* (2002), Wanner (2006), and Mark Collins (South Carolina Department of Natural Resources, pers. comm.) practiced gastric lavage on captive fish with no delayed mortality prior to conducting lavage in the field. And in Collins *et al.* (2008), three Atlantic sturgeon were sacrificed to monitor adverse effects from lavage on wild fish. No adverse effects were

discovered. Brosse *et al.* (2002) reported all lavaged sturgeon were in poorer condition than control fish after 60 days due to weight loss. However, Collins *et al.* (2008) recaptured fish (over 70 days apart) and documented normal weight gains in the intervals between capture and re-lavage. Other researchers have reported successful gastric lavage work in the field with no immediate mortalities (Haley 1998, Brosse *et al.* 2002, Savoy and Benway 2004, Nilo *et al.* 2006, Guilbard *et al.* 2007, Nellis *et al.* 2007, Savoy 2007, Collins *et al.* 2008). Even if mortality is prevented by using appropriate lavage techniques on sturgeon, NMFS recognizes the potential risks to individual sturgeon from anesthesia, improper lavage technique, and individual sturgeon reacting negatively to the procedure.

Recommendations

- NMFS recommends researchers follow the methods presented in this document and Haley (1998) when conducting gastric lavage on Gulf, green, Atlantic, or shortnose sturgeon. Other documents detail acceptable ways to deliver larger volumes of water for adult Atlantic, Gulf, and green sturgeon.
- NMFS recommends using soft, flexible tubing (polyethylene tubing such as is used in hospitals) to maneuver the bend in the alimentary canal during gastric lavage procedures.
- NMFS recommends practicing on non-listed or hatchery-reared sturgeon before attempting the procedure in the wild.
- Sturgeon must be anesthetized to ensure relaxation of the gut walls to properly position gastric tubes during the procedure.

Sex Identification

The validation of techniques to accurately identify the sex and stage of maturation of sturgeon that leads to the conservation of Gulf, green, Atlantic, and shortnose sturgeon should be a priority. All sturgeon biologists should use safe and effective methods of sexual identification and maturity with the fewest adverse effects to the fish's health. Ideally, the sex of a sturgeon could be identified externally. A study by Vecsei *et al.* (2003) examined the urogenital openings of a variety of species of male and female sturgeon and was able to determine the sex correctly 82% of the time. However to date, the sample size is too small to be confident in the methods described.

Methods commonly used by sturgeon researchers to identify the sex and stage of gametogenesis of sturgeon include borescope (endoscope in the gonoduct), laparoscopy (endoscope through an incision in the ventral body wall), surgery and gonadal biopsy, ultrasound, and blood plasma. These techniques collect different information and have different success rates posing different risks to sturgeon.

The safest forms of sexual identification are methods not requiring anesthetic, like ultrasound (Moghim *et al.* 2002, Colombo *et al.* 2004, Wildhaber *et al.* 2006), borescoping (Kynard and Kieffer 2002), plasma lipophosphoprotein analysis (Craik and Harvey 1984), and plasma vitellogenin analysis (Wildhaber *et al.* 2006). However these methods are time or labor intensive, as with blood and plasma analyses where researchers may not receive the results of their analysis until weeks later.

Endoscopy

Borescope

Borescopic examination has proven an effective method for sexing sturgeon using fiber optic technology. Kynard and Kieffer (2002), Wildhaber and Bryan (2006), and Wildhaber *et al.* (2006) described the technique using a flexible borescope on shortnose, pallid, and shovelnose sturgeon where the head and body of the fish is examined under a lightly anesthetized condition. This procedure, lasting one to two minutes, is conducted with a flexible fiber optic endoscope (16cm long x 4mm diameter) inserted carefully through the urogenital opening and into place within the urogenital canal (Kynard and Kieffer 2002). Sampled females are verified by positively identifying eggs through the urogenital wall. Developed eggs are staged as either "early stage" or "late stage" individuals to identify potential spawners for the coming spring. This is done by carefully comparing the coloration and separation of oocytes viewed through the urogenital wall. Undeveloped eggs are often almond or cream-colored and sometimes indistinguishable from male testes, while mature eggs appear darker, separated, and well formed. It is noted that there are variations of this technique using a trocar to first pierce the genital canal to view and/or biopsy the gonads with an inserted fiber optic borescope; however, NMFS does not recommend this procedure on listed sturgeon.

The above borescope is easily passed through the urogenital opening (average 7.6mm) of adult shortnose, juvenile Atlantic, and other sturgeon species, although there are no

similar morphological data for green sturgeon reported. Van Eenennaam *et al.* (2008) have suggested that the diameter of the urogenital canal of green sturgeon is smaller than other sturgeon species. The greatest potential for injury with this procedure, according to Kynard and Kieffer (2002), is internally at the juncture of the oviduct and urogenital canal, located approximately 9 to 20% of a sturgeon's body length from the vent, regardless of species. The borescope must be maneuvered carefully beyond the oviduct to clearly see and stage eggs. However, when using a 16-cm long borescope, the probe tip will reach beyond the oviduct in most sturgeon of one meter length or less. Further, Kynard and Kieffer (2002) reported repeated probing of the oviduct valve by 4-mm and smaller diameter probes did not penetrate the oviduct valve or damage the urogenital canal regardless of species or fish length. They concluded that careful use of a properly sized borescope would not harm reproductive structures and would be suitable for most sturgeon species.

Kynard and Kieffer (2002) examined 443 adults using a boroscope over six years. Of those viewed, 173 were identified as female and 270 were unidentified — either as females with immature eggs or identified as males. However, Wildhaber *et al.* (2006) was able to correctly identify 85% (93% accurate for males, 63% for females) of shovelnose and pallid sturgeon examined using a similar borescope. During their work, Wildhaber and Bryan (2006) and Wildhaber *et al.* (2006) did not document any injuries or mortalities associated with their borescope activities.

Borescopy requires less time than more invasive surgery, making it a safer alternative to laparoscopy (described below) for field use when handling large numbers of sturgeon under adverse conditions. However, the borescope has limited ability to distinguish between females with immature eggs and male fish as compared to laparoscopy or biopsy.

Laparoscope

Several sturgeon researchers have described using laparoscopic procedures in the lab and field to identify the sex and egg maturity of individual sturgeon. The method for laboratory laparoscopy is described thoroughly by Mohler (2003), Hernandez-Divers *et al.* (2004), and Matsche and Bakal (2008). As with borescopy, the sturgeon should be anesthetized and held in water as much as possible. An incision (approximately 4 mm) is made on the ventral (Hernandez-Divers *et al.* 2004, Wildhaber *et al.* 2006) side or between the lateral scutes (Conte *et al.* 1988) of the sturgeon and the endoscope is inserted through the incision and maneuvered internally to allow the researcher to view the gonads. In Hernandez-Divers *et al.* (2004), the body cavities were insufflated and the swim bladders collapsed, but NMFS recommends avoiding either of these procedures when conducting laparoscopy on Gulf, shortnose, Atlantics, or green sturgeon. Although NMFS considers laparoscopy a more invasive endoscopic procedure than boroscopy, it is a more reliable method for determining the sex and stage of maturity of sturgeons (Wildhaber *et al.* 2006) and therefore recommends laparoscopy as the endoscopic procedure of choice.

Hernandez-Divers *et al.* (2004) laparoscoped 17 Gulf sturgeon. During these procedures, seven fish were positively identified by endoscopy alone and the other 10 were identified by biopsy samples of the gonad tissue. Wildhaber and Bryan (2006) examining 34 pallid sturgeon with both ultrasound and endoscope, positively identified the sex of 100% of the fish. Wildhaber *et al.* (2006) found that laparoscopy could positively identify the sex of shovelnose sturgeon 93% of the time (93% for males, 92% for females).

Adverse effects were not reported in any of the papers discussing laparoscopy. Hernandez-Divers *et al.* (2004) reported 100% survival after extensive surgeries (45 minutes to an hour) for their 17 Gulf sturgeon. Unfortunately this work was conducted in a controlled laboratory setting by three surgeons and does not represent typical field research conditions. Additional research determining adverse effects associated with laparoscopic procedure still need to be documented, particularly on gravid females captured prior to initiating a spawning run. Several researchers have reported capturing sturgeon can may be related to abandoned spawning runs (Moser and Ross 1995, Kynard *et al.* 2007), but there have been no studies addressing the effects of anesthesia or laparoscopy on mature, late stage females still occupying their winter staging habitat prior to spawning.

Surgical Biopsy

Surgical biopsy and histological examination of a sturgeon's gonadal tissue is the most accurate while also the most invasive way to identify the sex and stage of maturity of a sturgeon (Van Eenennaam *et al.* 1996, Van Eenennaam and Doroshov 1998, Fox *et al.* 2000, Webb and Erickson 2007, Flynn and Benfey 2007). Chapman and Park (2005) conducted gonad biopsies on Gulf sturgeon by anesthetizing them and placing them in a sling on their backs. A two to four cm ventral incision was made, after which, a small gonadal biopsy was removed (Chapman and Park 2005, Webb and Erickson 2007). Surgical biopsy, usually removing about 1 cm^3 of tissue (Fox *et al.* 2000, Webb and Erickson 2007), lasts two to three minutes (Chapman and Park 2005). After biopsies are completed, the gonadal tissue is microscopically examined to verify the sex as well as the precise stage of maturation of sturgeon (Van Eenennaam *et al.* 1996, Van Eenennaam and Doroshov 1998).

As with other forms of surgery, the risks are minimized when performed in the laboratory but there is little to no information available on the extent of infection or delayed mortality. Although there is documentation of surgically sterilized sturgeon regenerating gonadal tissue, there is little information regarding the loss of reproductive potential due to the removal of small samples of gonadal tissue (Kersten *et al.* 2001, Hernandez-Divers *et al.* 2004). And, while it is known that the gonads deliver hormones to the fish that influence behavior (Hernandez-Divers *et al.* 2004), there have been no studies dealing with potential changes in behavior from small losses of gonadal tissue. Chapman and Park (2005) monitored Gulf sturgeon for 30 days following biopsy and reported no mortality.

In situations when knowing the stage of gametogenesis could lead to recovery of the listed species, laparoscopy or biopsy would be appropriate, but due to the increased risk

of these procedures, NMFS only recommends using these procedures in a laboratory setting. If there are situations when these methods would be more likely to contribute to the recovery of these species than other available methods, NMFS would recommend their use under limited circumstances. Gonadal biopsy should only be performed in the field opportunistically while a researcher is implanting an acoustic tag.

Ultrasound

One of the safest and least invasive methods of sexual identification is the use of ultrasound. These devices, although costly, allow researchers to observe the sex organs of sturgeon without surgical incision or sedation. Ultrasound is the technique with the most potential and is becoming more accurate as both technologies improve and readers become more experienced (Joel Van Eenennaam, University of California Davis, pers. comm.).

When conducting ultrasound analyses, the procedures described by Wildhaber *et al.* (2006), or slight variation of these techniques, appear to be the safest described in the literature. Sturgeon are placed in a prone position in a tank of water with their ventral surfaces exposed to air. The ultrasound transducer is coated with ultrasound gel and then covered in a protective plastic sheath to prevent any scratches to the ultrasound from the sturgeon's scutes. During scanning, output power, focus depth, and frame rate are kept constant. The transducer is maneuvered along the abdomen between the gills and the anus, keeping the wide end of the transducer facing the head and tail. The ultrasound cannot penetrate the hard calcium of the scutes, so there is no reason to attempt to ultrasound the sides or back of the sturgeon (Wildhaber *et al.* 2006).

Moghim *et al.* (2002) examined 249 anesthetized stellate sturgeon with ultrasound and then performed necropsies to verify the accuracy of the ultrasound. Overall, ultrasound was 97.2% accurate in determining sex with the procedure taking only 30 seconds to complete. Mature females were the easiest to identify (100%), followed by immature females (99.3%), mature males (96.5%), and then immature males (76.2%). Colombo *et al.* (2004) examined 51 euthanized shovelnose sturgeon and determined ultrasound was a viable method of sex identification. They were able to correctly identify the sex of sturgeon 88% of the time, though only 40% of post-spawned females were accurately identified. Excluding post-spawned female sturgeon, the ultrasound correctly identified the sex of sturgeon 94% of the time. Additionally, Wildhaber and Bryan (2006) accurately identified the sex of 100% of pallid and shovelnose sturgeon using ultrasound coupled with borescope. In another study, Wildhaber *et al.* (2006) correctly identified only 68% of fish in the field and 70% of fish in the laboratory. In both of these cases, males were more often correctly identified, which is similar to the results from Colombo *et al.* (2004) but opposite the findings from Moghim *et al.* (2002).

When performed without anesthesia, there are no risks associated with ultrasound examination of sturgeon. However, while ultrasound is able to identify gender, it is not a promising method for determining the stage of eggs. When working with listed Gulf, shortnose, Atlantic, and green sturgeon, NMFS generally recommends using ultrasound for instant sexual identification of fish in the field. This method is the least stressful and

comparably accurate to other available methods that provide immediate identification. Due to the expense of ultrasound technology, boroscoping shortnose, Gulf, and Atlantic sturgeon is an acceptable alternative. More research is needed to determine if boroscoping is safe for green sturgeon.

Blood Plasma

Potentially one of the most promising, most accurate, and least stressful procedures used to sex sturgeon is an analysis of blood plasma. Researchers have used vitellogenin or sex steroids such as testosterone, 11-ketotestosterone, and estradiol to assess the sex and stage of maturity for pallid, shovelnose, hybrid bester, and white sturgeon (Amiri *et al.* 1996, Webb *et al.* 2002, Wildhaber *et al.* 2006).

Blood samples are obtained from the caudal vein (Figure 5) and centrifuged to isolate the plasma where it is then analyzed by radioimmunoassay or frozen for later analysis. In initial studies, testosterone was used to discern sexual maturation (79% accuracy for males, 85% for females), as it is significantly elevated in mature male and female sturgeon (Webb *et al.* 2002). If testosterone indicates the sturgeon is maturing, estradiol levels of female white sturgeon exceed 2 ng/ml 93% of the time, while males and immature white sturgeon estradiol levels never exceed 2 ng/ml (Webb *et al.* 2002), resulting in reasonably accurate identification of immature males (72%), immature females (88%), mature males (96%), and mature females (98%). Later, researchers studied vitellogenin along with the sex steroids testosterone and estradiol (Wildhaber *et al.* 2006). At all stages of development, vitellogenin was significantly elevated in females when compared to males, predicting the sex of the sturgeon with over 99% accuracy. After sex determination, the same steps taken by Webb *et al.* (2002) can determine whether each gender of fish is sexually mature, as estradiol is significantly higher in maturing females and ketotestosterone is significantly higher in maturing males.

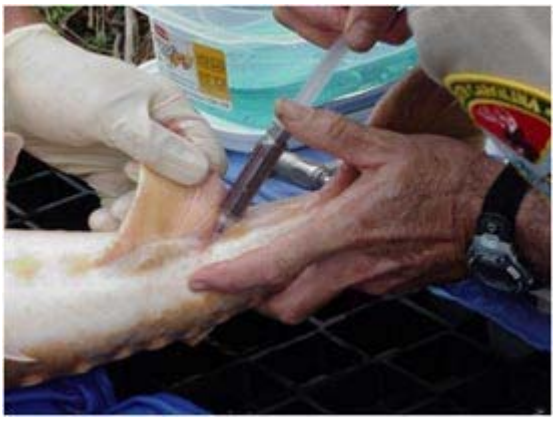


Figure 5: Blood collection from a shortnose sturgeon.
Photograph by J. Gibbons, SCDNR

Techniques for blood plasma analysis show promise in identifying sex and egg maturation of sturgeon, and should continue to be evaluated for use on Gulf, shortnose, Atlantic, and green sturgeon. However, this technique can only identify the sex and stage of maturity of a sturgeon after the sturgeon has been captured and released. Therefore

this technique is not useful if researchers only need to know the sex of a sturgeon to identify optimal fish for an acoustic tag. If the sex of the fish is not needed immediately, but rather for later population analyses, blood samples are the preferred method. Ultrasound would also be an acceptable method even if the results are not needed immediately. These methods are least stressful and highly accurate in this situation.

Recommendations

Endoscopy

- During borescope procedures, NMFS does not recommend using a trocar to first pierce the genital canal to view and/or biopsy the gonads.
- Althought NMFS considers laparoscopy a more invasive endoscopic procedure than boroscopy, it is a more reliable method for determining the sex and stage of maturity of sturgeons (Wildhaber *et al.* 2006) and therefore recommends laparoscopy as the endoscopic procedure of choice.

Gonadal Biopsy

- NMFS does not recommend the use of laparoscopy or biopsy on wild Gulf, green, Atlantic, or shortnose sturgeon, but does recommend their use on hatchery and laboratory sturgeon. However, if there are situations when these methods would be more likely to contribute to the recovery of these species than other available methods, NMFS would recommend their use under limited circumstances.
- Gonadal biopsy should only be performed in the field opportunistically while a researcher is implanting an acoustic tag.

Ultrasound

- NMFS generally recommends using ultrasound for instant sexual identification of fish in the field.

Blood Plasma

- Blood samples are the preferred method for determining the sex and stage of maturity of sturgeon when that information is not needed at the time of sampling.

Age Estimation

Age estimates of sturgeon populations help researchers and managers understand sturgeon growth rates, ages at maturity, mortality rates, productivity, longevity, and year class strength (Campana 2001, Paragamian and Beamesderfer 2003). Such knowledge is critical for designing appropriate fisheries management policies.

Bony structures form opaque and transparent age rings each year in most fish species in response to changes in temperature or other annual cycles. These rings, or annuli, are roughly correlated to sturgeon age. Unfortunately, most bony structures, such as clavicles, cleithra, opercles, and medial nuchals are not options for listed species of sturgeon because such sampling is lethal (Brennan and Cailliet 1989, Stevenson and Secor 1999, Jackson *et al.* 2007). Other structures such as dorsal scutes and pectoral fin spines, so named because of a dermal bone sheath (Feindeis 1997), are more viable options, but scutes are more difficult to read than fin spines (Huff 1975, Brennan and Cailliet 1989, Stevenson and Secor 1999, Jackson *et al.* 2007).

Pectoral fin spines are sampled by researchers similarly across the United States. The following methodology is therefore recommended for sampling pectoral fin spines of Gulf, green, Atlantic, and shortnose sturgeon (Figure 6).

> Using a hacksaw or bonesaw, two parallel cuts are made across the leading pectoral fin spine approximately 1-cm deep. The blade of the first cut is positioned no closer than 0.5-cm from the point of articulation of the flexible pectoral base to avoid an artery at this location (Rien and Beamesderfer 1994, Rossiter *et al.* 1995, Collins and Smith 1996). The second cut is made approximately 1-cm distally (Everett *et al.* 2003, Fleming *et al.* 2003b, Hurley *et al.* 2004, Hughes *et al.* 2005), where a pair of pliers can be used to remove the resulting fin spine section. The section is then placed in an envelope and air-dried for several days or weeks. Later it is cut into thin slices (usually about 0.5 to 2 mm thickness) typically using a jeweler's saw or a double bladed saw (Stevenson and Secor 1999, Everett *et al.* 2003, Fleming *et al.* 2003b, Hurley *et al.* 2004, Hughes *et al.* 2005, Johnson *et al.* 2005, Collins *et al.* 2008). The sections are then mounted onto the substrate of choice including clear glue, fingernail polish, cytosel, or thermoplastic cement. The cross-section detail of the fin spine annuli are then studied using stereoscopic readers.

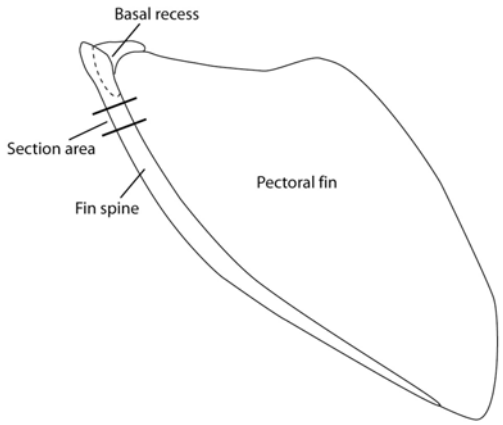


Figure 6: Diagram of the appropriate method for removing a small section of fin spine for age analysis.

Accuracy and Precision of Estimates

Accuracy and precision of the fin spine age estimates are concerns of fishery biologists and management agencies. Precision is a measurement of the distance between two reader's interpretations of the same fin spine sample, while accuracy is a measurement between the reader estimate of a sturgeon's age and the actual age (Beamish and MacFarlane 1983, Campana *et al.* 1995, Campana 2001, Hurley *et al.* 2004). To estimate precision, mark-recapture studies, oxytetracycline chemical marking studies, hatchery release studies, and in hatchery studies have been conducted to validate the age estimation process and also verify the assumption of one opaque and one translucent ring are formed each year (Clugston *et al.* 1990, Rien and Beamesderfer 1994, Campana *et al.* 1995, Rossiter *et al.* 1995, Stevenson and Secor 1999, Campana 2001, Paragamian and Beamesderfer 2003, LeBreton and Beamish 2004, Hurley *et al.* 2004, Jackson *et al.* 2007). Most studies of age estimates measure precision using at least two individual readings of the same slide. Subsequently, either the variability is recorded between readers, or the differences in reader's estimates are reconciled immediately after measurement.

Most age estimation studies suggest the results obtained should be used with caution because, while fin spines may provide the safest and most accurate estimation of age, they also consistently underestimate the actual age. The typical sources of error reported have been: 1) the rings are too close together or not clearly differentiated; 2) the original ring is difficult to identify; 3) the rings are missing within deteriorating sections; or 4) secondary fin spines, split rings, false rings, or spawning bands tend to create more or fewer rings than the actual age (Nakamoto 1995, Rossiter *et al.* 1995, Lai *et al.* 1996, Stevenson and Secor 1999, Farr *et al.* 2001, LeBreton and Beamish 2004, Whiteman *et al.* 2004). Moreover, fin spines from hatchery fish are often shaped differently, resulting in a more difficult age comparison control.

<u>Accuracy of Estimates</u>

The accuracy of fin spine estimates has been measured for Atlantic, pallid, shovelnose, white, lake, and Gulf sturgeon. Rossiter *et al.* (1995) and Stevenson and Secor (1999) monitored fish after one to three years between capture and found for lake and Atlantic

sturgeon respectively, growth rings did develop once a year. But LeBreton and Beamish (2000) determined only five of seven populations of lake sturgeon exhibited a series of one opaque and one translucent ring formed per year. This was also seen by Morrow *et al.* (1998) who documented two bands forming annually in shovelnose sturgeon fin spines during warmer years. Van Eenennaam *et al.* (1996) showed reader error of one to two years underestimation for Atlantic sturgeon at true ages ranging between 15 and 30 years. For shovelnose sturgeon and Atlantic sturgeon, age estimates underestimate actual age. The age underestimation was 1.6 years for fish under 15 years, 1.7 years for fish between 16 and 20 years, and 4.3 years for fish over 21 (Whiteman *et al.* 2004). A similar result was reported by Paragamian and Beamesderfer (2003) and also by Rien and Beamesderfer (1994) for white sturgeon, each finding age underestimation for white sturgeon under 60cm was over 70%, while the accuracy fell to below 60% for fish above 100cm. Moreover, using length to estimate age of sturgeon has proven unreliable. Clugston *et al.* (1990) recorded lengths of Gulf sturgeon after one year in the laboratory and then noted the inconsistent growth of fish in the wild during all months of the year. They concluded fish of similar sizes captured in the wild yield variable growth rates, suggesting length at age charts are flawed because growth is not constant among individuals. Paragamian and Beamesderfer (2003) provided additional evidence of invalid length at age charts using wild sturgeon. However, Peterson *et al.* (2000) and Schueller and Peterson (*in press*) demonstrated that juvenile sturgeon younger than three can be aged using length at age charts.

In the most extensive mark-recapture study to date, analyzing sturgeon at large over five years, Paragamian and Beamesderfer (2003) examined 760 marked (known age) white sturgeon recaptured up to 23 years later. They found ages were underestimated between 30 and 60%, depending on the time spent at large, meaning that age estimates were 1.5 to 2 times below the actual age of the fish. For marked-recaptured shortnose sturgeon, there was 96% accuracy between the readers' age estimates and the time the sturgeon spent at large. However, when using known-age fish, only 34% of the readers' estimates were accurate within one year (Collins *et al.* 2008). Also, when using multiple slides from the same fin spines of known-age hatchery fish, Hurley *et al.* (2004) reported only 28% of the estimates were correct, while 56% were within one year and 89% were within two years.

Precision of Estimates

As discussed previously, when measuring the precision of fin spine aging estimates, multiple readers estimate the age of identical sturgeon fin spines and then their results are compared to determine the variance between readers' estimates. Fleming *et al.* (2003b) studied 88 shortnose sturgeon fin spines where multiple readers were able to reach an agreement after consultation 100% of the time. Everett *et al.* (2003) analyzed shovelnose sturgeon using multiple readers and found the readers could not reach agreement on 26 of 736 (3.5%) of the samples when they attempted to reconcile measurements. Rossiter *et al.* (1995) also showed agreement between reader measurements while analyzing 20 lake sturgeon. They found high precision between readers for fish under 15 years old; however, for fish over 18 years old, reader agreement dropped to 80%. In the first two studies mentioned above, the readers reconciled measurements when there was a

disagreement in age estimation, while the latter study was conducted on only 20 samples without reconciliation of estimates.

While some studies have found general precision and agreement between readers, others were less successful. Van Eenennaam *et al.* (1996) showed multiple readers agreed on readings of Atlantic sturgeon fin spine samples approximately 33 to 40% of the time. Stevenson and Secor (1999) also evaluated reader agreement of Atlantic sturgeon fin spines and found no significant difference, but the disagreement error was approximately 1.2 years on average between readers. Nakamoto (1995) analyzed 154 green sturgeon fin spines and found readings from 34% differed by fewer than two years and 66% of the readings differed by fewer than five years. Rien and Beamesderfer (1994) measuring 935 white sturgeon fin spines twice, found only 37% agreement between readers and 68% agreement within one year. Jackson *et al.* (2007) found 80% of the time multiple readers estimated the age of shovelnose sturgeon within one year and 100% were estimated within two years. However Whiteman *et al.* (2004) found reader agreement on 234 shovelnose sturgeon samples of only 18% and within one year was still only 46%. NMFS recommends all sturgeon age estimates derived from fin spine analysis should test for precision between readers.

As discussed above, one major assumption for fin spine age estimation is each fin spine develops a ring each year; but there is evidence to suggest each fin spine may be different. Jackson *et al.* (2007) simultaneously removed both fin spines from shovelnose sturgeon and showed the spines from the same fish resulted in the same estimated age 36% of the time, within one year 66% of the time, and within two years 84% of the time. But this could be a result of how the fin spines are prepared, as measurements of 64 slides made from 16 pallid sturgeon fin spines resulted in only 25% agreement from the same spines (Hurley *et al.* 2004). Jackson *et al.* (2007) concluded the preparation of fin spines must be standardized so results can be reproducible.

Age Validation

Several researchers have suggested slow growth of adult and pre-spawn females may explain why some fin spine rings are closely spaced and become more closely spaced as fish get older (Beamish and MacFarlane 1983, Nakamoto 1995). It is thought the distance between rings is influenced by changes in food supply, metabolism, behavior, and environmental conditions as the sturgeon mature.

Accordingly, sturgeon researchers have begun to develop age estimate correction factors to validate age estimates of populations of different species. Bruch *et al.* (2009), while researching lake sturgeon, found growth increments on pectoral fin spine cross sections underestimated true age of fish older than 14 years and error increased with age, whereas otoliths accurately estimated true age up to at least 52 years. Increment formation in juvenile lake sturgeon pectoral fin spines was clearer and easier to interpret than otoliths. A power function developed by Bruch *et al.* (2009) provided a means for correcting existing age estimates obtained from lake sturgeon pectoral fin spines. For that reason, NMFS recommends using salvage specimens of Gulf, green, Atlantic, and shortnose sturgeon to establish age estimation correction factors.

Deleterious Effects of Fin Spine Sampling

Kohlhorst (1979) first reported potentially deleterious effects of fin spine removal from white sturgeon during a mark-recapture study where an incidence of mortality was recorded. The percentage mortality reported could have been magnified by a small sample size, but concern over this result triggered additional research in the laboratory.

Collins *et al.* (1995) and Collins and Smith (1996) monitored the effects of fin spine removal of juvenile shortnose and Atlantic sturgeon in a laboratory. Removing the entire leading fin spine from the base, a method not currently recommended for sampling fin-rays, they found wounds healed rapidly and that the remaining secondary pectoral fin spine grew in circumference until appearing very similar to the original fin spine. There were no significant differences for growth or survival between treatment and control sturgeon.

In other laboratory studies testing fin spine function, Wilga and Lauder (1999) found pectoral fins function by orienting the body vertically in the water column, but they are not used during locomotion. Following this study, Parsons *et al.* (2003) removed pectoral fin spines from shovelnose sturgeon placing them in tanks, where the current could then be increased to test their ability to hold position in a current. Without fin spines, treatment sturgeon were able to hold their position in a current as well as control sturgeon.

Most recently, while conducting mark-recapture surveys of Atlantic and shortnose sturgeon, Collins *et al.* (2008) discovered secondary fin spines had grown abnormally on older, mature Atlantic sturgeon after the leading fin spine had been taken months earlier. Concluding this regrowth could be due to slower growth of mature, adult fish and possibly become detrimental to the sturgeons' health, their team no longer samples fin spines from larger, adult sturgeon. Because of increased error in reading fin spines of older fish and evidence of abnormal regrowth, NMFS does not recommend taking fin spine samples from mature Gulf, shortnose, Atlantic, or green sturgeon.

Alternative Methods for Age Estimation

NMFS recommends developing newer, more accurate and precise methods of aging Gulf, green, Atlantic, and shortnose sturgeon. In recent years, Bruch et al. (2009) analyzed the use of radiocarbon bombing to estimate the ages of lake sturgeon using otolith cores. This is not a non-lethal technique, but if further testing indicates using other bony structures such as scutes for accurate and precise age estimates, this may become a useful method for age estimation. Likewise, telomeres have recently been used to estimate fish age. Hatakeyama *et al.* (2008), testing small teleost fish, found that telomere length shortens through the life of the fish and is inversely related to the length of the fish. However, no change in telomere length was noted for European sea bass between 12 and 94 months of age (Horn *et al.* 2009). Specific studies should be conducted on sturgeon to determine if telomere analysis could determine the age of sturgeon.

Recommendations

General

- NMFS recommends removing a 1cm portion of the pectoral fin spine from just above the point of articulation to estimate the age of Gulf, green, Atlantic, and shortnose sturgeon.

Accuracy and Precision of Estimates

- NMFS recommends fin spine derived age estimates be used with caution because they consistently underestimate the actual age.
- NMFS recommends all sturgeon age estimates derived from fin spine analysis should test for precision between readers.
- NMFS acknowledges the preparation of fin spines must be standardized so the results are reproducible and encourages future research to achieve this goal.

Age Validation

- NMFS does not recommend using lethal methods or length/age charts to estimate ages of Gulf, Atlantic, green, or shortnose sturgeon, except when working with juvenile sturgeon under three years of age.
- NMFS recommends using salvage specimens of Gulf, green, Atlantic, and shortnose sturgeon to establish age estimation correction factors.

Deleterious Effects of Fin Spine Sampling

- Because of increased error in reading fin spines of older fish and evidence of abnormal regrowth, NMFS does not recommend taking fin spine samples from mature Gulf, shortnose, Atlantic, or green sturgeon.

Alternative Methods for Age Estimation

- NMFS recommends developing newer, more accurate and precise methods of aging Gulf, green, Atlantic, and shortnose sturgeon.

Salvage Specimens

Dead or salvaged specimens can be invaluable for a number of basic and applied aspects of sturgeon biology and conservation. Scientific uses include, but are not limited to, morphology, genetics, histopathology, contaminants, age and growth, food habits, cryopreservation of sperm, and human impact/anthropogenic mortality. Educational uses of sturgeon collected include, but are not limited to, taxidermy, collection of hard parts (e.g., scutes, bones, and entire skeleton), necropsy, and development of sampling and necropsy procedures and manuals.

Although it is important to maintain salvaged specimens and their derivative tissues, making them available for future researchers and educators, listed sturgeon are protected and transfer of specimens must still be carefully documented under the ESA. Persons/laboratories receiving specimens must be authorized to possess listed species. All sturgeon research permits issued by NMFS currently include provisions for preserving incidental mortality resulting from research or found opportunistically.

If dead Gulf, green, shortnose, or Atlantic sturgeon are found or a researcher has a need for salvaged sturgeon or sturgeon parts, contact NMFS Headquarters in Silver Spring, Maryland at (301) 713-2289.

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