Biological Procedures and Protocols for Researchers and Managers Handling Pallid Sturgeon

Prepared by the Pallid Sturgeon Recovery Team

for

Region 6
U.S. Fish and Wildlife Service
Denver, CO

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Regional Director

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Date

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EXECUTIVE SUMMARY

Due to their endangered status and the fact that individual fish are important to recovery of the species, extra care is required in handling pallid sturgeon. The following protocol was developed by the U.S. Fish and Wildlife Service in cooperation with the Pallid Sturgeon Recovery Team for activities involving collecting, tagging, holding, handling, and transporting pallid sturgeon.

Prior to performing any work with pallid sturgeon, researchers and managers are required to obtain a Federal endangered species permit or sub-permit. In Louisiana, Mississippi, Arkansas, Tennessee and Kentucky contact 404-679-4176. In Missouri, Illinois and Iowa contact 612-713-5343. In Nebraska, South Dakota, North Dakota, and Montana contact 303-236-4256. Questions, comments or suggested changes to the protocol should be directed to, Pallid Sturgeon Recovery Coordinator, U.S. Fish and Wildlife Service, 2900 4th Ave North, Suite 301, Billings, MT 59101 or at (406) 247-7365. Proposed activities should also be coordinated with appropriate State agencies where a State permit may also be required.

Deviations from the protocol may be requested during the application or renewal process. Researchers and managers should use their best judgment in cases where guidelines are not directly applicable, or if in question, contact the Pallid Sturgeon Recovery Team Coordinator.

The following protocols will be followed to ensure that the best techniques are used regarding collecting, tagging, sampling, holding, culture, transporting, and data recording of pallid sturgeon in order to minimize loss of pallid sturgeon associated with permitted activities.

The primary intent of these guidelines and procedures is to reduce the risks of loss of pallid sturgeon by reducing the severity, duration, and the number of stressors, while still allowing for the data collection to expand our knowledge of these fish. All personnel that work with pallid sturgeon will be trained to handle the fish.

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This document was modified after signature to provide clarity on a few key points and to accommodate a change in address for the lab processing genetic tissue samples. All changes made after signature are identified via red text color.

Record Keeping
All permittees will maintain a copy of their Endangered Species Act 10(a) 1(A) permit and this protocol during all field operations as well as on file. Specific information must be recorded for each pallid sturgeon collected pursuant to activities authorized by a permittee's Endangered Species Act 10(a) 1(A) permit. To accomplish this, the pallid sturgeon data sheet (Appendix 1) must be completed as a minimum. Copies of all completed data sheets must be mailed to the Missouri River FWMAO attn: Project Leader, U.S. Fish and Wildlife Service, 3425 Miriam Ave., Bismarck, ND 58501 no later than December 31 of the year the fish were collected.

Personnel and Training Requirements
Collection: Minimum qualifications include training in appropriate fisheries management collection techniques. Additional activities may also require specific experience and knowledge such as implanting transmitters, culturing, and sexing.

Tagging and sampling: Minimum qualifications include training in fisheries management tagging and sampling techniques and stress mitigation. Specific training will be required for genetic sampling.

Fish Culture: One FTE will be designated and required to care for pallid sturgeon at Garrison Dam NFH, Gavins Point NFH, Natchitoches NFH, Blind Pony SFH and Miles City SFH, or in any facility that maintains pallid sturgeon in culture conditions. The minimum qualifications include training in warmwater fish culture and stress mitigation.

Handling and transportation: All personnel must be trained in the collecting and handling procedures described in this protocol. Drivers should be knowledgeable of proposed routes and coordinate with receiving station with anticipated routes and timelines. Personnel at the receiving point must be informed to expect the shipment. Before transporting, the shipper should make detailed arrangements with the receiver. Arrangements should include where and when fish will be delivered and the need for any specialized equipment at the receiving point. Arrangements should be verified before the vehicle leaves the site and again while in route, if possible. Water quality information from the collection site should be exchanged and matched as closely as possible at the receiving facility.

Trainees: Those individuals not meeting minimum qualifications for fisheries professionals will be considered to be trainees and will not be allowed to independently work with pallid sturgeon. They will be trained in protocols and procedures under the direct supervision of a qualified biologist, until deemed capable by their crew leader or supervising biologist.

Collection Methods
Two weeks prior to actual field work, all field personnel, the Regional Fish Health Center, and hatchery personnel will be notified. All pallid sturgeon are to be collected non-lethally. A fish holding container on the boat shall be of sufficient size to completely submerge the fish.

Gill Nets/Trammel Nets - Monofilament and multi-filament mesh nets may be used to collect pallid sturgeon. There are no mesh size restrictions for gill and trammel nets. Drifting sets should be monitored continuously. Time, date, duration and position of net sets should be recorded. Global positioning system (GPS) data should be used when recording location data. This will provide positional data and time for each set. Total numbers of each species is then noted and recorded with the GPS way points to apply to a Geographic Information System (GIS). Drift distance starts and stops with the clock. Indicate net length, mesh size, and mesh type in reports.

If water surface temperatures are 55 ^{o}F (12.8 ^{o}C) or less, then 24 hour static net sets may be used, and frequent checking for entangled pallid sturgeon is encouraged. When water surface temperatures are between 55 ^{o}F (12.8 ^{o}C) and 60 ^{o}F (15.6 ^{o}C) then overnight sets may be used cautiously, but for no more than 16 hours (i.e., dusk sets and retrieved at or near dawn). Weather conditions must be watched to insure that nets can be picked up as soon as possible the next day. As surface temperatures exceed 60 ^{o}F (15.6 ^{o}C) the nets must be checked for captured pallid sturgeon at regular and more frequent intervals. The following schedule shall apply at these warmer temperatures. Maximum net soak times should not exceed 10 hours when water surface temperatures range between 60.5 ^{o}F (15.8 ^{o}C) and 65 ^{o}F (18.3 ^{o}C). As water surface temperatures exceed 65 ^{o}F (18.3 ^{o}C), but are less than 70 ^{o}F (21.1 ^{o}C), static net sets should be checked for captured pallid sturgeon at a minimum of every 5 hours. At water temperatures above 70 ^{o}F, the use of static net sets is not encouraged and should be replaced with drifting sets and continuously monitored.

When static nets are deployed specifically for brood-stock collection purposes, the following restrictions apply to help ensure the highest probability of artificial propagation success. If water surface temperatures are 55 ^{o}F (12.8 ^{o}C) or less, then 24 hour static net sets may be used, and frequent checking for entangled pallid sturgeon is encouraged. When water surface temperatures are between 55 ^{o}F (12.8 ^{o}C) and 60 ^{o}F (15.6 ^{o}C) then overnight sets may be used cautiously but for no more than 16 hours (i.e., dusk sets and retrieved at or near dawn). As water temperatures exceed 60 ^{o}F (15.6 ^{o}C) then collection of brood stock should cease as recommended transportation temperatures are exceeded (see Handling and fish transportation section).

Calculate CPUE as fish per-net-hour or fish per-net-length/area for stationary sets. For drifting sets, CPUE shall be reported as fish per-net-hour and number of fish per-meter of the drifted area.

Trot Lines/Angling - Use appropriate sized hooks for the size of sturgeon being targeted. Mustad Tuna Circle Hooks in sizes up to 14/0 have proven successful in capturing larger pallid sturgeon in Montana. However, smaller 3/0 stainless steel Eagle Claw O' Shaughnessy hooks baited with a nightcrawler have proven successful in capturing a variety of pallid sturgeon sizes throughout much of the pallid sturgeon range.

In order to reduce risks from trot lines, this gear should be deployed in areas that will minimize hooked fish being excessively exposed to direct river current or while there is heavy debris loading. Trot lines must be checked at least once every 24 hours for hooked pallid sturgeon.

Calculate CPUE as fish per-hook-hour or fish-per-hook night. Indicate line length, dropper length, hook spacing and hook size/style, bait type, and number of hooks per set in reports.

Electrofishing - Electrofishing must not be used to purposefully stun and capture pallid sturgeon. Low power electrofishing (max. 100 volts DC and 3 amperes) may be used to move pallid sturgeon from heavy cover and direct them into nearby nets for capture.

SCUBA -Pallid sturgeon collected using this method are to be captured by hand. Contact should be made with the snout as quickly as possible after carefully grasping the fish by the caudal peduncle. Once in hand, the fish should be enclosed in a large, preferably small-mesh bag and brought slowly to the surface, while maintaining the fish in a horizontal position. SCUBA is used to capture pallid sturgeon primarily during the winter. Exposure of the fish to freezing air temperatures shall be avoided by keeping the fish submerged in water. Record sightings per hour of dive time in reports.

Trawls - Trawls have been effectively used to collect juvenile sturgeon. However, due to the nature of the trawling, a potential for serious injury to the fish is possible. Therefore, trawling efforts should be kept to a maximum of ten minutes under optimal conditions (low debris collection, sand substrate). When conducted in habitats with rock/cobble or when high densities of fish are present, trawling time should be reduced to limit incidental injuries. Calculate CPUE as fish per trawl and number of fish per-meter of the trawled area.

Data collected -The Pallid Sturgeon Data Sheet (Appendix 1), lists the physical data to be recorded for hatchery-reared and all unmarked specimens, as well as general data about the collection. Collecting morphological and meristic* data on all known hatchery fish is not mandatory; however these data should be collected by each sampling crew from a minimum of 5 known hatchery fish representing each year class stocked each year for a minimum of two years (2008 and 2009). At the end of each year, the data set will be evaluated to determine if additional morphometric and meristic data are necessary for hatchery fish released into the wild. This will insure adequate data are represented in the database. While collecting morphometric data, pallid sturgeon should be kept moist and held out of the water for no longer than 2 minutes, unless the gills are irrigated. It is preferred to hold the fish in the water in a stretcher or in a "stock" tank large enough to accommodate the fish. For procedures on taking measurements refer to: Bailey, R.M., and F.B. Cross. 1954. River sturgeons of the American genus Scaphirhynchus: Characters, distribution, and synonymy. Michigan Academy of Science, Arts and Letters, Vol XXXIX.

* Note: if dorsal and anal fin ray counts can not be collected in the field, a clear digital image can be substituted as long as the digital image can be linked back to the data sheet. A clear photo of both fins with identifiable rays, and a piece of paper indicating the PIT tag of the fish is one suitable option to accomplish this. (see Appendix 6)

Copies of all completed data sheets must be mailed to the Missouri River FWMAO attn: Project Leader, U.S. Fish and Wildlife Service, 3425 Miriam Ave., Bismarck, ND 58501 no later than December 31 of the year the sheets were completed for recording into the National Pallid Sturgeon Database. Copies of the Catch Record Database can be obtained from the above address.

Tagging, sampling methodologies and sampling protocols

Fish tagging and marking - All captured pallid sturgeon will be carefully examined for previously implanted PIT, elastomer, coded wire tags, external tags, scute marks, and evidence of external tag loss. Make several passes with the PIT and coded wire tag reader along both sides of the dorsal fin when checking for PIT tags and around the rostrum tip and scute area with the coded wire tag reader. Some fish may have two PIT tags, one on either side of the dorsal the fin with the left side being the primary location.

1) **Identification Tags**

a) PIT Tags - All adult pallid sturgeon must be implanted with a PIT tag prior to release. PIT tags should be inserted horizontally or front to back along the left anterior, fleshy base of the dorsal fin. A second PIT tag can be inserted on the right side of the dorsal fin if the first tag is unreadable. Tags should be scanned prior to implantation for recording and after to ensure it is working properly.

PIT tags provide reliable, long-term identification of individuals. Several companies are now providing tags and readers that work; Biomark (www.biomark.com), AVID (www.avidid.com) or Destron Fearing (www.destronfearing.com). There are basically two types of tags available; encrypted and un-encrypted.

In order to enhance recognition of recaptures and maintain consistency in readability of tags, only un-encrypted, 125 kHz tags will be used for pallid sturgeon work, unless a specific recovery area is already committed to a specific format.

b) External Tags - External tags have met with little success when applied to sturgeon and are therefore not recommended for mass marking. Various external tag types (dangler, cinch, dart, disc) have been used on shovelnose sturgeon and juvenile pallid sturgeon with limited success. Disc tags have had higher long-term retention on sturgeon than other external tags. However, the majority of recaptured adult pallid sturgeon that had previously been externally tagged exhibit tissue inflammation severe enough to be concerned about infection. In some cases, severe inflammation was still evident 2 years after the fish had been tagged. External tags can be used on shovelnose sturgeon, shovelnose X pallid hybrids, as well as on pallid sturgeon stocked for research purposes as well as wild caught pallid sturgeon. Utilization of external markers on wild-caught fish will be evaluated on a case by case basis.

c) Visual Implant Elastomer Tags – Colored elastomer tags are a mix of elastomer and curing agent available in a variety of colors. The mix is injected in rostrum and is visible from the ventral side through the translucent rostral tissue. Elastomer tags are suitable for batch marking hatchery-reared juvenile pallid sturgeon. Potential drawbacks include the limited life span of tag. As pallid sturgeon age, the tissue of the rostrum becomes more opaque making some elastomer tags difficult to discern. Use of UV LED flashlights and the amber glasses can increase detection of marks. In the field, a shade cover or box can be used, to improve the efficiency of the UV flashlight. Elastomer tags are suitable for use on juvenile hatchery-reared pallid sturgeon. Other applications for pallid sturgeon will be reviewed on a case by case basis.

d) Scute Removal – Surgical removal of lateral scutes, in specific patterns, can provide data on hatchery origin, brood year, family lot, or stocking site. Scute removal is suitable for use on juvenile hatchery-reared pallid sturgeon. Other applications for pallid sturgeon will be reviewed on a case by case basis.

2) **Radio/Sonic Transmitters**

a) Internal Transmitters - Internal transmitters are preferred over external transmitters; however, implanting should be performed only by individuals with experience in surgical procedures. During surgery, the head either should be placed in water or the gills flushed with water containing 60-100% Dissolved Oxygen (DO) or aerated such that DO saturation levels are 60-100%. Transmitters should have a biologically inert coating to help prevent expulsion. Prior to surgery, an anesthetic should be used. Limited experimentation at Garrison Dam NFH and Natchitoches NFH has demonstrated that 50-150 mg/l MS-222, in water buffered with sodium bicarbonate, can be a safe and effective anesthetic for pallid sturgeon. An incision, only slightly larger than the tag to be used, should be made in the ventral body wall, one to one and a half inch off the midline and anterior to the pelvic fins. Care should be taken to prevent severing blood vessels and damaging organs while making the incision. The incision should be closed with individually knotted sutures or surgical staples. Before and after surgery, the incision site should be wiped with an antiseptic to prevent infection. This same small incision should be used for sexing the fish.

For additional information and guidance on surgical procedures refer to: Conte et al. 1988. Hatchery manual for the white sturgeon. University of California, Division of Natural Resources, Cooperative Extension Publication 3322. The duration of surgical procedures should be limited to a maximum of 15 minutes per fish.

b) External Transmitters - Use of external transmitters are not recommended, but will be carefully reviewed and authorized on a case-by-case basis. Concerns are that attachment methods create inflammation and cause infection until the tag is shed.

3) **Coded Wire Tags**
Early hatchery-reared fish were marked with coded wire tags. Biologists and researchers operating in areas were these hatchery fish were released i.e., the Missouri River below Gavins Point Dam and the Mississippi River should scan all collected adult pallid sturgeon for the presence of coded wire tags to prevent erroneous classification of hatchery-reared pallid sturgeon as wild fish.

4) **Genetic Marks and Tissue collections**
Recent work has proved the efficacy of using genotype data to determine if an unmarked pallid sturgeon is wild or hatchery origin. Appendix 2 describes the procedures for collecting genetic tissue samples. At a minimum, a sub-set or portion of each pallid sturgeon genetic samples must be sent to the Conservation Genetics Lab at the USFWS Northeast Fishery Center or the Molecular Ecology Lab at Warm Springs Regional Fisheries Center (Addresses are available in Appendix 2) for inclusion in the pallid sturgeon genetic archive. Along with the genetic sample, a copy of the data sheet must be included for accurate cataloging.

Handling and fish transportation

Truck transport: When the objectives of field work are to capture pallid sturgeon broodstock, a hauling truck and tank should be on site for immediate transport. Use a circular hauling tank for larger specimens (>10 pounds), that is equipped with oxygen and a fresh-flow aerator system. Transportation times should not exceed 12 hours and may need to be less depending upon number of fish and water/air temperature. Maintain temperature of hauling-tank water within + 3 ^{o}F (± 1.6 ^{o}C) of ambient water temperature of origin. Temper the fish when moving them between bodies of water.

Pallid sturgeon should not be transported when ambient water temperatures are greater than 60 ^{o}F (15.6 ^{o}C).

To reduce stress during transport, non-iodized salt should be added to water in the hauling tank to provide a 0.25 percent salt solution for juveniles and 0.5 percent solution for adults.

For transport of pallid sturgeon that will exceed six hours, arrangements will be made to have a back-up vehicle and haul trailer available in the event of a mechanical breakdown. Pallid sturgeon should be visually inspected a minimum of every two hours on trips exceeding two hours.

Box and bag shipping equipment: Shipping of fish or eggs in boxes containing plastic bags is recommended for larval and juvenile sturgeon, exceeding 5 inches total length. Industry standard boxes and square bottomed shipping bags should be used. If possible, withhold food for 24 hours prior to shipment. Use two bags in the box. The box should be cardboard with a Styrofoam box insert with fit lid. Check the bags for leaks prior to use. Fill the inside bag with about 2 gallons of water, water additives, and fish. Deflate the bag of air and inflate the bag with oxygen. Twist the top of the bag to put pressure in the bag. Fold over the twisted top and seal with a docking ring (preferred) or two heavy duty rubber bands. Separately, twist the top of the outer bag and double it over prior to sealing

with a docking ring or two rubber bands. Place the styrofoam lid on the styrofoam box and seal with shipping tape. Then seal the cardboard box with two complete rounds of shipping tape. Load and ship with the 'up' arrows pointing up at the lid. If needed, temperature can be maintained by placing cold packs on the sides of the bags. Smaller plastic bags such as ziplock heavy duty freezer bags can be used but care must be taken to inflate and pack these in such a manner that the fish cannot be crushed or sharp edges are exposed to create a puncture. Bags used for shipping must not have corners that could trap and crush the fish. The water temperature should be similar to or slightly lower than that used to rear the fish and the bag temperature should be lowered to less than 60° F (15.6° C) prior to shipping. The hauling density should not exceed 0.5 pounds of fish per gallon of water.

Fish acclimatization and therapeutants

Following transfer from the field to a controlled environment, such as Garrison Dam NFH or other appropriate facilities, measures will be taken to mitigate for stress of transfer. Prior to transport, the following therapeutic agents may be used to combat infections.

oxytetracycline (LA200, Bio-Mycin) - shall be injected into muscle tissue of the pectoral fin or muscle tissue of the back at a rate of 0.045 cc/lb of body weight to provide the fish with some defense against bacterial infection due to stress. The injection should occur at the capture site prior to transport or immediately following significant handling.

fluorophenicol (Nuflor) - shall be injected into muscle tissue of the back at a rate of 0.03 cc/lb of body weight to provide the fish with some defense against bacterial infection due to stress. The injection should occur at the capture site prior to transport or immediately following significant handling.

tetracycline hydrochloride - Fry and fingerling pallid sturgeon can be treated with tetracycline hydrochloride soluble powder at a rate of 10 ppm and up to 60 ppm for up to four hours per day. This can be done daily for up to five consecutive days with no major problems when holding conditions or stress may be induce a systemic infection. Following transport, stress reduction techniques will include adding non-iodized salt at 0.5% (18.9 grams per gallon) levels to holding water for at least two days following transfer. Water temperatures will be similar to that at the location and time of capture. Water turnover rates will be between 2 and 4 times per hour in all culture tanks. If parasites have been found in the water supply, the supply will be filtered (15-20 micron) and disinfected using UV irradiation with a minimum of 100,000 microwatts per square centimeter of ultraviolet light intensity. Photo period will approximate levels similar to environmental conditions. Variations in photoperiod should be submitted in the permit application. Oxygen levels will be maintained at > 6.0 mg/L or saturation as measured with an oxygen meter. pH will range from > 6.5 to <7.5. Ammonia levels will be maintained at less than 0.0125 parts per million (ppm) and nitrite levels will be kept below 0.1 ppm for soft water and 0.2 ppm for hard water. Nitrogen supersaturation levels will be maintained below 100 - 102%.

Wound relief protocols and drugs and therapeutants will be administered as recommended by the Fish Health Center. Prophylactic drug and therapeutant treatments, other than salt, will be recommended by the Fish Health Center. Therapeutic protocols will be initiated prior to transport and assessed after arrival at the facility and shall follow strict recommended schedules.

Health plans will be initiated on a case by case basis. These health plans will consider physical check-ups, intervals between check-ups, personnel training, specific treatments, drugs, chemicals, and therapeutants to be used. The plan should also address salts to be used equipment decontamination, facility decontamination, immunization, vaccination.

The Fish Health Center will determine on a case by case basis if quarantine is required.

Fish Culture/Holding procedures

1) Short-term (1 week or less) Holding Facilities

   a) Field Holding Tanks - Holding tanks should be circular, covered, located in an area free from disturbances, and have provisions for fresh-water circulation. Pallid sturgeon should be maintained in water from the capture location, when possible. Holding tank water temperatures should be maintained within + 5°F (2.8°C) of ambient water temperature. A standby power supply must be provided in the event of a power failure, unless the fish are monitored every 3 hours.

   b) Modified Hoop Nets/Underwater Keeps - Modified hoop nets/underwater keeps can be used as a temporary holding facility, but for no more than 16 hours. Holding pallid sturgeon in hoop nets or keeps might be necessary for a short period if one or more pallid sturgeon are incidentally captured and field crews are not set up with a holding tank. Commercial fishermen, who are previously authorized by permit, may keep incidentally captured pallid sturgeon in hoop nets until personnel who are previously authorized by permit to obtain the pallid sturgeon arrive. Commercial fishermen must notify their contact within 2 hours of capturing a pallid sturgeon. Mesh size must be 1½-inch (3.81-cm) bar measure or smaller to prevent gilling and keeps should be circular. Hoop nets or keeps should be located such that adequate temperature and oxygen conditions vary little from ambient conditions at the capture location. Flow-through is very important if conditions permit and the structure will not be jeopardized. Hoop nets or keeps must be checked every eight hours and posted with a sign or float cautioning against disturbance.

2) Long-term Holding Facility Requirements and Rearing Facilities

   a) Hatchery or Aquarium - Pallid sturgeon have been held for more than 8 years in circular tanks with water circulation. Tanks should be covered and located in an area free from disturbances. An automatic standby power and water supply must be provided to maintain the fish in the event of a failure. These facilities must have a "contaminant-free" water supply. Fish

health must be regularly monitored. If signs of disease are noted or if a 20 percent loss of body weight occurs during holding, fish health personnel at the Service's Fish Disease Control Center in Bozeman, Montana (406-582-8656) should be contacted for treatment recommendations. Long-term holding facilities must be within the historical range of pallid sturgeon or be designed to prevent escapement. Water temperatures should be maintained between 40 and 70 degrees Fahrenheit. Densities for adults should not exceed 1.0 pound per square foot of surface area. Densities for juveniles should be maintained at less than 0.5 pounds per square foot of surface area.

Propagation and Stocking

Artificial propagation of pallid sturgeon is an important component for recovery. All activities associated with artificial propagation will be conducted in accordance with the most recent version of the Pallid Sturgeon Propagation Plan. Release of artificially propagated pallid sturgeon into the wild, will be conducted in accordance with the most recent version of the Pallid Sturgeon Range-Wide Stocking and Augmentation Plan. The latest versions of the propagation and stocking plans are available by contacting the Pallid Sturgeon Recovery Coordinator.

Disposal of incidental take

Pallid sturgeon mortalities should be left fully intact and frozen immediately to prevent decomposition. Legal chain-of-custody documentation (Appendix 3) should be maintained for each specimen to facilitate contaminant analysis reporting. Deaths should be reported to the Pallid Sturgeon Recovery Coordinator by phone and in writing as soon as possible. Describe all available information regarding the circumstances under which the fish died. The Service's Fisheries Assistance Office in Bismarck, North Dakota, will coordinate the transfer of specimens to the University of Alabama repository. If personnel are trained in the collection of tissue samples and if equipment for collection is available, the following samples shall be collected prior to freezing.

Fish Health Samples

Refer to Fish Health Protocols (Appendix 4) for proper procedures and data sheet. These samples are only to be taken if part of another study evaluating fish health. All samples shall be labeled with the PIT tag number. Please notify before shipping and forward all samples labeled with the PIT tag number to:

Bozeman Fish Health Center
U.S. Fish and Wildlife Service
920 Technology Blvd., Suite G
Bozeman, MT 59718
406-582-8656

Contaminants Samples

Refer to Standard Operating Procedures for Collection, Storage, and Shipment of Pallid Sturgeon Tissue Samples for Analysis of Organic and Trace Element Contaminants (Appendix 5). These samples should only be collected if on a mortality and part of a study evaluating contaminant levels. All samples shall be labeled with the PIT tag number and sent to:

U.S. Fish and Wildlife Service
Ecological Services Contaminants
3425 Miriam Ave Bismarck, ND 58501,
701-250-4481

Age Analysis (mortalities)

All morphological and meristic data will be collected along with PIT number. The right pectoral fin and spine will be cut off at or below the hinge point of the 1st spine for age analysis before freezing. Fin samples and data shall be shipped to the Service's Fisheries Assistance Office in Bismarck, North Dakota. All samples shall be labeled with the PIT tag number and include a copy of data sheet.

Appendix 1 Pallid sturgeon data sheet

Pallid sturgeon data sheet (02/07)

Date ☐☐ ☐☐ ☐☐

PIT 10 ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ R/N ☐

Lat. ☐☐ ☐☐☐☐☐
Decimal degree

Long. - ☐☐☐ ☐☐☐☐☐
Decimal degree

ER		EL	
H/V	color	H/V	color

Elastomer Colors

Flouresces	Non-Flouresces
G - Green	W - White
O - Orange	V - Purple
R - Red	B - Brown
P - Pink	K - Black
Y-Yellow	U - Blue

Scute

Loc.	#

CWT ☐

Recapture Y/N ☐

River _____R.M. _____ State _____

Location _____

Other tag information _____

Method: Gill, Trammel, Hoop net, Beam trawl, Otter trawl, Angling, Other _____

Genetics vial # _____ Sex M / F / U – Stage _____ / U

Duration of set _____ Mesh size _____

Fork Length _____mm Weight_____gm/kg

Temp. _____ºC Turbidity _____ntu

Picture Y/N ☐

Released – Taken to hatchery _____

Depth _____ m Velocity _____ m/sec

Captured by:_____

Comments _____

Substrate _____

Head Length _____mm

Snout to Mouth _____mm

Inter _____mm

Mouth Width _____mm

MIB _____mm

L-OB _____mm L-IB _____mm

R-OB _____mm R-IB _____mm

Anal _____rays Dorsal _____rays

Morphometric Measurements

Head Length– Tip of the rostrum to the posterior margin of the operculum.
Snout to Mouth – Tip of the rostrum to the anterior mouth midline.
Inter – Tip of rostrum to anterior insertion point of the R-OB.
Mouth-Width – Widest measurement on the outer edge of lips.
MIB (Mouth to Inner barbel**)** – Anterior point of insertion of the R-IB to the anterior midline of mouth.
L-OB (Left outer barbel)- Anterior insertion point to barbel tip.
L-IB (Left inner barbel)- Anterior insertion point to barbel tip.
R-OB (Right outer barbel)- Anterior insertion point to barbel tip.
R-IB (Right inner barbel)- Anterior insertion point to barbel tip.
Anal/Dorsal – Number of rays counted at fin base.

Field Descriptors

PIT – Pit tag number
R/N – R if recap with pit tag / N if new pit tag is inserted
ER (Elastomer Right) – Horizontal / Vertical position
ER (Elastomer Right) – Color code from elastomer box
EL (Elastomer Left) – Horizontal / Vertical position
EL (Elastomer Left) – Color code from elastomer box
Scute – Location (R=right, D=dorsal, L=left, or N=none)
Scute # - Scute number removed from the anterior
CWT – Y if tag is present / N if tag is not present
Recapture – Y if any marks present / N if not
Picture –Y if picture is taken for morphometrics / N if not

Appendix 2 Protocol for Taking Sturgeon Genetic Samples

Equipment you will need:

1) Two screwcap tubes filled with 95% NON-denatured ethanol
2) Surgical scissors and forceps
3) Sturgeon genetic card

Procedure:

1) Record genetic vial # and corresponding PIT # on the genetic card (this step is critical for pallids). Record all biological data. Please note if the fish is a recapture. Be sure to indicate why the samples are being sent in (genetic analysis needs), i.e. for broodstock analysis, unknown origin pallid sturgeon to check against parental database, sample for archive, etc.
2) To avoid sample contamination keep your hands, sampling instruments and work area clean. Vigorously wash scissors and forceps in fresh water prior to taking each genetic sample. Wipe the scissors and forceps with the clean section of a rag or a new tissue to ensure residual tissue from the last sampled fish is removed.
3) Use the scissors to cut two small pieces of tissue off of the caudal fin (approximately $1cm^2$ each). When it is not possible to obtain samples as large as 1 cm^2 a smaller piece of $0.5cm^2$ should be adequate.
4) Place one piece of tissue into each of the two screwcap tubes (a & b) filled with alcohol and tightly screw on the caps (If the lids are not tight the alcohol will evaporate).
5) Place both samples back in the plastic bag with the completed genetic card. Samples should be stored at room temperature.
6) Contact William Ardren via e-mail before sending samples to the USFWS genetics repository. He will provide details on sending the samples via FedEx.
7) Please e-mail the biological data for each sample when you send the samples.

USFWS Northeast Fishery Center
Conservation Genetics Lab
Attn: Meredith Bartron or Jeff Kalie,
P.O. Box 75
227 Washington Ave.
Lamar, PA 16848
Phone: (570)-726-4995
e-mail: Jeff_kalie@fws.gov or Meredith_Bartron@fws.gov

Tissue samples (or subset) collected from within the Mississippi River basin (includes Atchafalaya R.) should be sent to:

Greg Moyer,
U.S. Fish and Wildlife Service
Warm Springs Conservation Genetics Lab
5151 Spring Street, Warm Springs, Georgia 31830-9712,
Phone (706) 655-3382 ext 231
e-mail: Greg_Moyer@fws.gov

Appendix 2 continued

Genetic data card example (Missouri River samples):

Sturgeon Genetic Card

Circle **Pallid** **Shovelnose** **Lake**

Genetics vial # Strug-_____ PIT Tag #_____
(For pallid samples include photos head w/side and ventral views)

Capture Location_____
Latitude _____ Decimal degrees Hatchery Origin
Longitude _____ Decimal degrees Yes No Unknown
River _____ River Mile_____
State _____ Date _____

Interrostral Length _____mm Mouth - Inner Barbel _____mm
Outside Barbel _____ mm Inside Barbel _____mm
Head Length _____ mm Fork Length _____mm
Weight _____ lbs/kg Sex Male Female Unknown

Captured by_____

Genetic Analysis Needs_____

USFWS Northeast Fishery Center
Conservation Genetics Lab
P.O. Box 75
227 Washington Ave.
Lamar, PA 16848
Phone: (570)-726-4995

Appendix 2 continued

Genetic data card example (Mississippi River samples):

Sturgeon Genetic Card

Circle **Pallid** **Shovelnose** **Lake**

Genetics vial # Strug-_____ PIT Tag #_____
(For pallid samples include photos head w/side and ventral views)

Capture Location_____
Latitude _____ Decimal degrees Hatchery Origin
Longitude _____ Decimal degrees Yes No Unknown
River _____ River Mile_____
State _____ Date _____

Interrostral Length _____mm Mouth - Inner Barbel _____mm
Outside Barbel _____ mm Inside Barbel _____mm
Head Length _____ mm Fork Length _____mm
Weight _____ lbs/kg Sex Male Female Unknown

Captured by_____

Genetic Analysis Needs_____

USFWS Warm Springs Conservation Genetics Lab
5151 Spring Street
Warm Springs, Georgia 31830-9712
Phone: (706) 655-3382 (x 231) Fax: (706) 655-9034

Appendix 3 Chain of Custody Record

FILE NO. INV.

CHAIN OF CUSTODY RECORD			FILE NO. INV.
DATE AND TIME OF SEIZURE:	**REGION:**	**EVIDENCE/ PROPERTY SEIZED BY:**	
SOURCE OF EVIDENCE/PROPERTY (person and / or location) **TAKEN FROM:** **RECEIVED FROM:** **FOUND AT:**		**CASE TITLE AND REMARKS:**	

ITEM NO.	DESCRIPTION OF EVIDENCE/PROPERTY (include Seizure Tag Numbers and any serial numbers):			
ITEM NO.	**FROM:**	**RELEASE SIGNATURE:**	**RELEASE DATE:**	**DELIVERED VIA:** **U.S. MAIL** ☐
	TO:	**RECEIPT SIGNATURE:**	**RECEIPT DATE:**	**IN PERSON** ☐ **FEDEX:** ☐ **OTHER** ☐
ITEM NO.	**FROM: (PRINT NAME, AGENCY)**	**RELEASE SIGNATURE:**	**RELEASE DATE:**	**DELIVERED VIA:** **U.S. MAIL** ☐
	TO: (PRINT NAME, AGENCY)	**RECEIPT SIGNATURE:**	**RECEIPT DATE:**	**IN PERSON** ☐ **FEDEX** ☐ **OTHER** ☐

CHAIN OF CUSTODY RECORD (continued)

ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA: U.S. MAIL IN PERSON FEDEX OTHER
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE:	
ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA: U.S. MAIL IN PERSON FEDEX OTHER
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE:	
ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA: U.S. MAIL IN PERSON FEDEX OTHER
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE:	
ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA: U.S. MAIL IN PERSON FEDEX OTHER
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE:	
ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA: U.S. MAIL IN PERSON FEDEX OTHER
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE:	

Appendix 4 Fish Health Tissue Collection Protocols

The initial detection of an iridoviral agent in cultured shovelnose and pallid sturgeon prompted the development of specific guidelines for health sampling. Due to the tropism of the iridovirus for epithelial cells, it is extremely important to handle fish samples delicately. All samples should be handled to ensure that skin surfaces have as little contact with equipment and sampling surfaces. This outline will provide detailed instruction for health sampling of both juvenile and adult sturgeon. The primary means of sampling pallid sturgeon as an endangered species will be by non-lethal methods. However, lethal sampling instruction will also be provided for situations or facilities requiring inspection sampling.

NON-LETHAL SAMPLING TECHNIQUES: (Please contact USFWS Fish Health Biologist for specifics)

Collection of fin punches, barbel clips:

General:
* Label and track each fish individually with unique numbers (i.e. PIT #) for easy reference.
* Utilize only sterilized dissection equipment for collecting samples.
* Disinfect dissecting tools and DNA sampling tools between fish samples.
* Make sure fish are well oxygenated during fin punch collection.

Collection for histology:
* Individual fin punches will be collected from pectoral and caudal fins using a small paper hole puncher. Fins can also be clipped or notched using scissors or pig ear notcher. Refer to sturgeon anatomy picture for proper location of fin samples.
* Barbel clips may be collected by clipping the distal end of the barbel with sharp scissors.
* Both fin punches and barbel clips will be immediately placed into Davidson's fixative for a minimum of 48 hours, followed by immediate transfer to 70% ethanol.
* Place fish tissues into the Davidson's fixative at a ratio of 1 part tissue to 5 parts fixative.
* All histology samples should be collected in chemically resistant plastic containers or glass collection jars for transportation and storage. Seal jars tightly before transport.

Appendix 4 continued

Collection for Viral DNA analysis:

* Collect fin punches from the caudal and pectoral fins using a paper hole punch. Scissors may be used to clip the edge of the fins.
* Collect a portion of barbels with sharp scissors.
* Place each tissue type from individual fish in small 1 ml plastic tubes.
* These samples should be immediately frozen for transportation and then maintained at -70 F ultra-cold temperature for DNA analysis.
* Change gloves between each fish to be sampled.
* Disinfect sample collection instruments between fish.
* Refer to sturgeon diagram for sample locations.

Collection of Virology Cell Culture Samples:

* Collect both fin punches and barbel clips aseptically with sterilized dissection tools. Sample collectors should wear protective examination gloves.
* Refer to sturgeon diagram for sample location.
* Sample collection for virology may be as individual fish or pooled not to exceed a five fish pool.
* Samples will immediately be placed in small whirlpak sample bags. These bags should be chilled, not frozen. They can be kept in the refrigerator before transportation and should be transported chilled, insulated from ice packs. At no time should samples be allowed to become warm.
* These samples must be forwarded to receiving laboratory within 48 hours from collection.
* It is very important to sterilize dissecting tools between fish samples. An appropriate virucidal agent should be used.

Appendix 4 continued

LETHAL SAMPLING TECHNIQUES
(Only on mortalities): Collection of complete internal and external fish tissue samples.

General:
* Label all containers, showing species, and date collected.
* Maintain fish sample collection report with:

** fish source
** fish condition
** water temperature
** fish handling
** fish culture information
** mortality records

All dissecting tools should be sterilized prior to collection and should be disinfected between individual fish.

Sample collectors should wear protective gloves during collection procedure.

Fish should be euthanized with Tricaine Methane Sulfonate (MS-222) prior to sampling.

Collection of Histology Samples:
* Fish should be dead no longer than 15 minutes for good histological sample collection.
* Fish smaller than 60mm can be preserved as whole fish. Slit fish ventrally along the belly, from the vent to the gills. Pull viscera away from the kidney area and puncture the air bladder to facilitate fixation of the kidney.
*Fish larger than 100mm will require thin sections of each organ for fixation. Tissues for histology: gill, heart, liver, spleen, kidney, muscle, ceca, digestive tract, fins, barbels, nares, rostrum, mouth parts, any lesions that are visible.
* The tissue pieces may be as large as 25 mm (1 inch square), but no thicker than 5 mm (about 1/4 inch).
* Histology tissues should be immediately placed in Davidson's fixative. One fish per collection jar. Do not combine tissues from other fish.
* Sample tissues should be placed in fixative at a ratio of 1 part fish to 10 parts fixative.
* After specimens have been in fixative for 48 hours, transfer to 70% ethyl alcohol.
* Samples can be transported in ethyl alcohol and stored for histology processing.
* Sample containers can be glass or chemical resistant plastic.

Appendix 4 continued

Collecting Virology Cell Culture Samples:

* Collect both external and internal samples: caudal fin, pectoral fin, barbel, nares, rostrum, mouth, spleen, kidney, gill, ceca, heart, kidney, gut.

* Maintain separate virology bags for external and internal samples. Samples can be taken individually or five fish pooled.

* Always use sterilized dissecting tools. Wear appropriate gloved protection while sampling.

* Collect in whirlpak plastic bags and immediately chill samples. Do not freeze. Do not allow samples to become warm.

* Transport samples to receiving laboratory within 48 hours.

Appendix 5 Contaminant Sample Collections

STANDARD OPERATING PROCEDURES FOR COLLECTION, STORAGE, AND SHIPMENT OF PALLID STURGEON TISSUE SAMPLES FOR ANALYSIS OF ORGANIC AND TRACE ELEMENT CONTAMINANTS (mortalities)

1. Wash hands throughly and rinse completely. Wear vinyl or latex gloves (powder less). Final rinse with distilled water.

2. Rinse fish clean of any debris.

3. Dissection surface should be a chemically inert substance such as a stainless steel solvent (pesticide grade acetone, hexane, or isopropanol) rinsed pan, or solvent rinsed heavy duty aluminum foil placed shiny side down and dull side towards fish. Take care that sample does not contact potentially contaminated surfaces (plastics, identifying labels, printed papers, uncleaned work surface or tools, etc).

4. Use previously cleaned dissection tools which were decontaminated under the following guidelines: 1) non-phosphate detergent wash. Liquinox or Alconox brand detergents are recommended. 2) tap water rinse. 3) distilled/deionized water rinse. 4) solvent rinse (pesticide grade acetone, isopropanol or hexane). 5) air dry. 6) distilled/deionized water rinse. 7) wrap instruments in aluminum foil (shiny side out) for storage until use. Scales for sample weights should also be clean or covered with solvent rinsed aluminum foil.

5. Separate, clean dissection tools are to be used for each individual fish. And instruments used to collect tissue samples should be separate from instruments used to make initial opening in abdominal cavity.

6. Complete a Fish Health Examination Sheet (attached)

7. Do not let dissected samples remain exposed to the air. Exposure can dry samples and reduce the natural percentage of moisture. Prepare each dissected sample for shipping or freezing as it is dissected.

8. Tissue samples to be collected should include: kidneys, gonads, liver, and muscle with skin.

9. Samples should be placed in a chemically-cleaned glass jar and sealed with a teflon-lined lid. Lids are then to be sealed with tape (electrical or packing). Jars should be pre-labeled with a permanent, waterproof marking pen. As an alternative, solvent (pesticide grade acetone, hexane or isopropanol) rinsed, heavy-duty aluminum foil may be used to wrap the sample (remember, shiny side out). After double-wrapping, place the sample (with sample identification label) inside an air-tight zip-lock or whirl-pak bag.

10. Complete a Chain of Custody Record (Appendix 3)

Appendix 5 continued.

11. Samples are to be sent to US Fish and Wildlife Service, Ecological Services, 3425 Miriam Ave., Bismarck, ND 58501 (701) 250-4481. All coolers should be shipped via OVERNIGHT service. Always call before shipping to ensure personnel will be available to handle incoming samples. Upon receipt in Bismarck, samples will be stored in an Environmental Contaminants freezer until authorization to ship samples to a pre-approved analytical laboratory.

12. Samples not shipped to Bismarck within 24 hours after collection need to be frozen and then shipped on dry ice. For frozen samples, dry ice to sample weight ratio should be 1 to 1. Samples shipped to the Bismarck Field Office within 24 hours of collection need to be chilled immediately and can then be shipped on wet ice. However, chemical coolants such as blue ice packs are preferable to wet ice because their packaging prevents leakage should they thaw. Regardless, coolants such as wet ice or blue ice should be sealed in plastic bags. Sample containers (jars or whirl-paks) should also be separately contained in plastic bags. Samples should be properly packed in the cooler with bubble wrap.

Appendix 6 Meristic count guidance

Example photo of pallid sturgeon dorsal fin
The 3 anterior rudimentary rays in the photo would be counted for a total of 30 dorsal fin-rays (rays individually marked with black dots to aid identification).

In the dorsal and anal fin-ray counts, all anterior rudiments behind the predorsal and preanal plates are included. The last ray in those fins, as counted, is double at its base.

Recommended equipment list

The following three lists contain items that you may find useful when working with pallid sturgeon in the field. Individual activities may need additional items necessary for particular work dependant on field conditions and activities, therefore these lists should only serve as a guide.

List for Field Collection

- ☐ Crew trained in netting and trawling procedures
- ☐ Crew trained in best handling procedures
- ☐ Nets and sampling gear
- ☐ Holding tank on boat, must be at least six feet in length for larger specimens
- ☐ Bucket or bilge pump available for filling holding tank and for circulating water
- ☐ PIT tag reader, tag injectors, and tags
- ☐ Spare PIT tag reader
- ☐ Coded Wire Tag reader
- ☐ Crews trained in proper tagging procedures
- ☐ Water proof field notebooks and data sheets
- ☐ Cloth measuring tape (a quilting tape works well) and weighing scale
- ☐ Stretcher for moving fish and weighing
- ☐ Cellular phone for emergencies
- ☐ Appropriate therapeutic antibiotics, syringes and dosage chart
- ☐ Global positioning system
- ☐ Black light for examination of elastomer tags in stocked fish

List for Genetic Samples

- ☐ 95% NON-denatured alcohol
- ☐ Tissue Forceps
- ☐ Scissors
- ☐ Screw-cap tubes
- ☐ Permanent marker
- ☐ Data sheets
- ☐ Butane lighter
- ☐ Latex gloves
- ☐ Single use razor blades

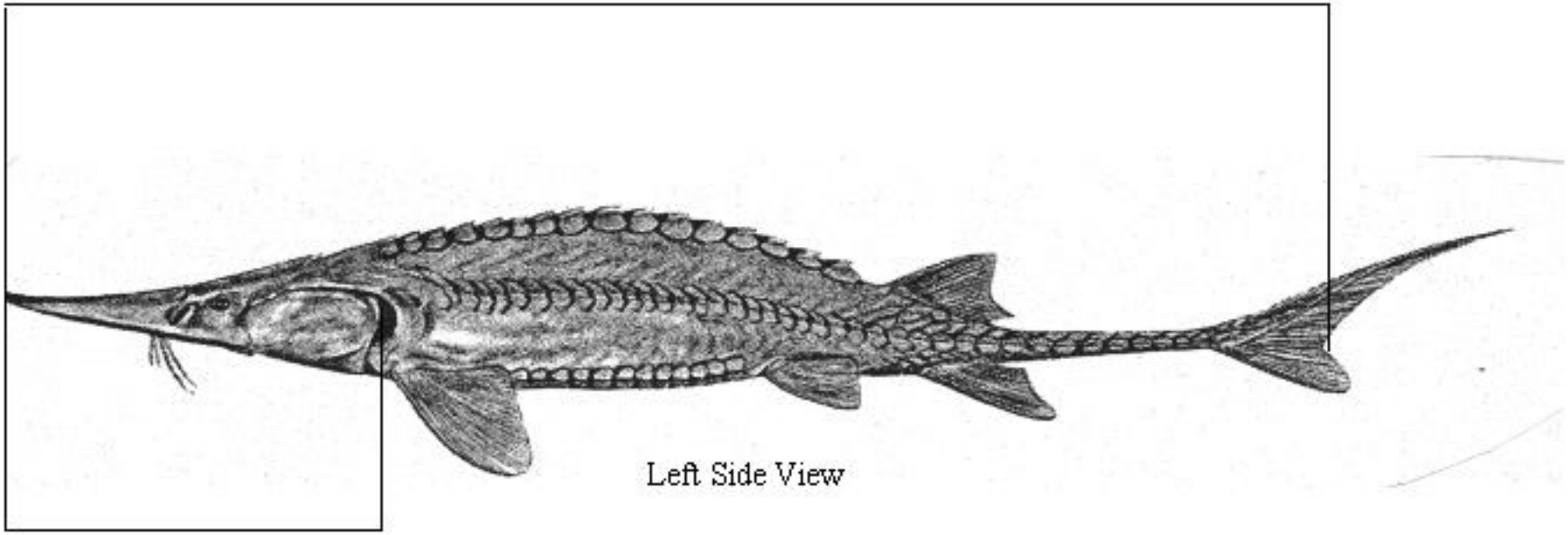
Hauling truck check list

- ☐ Crew trained in hauling procedures.
- ☐ Loading crew trained in best handling procedures.
- ☐ Drivers know the route and maps available.
- ☐ Personnel at receiving point are expecting shipment.
- ☐ Cellular phone and necessary phone numbers.
- ☐ Adequate fuel, spare tires and emergency equipment.
- ☐ Oil and other fluid levels checked.
- ☐ Haul tank filled to proper level with water and water temperature in tank similar to host water (within 3 degrees Fahrenheit) and securely attached.
- ☐ Water additives in tank water (salt).
- ☐ Stretchers and nets in place.
- ☐ Oxygen/temperature meter calibrated, in place, and operating.
- ☐ Primary aeration system functioning oxygen bottles full - adequate supply for trip.
- ☐ Emergency aeration system in place and workable.
- ☐ Filling pump present and functioning.
- ☐ Receiving facility/tanks ready and filled.
- ☐ Two large buckets available.
- ☐ Salt bucket pre-marked for non-iodized NaCl.
- ☐ Pit tag reader, injectors and tags waterproof field notebooks and data sheets

01/03/2003

Required Morphological Measurements for Pallid Sturgeon

A



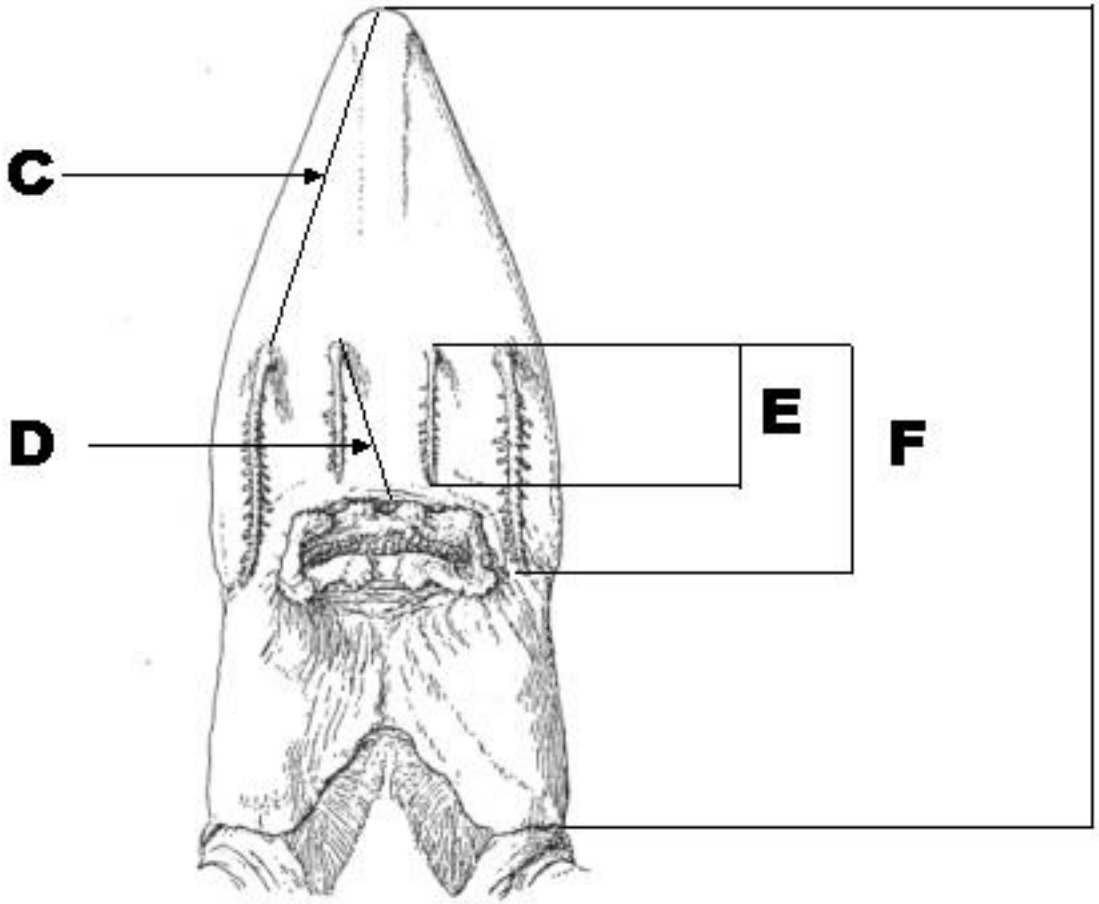
Left Side View

B

A – Fork Length – Tip of snout to the median of the caudal fin rays. (Note: on larger fish, it may be easier to lay tape along bottom of tank to get a straight line measurement)

B – Head Length – Tip of snout to back edge of opercle flap.

01/03/2003



Line drawing taken from:

S. A. Forbes and R. E. Richardson. On A New Shovelnose Sturgeon from the Mississippi River. Bulletin Illinois State Laboratory of Natural History 737-44, 1905.

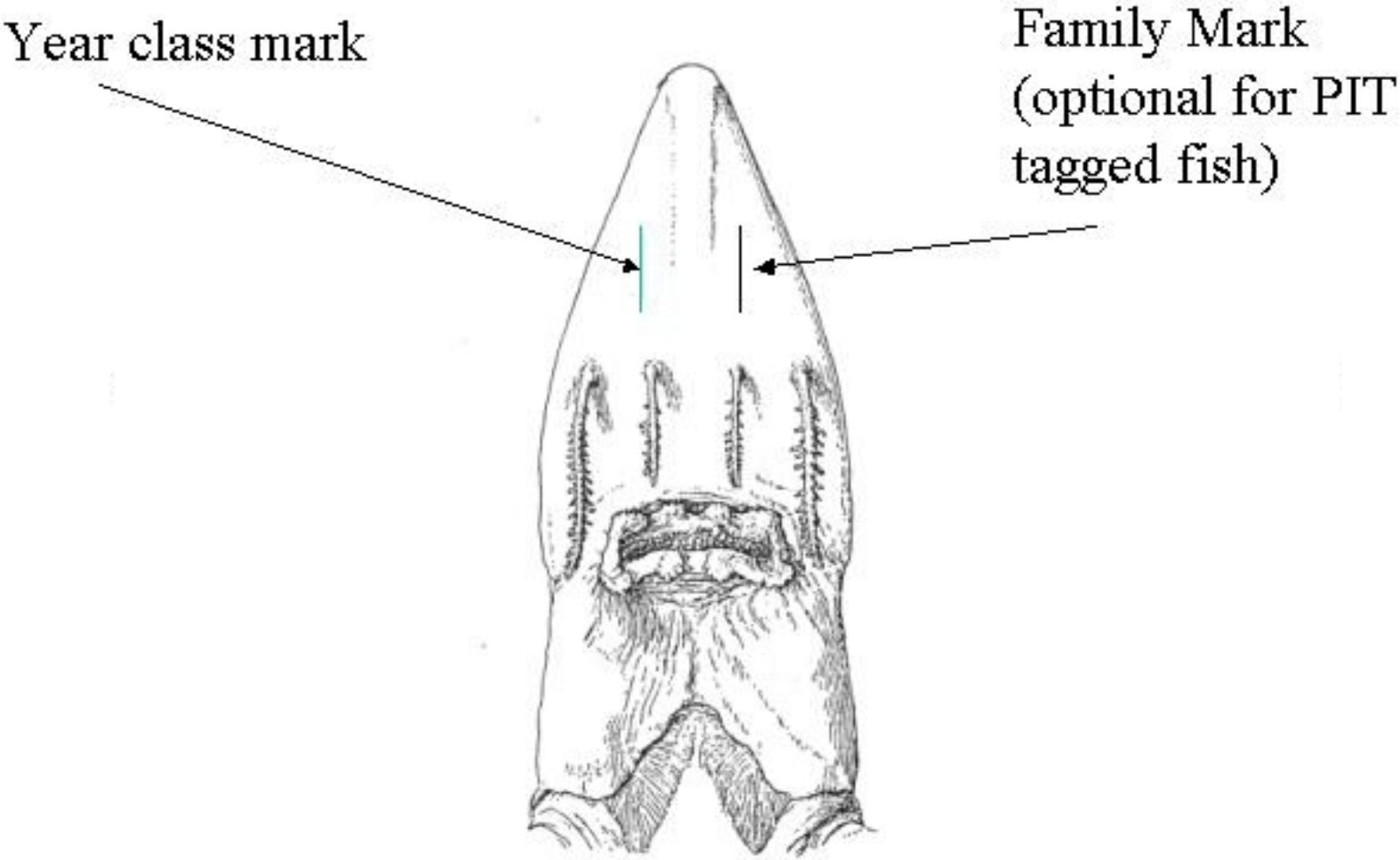
B – Head Length (see previous page)

C – Interrostral Length – Tip of snout to front edge of the outer barbel.

D – Mouth to Inner Barbel Length – Leading edge of mouth to front edge of inner barbel.

E – Inner Barbel Length – Front leading edge of inner barbel to it's tip.

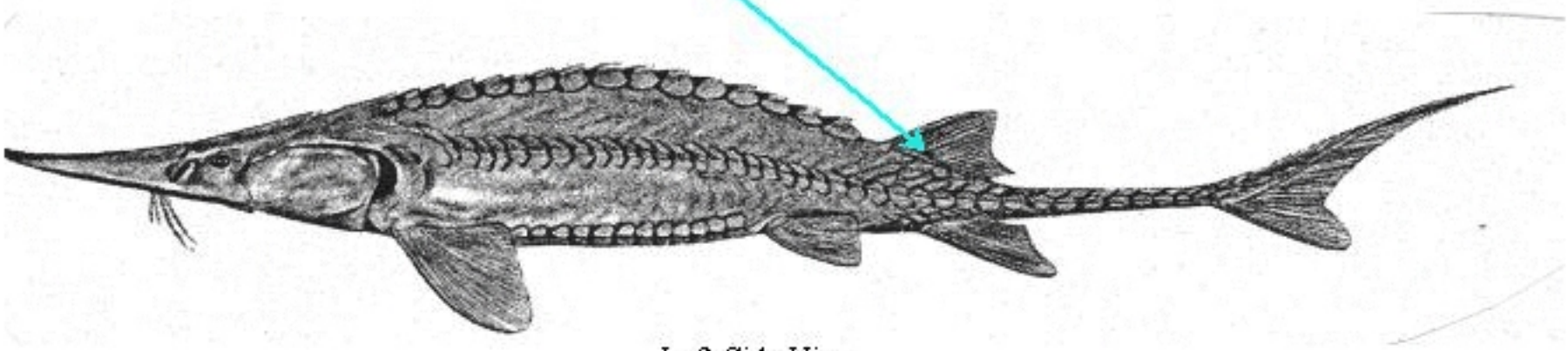
F – Outer Barbel Length – Front leading edge of outer barbel to it's tip.


Year class mark
Family Mark
(optional for PIT
tagged fish)

01/03/2003

Required Tagging Location for Passive Integrated Transponder (PIT) for Pallid Sturgeon

Insert tag from front to back on fishes left side, into tissue at base of dorsal fin.



Left Side View

01/03/2003

Ventral view of pallid sturgeon photo for genetic sample.

01/03/2003

Side view of pallid sturgeon photo for genetic sample.