Range-wide
Pallid Sturgeon
Handling Protocols and Procedures

Originally Biological Procedures and Protocols
for Researchers and Managers Handling Pallid Sturgeon 2012

for

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

Final May 2019

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RANGE-WIDE
PALLID STURGEON
Handling Protocols and Procedures

Prepared by the Pallid Sturgeon Recovery Team,
Fish and Aquatic Conservation Staff and Pallid Sturgeon Recovery Coordinators

for

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

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Regional Director 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 and the Basin Pallid Sturgeon Workgroups 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 404-679-7313 or email to *permitsR4ES@fws.gov*. In Missouri, Illinois and Iowa contact 612-713-5343 or email to *permitsR3ES@fws.gov*. In Nebraska, South Dakota, North Dakota, and Montana contact 303-236-4256 or email to *permitsR6ES@fws.gov*. Questions, comments or suggested changes to the protocol should be directed to, Pallid Sturgeon Recovery Coordinator, U.S. Fish and Wildlife Service, 420 South Garfield Avenue, Pierre, SD 57501 or at (605) 224-8693. 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 or amended application submitted. 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 in order to minimize stress and loss of Pallid Sturgeon and standardize data collected 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 and insure that all appropriate data is collected, to expand our knowledge of these fish. All personnel that work with Pallid Sturgeon are required to be knowledgeable and trained in these procedures to handle the fish.

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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; either hard copy or electronically. Copies of all completed data sheets or an electronic equivalent must be sent to the Missouri River FWCO attn: Project Leader, U.S. Fish and Wildlife Service, 3425 Miriam Ave., Bismarck, ND 58501(701-250-4419) no later than December 31 of the year the fish were collected.

Personnel and Training Requirements

Activities conducted for Pallid Sturgeon research and sampling will be categorized into Tier 1 and Tier 2 activities. Tier 1 activities will be generally those activities that are low risk to the fish as outlined in this document and can be conducted with minimal training in appropriate fisheries management collection techniques. Additional activities, categorized as Tier 2 activities, require specific training and experience such as implanting transmitters, culturing fish, collecting tissue biopsies, and assessing reproductive condition. All personnel must have documented experience or be trained in the collecting and handling procedures described in this protocol. Training can be accomplished through formal instruction at a U.S. Fish & Wildlife Service sponsored Basin Workgroup workshop or under the supervision of an experienced biologist already permitted for that activity. Those applying for a permit will be required to document their experience. Those transporting live fishes should be knowledgeable of proposed routes and coordinate with receiving stations with anticipated routes and timelines. The appendices (1-9) at the back of this document are guidelines for standard procedures that have been accepted. However, these guidelines should not be a substitute for the hands-on training and experience gained either through formal training from one of the workgroups or through instructions by an experienced biologist.

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 and work under the direct supervision of a qualified biologist (Tier 1 or 2), until deemed capable by their crew leader or supervising biologist.

Tier 1 certification: Those persons who are knowledgeable through experience or education to meet the minimum qualifications for fisheries professionals. These persons can either gain the experience through an accepted workshop or by working under the direct guidance and direction of a Tier 2 individual.

These activities will include the sampling of fish, collection of morphometric data, scanning and implanting of identification tags, collection of genetic samples,

Tier 2 certification: Those persons who are very knowledgeable about handling procedures through experience or education that exceed the minimum qualifications for fisheries professionals. These persons will typically have multiple years of directly conducting these activities in addition to formal training.

These activities will typically include those procedures that require additional experience to include the collection of internal tissue samples, any procedure involving biopsy, implantation of transmitters, gastric or colonic lavage, egg collections, blood samples. Available standard accepted procedures for these activities will be included in the appendix for reference.

Propagation Activities

Fish cultures procedures should follow the most current Pallid Sturgeon Propagation Guidelines (2018).

Coordination: Two weeks prior to actual field work for collection of broodstock, all field personnel, the Regional Fish Health Center, and hatchery personnel will be notified. A fish holding container on the boat shall be of sufficient size to completely submerge the fish and prevention of direct exposure to sunlight and ambient air temperatures. Transport equipment to the hatchery facility will either be onsite or readily available.

Fish Transportation/Translocation Protocols

Personnel at the receiving point must be informed 24 hours in advance of activities to potentially expect the shipment.

Transportation protocols are outlined and should follow the most current procedures in the Pallid Sturgeon Propagation Guidelines (2018).

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 (>15 pounds); whereas, rectangular tanks are approved for smaller broodfish. All tanks should be equipped with oxygen tanks 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°F (± 1.6°C) of ambient water temperature of origin. Temper the fish when moving them between bodies of water. Temper the fish if the fish capture and distribution site's water temperatures vary by more than 5°F (3°C). The temperature change should not exceed a 4°F (2°C) change per hour.

Broodstock: Pallid Sturgeon to be utilized for spawning activities should not be transported when ambient water temperatures are greater than 60°F (15.6°C).

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.

Collection Protocols

Gill Nets/Trammel Nets

Monofilament or multi-filament mesh nets may be used to collect Pallid Sturgeon. There are no mesh size restrictions for gill and trammel nets. Drifting net sets should be monitored continuously. Time, date, duration and position (Global positioning system [GPS]) of net sets should be recorded. Total numbers of each species are recorded. Drift distance is recorded in meters. Indicate net length, mesh size, and mesh type in reports.

If water surface temperatures are 55 °F (12.8 °C) or less, 24-hour static net sets may be used. When water surface temperatures are between 55 °F (12.8 °C) and 60 °F (15.6 °C) then shorter duration or 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 ensure that nets can be picked up as soon as possible the next day. As surface temperatures exceed 60 °F (15.6 °C) 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 °F (15.8 °C) and 65 °F (18.3 °C). As water surface temperatures exceed 65 °F (18.3 °C), but are less than 70 °F (21.1 °C), static net sets should be checked for captured Pallid Sturgeon at a minimum of every 5 hours. At water temperatures above 70 °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 °F (12.8 °C) or less, then 24-hour static net sets may be used. When water surface temperatures are between 55 °F (12.8 °C) and 60 °F (15.6 °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 °F (15.6 °C) then collection of brood stock should cease as recommended transportation temperatures are exceeded (see Handling and fish transportation section).

Calculate Catch Per Unit Effort (CPUE) as fish per-net-night 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.

Trotlines/Angling

Use appropriate sized hooks for the size of sturgeon being targeted. Circle hooks in sizes up to 14/0 have proven successful in capturing larger Pallid Sturgeon in Montana. However, smaller 3/0 stainless steel circle hooks baited with a nightcrawler have proven successful in capturing a variety of Pallid Sturgeon size classes.

Water temperatures and water velocities at the set location can both play a role in increasing stress in the fish. Increased temperatures or increased flow velocity need to be a consideration for set duration. A good rule of thumb is that with increased temperatures or increased velocities; set duration should be reduced. The following guidelines will be used until additional analysis of existing data can be accomplished.

If water surface temperatures are 65° F (18.3° C) or less, a maximum of 24-hour sets may be used, and frequent checking for entangled Pallid Sturgeon is encouraged. Weather conditions must be watched to ensure that sets can be picked up as soon as possible the next day. Maximum soak times should not exceed 18 hours when water surface temperatures range between 65 ^{o}F (18.3 ^{o}C) and 70 ^{o}F (21.1 ^{o}C). As water surface temperatures exceed 70 ^{o}F (21.1 ^{o}C), the use of trotlines should be limited to less than 4 hours.

In order to reduce risks from trotlines, 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.

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 cannot be used to purposefully stun and capture Pallid Sturgeon.

SCUBA

Pallid Sturgeon collected with 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. If there is danger of breaking the caudal peduncle, the fish should be released immediately. 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 has been used to capture Pallid Sturgeon, primarily during the winter. Record number of Pallid Sturgeon captured and 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 higher densities of fish are present, trawling time should be reduced to limit incidental injuries. Calculate CPUE as fish per trawl hour and number of fish per-meter of the trawled area.

Data collected
The Pallid Sturgeon Data Sheet (Appendix 1; Figures 2-3, Table 1), lists the required physical data to be recorded for all specimens, as well as general habitat data about the collection. This will ensure adequate data are represented in the database. Collecting previously required morphological and meristic data on captured fish is no longer mandatory.

General Handling Protocol
Handling Fish - The general handling protocol addresses all activities and procedures performed on a fish from the time it is removed from its natural environment until it is returned to the river or holding facility. This guide refers to all handling not addressed elsewhere in this document and specifically addresses handling precautions associated with cold weather and photography.

Critical issues to reduce or prevent stress while holding a fish include water quality, water temperature, holding duration, and stress due to handling itself. Water quality can be maintained by changing water frequently, adding salts, or electrolytes to improve osmotic regulation. Safe handling requires proper body support of the fish in a horizontal hold if possible and techniques that prevent injury such as using wet hands (bare or gloved) and not holding fish by the eyes, jaws, or gills. Time out of the water should be minimized as much as possible. Holding tanks should be large enough to submerge and safely hold fish between the capture event and either return to the river or transfer to other holding facilities. Special care should be taken in handling fish during the course of data collection.

Handling fish when air temperatures are below 32 ^{o}F (0 ^{o}C), presents a high threat of injury. Specifically, working with fish in freezing air temperatures can cause permanent or lethal damage to tissues. The dangers include, but are not limited to skin, eyes, fins, barbels, gill damage, reduced immune response, and reduced energy reserves. Effort must be taken to avoid damage to the fish. The dangers of handling Pallid Sturgeon at cold water and air temperatures should be discussed annually with field and hatchery crews to increase awareness and reduce stress and damage due associated with cold-weather handling. Field and hatchery crews should be conscious of signs for potential frostbite. The protocol for handling fish during sub-freezing temperatures is as follows:

1) Gill nets should be placed in a tub of water while sturgeon is removed from the net.
2) If forecasted air temperatures are to be less than 32 ^{o}F (0 ^{o}C), handling Pallid Sturgeon (and other native fishes) when air temperature is below freezing should be avoided. Crews should watch the predicted hourly weather forecast to restrict (cancel sampling efforts) or adjust (start later in the morning) sampling efforts to avoid pulling gear in freezing air temps. Note that this is not meant to preclude the pulling of nets set overnight given a weather front that has moved in unexpectedly.
3) Sturgeon should remain in a tub of water while collecting morphometric data and other biological information. When a biopsy or other surgical procedure is necessary the incision site can remain in the ambient air.
5) Avoid all sturgeon flesh-to-metal contact.

Guidelines for Photographing Pallid Sturgeon - Photography is an essential tool for documenting collection of Pallid Sturgeon and communicating recovery activities to the scientific

community and public. When collecting photographic images of Pallid Sturgeon, it is important to be aware as to how that image may be interpreted. Digital images may spread rapidly and persist with unintended or unanticipated consequences. It is important to the credibility of the Recovery Program that images of Pallid Sturgeon collected by scientists and managers convey to a viewer the level of sensitivity appropriate to a rare and endangered fish species.

Field photography, as with any type of scientific data collection procedure, should be planned. Careful thought should be given to the safety of the subject, the quality of the image captured and what the image is intended to document. Following a few simple guidelines should produce effective images with minimal stress to the fish.
When photographing Pallid Sturgeon, it is important to remember that any time the fish is out of the water, it is perceived to be vulnerable and stressed. Therefore, minimize the amount of time the fish is out of the water. Keep the fish in contact with or as close to the water as possible. Wet all surfaces and equipment that come in contact with the fish to prevent tissue damage and reduce the loss of protective mucous. Avoid photographing fish entangled in gear. Fish entangled in gear are obviously stressed and viewers perceive the subject to be in distress. Avoid photographing fish on metal surfaces. Aluminum boat decks can be hot enough to cause burns on warm sunny days, and can be cold enough to freeze exposed tissue in the winter. Do not lift or hold the fish by the gills or the tail. Gill tissue is sensitive to physical damage and the caudal peduncle of Pallid Sturgeon is slender and weak. Instead cradle the fish close to the body and low to the water surface or boat deck. Avoid images that give the appearance of the fish as a “trophy.”

Photographic images are mere instants in time. It is difficult for the viewer of a photograph to know how long the fish was out of water or how stressed it may be. By holding the fish close to the water or low to the boat deck near the gunnel, the image takes on an element of “action,” capturing a “transitional moment” as it is being taken out of the water or as it is about to be released (figure 1). An awareness of the background in the photograph is similarly important. Turning the subject so that water is in the background creates a much better photograph than one that has a concrete boat ramp or parking lot behind the subject. A background of pavement and asphalt suggests to the viewer that the fish has been out of the water for an extended time and is in peril.

Excellent quality photographs of Pallid Sturgeon and recovery activities are always in demand. Before releasing photographs illustrating recovery activities have the photographs reviewed by a colleague, supervisor, agency representative, or the Recovery Coordinator. A photographic review ensures that images displaying inappropriate handling of Pallid Sturgeon or unsafe practices are not released to a broad audience. When providing photographic images be sure to provide the photographers name, date and location of the image, and the Agency employing the photographer. Print and online publications cannot use the images without the appropriate permission and attributes. In addition, publication editors may refuse images that are not appropriately sensitive to the species captured or images where scientists are not wearing personal flotation devices (PFD).

Figure 1. This photograph incorporates many of the guidelines for good Pallid Sturgeon photography. The biologist in the photograph holds the fish close to the body, low to the deck of the boat near the gunnel, while avoiding holding the fish by the gills or grasping the caudal peduncle. The river is in the background and the photograph appears to capture a transitional moment just before the sturgeon is released. Photograph courtesy of the USGS.

Tagging protocols

Fish tagging and marking - All captured Pallid Sturgeon will be carefully examined for previously implanted Passive Integrated Transponder (PIT), elastomer, coded wire tags, external tags, scute marks, and evidence of external tag loss. Tagging protocols will follow respective regional Tagging Guidelines. 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.

Identification Tags / Marks

PIT Tags - All collected Pallid Sturgeon of suitable size (>230 mm) that do not have a PIT tag must be implanted with a PIT tag prior to release. PIT tags should be inserted horizontally along the body and front to back along the fish's left side at the anterior, fleshy base of the dorsal fin (Appendix 1; Figure 4). Tags should be scanned prior to implantation for recording and after implantation to ensure it is working properly.

In order to maintain consistency in recognizing and identifying recaptured fish, only un-encrypted tags will be implanted in Pallid Sturgeon, Since PIT tag technology has improved, the program recognizes that adoption and incorporation of better technology is important.

At this time, a full-scale conversion to 134.2 kHz (12 mm 134.2 kHz ISO FDX-B) tags will begin being implemented during 2018 and into 2019. The Recovery Team has identified and the Fish and Wildlife Service supports a transition strategy consisting of a phased approach. The first phase is to transition to dual frequency PIT tag readers, range-wide, capable of reading both the 125 kHz and 134.2 kHz tags. Transition with the new tags will begin by incorporating the new 134.2 kHz tags with tagging of new or untagged fish and as supplies of 125 kHz tags are depleted or exchanged. The new frequency tags will only be implanted on new insertions such as with stocking events or as untagged fish are encountered or captured.

External Tags – Use of external markers on wild-caught fish will be evaluated on a case by case basis. External tags have been 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 varying 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 exhibited tissue inflammation severe enough to be concerned about infection. In some cases, severe inflammation was still evident two years after the fish had been tagged. External tags can be used on shovelnose sturgeon, shovelnose sturgeon X Pallid Sturgeon hybrids, and Pallid Sturgeon stocked for research purposes, as well as special cases for wild caught Pallid Sturgeon after approval from the Recovery Team Coordinators. Use of external markers on wild-caught fish will no longer be implemented unless their use can be justified to the Recovery Team.

Visual Implant Elastomer Tags – 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. 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 when used in accordance to the upper and middle basin marking and tagging plans. Other applications for Pallid Sturgeon will be reviewed on a case by case basis.

Scute Removal – Scute removal is suitable for use on juvenile hatchery-reared Pallid Sturgeon when used in accordance to the upper and middle basin marking and tagging plans. Other applications for Pallid Sturgeon will be reviewed on a case by case basis. Surgical removal of lateral scutes, in specific patterns, can provide data on hatchery origin, brood year, family lot, or stocking site.

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 fish for the presence of coded

wire tags around the tip of the rostrum and the first several dorsal scutes to help prevent erroneous classification of hatchery-reared Pallid Sturgeon as wild fish.

Radio/Sonic Transmitters

Internal Transmitters - Internal transmitters are preferred over external transmitters; however, implanting should be performed only by Tier II individuals with experience in surgical procedures and listed on their Agencies collectors permit. During surgery, the head 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, no anesthetic should be used. An incision, only slightly larger than the tag should be made in the ventral body wall, 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 assessing reproductive condition. The duration of surgical procedures should be less than 15 minutes and minimized to reduce stress to the fish For additional information and guidance on surgical procedures refer to Appendix 7:

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

Genetic Marks and Tissue collections

A tissue sample of all Pallid Sturgeon brood stock used for captive propagation and wild caught Pallid Sturgeon should be taken for future genetic analyses (note that it is not necessary to take a genetic sample from a recaptured Pallid Sturgeon – see Appendix 2 for more detail). Appendix 2 describes the procedures for collecting genetic tissue samples and relevant data to record for submission to genetic repositories. Each Samples collected from the Missouri River will be sent to the genetic repository located at the Conservation Genetics Lab at the USFWS Northeast Fishery Center; alternatively samples collected from the Mississippi River will be sent to the genetic repository housed at the USFWS Warm Springs Regional Fisheries Center's Conservation Genetics facility (addresses available in Appendix 2). Note that all genetic samples along with a copy of the data sheet must be included for accurate cataloging.

Fish acclimatization and therapeutants

Prior to transport, when water temperatures between the environment and a haul tank differ by greater than 3° C (5.4°F), fish should be tempered. Following transfer from the field to a controlled environment, measures will be taken to mitigate for stress of transfer. The most current version of the Pallid Sturgeon Propagation Plan should be followed for specifics of transport, acclimation, and use of therapeutants.

Wound relief protocols and drugs and therapeutants will be administered as recommended by the Bozeman Fish Health Center. Prophylactic drug and therapeutants 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.

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. Deaths must be reported to the Pallid Sturgeon Recovery Coordinator by phone and/or in writing as soon as possible. Describe all available information regarding the circumstances under which the fish died. The Service's Pallid Sturgeon Recovery Coordinator will coordinate carcass disposal or the transfer of the specimens to the University of Alabama repository if needed. 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 (Appendix7) 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
1805 South 22 Avenue, Suite #1
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 6). 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)

Length and weight 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-ray samples, copies of cross sectional imagery, and copies of data shall be shipped to the Service's Fisheries Aquatic Conservation office in Bismarck, North Dakota. All samples shall be labeled with the PIT tag number and include a copy of the data sheet.

U.S. Fish and Wildlife Service
Missouri River FWCO
3425 Miriam Ave Bismarck, ND 58501,
701-250-4419

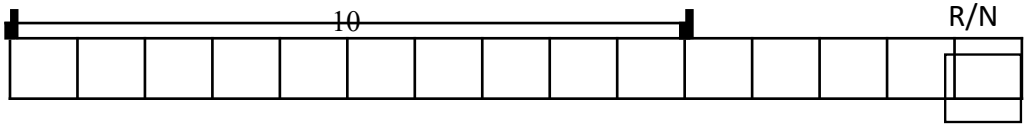
Appendix 1 - Pallid Sturgeon data sheet (03/18)

Date ☐☐ ☐☐ ☐☐

Lat. ☐☐ ☐☐☐☐☐
Decimal degree

Long. ☐☐☐ ☐☐☐☐☐
Decimal degree

PIT



ER		EL	
H/V	color	H/V	color

Elastomer Colors

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

Scute ☐

Loc.	#	Loc.	#

Scute

River_____R.M._____State _____

Location_____

Method: Gill, Trammel, Hoop net, Beamtrawl,
Otter trawl, Angling, Trotline, Other_____

Duration of set_____ Mesh size _____

Temp._____ºC Turbidity_____ntu

Depth_____m Velocity_____

_____m/sec Substrate _____

Picture Y/N ☐

Fork Length_____mm Weight_____g/kg

Sex M / F / U Stage_____Ripe / Green / U

Radio Tag Code_____Frequency_____

Other Tag Information_____

Genetic Vial #STURG-_____

Blood Sample #_____

FATE: Released/Taken to hatchery_____

Captured by_____

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

Comments_____
_____
_____
_____
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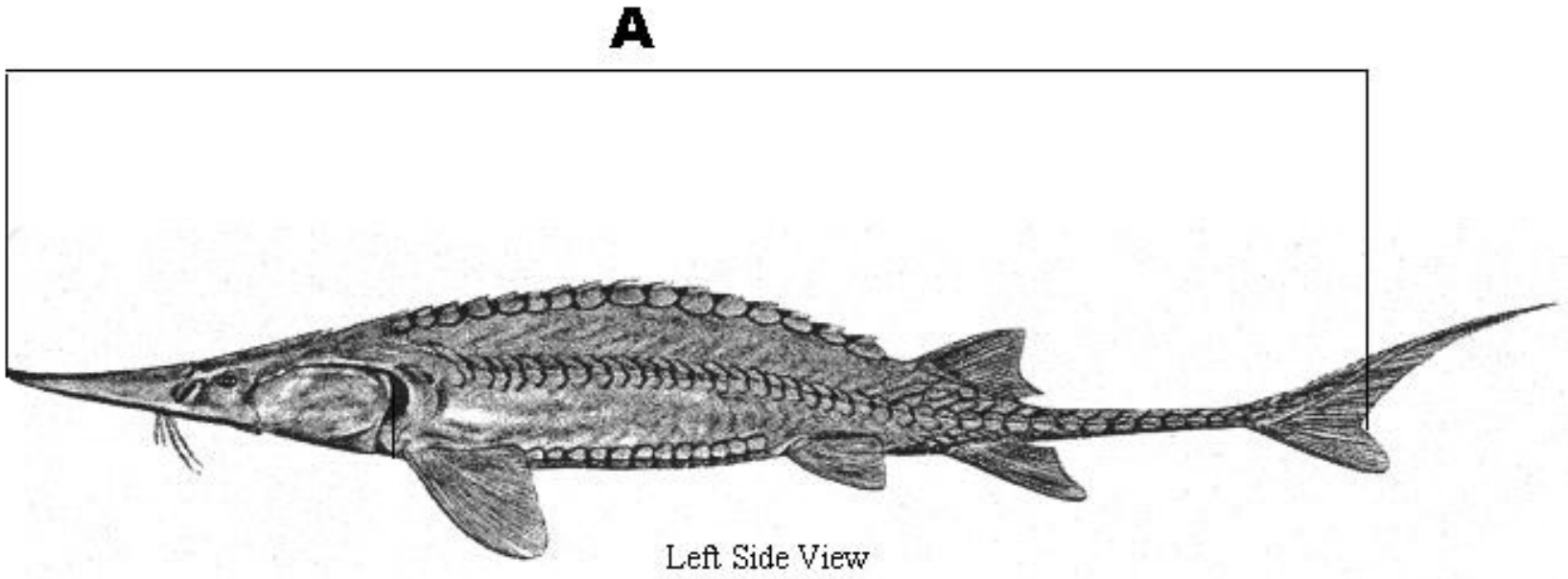
Table 1. Data Fields Required for Pallid Sturgeon Captures			
Data	**Description**	**Data**	**Description**
Date	Date of Capture	Radio Tag	Code & Frequency
PIT	Full PIT tag number	Other Tag Information	Any other tag info
Lat.	Capture Location Decimal degree	Genetic Vial #STURG	
Long.	Capture Location Decimal degree	Blood Sample #	
Scute	Note Left or Right and number in order	Comment	
Scute	Note Left or Right and number in order	Duration of set	
R/N	Recapture or New Tagged Fish	Mesh size	
C W T	If Coded Wire Tag is present	Temp.	
River	Name of River of capture location	Turbidity	
R.M.	Estimated River Mile of Capture	Depth	
State	State of capture	Velocity	
Location	Name of Capture Location	Substrate	
Method:	Gill, Trammel, Hoop net, Beam trawl,Otter trawl, Angling, Other		
FATE: Released/Taken to hatchery	Note what was done with fish: Mortality, Released, Taken to the hatchery		
Captured by	Crew Leader		
Fork Length	Fork Length of fish captured		
Weight	Total Weight of fish captured		
Sex M / F / U	Note if gender is known otherwise note as unknown		
Stage	Stage if known		

Required Morphological Measurements for Pallid Sturgeon



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)

Figure 2. Fork Length Measurement Required

01/03/2003

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

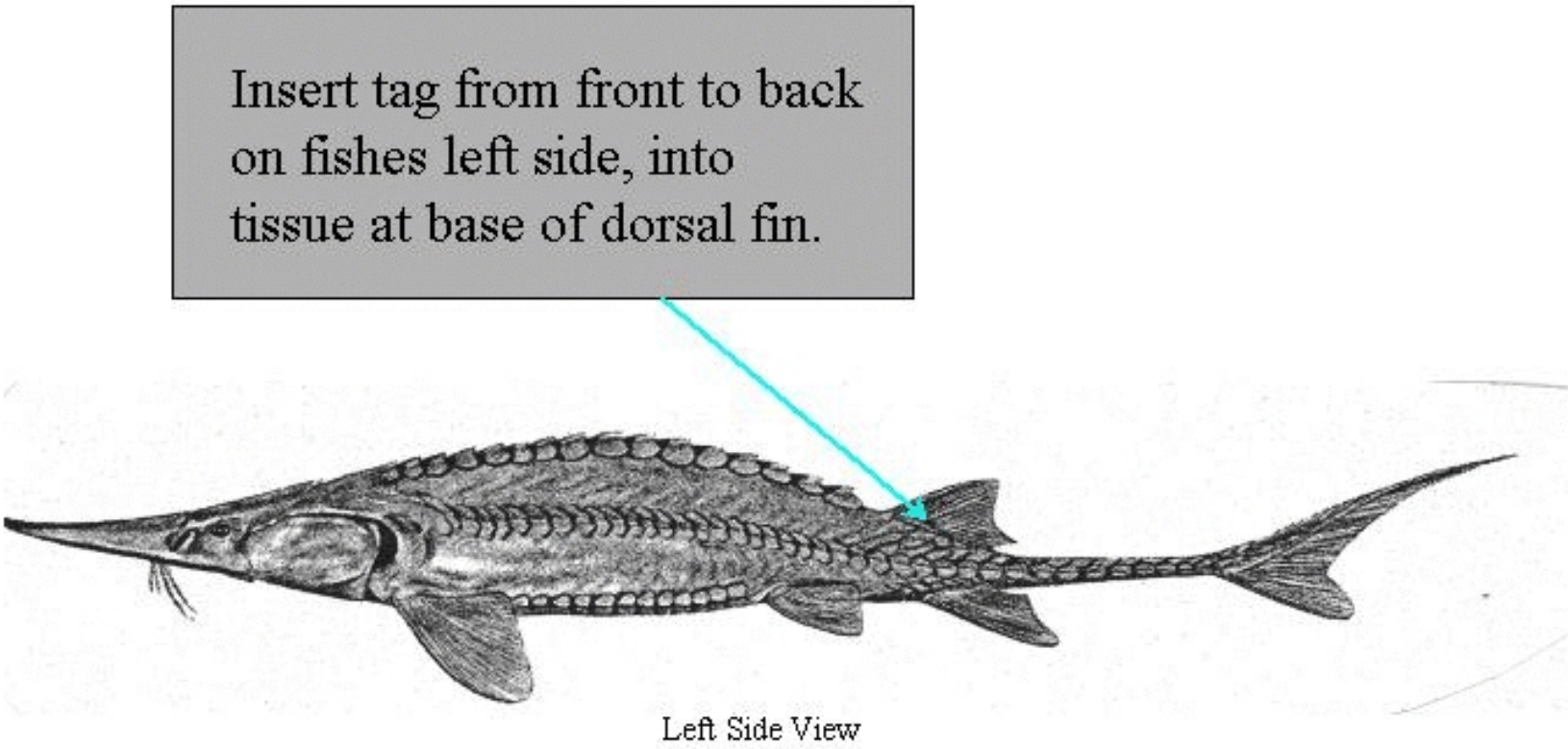


Figure 3. PIT Tag Location

Appendix 2 Protocol for Taking Sturgeon Genetic Samples

Equipment you will need:

1) Genetic Sample Kits (which contain the following)
    a. Two screwcap tubes filled with 95% NON-denatured ethanol or desiccant
    b. Sturgeon genetic card
2) Surgical scissors and forceps

Procedure:

1) Record genetic vial # and corresponding PIT # on the genetic card (**this step is critical for Pallid Sturgeon samples**). 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, or sequential numbered tubes**) 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 Meredith Barton, Jeff Kalie, or Nathan Wheelan via e-mail before sending samples to the USFWS genetics repositories. They will provide details on sending the samples via FedEx.
7) Please e-mail the biological data for each sample when you send the samples.

**If a Pallid Sturgeon is collected as a potential broodfish, the two genetic vials are divided. One sample is submitted to your basins respectively genetic lab while the other is submitted to Dr. Ed Heist (INSERT CONTACT INFORMATION HERE). Include a copy of the genetic card to Dr. Heist along with the genetic vial and contact him prior to shipping the samples.

Appendix 2 Protocol for Taking Sturgeon Genetic Samples Tissue samples (or subset) collected from within the Missouri River basin must be sent to:

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

Genetic data card example (Missouri River samples):

Sturgeon Genetic Card

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

Genetics vial # Sturg-_____ 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 _____

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 Protocol for Taking Sturgeon Genetic Samples: Tissue samples (or subset) collected from within the Mississippi River basin (including Atchafalaya R.) should be sent to:

Nathan Whelan - Regional Geneticists,
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: Nathan_Whelan@fws.gov

Genetic data card example (Mississippi River samples):

Sturgeon Genetic Card

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

Genetics vial # Sturg-_____ 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 _____

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 (x1231) Fax: (706) 655-3389

Appendix 3 - Removing Scutes from Pallid Sturgeon

Specific patterns of removed scutes can provide a rapid assessment of hatchery origin, brood year, family lot, and stocking site. Lateral scutes can be removed using a scalpel or bladed knife. A No. 3 Scalpel handle and No. 12 hooked surgical blade works well for smaller hatchery aged fish; leather finger and thumb guards may be helpful if doing many markings in a hatchery production program. A flexible paring knife is often preferred for larger fish. Determine the pattern appropriate prior to scute removal. A deft touch is required to remove a single scute on small sturgeon; care must be taken to avoid removing more scutes than targeted. Typically, it is easiest to remove a scute going against "the grain" or pulling the blade toward the overlapping pattern; be certain to remove the entire scute. Ideally a scute will be removed without removing muscle tissue. The fish can be held with its head pointing towards or away (tagging persons' preference) from tagging person's body in one hand to immobilize the fish. Scutes are counted from head toward the tail in ascending order, with number 1 being the first movable scute; left and right refer to the fishes left and right sides. To remove a scute place the blade under the posterior end (tail) of the scute you intend to remove with the blade facing the anterior (head). Draw the blade underneath and toward the anterior (head), gently removing the scute from the tissue. Replace blades when needed. A single blade can be used on multiple fish.

Appendix 3 – Continued Removing Scutes from Pallid Sturgeon

Appendix 4 Blood Collection for determining sex and stage of maturation

Plasma sex steroids (testosterone, 11-ketotestosterone, and estradiol) can be used to determine the sex and stage of maturity of Pallid Sturgeon.

Blood Collection and Storage of Plasma

Gloves should be worn when handling blood and plasma. A 20-gauge x 1 ½ inch (0.9 x 40 mm) needle and 3-5 ml syringe or vacutainer is recommended for blood collection. Blood is collected from the caudal vasculature just behind the anal fin (caudal peduncle area; Figure 1). Typically, 3 - 4 ml of blood is collected in a sample; a minimum of 0.5 ml of blood is needed to provide enough plasma to perform the assay. Insert the needle holder or syringe at a slight angle at the exact mid-line of this region just posterior of the anal fin. If there are scutes present position the needle slightly posterior or anterior of the scute. Press gently through the skin while inserting the needle; if using a vacutainer holder and needle, once through the skin place a vacutainer onto the vacutainer holder; if using a syringe and needle, once through the skin begin to withdraw the plunger slowly thus filling the syringe. If blood does not flow into the vacutainer or syringe reassess the needle alignment and reposition the needle for another attempt. If using a vacutainer, once the blood has been collected, invert the vacutainer several times, and place on wet ice. If using a syringe, immediately transfer to a heparinized tube and place on wet ice. Blood plasma can be separated via centrifuged (3400 rpm for 5 minutes) or left standing on wet ice or in a refrigerator for 12 h until separated; once the plasma (the clear/whitish fluid on the top of the red blood cells) is separated it can be transferred from the vacutainer via pipette into a labeled vial. A new pipet should be used for each individual fish. Plasma may be stored long term for steroid analysis at -20 or -80ºC. As soon as possible, send the plasma to the laboratory where analyses will be conducted. If the plasma samples are frozen, ship the plasma on dry ice to ensure that it does not thaw.

Figure 1. Blood collection site located directly midline just behind the anal fin.

Appendix 5 - Telemetry Transmitter Implantation Standard Operating Procedure

Equipment List

- A small container to hold the surgery tools in disinfectant
- A small "sharps" container for the used blades and suture needles
- Alcohol (disinfect equipment)
- Sterile saline solution (rinsing tools prior to surgery)
- Scalpel (#3) and # 10 (large fish) # 15 (small fish scalpel blade)
- Textured Jawed Forceps with scissors (these forceps will allow holding the suture needle and cutting of suture material with one tool)
- Tissue forceps or tweezer (this allows holding the tissue around the incision during suturing)
- Large size reversed cutting needle (for threading antennae)
- Telemetry tag
- Suture material – (Ethicon PDS-II size 0 with OS-6 half-circle reverse cutting needle or CP-1 or CP-2 half-circle reverse cutting needles).

Procedure

In field telemetry tagging situations sturgeon are often not anesthetized prior to surgery because a proven and widely-accepted method for anesthetizing adult sturgeon is not available.

Typically, individuals implanted with tags are immobilized ventral-side up in immersed in a tank continuously supplied with river water while keeping the head and gills submersed. A stretcher or mesh webbing can be used to help immobilize the fish.

Disinfect surgical tools, telemetry tags, and antennae threading needle in a 70% or greater concentration of isopropyl alcohol (there are other appropriate solutions available) before and in between procedures. Ensure any tissue on instruments is wiped clean between procedures. Rinse equipment with sterile saline solution prior to surgery, to avoid irritation to the tissue caused by the alcohol.

An ideal site of the incision (as the sturgeon lays abdomen-up) is 3 to 5 ventral scutes anterior from the pelvic fin and 2.5-5.0 cm off the ventral mid-line. Exact position varies between individuals and sturgeon species. This places the incision lateral to the mid-line in well vascularized and thicker muscle tissue to ensure proper healing.

The transmitter is inserted into the peritoneal cavity through an incision that will be closed with 3–4 sutures evenly spaced along the length of the incision.

Using the scalpel and tissue forceps, use one to two firm strokes to create a 3 - 4 cm long incision (approximately 2 - 4 cm long). One can see the gonad through the incision and determine sex of the fish at this time prior to inserting the tag and before blood from the incision obscures visibility.

Position the veterinary needle so that the sharp tip is protected inside the PVC tube sheath and the needle eye protrudes from the other end of the PVC sheath. Insert the sheathed needle tip posteriorly into the incision while holding onto the needle eye and tubing at the other end (ensure that the sharp needle point does not become exposed). Move the sheathed needle tip along the inside the peritoneal cavity of the fish until the needle point is 5 - 9 cm posterior to the incision and on the midline. By pushing the sheathed tip of the needle upwards (against the inside of the body wall) the position of the needle inside the fish can be identified. When the tip of the sheathed needle is pressing up in the correct location, slowly push the tip of the needle out of the PVC sheath (by sliding the needle eye into the sheath) and pierce the body wall. Once the tip of the needle exits the skin, stop and slide the sheath off of the needle in the direction of the needle eye. The needle eye should still be protruding from the incision. Now thread the radio-transmitter antenna through the needle eye and pull the needle (with the antenna) all the way out of the peritoneal cavity. Remove the needle from the antenna which is now protruding from the ventral midline. Insert the transmitter into the incision, this is best done by inserting the rounded end of the transmitter (end without the antenna) into the incision first. After the transmitter has been inserted, pull the tip of the protruding antenna posteriorly so that the remaining segment of the antenna nearest to the transmitter slips into the incision and the transmitter slides posteriorly and ends up snug against the inside of the body wall where the antenna protrudes. The incision is now ready to be closed with sutures. Use a simple interrupted suture or an interrupted cruciate (also called cross or X- mattress) suture to close the wound. Small incisions can be closed with a couple of sutures. Longer incisions may require more.

We recommend and describe a modified instrument surgeons' knot that is made by following these steps

Step 1) With the needle holder in your right hand, grasp the needle, and pass the needle through both sides of the incision, (either in one complete motion or go through one side and pull the needle up through the incision, reposition the needle into the needle holder, and then go through the other side). Pull the suture through leaving a 2 cm free end ("free end" refers to the end without the needle). Beginners may choose to leave a slightly longer free-end to avoid accidentally pulling the end through the incision during throws.
Step 2) Release the needle from the holder and grasp the needle end of the suture with the left hand. Position the needle holder parallel to the incision and against the far side of the suture, and with the left hand wrap the needle end of the suture around the needle holder 2-3 times (we usually use three wraps so when tightened it will not slip, although two throws can also have minimal slippage).
Step 3) Grasp the 2 cm free end with the needle holder and the wrapped suture will slide off the holder to encircle the 2 cm end.
Step 4) To tighten the throw, pull the 2 cm free end (grasped in the needle holder jaws) toward you and the needle end (held in your left hand) away from you making sure the suture lies flat.
Step 5) The second throw begins with releasing the 2 cm free end from the needle holder and position the needle holder parallel to the incision and against the near side of the suture and wrap the long end twice around the needle holder with the left hand (in the opposite rotation as Step 2), grasp the 2 cm free end in the needle holder and pull it through the wrap, and tighten the throw by pulling the 2 cm free end away from you and the needle end toward you.
Step 6) The third throw is a single wrap following steps 2, 3, and 4.
Step 7) The fourth throw is also a single wrap, following step 5.

We call this the 3,2,1,1 or 2,2,1,1 knot, referring to the number of wraps in each throw. The needle holder should remain parallel to the incision when tying knots and should be moved back and forth perpendicular to the incision. Because the monofilament suture has a low coefficient of friction it requires four or more throws to prevent knot slippage. The distance of the needle puncture from the edge of the incision should be about equal to the depth of the layer of tissue being sutured (about 0.6-1.2 cm in white sturgeon) and include equal amounts of tissue on either side of the incision; different amounts of tissue or different suture depths can cause the skin edges to overlap.

The knot of the interrupted sutures should be offset slightly so as not to rest directly on top of the incision, and should be snug but loose enough to slide the tip of your needle holder underneath. If the knot is too tight it may cause tissue strangulation or may tear away, depending on the amount of edema or tissue necrosis that occurs after suturing. After tying the suture material, it should be trimmed to about 3-5mm. If you trim too close the knot may unravel but excess material increases foreign material tissue reaction.
Post-operative fish can be held for a few minutes after surgery and then released. Total time from capture to release should range 10 - 25 min. Developing speed and expertise will reduce stress to the fish.

Additional Reference Resources

Berg, J. 1993. Sterilization. Pages 124-129 in D. Slatter, editor. Textbook of small animal surgery. W.B. Saunders Company, Philadelphia, Pennsylvania.

Boothe, H.W. 1998. Selecting suture materials for small animal surgery. Small Animal 20(2): 155-163.

Chapman, F. and C. Park. 2005. Comparison of sutures used for wound closure in sturgeon following a gonad biopsy. North American Journal of Aquaculture 67:98-101. Giddings, F.D. 1997. Surgical knots and suturing techniques. Giddings Studio Publishing, Fort Collins, Colorado. 37pp.

Gilliland, E. 1994. Comparison of absorbable sutures used in largemouth bass liver biopsy surgery. The Progressive Fish-Culturist 56: 60-61.

Knecht, C.D., A.R. Allen, D.J. Williams, and J.H. Johnson. 1987. Fundamental techniques in veterinary surgery. 3rd edition. Saunders, Philadelphia. Summerfelt, R.C., and L.S. Smith. 1990. Anesthesia, surgery, and related techniques. Pages 213-272 in C.B. Schreck and P.B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Appendix 6 Oocyte Sampling utilizing extractor

Materials List:

- Precision miniature stainless steel tubing; 9 gauge 0.135" ID, 0.148" OD; 1 ft. piece makes 3 needles
- Miniature diamond needle file; half-round, Fine (170/200 grit)
- PVC tubing 1/8" ID, ¼" OD; 5/32" ID x ¼" OD airline tubing works also just doesn't grip as tight and can work loose over time
- Syringes – slip tip syringes work better than luer-lock; tubing doesn't hold on the luer-lock very well

Directions:

The oocyte sample is collected through the abdominal wall on the fish, adjacent to the midline on the posterior half of the abdomen (figure 1). To use the device properly:

1) Sterilize the needle in 95% ethanol.
2) Fill the needle, tubing and syringe up to 5 ml with Ringer's solution (6.78g NaCl, 0.216g KCl, 0.2g Ca Cl_2, 1.192g HEPES, 1L distilled water) or physiological buffered saline so that all air bubbles are removed from the needle and syringe.
3) Insert the point of the needle at a 35° angle, with the beveled side away from the fish, so that the bevel cuts the flesh allowing only three-fourths of the beveled edge to enter the flesh (figure 2).
4) Remove the needle and rotate the bevel 180° so that it is now facing the abdomen. Failure to rotate the bevel will cause the needle to cut a hole in the abdominal wall instead of leaving a flap of skin and could potentially damage other organs.
5) Lift the small flap of skin created by the first puncture gently and reinsert the point of the needle into the same hole, approximately two centimeters into the fish (figure 3)
6) Pull up on the syringe's plunger until an air bubble appears in the PVC tubing or until the plunger does not move easily.
7) Stop applying pressure to the plunger and remove the needle from the fish.
8) Expel the oocyte sample from the needle by pushing down on the plunger and push the flap of skin back into place for proper healing (figure 4). Occasionally the tip and cutting edge needs to be re-sharpened using a small needle file.

Appendix 6 continued - Egg Sampling utilizing extractor

Figure 1.

Figure 2.

Figure 3.

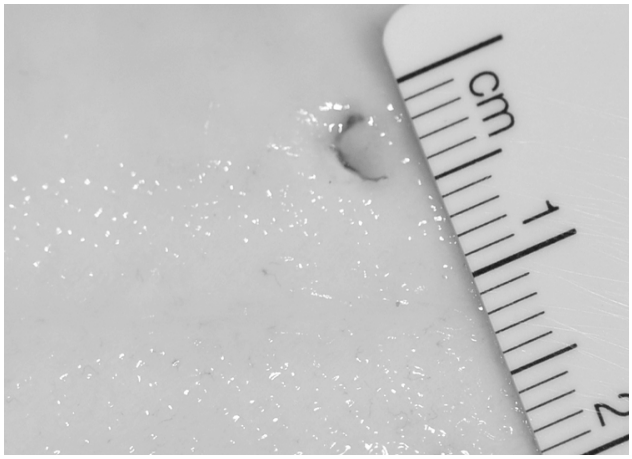


Figure 4.

Appendix 7 Fish Health Tissue Collection Protocols

The initial detection of an iridoviral agent in cultured shovelnose sturgeon 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.
* Utilize new or sterilized sampling tools and change gloves 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 Dietrich's fixative.
* Place fish tissues into the Dietrich's fixative at a ratio of one (1) part tissue to 10 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.

Collection for Viral DNA analysis:

* Collect fin punches from the caudal and pectoral fins using a paper hole punch. Sterilized scissors may be used to clip the edge of the fins.
* Collect a portion of barbels with sharp sterilized 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.
* Utilize separate tools for each sample. If using the same instruments between samples, disinfect with 10% bleach solution with multiple rinses in fresh water to remove the bleach.
* 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 such as 70% Isopropanol or 70% Ethanol is preferred.

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 Dietrich'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
* Sample containers can be glass or chemical resistant plastic.

Collecting Virology Cell Culture Samples:
* Collect internal samples mainly from the kidney and spleen for virology testing.
* Samples can be taken individually or five fish pooled.
* Always use sterilized dissecting tools. Disinfect between sample pools. 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 8 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 thoroughly 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 10)

Appendix 8 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 10 Chain of Custody Record

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:**	

TEM 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** ☐ **IN PERSON** ☐ **FEDEX:** ☐ **OTHER** ☐
	TO:	**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:**	

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:**	