# Basin-wide Pallid Sturgeon Propagation Plan

## Prepared by the
## Basin-Wide Pallid Sturgeon Propagation Committee
## 30 September 2016

Molly Webb[1], Bob Snyder[2], Jake Colehour[3], Rob Holm[4], Lacey Hopper[5], Kevin Kappenman[1], Roderick May[6], Jeff Powell[7], Mike Rhodes[8], Ken Staigmiller[9], and Matt Toner[1]

[1]Bozeman Fish Technology Center, USFWS
[2]Private Contractor
[3]Blind Pony State Fish Hatchery, Missouri Department of Conservation
[4]Garrison Dam National Fish Hatchery, USFWS
[5]Bozeman Fish Health Center, USFWS
[6]Neosho National Fish Hatchery, USFWS
[7]Gavins Point National Fish Hatchery, USFWS
[8]Miles City State Fish Hatchery, Montana Fish Wildlife and Parks
[9]Great Falls Fish Health Laboratory, Montana Fish Wildlife and Parks

Photo Credit: Christopher S. Guy

**Table of Contents**

## Introduction

First described by Forbes and Richardson in 1905, the pallid sturgeon (*Scaphirhyncus albus*) was listed by the United States Fish and Wildlife Service as an endangered species on September 6, 1990. The pallid sturgeon is one of the rarest and largest fish species found in the Mississippi and Missouri rivers, attaining a length of 6 feet and weight of 80 pounds. Pallid sturgeon are genetically similar to the more common shovelnose sturgeon (*Scaphirhyncus platorynchus*) and hybrids have been documented in wild populations.

While limited specific knowledge exists about its life history, the pallid sturgeon is known to be a long-lived, late-maturing iteroparous species. Although the life expectancy of the pallid sturgeon is unknown, individuals older than 50 years have been documented and the maximum age is estimated to be between 50 and 60 years old.

Age at first sexual maturity was determined for pallid sturgeon by Keenlyne and Jenkins (1993) using "spawning bands" which are narrow annuli formed in the leading ray of the pectoral fins. Using this technique, age at first maturity for female pallid sturgeon was determined to be 9-12 years with first spawning at age 15, and male pallid sturgeon maturity was determined to occur at 5-7 years. However, this method may be inaccurate as other factors such as growth and health may influence annuli formation. Hatchery-reared male and female pallid sturgeon released in the lower Missouri River were first documented in spawning condition in 2006 at 9-14 years of age (DeLonay et al., 2009). Hatchery-reared pallid sturgeon males in the upper Missouri River reached sexual maturity at 10 to 17 years old (773-1170 cm fork length and 1.775-7.380 kg body weight), while females reached first maturity at 18 years old (1070-1151 cm fork length and 6.2-6.8 kg body weight; Webb et al., 2016). The spawning periodicity of the pallid sturgeon is estimated to be 1 to 3 years for males and 2 to 6 years for females.

The limited knowledge about the specific dietary, rearing and spawning requirements, in addition to the species' large size, late maturity, longevity, and relatively long spawning periodicity, create challenges for broodstock management and propagation of this endangered species.

Both endogenous and environmental factors control the biological clock in sturgeons (see Webb and Doroshov 2011). The relative importance of the environmental factors controlling reproduction and the magnitude of change of these factors required to initiate key gametogenic stages and a spawning event have not yet been well defined for pallid sturgeon. Photoperiod, water quality, water velocity, water temperature (temperature units, maximum, minimum, rate of change), turbidity, spawning substrate (hard substrate for egg to adhere to), presence of other pallid sturgeon (pheromones stimulate/coordinate maturation), diet (forage availability and quality, effects on body condition and gamete quality due to nutrition) are all suspected to affect gamete production, gamete quality and spawning success.

Because pallid sturgeon populations are limited in number and declining, with rare natural spawning and insufficient recruitment, the Pallid Sturgeon Recovery Plan recommends a propagation program as a short-term recovery objective to perpetuate the species until habitat modifications can occur to allow natural spawning and recruitment.

## Objective

The objective of this *Basin-Wide Pallid Sturgeon Propagation Plan* is to describe, document, and guide the fish culture methods that will be used to propagate the species throughout the species' range. This plan outlines the processes and procedures that will minimize anthropogenic mortality of wild and captive fish, and provide healthy, hatchery-produced fish that will meet recovery goals. Stocking of hatchery reared pallid sturgeon will be conducted in accordance with the *Pallid Sturgeon Range-wide Augmentation and Stocking Plan* (USFWS 2008).

This plan can be revised at any time by the Basin-Wide Pallid Sturgeon Propagation Committee (Propagation Committee) to reflect new information or needed modifications in methods, techniques or culture parameters. However, significant deviation from the methods and parameters established in this plan can occur only if reviewed and approved by the Propagation Committee. The guidelines for the culture of pallid sturgeon established in this plan should be used when evaluating the fish culture practices at existing facilities, including experimental facilities, or in determining the suitability of a facility for the culture of pallid sturgeon.

## Changes from Previous Plan

The previous Pallid Sturgeon Propagation Plan was written to identify those management practices for Upper Basin pallid sturgeon hatcheries that promoted successful spawning and produced healthy pallid sturgeon. This current manual expands its coverage to include Middle Basin hatcheries. At the time this plan was written, the following hatcheries are, or recently have been, involved in pallid sturgeon propagation:

Miles City State Fish Hatchery (MTFWP)
Garrison Dam National Fish Hatchery (USFWS)
Gavins Point National Fish Hatchery (USFWS)
Blind Pony State Fish Hatchery (MDOC)
Neosho National Fish Hatchery (USFWS)

The Lower Basin does not have a pallid sturgeon propagation program at this time (2016). Should the Lower Basin initiate the propagation of pallid sturgeon for conservation stocking using Lower Basin hatcheries, this plan will be expanded to include these hatcheries.

The iridovirus originally identified as the Pallid Sturgeon iridovirus has been found to be endemic in wild populations of shovelnose and pallid sturgeon in the Upper Missouri basin and has been renamed Missouri River Sturgeon iridovirus (MRSIV). A Polymerase Chain Reaction (PCR) test has been developed to detect the presence of MRSIV.

Since the development of the previous iteration of this manual, more is known about the spawning and propagation of pallid sturgeon and pallid sturgeon propagation has made advances. Spawning protocols have been improved and yield more consistent identification of adult spawning readiness and the production of viable gametes. Through the process of hatchery technical reviews, Upper Basin pallid sturgeon facilities have identified and implemented changes that have addressed fish health, water quality, and infrastructure problems. These changes have resulted in more consistent production of healthier fish for conservation stocking,

avoidance of epizootic events, and management of pathogens that had previously caused catastrophic losses.

Middle Basin pallid sturgeon hatcheries have recently experienced fish health problems that have impacted their stocking programs. Epizootic outbreaks of *Ranavirus* have caused catastrophic losses of pallid sturgeon at Blind Pony State Fish Hatchery (SFH) and changes in water sources and apparent changes in water source chemistry are causing fin curl in juveniles produced at Neosho National Fish Hatchery (NFH). Hatchery technical reviews of these Middle Basin pallid sturgeon hatcheries have been completed in 2016 with the goal of remediating these issues.

**Gamete Biology**

Like teleosts, sturgeons have a biological clock controlled by endogenous and environmental factors (e.g., Doroshov et al. 1997; Webb et al. 2001; DeLonay et al. 2007; Tripp et al. 2009). The clock may begin with sex differentiation, but knowledge of this event in sturgeon is largely limited to morphological studies (Persov 1975; Fedorov et al. 1990; Grandi et al. 2008). Puberty is a hallmark in the onset of a reproductive cycle and the timing of puberty depends, as in teleosts (Taranger et al. 2010), on age and body size, accumulated energy (e.g., lipids), and environmental cues. The first reproductive cycle is activated by neuroendocrine signals, with endogenous factors acting as the gates to first maturation (Doroshov et al. 1997). Gametogenesis and spawning are controlled by energy balance, environmental factors, and the neuroendocrine reproductive axis (Moberg et al. 1995; Doroshov et al. 1997; Bruch and Binkowski 2002; Paragamian and Wakkinen 2002; DeLonay et al. 2007; Erickson and Webb 2007). The relative importance of the environmental factors controlling reproduction and the magnitude of change of these factors required to initiate key gametogenic stages and a spawning event have not yet been well defined for many chondrostean species. It will be important to understand the specific role of environmental factors in driving gametogenesis and spawning to address the mismatch that may occur with global climate change.

There are multiple guides or published papers describing stages of gonadal maturity in sturgeon (e.g. Dettlaff et al. 1993; Doroshov et al. 1997; Van Eenennaam and Doroshov 1998; Bruch et al. 2001; Flynn and Benfey 2007; Webb and Erickson 2007; Wildhaber et al. 2007). The stages of maturity described in these papers and guides differ, but regardless of the number of stages, the stages of maturity can be directly applied to pallid sturgeon.

**Broodstock Management**

Although pallid sturgeon recovery ultimately requires natural spawning and recruitment within all or a portion of the species' range, the recovery plan identifies artificial propagation as the only option to perpetuate the species through the existing reproduction/recruitment bottleneck. Artificial propagation will be used to increase the current abundance of pallid sturgeon within the species' historic range until river function and habitat changes allow sufficient natural reproduction and recruitment to maintain the species. The broodstock and stocking programs will have to continue beyond that time when suitable flows and habitats are made available until the reestablished hatchery-based populations are proven to provide the necessary recruitment in those areas where recovery is possible. In areas where pallid sturgeon are not recoverable due to insurmountable problems with spawning and recruitment, long-term stocking programs will be required to maintain the presence of pallid sturgeon.

Little was known about the abundance and distribution of the pallid sturgeon when the original Pallid Sturgeon Recovery Plan was written in 1993 (USFWS 1993). Based on the assumption that there were only a few surviving wild pallid sturgeon, the Pallid Sturgeon Recovery Plan calls for the establishment of three separate broodstocks, each composed of ten to fourteen captive wild fish. These captive broodstocks would then serve as a source of gametes and fish for recovery stocking efforts. The discovery of larger-than-expected numbers of surviving wild fish and the limited available hatchery space made this strategy impractical. Fortunately, the development of relatively effective capture and spawning techniques permitted an optional strategy of capturing, spawning and releasing wild pallid sturgeon to obtain fertilized eggs for the creation of a broodstock population and for production of fish for restoration stocking.

The current pallid sturgeon propagation program includes a dual strategy:

1) use the offspring of artificially spawned wild pallid sturgeon to provide fish for conservation stocking and research and,
2) use a captive broodstock (in the Upper Basin) and cryopreserved milt repositories to serve as genetic reserves and produce gametes and fish for conservation stocking and research.

The spawning protocols described in this Plan are designed to capture and preserve as much of the wild genome as possible for representation in the captive broodstock program. However, the wild populations of pallid sturgeon consist of old-aged (>50 years old) fish (Braaten et al, 2015). Eventually, the wild pallid sturgeon populations in the Upper Basin will cease being reliable sources of gametes due to decreasing numbers or their extinction. Exactly when this will occur is currently unknown, but is expected to be within the next decade.

With the demise of wild pallid sturgeon as a source of gametes in the Upper Basin, the captive broodstock, currently held at Gavins Point NFH will, with the populations established in the Upper Basin through conservation stocking, become the only sustainable, genetically-diverse source of gametes and fish for the long-term maintenance of pallid sturgeon. Habitat and other environmentally-limiting factors are being addressed through conservation recovery actions with the goal that natural recruitment will occur again in the wild.

The Middle Basin wild pallid sturgeon population is also at risk due to the impacts of low body condition on gamete production. Wild fish captured for use as broodstock often require conditioning prior to reproductive cycling to promote gamete production. It is not the intent of this program to permanently remove these wild donors from their environment. Captured wild fish will be kept alive and returned to the river reaches where they were captured.

## Wild Broodstock Program

*Donor Populations*

Only genetically pure pallid sturgeon will be used for establishing the captive broodstock and for restoration stocking. It is recommended that only fish within their own management unit may be used as donors when supplementing a population with hatchery-released pallid sturgeon to reduce outbreeding depression.

Wild donor fish captured from the Great Plains Management Unit (GPMU) will be used to produce progeny for the establishment of the captive broodstock population maintained at Gavins Point NFH and for conservation stocking in the GPMU. Wild fish from the Central Lowlands Management Unit (CLMU) and Interior Highlands Management Unit (IHMU) will be used to produce offspring for conservation stocking in the Middle Basin. There is currently no captive broodstock population for the Middle Basin. No source of pallid sturgeon for the Lower Basin has been identified at this time, as there are no current plans for conservation stocking in the Coastal Plains Management Unit (CPMU).

*Handling & Stress*

Capture, handling, transportation and spawning are all stressful to pallid sturgeon. The need to minimize the stress of capture cannot be over-emphasized. Stress reduces the probability of survival of the fish, compromises the fish's immune system, and can precipitate fish health problems and disease. It is important to eliminate as many sources of stress and reduce the incidence of stress as much as possible. Multiple stressors can significantly decrease the ovulatory success, increase the chances of follicular atresia, and increase the variability in embryo survival to hatch. Further work is needed to identify and reduce the sources of stress in the captured broodstock. All propagation procedures should be periodically reviewed by the Propagation Committee to identify and mitigate sources of stress.

The current version of the *Biological Procedures and Protocols for Researchers and Managers Handling Pallid Sturgeon* (USFWS 2012) will guide the capture, handling, transportation, holding, spawning and release of wild adult pallid sturgeon used as broodstock. The current version of this protocol can be found on the USFWS's Missouri River Fish and Wildlife Management Assistance Office's website at https://www.fws.gov/mountain-prairie/endspp/protocols/PallidSturgeonHandlingProtocol.pdf. This protocol will be reviewed and updated when necessary.

*Collection of Adults*

It is extremely important that the maximum amount of the existing wild pallid sturgeon genome be captured and preserved within the captive broodstock population and be represented in the reestablished populations within the recovery areas. In order to accomplish this, captured adult pallid sturgeon will be selected based on the following prioritization:

1) Fish that have not previously contributed to the creation of progeny (i.e. "new" fish).

2) Recaptured fish that have been spawned but are significantly underrepresented in the population of released fish or captive broodstock population.

3) Recaptured males that have been spawned, but are not represented in the cryopreservation repository.

4) Should wild pallid sturgeon numbers decrease to the point where no or few new fish are captured, but it is determined by the Propagation Committee that it is necessary or

desirable to reuse previously-spawned wild pallid sturgeon to create new, unique matings, recaptured fish may then be brought into a spawning facility.

All fish that are adequately represented in the captive broodstock population, the cryopreservation repository and at least one reestablished population in the wild, will be released immediately.

The USFWS will maintain the database for captured pallid sturgeon. Copies of the fish database will be distributed to each capture boat and transport truck to facilitate a quick determination of whether a fish should be released or held as a donor based on its PIT tag. In order to facilitate the selection of captured pallid sturgeon for spawning, this database will be sorted by PIT tag number and by second PIT tag, if any. Entries for previously captured fish will be color-coded to indicate where the fish ranks within the selection priorities.

Immediately before capture operations begin, there will be a pre-collection coordination meeting to review the pallid sturgeon handling protocol, to distribute the current pallid sturgeon database used to determine what fish are to be kept and to make sure all capture teams have all of the appropriate equipment for sampling.

The collection of wild adult pallid sturgeon for use as broodstock occurs in the Upper Basin in the spring. The adults collected are spawned that summer. The Middle Basin wild broodstock collections occur in the fall and spring. Fish that will not spawn in the year they are captured are usually held until they are reproductively ready. This also provides the opportunity to improve the condition of often emaciated fish to produce viable gametes.

Adult broodstock will be collected from the river in a manner consistent with the most recent version of the *Biological Procedures and Protocols for Researchers and Managers Handling Pallid Sturgeon* (USFWS 2012) and in compliance with specific state or federal permit conditions. Stress during capture can be reduced by minimizing handling and the time a fish is kept out of water, maintaining adequate water quality in holding tanks, and keeping transportation times as short as possible. Fish must not be held out of water for longer than 2 minutes, unless the gills are irrigated. If a pallid sturgeon is extremely tangled in the trammel net, gill net, or trot line, the net or line must be cut to minimize handling stress and the time the fish is held out of water. Holding tanks in the capture boats should be a minimum of 6 feet in length and made of plastic or other non-abrasive material (if metal, tanks must be lined with a spray-on bed-liner compound or a similar substance). Holding tanks must be covered when transporting fish (although a tarp is preferred, a raincoat works in an emergency). Holding tank water should be exchanged at least once every 15 minutes using an electric aerator, a bilge pump, or a bucket. A non-abrasive cradle, preferably with a hood, should be used to move fish.

All capture boats must have a PIT tag reader and a coded wire tag reader. The use of heavy-duty alkaline batteries in all PIT tag readers is required, as weak batteries in PIT tag readers can cause problems with the detection of PIT tags. Therefore, all boats should carry fresh additional batteries. Fish should be inspected immediately upon capture for internal and external tags. Extreme diligence is needed when searching for PIT tags. Tag location and depth within the fish, reader orientation, and false readings as the result of conflicting signals when there are two

PIT tags can affect the detection and reading of PIT tags. Fish that have not been previously PIT-tagged will be immediately PIT-tagged according to the *Protocols for Tagging and Marking Hatchery Reared Pallid Sturgeon in Recovery Priority Management Areas 1, 2, and 3* (Jaeger et al. 2007) for Upper Basin fish. The Middle Basin uses a Middle Basin Broodstock Collection Datasheet that lists the Middle Basin collection protocols and records capture data. Two 1 $cm^2$ samples of caudal fin from each "new" fish will be collected for genetic analysis. One fin clip will be placed in properly labeled vials containing 95% denatured ethanol according to the protocol described in Appendix A. The second fin clip will be collected and placed in a pre-labeled vial with ATL buffer for an iridovirus screen according to the protocol described in Appendix B. Physical measurements and collection information will also be taken and recorded at this time according to the protocol described in the *Biological Procedures and Protocols for Researchers and Managers Handling Pallid Sturgeon* (USFWS 2012).

To provide protection against stress-induced bacterial infections, trained personnel can administer a prophylactic intramuscular antibiotic injection to each pallid sturgeon prior to their transport to the spawning facility. Fish that will be released into the river will not be injected unless urogenital catheterization or gonadal biopsy has been conducted. Antibiotic injection will be administered into the dorsal musculature according to the protocols described in "Use of Injectable Drugs" in Fish Health section of this document.

*Sexing and Staging of Potential Donors*

Initial determination of the sex and stage of maturity of a captured pallid sturgeon should occur at the time of capture. As many of the wild pallid sturgeon have been previously handled, the database should be referenced for information on the sex and stage of the fish as well as the use of the fish for the season in the broodstock program. Urogenital catheterization, gonadal biopsy, or ultrasound is performed by trained personnel while the fish is held in a holding tank in the capture boat or on the transportation truck. If the fish is new or has been previously identified as a fish that will be spawned in the broodstock program, the sex and stage of maturity may be determined at the spawning facility. Upper Basin females with immature ovarian follicles that will not reproduce that season will be released immediately. Non-reproductive Middle Basin females may be held for conditioning and spawning in subsequent years as needed. Occasionally, the catheterization of testes confirms the fish is a male, however, stage of maturity is not visually identifiable. Given the age of the wild pallid sturgeon, males are taken into a spawning facility based on the selection criteria described below, and plasma sex steroids may be used to assess spawning readiness in males. Sex and stage of maturity can be determined in females visually and gonadal development will be classified according to Table 1.

Table 1. Stages of female gonadal development with macroscopic and histological descriptions.

| | Developmental Stage | Description |
|---|---|---|
| Differentiation | 1 | Ovarian groove starts to develop into the small and very thin ovigerous "ribbon" which contains clusters of oogonia and potentially a few very small oocytes just beginning the cytoplasmic growth phase. |
| Previtellogenic | 2 | Obvious ovigerous folds with small translucent oocytes. Histology reveals previtellogenic oocytes in the endogenous growth phase. The follicular epithelium (granulosa) in the larger sized oocytes begins mitotic proliferation, and the outer follicular layer (theca) has some vascularization. (~ 0.2-0.4 mm). |
| Early vitellogenic | 3 | Ovigerous folds contain small white oocytes. The distinguishing histological features are the differentiation of the first layer of the egg chorion, zona radiata 1. The granulosa cells increase in thickness and become cuboidal, and the thecal layer is vascularized. There are some small yolk platelets in the cytoplasm (~ 0.5-1.0 mm). |

| | | |
|---|---|---|
| Mid-vitellogenic | 4 | Ovigerous folds contain ovarian follicles that are white to cream to yellowish in color. Differentiation of the second and third layers of the chorion has occurred. As ovarian follicle size increases, the density and size of yolk platelets also increases (1.0-1.5 mm). |
| Late-vitellogenic | 5 | The ovarian follicles darken in color as melanin pigment is deposited in the egg cortex. Grey to black ovarian follicles are visible (1.5-2.5 mm). |
| Post-vitellogenic | 6 | Fully grown ovarian follicles are usually black (but can vary from olive brown to greyish). The nucleus (germinal vesicle) moves off-center towards the animal pole. The animal pole contains small, round yolk platelets, while the vegetal hemisphere contains larger, oval-shaped yolk platelets and numerous medium-sized oil droplets (>2.5 mm). |
| Oocyte maturation/Ovulation | 7 | Oocytes have undergone the final stages of maturation (i.e., germinal vesicle breakdown) and are ovulated. Eggs are freely flowing from the vent went captured in the wild or from a hormonally induced captive fish. |

| | | |
|---|---|---|
| Post-ovulatory | 8 | Ovaries contain numerous postovulatory follicles and the next generation of oocytes similar to Stage 2 and sometimes Stage 3. The often reddish appearance of ovarian tissue is a result of vascularization for weeks after spawning. |
| Atretic | 9 | Oocytes in the ovary are soft, crush easily, and have a marbled appearance. Histology reveals atretic vitellogenic or mature follicles containing residual yolk and/or melanin pigment. |

Spawning readiness in post-vitellogenic females can be determined by the position of the nucleus (germinal vesicle) in relation to the animal pole and germinal vesicle breakdown in response to progesterone *in vitro*. As a pallid sturgeon's eggs approach ovulation, the germinal vesicle migrates toward the animal pole of the egg. The distance of the germinal vesicle to the animal pole relative to the egg diameter is the oocyte polarization index (oocyte PI; Figure 1). Ovarian follicles are collected to monitor development by urogenital catheterization or gonadal biopsy. During catheterization, a 1/8" ID, semi-rigid translucent tube (ice machine tubing works well), disinfected with sterilizing solution such as Cidex Advanced Sterilization Products or 70% ethyl alcohol and then rinsed in sterile saline prior to use. The tubing is inserted through the urogenital pore into the egg mass. Ovarian follicles are aspirated into the tube and placed in Ringers solution. During gonadal biopsy, ovarian follicles are collected by making a small abdominal incision (0.5 cm) just off of the ventral midline. A sterile catheter (4 mm inner diameter) is inserted into the incision and ovarian follicles are removed by gentle suction. The incision is closed with a single cross stitch or two single stitches using monofilament Polydioxanone (PDS) or the multifilament vicryl (polyglactin 910) suture with a Cutting CP-2 needle. Both PDS and vicryl have an anti-bacterial "Plus" version which is highly recommended. Ovarian follicles may also be removed using an oocyte-extracting device described in Candrl et al. (2010). A minimum of 80 eggs are required for calculation of oocyte polarization index and the oocyte maturation assay. Twenty to thirty ovarian follicles are boiled for 5 minutes in Ringers solution, cooled on ice for 20 minutes, fixed in 10% phosphate buffered formalin for at least 12 hours, and bisected along the animal-vegetal pole axis for calculation of oocyte PI. If collection of ovarian follicles occurs in the field, follicles may be placed directly in 10% phosphate buffered formalin for calculation of oocyte PI. Sixty eggs are required for the oocyte maturation assay as described below.

Monitoring oocyte PI and performing the oocyte maturation assay have been used to determine the proper time to induce ovulation by injecting luteinizing hormone-releasing hormone analog (LHRHa), a hormone analog that promotes oocyte maturation and ovulation. A female is considered to have reached spawning readiness when the oocyte PI is less than 0.10, however a lower oocyte PI is preferred prior to hormonal injection to induce ovulation. See Appendix C for the protocol for calculation of oocyte PI.

Oocyte maturation assays (see Appendix C) in combination with oocyte PI have been used to monitor the egg development of pallid sturgeon in order to determine spawning readiness. The use of progesterone assay is not recommended as a stand-alone technique for accurately staging pallid sturgeon ovarian follicles.

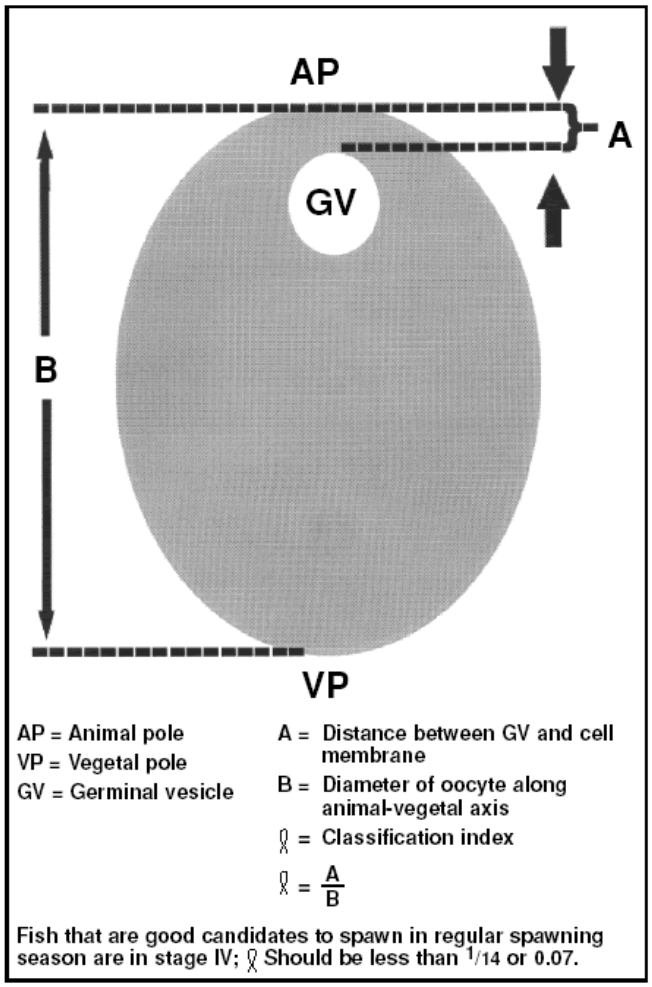



*Figure 3. Diagram of a sturgeon oocyte showing the position of the germinal vesicle (GV) at the animal pole and the formula for determining the oocyte polarization index (PI) (modified from Detlaf et al., 1981, and Conte et al., 1988).*

Figure 1. Oocyte polarization index (from Mims, S.D., A. Lazur, W.L. Shelton, B. Gomelsky and F. Chapman. 2002).

*Post-capture Transportation to Hatchery*

Round tanks are best for transportation of large pallid sturgeon. Pallid sturgeon should be transported in river water obtained near the capture site. During transportation, tank water temperature should be maintained within ± 5ºF (3ºC) of ambient river water temperature. Gas supersaturation causes gill embolisms in pallid sturgeon, therefore tank water oxygen levels should be kept above 5 ppm, but less than saturation. Electric agitators can help reduce oxygen supersaturation. The use of an oxygen meter should be used during transportation to monitor gas saturation levels.

To reduce the osmotic potential of the hauling water, to stimulate mucous production, and to provide some protection from parasites and bacteria, non-iodized salt can be added to the hauling water to provide a 0.25-0.5 percent salt solution (2-4 pounds of salt per 100 gallons).

If possible, adjust the water temperature at the spawning facility to approximate tank temperature. If this is not practical, temper the fish if the tank water and hatchery water temperatures vary by more than 5°F (3°C). The temperature change should not exceed a 4°F (2°C) change per hour.

*Holding Wild Adult Fish in Hatcheries*

Hatchery spawning facilities should have filtered, disinfected (UV or ozonated) water supplies to improve water quality and reduce pathogen loads. Disinfection units should be designed to handle a facility's historic pathogens and typical flows. A chart showing the recommended minimum applied ultra-violet radiation dosages to control common fish pathogens appears in Appendix D. Added security can be achieved if disinfection systems are designed with redundant disinfection units and independent, backup power supplies. Even if redundant units are only sized for part of the total available flow, they allow the continued disinfection of incoming water should the main unit fail or require maintenance or repair. An independent power supply assures that filtration and disinfection are maintained in the event there is a power failure or other electrical problem.

Tanks should be round and have smooth (gel coated or lined) bottoms and sides to minimize abrasion. It is recommended that no more than 10 adult pallid sturgeon be kept in a 20 feet diameter tank. The numbers of pallid sturgeon kept in tanks with other diameters should be scaled according to tank size. All tanks should contain both males and females to take advantage of any possible pheromone or behavioral cues that might stimulate hormonal responses and gamete maturation.

Tank room light levels should be kept low, while providing a natural photoperiod. Tanks can be covered to reduce light levels and to eliminate potential injury or mortality caused by fish jumping out of tanks. Handling and human contact should be minimized as much as practical to reduce stress and injury.

It is important that captured pallid sturgeon be given the opportunity to feed during captivity. This can best be achieved by the use of fish or other live forage. In order to reduce the possibility that a fish pathogen is introduced with the live forage, it is preferable to have the live forage raised on site. If on-site forage is unavailable, live forage should be obtained from a "disease free" source. Live forage should be administered "by eye". Forage density should be held at a level where it is easily utilized by the pallid sturgeon, however live forage can be overstocked. Experience with rainbow trout used as forage has demonstrated that if rainbow trout are stocked too densely, they will pick on and damage the exposed gill filaments of the pallid sturgeon. As a guideline, Gavins Point NFH feeds 1000 4-5" rainbow trout per tank per month with 6-7 adults per tank.

The health and condition of the captured fish will be monitored regularly. Signs of disease, behavioral changes, changes in feeding patterns or extreme (typically >20%) loss of body weight will initiate contact with fish health personnel for health inspection, diagnosis, and treatment.

Experience indicates that a suitable temperature range for spawning pallid sturgeon is 61-68°F (16-20°C) in the Upper Basin and 54-68°F (12-20°C) in the Middle Basin. Because warm water temperatures can induce follicular atresia, water temperatures throughout the spawning process should be maintained at approximately 61-64°F (16-18°C) in the Upper Basin and 59-64°F (15-18°C) in the Middle Basin. Immediately after spawning is completed, water temperatures should be gradually reduced 5-7°F (3-4°C) to slow pathogen reproduction and minimize stress in the fish.

Captured pallid sturgeon occasionally die when held in captivity. Pallid sturgeon have difficulty tolerating handling and the accumulated stressors of artificial spawning and captivity. It is important to understand why adult pallid sturgeon die in captivity in order to improve culture processes, to improve the overall success of the pallid spawning and culture program, and to improve our understanding of pallid sturgeon physiology. To better identify the causes of mortality in captured fish, it is recommended that a fish health specialist inspect adult sturgeon that become moribund (defined as "are dying"). Section 10(a)1(A) permits for each spawning facility should reflect the need to sacrifice fish immediately before their death for diagnostic purposes. When an adult pallid sturgeon becomes moribund or dies, the Recovery Team Leader and a regional fish health biologist must be notified. Fish health or trained hatchery personnel can take tissue samples following the established *Protocol for Collection of Biological Samples from Moribund Pallid Sturgeon* (Appendix E). Because tissue samples deteriorate rapidly due to post-mortem changes, tissue samples must be collected within 20 minutes of death to be of value. A typical mortality report should contain the tag number of the fish, the estimated date and time of death, the circumstances that lead to the mortality, and any actions taken (if applicable) to prevent other mortalities. As a last resort, if tissue samples aren't collected, fish should be kept whole and placed on ice or frozen.

*Returning Adults to the Wild*

Post-spawn adults will be returned as soon as they are determined to be healthy enough and the receiving waters' temperatures are adequate. The white sturgeon recovery program releases their hand-stripped females one week after spawning. If fish are to be tagged, tagging should occur as soon as possible after spawning has been completed to minimize the time the fish are held. If it is necessary to hold post-spawn brood fish, their holding water temperature should be gradually reduced 5-7ºF (3-4ºC) to slow pathogen reproduction and reduce stress. Adults can be released at any available site within the RPMA from which they were collected.

Tagging adult pallid sturgeon using Combined Acoustic/Radio Transmitters (CART tags) is necessary for movement and habitat use research. While captured pallid sturgeon broodstock offer a readily accessible source of adult fish for tagging, the additional stress of holding them until they can be tagged and the implantation of CART tags immediately post-spawn may be sufficient to cause mortality in brood fish. If an individual fish's post-spawn health allows, adults can be implanted before their release. The manager of the hatchery holding the post-spawn adults will make the final determination of whether the adults can withstand the additional stress of transmitter implantation. Instead of tagging post-spawn pallid sturgeon adults, it is preferable to CART tag adult pallid at other opportunities such as when adult sturgeon are captured during either the spring or fall brood fish collections but not held for spawning.

## Captive Broodstock

*History*

In 1991, it was determined that the pallid sturgeon recovery program should establish a minimum of three captive broodstock populations, each genetically representative of the wild populations from which they came. Each population was to be maintained at a separate facility to guard against a catastrophic loss, conserve unique genetic material, and preserve options for future recovery activities. Wild broodstock were to be removed from three regions or reaches of the Missouri River spanning the pallid sturgeon's range. They were to be spawned with the resultant progeny (year-classes and families) used to establish a captive broodstock that will preserve the maximum amount of remaining genetic variability. Once the captive broodstock had reached maturity within the hatchery environment, they could be crossed according to the strict spawning protocols described in the *Population Genetics Management Plan for Pallid Sturgeon in the Upper Missouri River Basin* (Heist et al. 2013) to obtain progeny for future recovery efforts.

The only captive broodstock ever established is the one at the Gavins Point NFH, which holds Upper Basin offspring of adults representing the genome of pallid sturgeon from RPMAs 1 and 2 in the Missouri River (above Fort Peck Dam to Lake Sakakawea including the Yellowstone River). No captive broodstock have been developed from any other RPMA. Gavins Point NFH was selected as the site for the pallid sturgeon broodstock program because it had previously been designated as the lead facility for culturing declining fish species within the Missouri River system, the facility has optimum water quality and quantity parameters, the hatchery has excellent sturgeon culture facilities, and, since there is no need to heat or chill water to obtain optimum growth, the culture of pallid sturgeon at this facility is very efficient.

*Captive Broodstock Management*

At this time (2016), only the Upper Basin utilizes a captive broodstock as a genetic reserve of the existing genome of pallid sturgeon in the Upper Basin and as a source of genotypes not represented or under-represented in the stocked population in the Upper Basin. The captive broodstock held at Gavins Point NFH uses genetic procedures, guidelines, and recommendations outlined within this document and the *Population Genetics Management Plan for Pallid Sturgeon in the Upper Missouri River Basin* (Heist et al. 2013) and annual spawning matrices provided by the USFWS Northeast Fishery Center.

The mating design incorporated into the program provides for, as much as possible, the equalization of the contribution of parents to the populations of stocked fish in the Upper Basin. Typically, 1:3 matings are used for Upper Basin fish and 1:2 matings are used by the Middle Basin. These ratios approximate the proportions of each sex captured during adult collections, assure that there will be sufficient milt from "new" males over the time of the conservation stocking program, and equalize parental contribution by standardizing the contribution of females to yield higher effective population size. If more than one female and more than one male spawn simultaneously, they should be injected with the appropriate hormone, spawned, and the green eggs divided into the same number of sublots (cells) as there are spawning males (di-allele mating). There should be matings between all possible parents. Gametes from different individuals will not be mixed prior to fertilization. This type of activity will be continued each year until the desired number of year-classes has been developed. A goal of the pallid sturgeon

recovery program was to have a minimum of 5-10 pairs of adults contributing to each year-class. However, when working with very low numbers of individuals in an endangered species population located within a large river system where adults may spawn every 3-5 years, these numbers may be unattainable. Another goal is to have a minimum of 25-50 mated pairs contributing to the future broodstock at Gavins Point NFH in order to maximize the effective population size. Experimental, reintroduction, or augmentation stocking will be accomplished once progeny have been produced that are genetically representative of the wild population.

Fertilized eggs from each mating (sublot or family) should be kept separate. Progeny should be reared in separate groups until they are large enough to tag (PIT, elastomer, or coded wire). By the time each lot is large enough to PIT tag (10"-14" fork length), equal numbers of fish should be retained out of each sublot for future broodstock. Once PIT tagging (or other selected tagging) has occurred, sublots can then be mixed for further rearing and culture purposes. The rearing regime for these future broodstock will parallel that for any production fish, except there should be no mixing of these two uses of sturgeon. There may, on occasion, be surplus broodstock that may not be needed for that purpose. If this does occur, then this surplus group can be tagged and stocked according to accepted sub-basin stocking plans. There will be a great amount of effort incorporated into the rearing regime to avoid traits introduced due to culture methods or domestication influences. Broodstock retention must be done in a random fashion to avoid any type of selection.

Once families from each year-class have been established, there are a couple of options for choosing the broodstock numbers needed for the captive program. If advanced young-of-the-year fish (< 6 months old) will be used for future broodstock, then approximately 50-100 fish will be randomly selected from each family for year-class participation. A year later this number can be randomly reduced to 30-40 individuals until these fish are mature in 10 to 15 years. If yearling fish (>12 months old) are chosen for broodstock, then 30-40 fish can be randomly selected at that time from each family. Thus, no matter when future broodstock are selected, there should be a sizeable group of fish set aside so that a genetically representative number of fish will be available for spawning when sexual maturation occurs. When reducing numbers from year to year, any fish surplus to the captive program can be stocked or used for other approved purposes.

All fish can be fed a commercially available, appropriate fish food, live forage, experimental diet, or any combination of these foods to maximize survival, preserve a disease-free status, maximize relative weights and condition, and provide for a healthy fish that will provide quality eggs that contribute to future generations of genetically diverse progeny. Fish will be fed at a rate of 0.10-0.25 percent of body weight during the coldest part of the year and approximately 1.0 percent of body weight during the warmest part of the year. At the present time at Gavins Point NFH, live forage fish are provided to the larger broodstock to supplement their diet. Broodstock densities will be similar to those used for the production of stocked fish. If a need arises to deviate from these density parameters later in the broodstock program, then adjustments will be made for the benefit of the fish and the future egg production and progeny.

Continuous genetic evaluation and monitoring of the captive broodstock should be conducted and is a central feature of a well-designed hatchery recovery program. All genetic, spawning,

crossing, and PIT tagging information should be recorded and maintained using a digital format with backup.

Injection procedures, hormone use, egg and milt processing, egg enumeration, and incubation will be very much the same as that outlined for wild sturgeon spawning. Newly fertilized, de-adhesed eggs may be water hardened with up to 200 ppm active ingredient buffered iodophore for up to one hour in order to prevent disease transmission. Pertinent information, such as female PIT tag number, female size, volume of eggs, egg size, total egg number, eggs per female, percent neurulation, percent hatch, fry size, stocking densities, and survival will be noted. Any other important incubation and rearing characteristics will be documented. Rearing parameters follow that used for fish produced for conservation stocking.

*Selection of Fish Destined for Captive Broodstock Program and Release into Recovery Areas*
The numbers of fish from each mating to be stocked into an RPMA or incorporated into the captive broodstock population will be identified within stocking plans. As these fish will be the basis for the future wild and captive broodstocks, they must represent as much of the original pallid sturgeon genome and available genetic variability as possible. Fish should be chosen randomly to include as much genetic variability as possible. While the culling of fish with obvious physical deformities or health problems is permissible, selecting fish for size, disease resistance, or any other attribute is prohibited. The number of fish needed should be randomly removed. All tanks holding the progeny of any single mating should be included in the sample of that mating. If a group of fish to be stocked contains fish that are too small to be physically tagged, the small fish should be deemed genetically marked (DeHaan et al. 2005) and stocked in accordance with stocking plans, along with the lot or grown to a larger size, physically marked, and stocked at a later date. Small fish should also be included in the random, representative samples destined to be included in the captive broodstock program and kept until it is determined that they are unsuitable for this purpose.

Future brood fish are typically selected at approximately one year old. A 50 fish sample from each family is initially taken. A 100 fish sample may be used if the future brood fish are selected as fingerlings.

**Spawning**
It is recommended that water temperatures throughout the spawning process be kept at approximately 61-64°F (16-18°C) in the Upper Basin and 54-68°F (12-20°C) in the Middle Basin. Though pallid sturgeon have been successfully spawned at Gavins Point NFH at 53°F (11°C). Diel fluctuations in water temperature should be avoided during spawning.

Once a female has reached spawning readiness (PI$\leq$0.10 and greater than 80% GVBD in response to progesterone *in vitro*), hormonal injections of LHRHa are used to stimulate oocyte maturation and ovulation in females and spermiation in males.

The total dosage of LHRHa administered to females is 0.05-0.10 mg/kg of fish weight. The majority of hatchery personnel use 0.05 mg/kg of female weight. LHRHa is given to females in two injections: a priming dose equal to 10% of the total dosage, and a resolving dose equal to 90% of the total dosage that is administered 12 hours after the priming dose. At 18°C (65°F),

ovulation can occur 10-24 hours after the resolving dose is administered. Males are given a single LHRHa injection at a dosage of 0.01-0.02 mg/kg. Spermiation begins approximately 10 hours after injection, and milt is generally collected 18-24 hours after hormonal injection. It is recommended that males be injected at least 12 hours before females to allow time for determination of sperm viability before ovulation occurs.

The abdomen of the male must be patted dry prior to milt collection. Sperm should be collected via syringe and sterile tubing and then transferred into dry sealable plastic bags for storage. A sperm sample from each male must be evaluated for potential fertility by checking motility at the time of collection and immediately prior to use. Milt with zero sperm motility should not be used. It is essential to ensure that the sperm not come in contact with water prior to its use. Collected milt should be kept between 2-4°C (35 and 40°F). Sperm can be refrigerated or placed in a cooler with wet towels or cardboard separating the zip lock freezer bags from ice placed in the cooler bottom. Sperm refrigerated for up to two weeks has remained viable, however a loss of motility and viability does occur over time. Sperm should not be frozen for short-term use.

Eggs are expressed from the females by hand-stripping. The eggs within a female ripen over time, with eggs posterior in the ovary ripening first. This requires that eggs from a female be collected periodically, typically at 20 minute to 2 hour intervals, throughout the spawning period. The timing of egg collection should be based on the individual female's ovulatory progression. It is generally believed that egg collection should be made as often and quickly as possible until hand stripping becomes unproductive. In theory, this promotes egg quality and minimizes stress to the fish. Generally, the use of an incision to release the eggs from female pallid sturgeon is not recommended due to the stress of surgery, increased risk of infection, and extended recovery time required for incisions to heal. Also, the use of catheterization to express the eggs from a female should not be conducted as a standard practice. If in the rare case that a female will not be able to release eggs naturally following ovulation and upon stripping, a C-section may be performed.

Eggs are collected in a dry pan. Sperm of good quality (~80% active for 3 minutes) from a single male is diluted 1:200 by adding 5 mls of sperm to 1,000 mls of ambient temperature hatchery water and quickly but gently added on top of the eggs. In cases where sperm motility is low, the amount of sperm may be increased proportionally. The egg/sperm mixture is stirred for 3 minutes at which time excess sperm is rinsed from the eggs. The fertilized eggs are then drained of excess water and a supersaturated aqueous solution of Fullers Earth (ambient temperature hatchery water) is added. When pallid sturgeon eggs come in contact with water the chorion becomes sticky causing the eggs to adhere to one another or to any surface with which they come into contact. The Fullers Earth solution is added at the rate of approximately 2-4 times the egg volume. The eggs are continuously and gently stirred with a feather until deadhesion is complete, usually 20 minutes. Egg stickiness can be variable and there are times when it may take an hour or longer in Fullers Earth solution to deadhease the eggs. Also, if clumping occurs immediately after placing eggs in jars, remove the eggs and treat them with Fullers earth again. It is generally best to pour off the Fullers Earth solution and flush with hatchery water prior to placing the eggs in jars, as the volume of water may not sufficiently clear the jars of excess sediment during the initial incubation.

## Embryo Care

*Embryo Disinfection*

Pallid sturgeon embryos can be disinfected by water-hardening in a 100-200 ppm buffered iodophore for 30-60 minutes. A 60-minute treatment using 100 ppm iodophore is recommended. Iodophores have limited potential to eliminate an iridovirus within a pallid sturgeon embryo as iodophores are proven bactericides but are only incomplete viricides.

*Embryo Enumeration*

Embryos are initially enumerated by using Von Bayer egg counts and displacement when they are placed into incubation jars. Typical counts for pallid sturgeon embryos are 47,000 to 51,000 embryos per quart.

The extreme variability in mortality at both gastrulation and 4-6 days post-hatch in current pallid sturgeon culture makes estimating the number of progeny produced from an initial egg inventory impractical. It is more effective to make the estimates of expected progeny 7-10 days post-hatch, when mortality usually stabilizes. It is recommended to assess fertilization success, percent neurulation, and 1-3 days post-hatch to collect critical information about survival at these difficult developmental stages.

*Incubation*

Pallid sturgeon embryos are incubated and hatched in upwelling hatching jars similar in design to McDonald, Eagar, or grayling jars. Flow through each jar is initially adjusted so that the incubating embryos are suspended and mildly rolling. After approximately 48 hours (depending on water temperature) or after neurulation occurs flows can be increased to vigorously roll the embryos. While the actual flow through a jar is based upon the size of the jar and the volume of embryos within the jar, typical flows are 1 to 1½ gpm. Based upon experience incubating pallid sturgeon embryos at various water temperatures, the acceptable temperature range for incubating pallid sturgeon embryos from adults collected in Montana and North Dakota appears to be 55-65°F (13-18°C). Middle Basin fish are usually incubated within the range of 60 to 65°F (15.5-18.3°C). There is concern within the Propagation Committee that accelerating embryo development by incubating pallid sturgeon embryos above this range can be harmful. It is recommended that 65°F be considered an upper bound for the successful incubation of pallid sturgeon embryos until new evidence is available.

Fungal infections - primarily Saprolegnia - can be a serious problem, killing embryos either by invasive damage to the embryos structure or by causing the embryos to form clumps that lead to suffocation. Rolling the embryos during incubation is an effective technique to keep embryos from clumping. Incubating embryos at temperatures near the upper limit of the accepted range of incubation temperatures reduces embryo incubation time and, therefore, embryo exposure to fungus. When necessary, dead embryos should be siphoned from incubation jars to prevent them from becoming a medium for fungal growth. Based on the results of its use on paddlefish embryos at Gavins Point NFH, the use of formalin to control fungus during pallid sturgeon embryo incubation is not recommended.

*Embryo Allocation, Shipping, and Use for Stocking*

Neurulated embryos are distributed among hatcheries based on the relative size of egg lots, neurulation and survival estimates, the number of families a hatchery can keep separate, and total hatchery capacity. Survival from neurulation to fingerling is highly variable among egg lots and also among the same lot of embryos raised at different facilities. This variability in survival, in conjunction with various fish health issues, makes it difficult to predict the useable numbers or lots of fish at any hatchery. To reduce the potential impacts of this uncertainty on meeting stocking goals, embryo production should be maximized, (i.e. collect the most eggs safely possible from each female spawned), and neurulated embryos should be distributed among hatcheries in such a manner as to maximize the genetic variability (i.e. the number of families) represented in the fish at each facility.

The shipping of neurulated embryos is the only safe and acceptable method to transfer pallid sturgeon between hatcheries. Because of the short period of time between fertilization and hatching, embryo shipping logistics need to be scheduled immediately after spawning is concluded so that facilities can get fertilized eggs when they are available. Handling and disturbance need to be minimized during embryos shipment. Although it may be more convenient to ship embryos immediately after spawning, and pallid sturgeon embryos have been successfully shipped the day after fertilization, it is best to ship embryos after neurulation occurs, as the embryos are less sensitive to physical shock at this time. Embryos should be completely water-hardened prior to shipping. Embryos are shipped in sealed plastic bags containing oxygenated water. The water in the shipping bags should be held at the ambient temperature of the sending hatchery's water. Upon arrival, the embryos should be tempered to the receiving hatchery's water temperature or the receiving hatchery's water temperature can be adjusted to the temperature of the embryos. Embryos should be disinfected with a 100 ppm iodophore solution for 10 minutes prior to being brought into the production area of the receiving hatchery.

Embryo surplus to a spawning facility's needs should be sent to other facilities for their use, offered for approved research or educational purposes, or held for stocking as fry as outlined in stocking plans.

## Fingerling and Yearling Production

*Rearing Environment*

Although round tanks are preferred for rearing pallid sturgeon, rectangular tanks have been successfully used to rear smaller fish. Round tanks have several advantages over rectangular tanks in the culture of pallid sturgeon. Round tanks with center drains are somewhat self-cleaning, improving tank hygiene and reducing the amount of disturbance (stress) the fish have to endure during tank cleaning. Water velocities in round tanks can be easily adjusted to provide the velocities that are appropriate for or preferred by the size of the fish. Tanks should have smooth (gel coated or lined) bottoms and sides to minimize abrasion of the fish and to improve ease of cleaning.

Although lighter tank colors make observing and cleaning pallid sturgeon easier during initial rearing and the initiation of feeding, sturgeon seem to prefer dark tank interiors over light-colored tanks, therefore the use of dark tank interiors should reduce the stress on the fish.

Pallid sturgeon prefer little or no light in their rearing environment and, if given the opportunity, avoid direct sunlight. Indirect sunlight is a better option than direct artificial lighting as it provides a more natural photoperiod. Partially covering tank room windows or covering them with a dark translucent material provides the low light levels preferred by pallid sturgeon. Most pallid sturgeon facilities keep overhead artificial lights off in the rearing areas except during cleaning, sampling, or moving operations.

It is important to keep tanks clean with daily cleaning to remove feces and wasted feed. Reducing feed levels to minimize feed wastage is preferred to twice-daily cleaning. This avoids the additional disturbance and stress of the additional tank draining and cleaning operations.

*Water Quality*

Pallid sturgeon rearing facilities should employ the same water supply guidelines established for pallid sturgeon spawning facilities. Water supplies should be filtered and disinfected (UV or ozonated). Disinfection units should be designed to handle a facility's historic pathogens and typical flows. A chart showing the recommended minimum applied ultraviolet radiation dosages to control common fish pathogens appears in Appendix D. Added security can be achieved if disinfection systems are designed with redundant disinfection units and independent, backup power supplies. Even if redundant units are only sized for part of the total available flow, they allow the continued disinfection of incoming water should the main unit fail or require maintenance or repair. An independent power supply assures that filtration and disinfection are maintained in the event there is a power failure or other electrical problem.

*Rearing Densities*

Newly hatched pallid sturgeon fry initially distribute themselves throughout a tank's water column. As pallid sturgeon larvae mature and begin to feed, they become more bottom-oriented. As rearing densities increase, sturgeon expand their distribution to the sides of tanks. Since pallid sturgeon distribution is limited to the water column immediately adjacent to the bottom, sturgeon culture uses pounds per square foot (area) for density calculations rather than the normal pounds per cubic foot (volume) used in most fish culture.

Regardless of the shape of the rearing tanks, pallid sturgeon distribution within rearing tanks can be inconsistent, with some hatcheries seeing both clumping and uniform distribution. This inconsistent behavior can confound, but doesn't invalidate, density calculations and the effects of density on growth rates and fish health.

Various factors determine optimum rearing densities. While low densities are preferable to high densities, there is a minimum density below which water quality, tank hygiene, and feeding efficiency may suffer. Without sufficient "sweeping" by swimming fish, waste feed and fecal material accumulate on tank bottoms, rather than being flushed toward drains or tailscreens. At low densities feeding behavior can decrease due to a lack of competition and feed wastage can increase, particularly with automatic feeders, as feed may not be distributed where the fish can best utilize it. Tanks used for rearing pallid sturgeon should be sized to provide densities as low as possible while achieving optimum tank function.

Although it is currently unknown which of the pallid sturgeon diseases are density-dependent, there should be a density threshold below which pallid sturgeon can tolerate intensive fish culture. This density threshold may be different for each facility depending on available water chemistry, quality and quantity; the water temperature profile; the accumulated stressors present; the pathogen load; and the age of the fish. There are risks in holding fish near or at this density threshold. Unplanned stressors or events such as breakdowns of filtration or disinfection equipment, power failures, decreases in water quality due to run-off events, disruptions in flows, or extreme changes in temperature can reduce the acceptable density threshold. Fish held at or near their acceptable density threshold would instantaneously be stressed by such events and, therefore, more prone to break with disease, if any of these events occurred. As an example, Miles City SFH experienced a bacterial gill disease epizootic in one lot of pallid sturgeon fish intentionally held at a high density (0.8 lbs/$ft^2$) when the incoming water quality degraded due to runoff.

Due to the potential risks of holding fish near their maximum density threshold, the potential impacts to the pallid sturgeon propagation program from health issues, and the endangered status of the pallid sturgeon, the Propagation Committee has established maximum rearing densities of 0.5 lbs/$ft^2$ for fingerling pallid sturgeon and 0.7 lbs/$ft^2$ for yearling pallid sturgeon, values which experience has demonstrated to be useful.

*Flow*

The distribution of pallid sturgeon reared in round tanks is partially determined by the water velocity within the tank. Pallid sturgeon prefer not to have to continually fight high water velocities. The fish typically spread out when velocities are low but move towards the center drainpipe when velocities are high. Experimental work at Garrison Dam NFH demonstrates that larvae in 30" diameter tanks use the entire bottom surface area of the tanks when water velocities at the tanks' circumference are between 0.1 to 0.2 ft/sec. When circumference water velocities reached 0.3 ft/sec, the larvae would begin to move towards the center drain where water velocities were lower. Fingerlings and advanced fingerlings (3-9") held in tanks with 4, 5 and 8 feet diameters used the entire bottom surface area of the tank when water velocities at the tank circumference are between 0.3 to 0.6 ft/sec. When circumference water velocities reached 0.7 ft/sec, the fish would begin to move towards the center drain where water velocities were lower.

For tanks with diameters less than 10 feet, exchange rates should be approximately 0.3-1.0 exchanges per hour. Large tanks can have lower exchange rates as fish are not required to continually orient themselves into the current.

*Rearing Temperatures*

Severely manipulating the growth rates of pallid sturgeon in intensive culture environments by radically altering rearing water temperatures can be detrimental to the health and development of the fish. Pallid sturgeon have been observed to stop feeding when water temperatures drop to approximately 45°F (7°C) and show signs of stress when water temperatures exceed approximately 68°F (20°C). Based on these observations, the acceptable temperature range for intensively cultured pallid sturgeon should be considered to be 43-70°F (6- 21°C).

After hatching, the water temperature for larval pallid sturgeon should be gradually increased from the temperatures recommended for incubation and hatching (55-65°F (13-18°C)) to 63-65°F (17-18°C) for initial rearing. After the fish are completely on feed, water temperatures can be gradually increased to the recommended summer/fall rearing temperature range of 63-68°F (17-20°C). Due to concerns about the possible effects of artificially induced high growth rates and observations of stress in pallid sturgeon exposed to water temperatures above 68°F (20°C), pallid sturgeon should not be reared in water temperatures above 70°F (21°C).

It is recommended that fish be kept on feed year round. Pallid sturgeon appear to go off feed at or slightly below 45°F (7°C). Over-wintered pallid sturgeon should be kept at or above the temperature at which they are observed to stop feeding. If a facility cannot keep its over-wintered pallid sturgeon above the minimum recommended temperature, it is recommended that that facility does not over-winter pallid sturgeon for spring stocking.

*Growth Rates*

Growth rates are dependent on various factors including the size and age of the fish, diet, feed rates, water temperature, conversions, rearing density, and water quality. During the first year of growth, pallid sturgeon can grow at rates of 0.040-0.085" per day. Feeding the current commercial diets available and typically used for pallid sturgeon culture at the high feed rates associated with rapid growth rates can cause potentially irreversible and fatal liver damage. Barrow's has concluded that the currently available commercial diets do not permit rapid growth with lean livers (Rick Barrows, pers. comm.). Further research relating diet, growth rates and liver condition needs to be conducted. While a range of optimum growth rates for pallid sturgeon have not been developed, it is unwise to significantly retard or accelerate the growth rate of pallid sturgeon by manipulating rearing water temperatures beyond the limits of the preferred temperature range.

*Handling, Enumeration, and Sorting*

All operations involving the handling or manipulation of young pallid sturgeon should be minimized, and when necessary, should be performed in ways to minimize stress as much as practical. It cannot be over-emphasized that pallid sturgeon should be handled or disturbed as little as possible. The use of knotless nets for handling all young pallid sturgeon too small to require a stretcher is encouraged.

Pallid sturgeon culturists must balance maintaining immaculate tank hygiene and minimizing the stresses caused by cleaning operations. While it is important to keep tanks clean by flushing wasted feed and feces from tanks, the increased light levels, broom harassment, tank draining and crowding associated with tank cleaning operations can be very stressful to pallid sturgeon. It is recommended that tanks be cleaned once per day, feed levels be adjusted through careful observation to minimize waste, and the velocity of incoming water be adjusted to maximize the self-cleaning action of circular tanks while allowing the fish to utilize the entire area of the tank bottom.

The first enumeration of pallid sturgeon larvae occurs approximately 30-45 days after hatch and is scheduled to coincide with other needs for handling, such as splitting or their transfer into

larger tanks. This eliminates the need for extra handling and, since the fish are usually 2 inches or longer, minimizes handling stress and reduces the physical damage to the fish from netting.

**Excess Hatchery Production and the Disposition of Surplus Embryos and Fish**

Throughout the rearing period, it is sometimes necessary for hatcheries to reduce their inventories in order to keep inventories below hatchery carrying capacities. Hatchery inventories are usually calculated and adjusted immediately after spawning, after initial inventory, in the fall, and in the spring. The description of these inventory adjustments appears below:

| Inventory Adjustment | When | Size of fish |
|---|---|---|
| Post-spawn | Late June, early July | Neurulated embryos |
| Initial inventory | Mid-July | Advanced larvae |
| Late fall | September-October | Fingerlings |
| Early spring | April-May | Yearlings |

Fish that must be removed from hatchery inventories to meet hatchery carrying capacity will be stocked as described within stocking plans or disposed of. Should it be necessary to dispose of pallid sturgeon, the guidelines established in *Disposition of Surplus Artificially Propagated Fishes* will be followed (Appendix F).

**Feeding and Nutrition**

Natural and prepared feeds are being used in the culture of pallid sturgeon. There is some evidence that pallid sturgeon initially fed live feeds have difficulty subsequently adapting to commercial diets. Sturgeon respond to external food stimuli before their mouths and digestive tracts are completely developed. Early familiarization with food scent has been found to improve the acceptance of feeds. The results from a study by Webb et al. (2007) suggest that pallid sturgeon larvae should be hatched and reared at 16°C and fed 5 days prior to yolk absorption (equivalent to day 8 post-hatch at 16°C) for at least the first 30 days for highest larval survival. For the highest survival rates at 20°C, pallid sturgeon should be exposed to feed 5 days prior to yolk absorption or 3 days post-hatch, while at 24°C, the highest survival rates were seen in fish fed 3 days prior to yolk absorption or 2 days post-hatch.

Commercial feeds successfully used in pallid sturgeon culture are primarily salmon and trout formulations from Otohime and Nelson's Silver Cup. These feeds are used separately or in combination. Feed size is increased gradually as the length of the fish increases. To help fish transition to new feed or diet changes, feed sizes and feed types are blended for 7-10 days. Vibrator and mechanical feeders are typically employed to present feed to the fish 24 hours a day. Feed levels are calculated to feed the fish to satiation while minimizing waste. Juvenile pallid sturgeon must be kept on feed all year long, with feed levels adjusted to meet the fish's intake. Tables 2 and 3 show the feeding regimes used at the pallid sturgeon hatcheries. For each feed size or type used, the approximate starting fish length and feed rate are shown.

Neosho NFH uses frozen bloodworms for pallid sturgeon feed. Fish on bloodworms are fed to satiation three times per day. Although there is little waste when using bloodworms, their use

can be problematic, and the water in which they are frozen may carry unidentified and undesirable fish pathogens. It is important to use reputable sources for any natural feeds.

The use of forage fish for general production of pallid sturgeon is cost prohibitive. There are also concerns over importing fish pathogens with forage fish. Any use of forage fish in pallid sturgeon culture will follow the recommendations established for their use with adult pallid sturgeon.

Table 2. Feed type and rates for pallid sturgeon at various sizes by hatchery.

| | **Gavins Point NFH** | |
|---|---|---|
| **Feed Type** | **Approx. Start Size** | **% Body Weight Feed** |
| Otohime B2 & Cyclop-Eeze 20% | Larvae | 15 |
| Otohime B2 & C1 | 1" | 10 |
| Otohime C1&C2 | 2" | 8-10 |
| Otohime C2 | 3" | 5-8 |
| Otohime C2 & Silver Cup Salmon #2 crumbles | 4" | 5-8 |
| Silver Cup Salmon #2 crumbles | 6" | 3-5 |
| Silver Cup Salmon #2 crumbles | 8" | 2-3 |

| | **Garrison Dam NFH** | |
|---|---|---|
| **Feed Type** | **Approx. Start Size** | **% Body Weight Feed** |
| Otohime B2 | Larve | Satiation |
| Otohime B2 & C1 | Advanced Fry | Satiation |
| Otohime C1 | 1.2" | Satiation |
| Otohime C1&C2 | 2.2" | Satiation |
| Otohime C2 | 2.5" | 12 |
| Otohime C2 & Silver Cup Salmon #2 crumbles | 3.7" | 7.4 |
| Silver Cup Salmon #2 crumbles | 3.8" | 6 |

| Feed Type | Miles City SFH<br>Approx. Start Size | % Body Weight Feed |
|---|---|---|
| BioDiet Starter #3 | Larvae | 1.5 Conversion |
| Silver Cup Trout #2 | 3" | 1.5 Conversion |
| Silver Cup Trout #3 | 5" | 1.5 Conversion |

| Feed Type | Neosho NFH<br>Approx. Start Size | % Body Weight Feed |
|---|---|---|
| One Day Old Brine Shrimp and Cyclop-Eeze (≈ 25%) | Larvae | Satiation |
| One Day Old Brine Shrimp and Cyclop-Eeze (≈ 25%) | 1" | Satiation |
| Graded Adult Brine Shrimp and Cyclop-Eeze (≈ 25%) | 2" | Satiation |
| Adult Brine Shrimp and Graded Brine Shrimp (≈ 25%) | 3" | Satiation |
| Adult Brine Shrimp | 4" | Satiation |
| Adult Brine Shrimp | 8" | Satiation |
| Adult Brine Shrimp | 10" | Satiation |
| Adult Brine Shrimp | 11" | Satiation |

| Feed Type | Blind Pony SFH<br>Approx. Start Size | % Body Weight Feed |
|---|---|---|
| Brine Shrimp Nauplii | Larvae | NA |
| Brine Shrimp Nauplii & Shredded Frozen Brine Shrimp | Larvae - 1" | NA |
| Shredded Frozen Brine Shrimp & Frozen Brine Shrimp | 1-2" | NA |

| | | |
|---|---|---|
| Frozen Brine Shrimp | > 2" | NA |

**Stocking**

*Transportation*

The transport of pallid sturgeon is discussed in the *Protocol on Collecting, Tagging, Holding, Transporting and Data Recording for Researchers and Managers Handling Pallid Sturgeon* (USFWS 2012). Those responsible for transporting pallid sturgeon are encouraged to review this document annually. Transportation of juvenile pallid sturgeon generally follows the guidelines established for hauling adults.

Round tanks are best for transportation of large pallid sturgeon. To maintain good tank water quality during transportation, taking fish off of feed 24 hours prior to transportation can minimize fecal waste and released ammonia. Loading rates during transportation should be kept as light as possible, with 0.5 pounds of fish per gallon of water as a suggested maximum loading rate.

Stocking should be scheduled to avoid releasing fish into extreme high and low water temperatures. If possible, hatchery and hauling tank water temperatures should be manipulated to approximate the temperature of the receiving water. The fish will require tempering if the tank water and receiving water temperatures vary by more than 5ºF (3ºC). During transportation, tank water temperature should be maintained within ± 5ºF (3ºC) unless the fish will be tempered to the receiving water temperature en route. Gas supersaturation causes gill embolisms in pallid sturgeon, therefore tank water oxygen levels should be kept above 5 ppm, but less than saturation. Electric agitators can help reduce the possibility of oxygen supersaturation. The use of an oxygen meter is also helpful in determining actual gas saturation levels.

To reduce the osmotic potential of the hauling water, to stimulate mucous production and to provide some protection from parasites and bacteria, non-iodized salt can be added to the hauling water to provide a 0.25-0.5 percent salt solution (2-4 pounds of salt per 100 gallons).

Fish are removed from transportation trucks by hand netting or through a discharge pipe. As released pallid sturgeon can distribute themselves into downstream habitats, the extra handling and additional stress induced by distributing of pallid sturgeon by boat should be avoided if possible. Boats used for the distribution of pallid sturgeon shall employ covered holding tanks fitted with oxygen induction systems.

*Coordination*

The timing and location of stocking in any RPMA will be determined by the biologist(s) responsible for managing pallid sturgeon in that RPMA. Hatchery managers will strive to meet these stocking requests. Stocking timing and location, the numbers and size of fish to be stocked, and the stocking goals for each RPMA will be are documented in the *Pallid Sturgeon Range-wide Stocking Plan* (USFWS 2008).

The time and location of stocking and any assistance with stocking will be coordinated well before the day of stocking occurs. Each transport truck is required to have a cell phone on board during transportation of pallid sturgeon.

Post-release growth rates and changes in condition factor or relative weights of stocked fish are important parameters to evaluate the pallid sturgeon stocking program. Prior to their release, individual lengths (to the nearest millimeter) and weights (to the nearest gram) will be collected and recorded for each individually tagged hatchery-reared pallid sturgeon.

## Fish Health

*Health Certification*

Prior to stocking, the health status of all lots of pallid sturgeon will be assessed. Certification of the health status of pallid sturgeon at a facility usually occurs during and through the hatchery's annual health certification process. Testing must be conducted within six weeks prior to the anticipated date of stocking in order to assure test results are available prior to stocking the fish. All hatcheries rearing pallid sturgeon must make arrangements for health testing with the appropriate fish health lab. Labs should be given as much notification as possible.

During an annual inspection, 60 random pallid sturgeon representing that year's production are randomly collected. Samples should come from all family groups, unless numbers preclude the loss to sampling, and from several tanks of fish. Kidney and spleen samples, or whole fish samples in the case of fish too small to dissect, are used to detect viruses using cell culture. Fin clips are used to detect specific viruses (MRSIV and ranvirus; Appendix B) that have caused epizootic outbreaks in pallid sturgeon using polymerase chain reaction (PCR) techniques. Annual hatchery health certification inspections that test all species reared at these facilities provide further confidence in the health status of pallid sturgeon hatcheries.

Testing for other pathogens may be required by state authorities responsible for import and fish transport oversight in the state into which the fish are planned for stocking. More involved health screening may also be requested by individual states in order to evaluate overall condition and health of fish being stocked. Refer to the Permitting section of this plan for further discussion and more information.

*Hatchery-to-hatchery Transfers of Fish*

Hatchery-to-hatchery transfers of fish are not recommended due to the risk of spreading disease to the receiving facility. Alternative means utilizing embryo transfers should be employed. In those situations, where live fish must be brought onto a hatchery, the incoming fish should be kept isolated from other fish on station to reduce the risk of potential infection. Where fish transfers cannot be avoided, transfers are best accomplished with fish that are as small as possible. The management authority responsible for the hatchery receiving fish and the appropriate state agency in which the receiving hatchery resides must be notified and approve of all fish transfers.

*Health Indices*

Indices of relative rates of disease among fish and lots of fish have been developed and used, with the goal of being able to stock pallid sturgeon from lots symptomatic for a disorder (primarily iridovirus and fin curl). The goal of a successful propagation program is to stock healthy fish that possess the expectation for high post-stocking survival rates. Given this goal,

hatcheries should strive to produce disease-free fish and only healthy fish should be stocked. Thus, the use of relative health indices is not necessary and is not recommended.

*Fish Health Testing and Monitoring*

No fish health screening is currently required prior to bringing adult pallid sturgeon into a hatchery facility. During their captivity, adult pallid sturgeon will be observed for signs of stress and disease. Fish health staff will be notified if problems are suspected.

For diagnostic testing ("Why are my fish sick?"), live samples of the sickest, worst-case fish should be sent to fish health specialists, as this aids in the detection of fish pathogens and the determination of specific fish health problems. Observations of the fish and their environment and information about the progression and suspected causes of the disease are helpful in determining the cause and treatment of a fish health problem. These include: changes in the fish's appetite, distribution, or behavior; the severity and progression of the disease; the current rearing conditions including any recently administered treatments; suspected sources of stress; and changes in water temperature, quality, flow or other environmental change.

For fish health evaluations ("Are my fish healthy?") for importation permitting, random, representative samples from each production lot should be used.

PCR diagnostic tools for the MRSIV and *Ranavirus* have been developed. However, care must be employed when evaluating PCR positives due the low thresholds of detection of these tests. The Bozeman Fish Health Lab usually provides fin clip sampling supplies as needed to Upper and Middle basin hatcheries or collects fin samples for virus evaluations. The Lab performs the both the PCR and cell culture detection processes on site. Suspected *Ranavirus* samples are sent to Dr. Tom Waltzek at the University of Florida for identification.

Fish health reports should be available to basin workgroup committee members. The Recovery Team Leader will be responsible for dissemination of this information when requested.

*Use of Injectable Drugs*

Injectable drugs are valuable tools to maintain the health of captured adult pallid sturgeon. Florfenicol, a broad spectrum bactericide with extended release action has been successfully used in pallid sturgeon culture for more than 15 years (Rob Holm, per.com.) both prophylactically, to prevent or minimize potential bacterial infections from handling, netting and tagging, and therapeutically, to stop or slow the development of disease by a diagnosed pathogen. The recommended dosage for pallid sturgeon is 0.07 ml/kg of fish body weight. Adult pallid sturgeon captured in the Middle Basin for use as broodstock receive prophylactic injections of oxytetracycline 0.045 mls/lb of fish body weight.

The Food and Drug Administration's Center for Veterinary Medicine has determined that the use of unapproved drugs in the culture of endangered species is a low enforcement priority. In order to comply with FDA regulations, extra label use of drugs is permitted either under the direction of a veterinarian or through coordination with the USFWS INAD office at the Bozeman Fish Technology Center.

It is important that injections are administered in such a manner as to minimize damage to the fish. The fish receiving an injection should be sufficiently restrained, or the injection appropriately timed, so that the fish is immobile during the entire injection process. If more than 1 cc. of an antibiotic is to be injected into an individual fish, the injection will be split between two injection sites. A 16-18 gauge needle, 1-1.5" long is recommended.

*Severe Pallid Sturgeon Pathogens*

The best method to control disease in artificially propagated fish is to use all means available to proactively avoid disease. Reducing or eliminating all conceivable sources of stress in the fish reduces the risk of disease. Providing favorable rearing conditions such as low light levels, minimized handling and disturbance, low densities, adequate water flows, and acceptable water temperatures reduce stress. Feeding appropriate diets can ensure that dietary deficiencies do not bring about disease. Filtering and disinfecting water supplies remove harmful irritants and reduce the numbers of pathogens in the incoming water. Prophylactic antibiotic injections may help to avoid disease outbreaks. Disinfecting gear and tanks and designating equipment for each rearing unit reduce the risk of contamination and spreading disease. These and other methods are currently being employed by the hatcheries propagating pallid sturgeon to control disease. Further refinements will come through the continual review of protocols and the development of new techniques, diets and drugs. A table that describes the use of chemotherapeutants used in pallid sturgeon culture appears in Appendix G.

Diseases in pallid sturgeon range from the benign to those that are usually fatal. In pallid sturgeon culture, as in any fish culture, there are pathogens that create fish health problems at specific hatcheries and also pathogens that affect all hatcheries that propagate pallid sturgeon. It is important to document the disease problems experienced during the culture of pallid sturgeon in order to identify effective treatments and avoid ineffective methods.

The culture of pallid sturgeon has improved since the first efforts in the 1990s. Much more is known about the environmental requirements of the fish; their susceptibility to pathogens, parasites, and fin deformities; and, most importantly, managing or avoiding epizootic outbreaks of disease. Minor bacterial infections (*Aeromonas*, bacterial gill disease, etc.) and exoparasites (*Saprolegnia* sp. and *Costia* sp.) can be easily treated and controlled with ready available therapeutants (see Appendix G). The major disease threats experienced at pallid sturgeon hatcheries are MRSIV, *Ranavirus* and fin curl. These are difficult to treat, can cause catastrophic losses of fish, have been demonstrated to affect post-stocking survival (Rotella 2015), usually require the disposal of infected lots, and disrupt the stocking of infected or suspect pallid sturgeon for conservation purposes.

MRSIV

MRSIV has caused high mortality in pallid sturgeon reared in Upper Basin hatcheries. Most lots of infected and exposed fish were destroyed and the disease affected pallid sturgeon stocking in the Upper Basin for several years. The pattern of infection appeared to be caused by horizontal transmission from a wild source. The reservoir of the pathogen was originally unknown until it was identified in wild shovelnose sturgeon and pallid sturgeon populations in RPMAs 1 and 2. Whether the pathogen is present outside these reaches is unknown.

Isolating Upper Basin hatcheries from sources of the pathogen by filtering and disinfecting incoming water has proven to be effective in preventing further outbreaks of the disease. Interestingly, MRSIV cannot be detected in adult fish in the Gavins Point NFH broodstock that had been exposed to MRSIV as young fish (Jeff Powell, pers. comm.). MRSIV is being successfully managed by Upper Basin hatcheries and rarely affects the Upper Basin propagation and stocking programs.

Ranavirus

Epizootic outbreaks of *Ranavirus* have resulted in the loss of entire lots of pallid sturgeon at Blind Pony SFH. The disease can cause high mortality in amphibians, reptiles, and fish and has been detected through PCR in the incoming water at Blind Pony SFH. *Ranavirus*, like MRSIV, is a genus in the Iridoviridae family and, as such, should respond to proper dosages of UV light. Recommendations from a recent (2016) technical review of the pallid sturgeon program at Blind Pony SFH include the installation of UV disinfection to the hatchery's infrastructure. The likelihood of successfully managing *Ranavirus* outbreaks at Blind Pony SFH with UV disinfection is high.

Fin Curl

Fin curl has been observed in many sturgeon species artificially reared in spring or well water. While the exact cause is unknown, it is suspected that some micronutrient is missing from these types of water supplies, deleteriously affecting the proper formation of the pectoral fins. Fin curl has been observed in pallid sturgeon cultured at Bozeman Fish Technology Center, Neosho NFH, and, in 2016 at Miles City SFH. The only known method to prevent fin curl is to use an "open" water source where the water is exposed to more complex assemblages of organisms, minerals and nutrients than is normally found in well and spring habitats. Neosho NFH is experimenting (in 2016) to see if spring water used in raceway and pond culture of salmonids will mimic water from and open source and, thus, avoid fin curl. Initial results seem encouraging.

*Post-stocking Hatchery Hygiene*

After pallid sturgeon are removed from their tanks, all tanks and associated equipment will be treated with a suitable disinfectant (typically Sterilize, Hyamine or chlorine bleach) and permitted to dry for a minimum of 1 day.

## Permitting

The propagation of pallid sturgeon will only occur under authority of an Endangered Species Act Section 10(a)1(A) permit or sub-permit. All permits and permission from state and federal agencies must be obtained before fish collection, possession, transport or importation occurs.

Most states require an import permit to bring pallid sturgeon into a hatchery if the adults are collected in another state. While not recommended, hatchery-to-hatchery transfers of live fish require the receiving facility to obtain approval from the appropriate USFWS regional fish health center and the state in which the receiving hatchery is located. The fish health personnel from a receiving hatchery's agency need to approve all shipments of embryos and live fish. The final decision to receive fish from any facility resides with the individual states.

Montana requires that an agency obtain a collection permit for the capture of fish from Montana's waters, unless the capture is performed under the auspices of Montana Fish, Wildlife and Parks. Montana requires an importation permit for the importation of live fish or embryos into Montana and requires certification of the health status of the fish and source hatchery. An aquatic nuisance species inspection of the sending facility is also required. Montana also requires that all releases must be reviewed and approved by FWP's Fisheries Division, and incorporated into its state stocking program.

South Dakota requires a Department of Game, Fish and Parks Fish Importation Permit to bring embryos or live fish that originated outside of the state into that state. Fish import permits are issued by the South Dakota Fish Health Coordinator. Fish health certification is required.

North Dakota currently requires an importation permit to stock fish in North Dakota. Permits are issued by The North Dakota Game and Fish Chief of Fisheries. Transfers to, from or between USFWS hatcheries do not need approval from North Dakota, although the Chief of Fisheries relies on hatchery managers to notify him/her if a problem arises or a controversial transfer is proposed.

The State of Missouri requires a Certificate of Veterinary Inspection health certification by a veterinarian and tested for viral hemorrhagic septicemia (VHS) before fish can be stocked.

Nebraska requires a fish importation permit issued by the Nebraska Game and Parks Commission. Imported fish must be tested using American Fisheries Society guidelines and certified to be free from VHS and spring viraemia of carp virus (SVCV).

**Genetics**

The Upper Basin propagation program operates under the guidance of the *Population Genetics Management Plan for Pallid Sturgeon in the Upper Missouri River Basin* (Heist et al. 2013). While the Middle Basin does not have a similar guiding document, the principles outlined in this plan are also used by the Middle Basin program.

Geneticists with the USFWS Northeast Fisheries Center and Southern Illinois University assist the propagation programs in the Middle and Upper basins. Their services are used to:

- determine the genetic status of wild adult sturgeon captured as potential broodstock for creation of progeny for conservation stocking and incorporation into the captive broodstock at Gavins Piont NFH;
- maintain a list of known wild pallid sturgeon to be targeted for capture during Upper Basin broodstock collection efforts;
- maintain a history of all adults used for matings and the relative representation of these matings in the populations of stocked pallid sturgeon;
- assess the effective population size ($N_e$) of individual and combined stocked populations;
- provide mating designs for spawning wild adults and adults from the Gavins point broodstock; and
- assist with the management of the Gavins Point broodstock by identifying which fish to cull as crowding requires the reduction of family representatives.

These geneticists also provide addition assistance to the assessment and management of wild pallid sturgeon across their range.

Only unhybridized fish are used as broodstock. Differentiating unhybridized from hybridized pallid sturgeon using meristics and morphometrics has proven unreliable. Instead, the use of genetic markers is the only sure method of selecting unhybridized wild pallid sturgeon for use as broodstock. Tissue samples (fin clips) from captured wild fish are tested to identify unhybridized adults. Hybrid fish are returned to the wild immediately upon their identification.

During the spring broodstock collection in the Upper Basin, two types of pallid sturgeon are targeted: "new" (previously uncaptured) adults and adults whose genotypes either haven't been successfully incorporated into the captive broodstock or in one or more stocked populations, or "under-represented" (progeny stocked below the median rate of representation of a female's genotype or whose post-stocking survival results in fewer than desired surviving in the wild) adults in stocked populations. Capture teams are provided a list of tagged adults that shows each fish's relative importance for capture. This increases the efficiency of the capture effort by avoiding retaining and transporting fish that are not needed as broodstock.

A record of the matings used during the history of pallid sturgeon propagation is maintained. By assessing the numbers of progeny stocked from each mating and the relative recapture rate of the progeny stocked from each mating, an estimate can be made of the $N_e$ of a population and a determination of the level of representation of a genotype. An additional benefit of this record is its value in assigning parentage to unidentified pallid sturgeon (untagged fish or fish that have lost their tags).

Annual mating designs are developed for spawning of captured wild adults and adults from the captive broodstock. The genotypes of the available fish are used to determine mating designs that minimize relatedness and conserve the genetic variability of pallid sturgeon populations.

The hatchery space available for the captive broodstock at Gavins Point NFH is finite. As fish in the captive broodstock grow, tank carrying capacities are approached requiring reducing the numbers of fish held in the affected family groups. In consultation with geneticists, a determination is made of which fish to hold and which to remove to maintain broodstock within recommended tank densities.

**Tagging**

Although it is preferable if pallid sturgeon are marked, hatchery propagated pallid sturgeon may not need to be physically marked prior to their release (DeHaan et al. 2005) in accordance with stocking plans, if their size at time of release cannot accommodate physical tags. Fish that can accommodate physical marks should use at least one physical mark. Pallid sturgeon that average nine inches in length or longer have been shown to accommodate PIT tags, coded wire tags, and elastomer tags. Fish of this size and larger should receive two physical marks whenever possible. The method of tagging, tag numbers and identifying elastomer colors used will be recorded for each fish tagged.

Tagging occurs under the guidance of *Biological Procedures and Protocols for Researchers and Managers Handling Pallid Sturgeon* (USFWS 2012) and, in the Upper Basin, *Protocols for*

*Tagging and Marking Hatchery Reared Pallid Sturgeon in Recovery Priority Management Areas 1, 2, and 3* (Jaeger et al. 2007).

**Data and Database Management**

Pre-release data from hatchery propagated pallid sturgeon will be collected using an accepted standardized program such as PTAGS. Each hatchery's manager is responsible for sending the data collected from his/her hatchery's fish to the biologists responsible for the RPMA into which the fish are released and to the Recovery Team Leader. Data can be sent in any spreadsheet format, although Excel and PTAG are preferred. Prior to its transfer, the hatchery generating the data will proof the data. Management biologists will verify the accuracy of the data. The USFWS is responsible for maintaining the database.

**References**

Bruch, R.M. and F.P. Binkowski. 2002. Spawning behavior of lake sturgeon (*Acipenser fulvescens*). Journal of Applied Ichthyology 18:570-579.

DeLonay, A.J., D.M. Papoulias, M.L. Wildhaber, M.L. Annis, J.L. Bryan, S.A. Griffith, S.H. Holan, and D.E. Tillitt. 2007. Use of behavioral and physiological indicators to evaluate *Scaphirhynchus* sturgeon spawning success. Journal of Applied Ichthyology 23:428-435.

Braaten, P. J., S. E. Campana, D. B. Fuller, R. D. Lott, R. M. Bruch, and G. R. Jordan. 2015. Age estimations of wild pallid sturgeon (*Scaphirhynchus albus*, Forbes & Richardson 1905) based on pectoral fin spines, otoliths and bomb radiocarbon: inferences on recruitment in the dam-fragmented Missouri River. Journal of Applied Ichthyology 31:821-829.

Candrl, J.S., D.M. Papoulias, and D.E. Tillitt. 2010. A minimally invasive method for extraction of sturgeon oocytes. North American Journal of Aquaculture 72:184-187.

DeHaan, P.W., D.E. Campton, W.R. Ardren. 2005. Genotypic analyses and parental identifications of hathery-origin pallid sturgeon in the upper Missouri River. Report to the USFWS. pp. 35.

Doroshov, S.I., G.P. Moberg, and J.P. Van Eenennaam. 1997. Observations on the reproductive cycle of cultured white sturgeon, *Acipenser transmontanus*. Environmental Biology of Fishes 48:265-278.

Erickson, D.E. and M.A.H. Webb. 2007. Spawning periodicity, spawning migration, and size at maturity of green sturgeon, *Acipenser medirostris*, in the Rogue River, Oregon. Environmental Biology of Fishes 79:255-268.

Fedorov, K.Y., S.E. Zubova, V.V. Semenov, and A.B. Burlakov. 1990. Secretory cells in the gonads of juvenile starlet sturgeon, *Acipenser ruthenus*, during sexual differentiation. Journal of Ichthyology. 30:1-3.

Grandi, G. and M. Chicca. 2008. Histological and ultrastructural investigation of early gonad development and sex differentiation in Adriatic sturgeon (*Acipenser naccarii*, Acipenseriformes, Chondrostei). Journal of Morphology 269:1238-1262.

Heist, E., M. Bartron, J. Kalie, and R. Leary. 2013. Population Genetics Management Plan for Pallid Sturgeon in the Upper Missouri River Basin. Final Report, Western Area Power Administration. pp. 40.

Jaeger, M., K. Kappenman, H. Bollig, R. Klumb, B. Gardner, S. Krentz, R. Holm, and R. Wilson. 2007. Protocols for tagging and marking hatchery reared pallid sturgeon in recovery priority management areas 1, 2, and 3. Report to the Upper Basin Pallid Sturgeon Recovery Workgroup. pp. 64.

Moberg, G.P., J.D. Watson, S.I. Doroshov, H. Papkoff, and R.J. Pavlick, Jr. 1995. Physiological evidence for two sturgeon gonadotropins in *Acipenser transmontanus*. Aquaculture 135:27-39.

Paragamian, V.L. and V.D. Wakkinen. 2002. Temporal distribution of Kootenai River white sturgeon spawning events and the effect of flow and temperature. Journal of Applied Ichthyology 18:542-549.

Persov, G.M. 1975 (in Russian). Sex Differentiation in Fish. Publishing House of Leningrad University, Leningrad.

Rotella, J. 2015. Upper basin pallid sturgeon survival estimation project, 2015. Report to the Upper Basin Pallid Sturgeon Recovery Workgroup. pp. 105.

Taranger, G.L., M. Carillo, R.W. Schulz, P. Fontaine, S. Zanuy, A.F. Felip, A. Weltzien, S. Dufour, Ø. Karlsen, B. Norberg, E. Andersson, and T. Hansen. 2010. Control of puberty in farmed fish. General and Comparative Endocrinology 165:483-515.

Tripp, S.J., Q.E. Phelps, R.E. Colombo, J.E. Garvey, B.M. Burr, D.P. Herzog, and R.A. Hrabik. 2009. Maturation and reproduction of shovelnose sturgeon in the middle Mississippi River, North American Journal of Fish Management 29: 730-738.

U.S. Fish and Wildlife Service. 1993. Pallid sturgeon (*Scaphirhynchus albus*) recovery plan. Denver, Colorado. pp. 55.

U.S. Fish and Wildlife Service. 2008. Pallid Sturgeon (*Scaphirhynchus albus*) range-wide stocking and augmentation plan. U.S. Fish and Wildlife Service. Billings, Montana. pp. 55.

U.S. Fish and Wildlife Service. 2012. Biological procedures and protocols for researchers and managers handling pallid sturgeon. U.S. Fish and Wildlife Service. Billings, Montana. pp. 37.

Webb, M.A.H., J.P. Van Eenennaam, G.W. Feist, J. Linares-Casenave, M.S. Fitzpatrick, C.B. Schreck, and S.I. Doroshov. 2001. Effects of thermal regime on ovarian maturation and plasma sex steroids in farmed white sturgeon, *Acipenser transmontanus*. Aquaculture. 201:137-151.

Webb, M., Kappenman, K., Talbott, M., Cureton, E. and C. Guy. 2007. Investigations to Improve Larval Pallid Sturgeon Survival during the Onset of Exogenous Feeding. Final Report to the Science Support Partnership.

Webb, M.A.H., P.A.C. Maskill, and L.J. Halvorson. 2016. Identification of reproductive indices and spawners in the captive pallid sturgeon population at Gavins Point National Fish Hatchery. Annual Report for the Upper Basin Pallid Sturgeon Workgroup.

## Appendices

### Appendix A. Genetic Sampling of Wild Broodstock.

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 pallid sturgeon). Record all biological data. Please note if the fish is a recapture.
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 and 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 Northeast Fishery Center or Southern Illinois University via e-mail before sending samples for analysis.

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_____

Comments_____

**Appendix B. Pectoral fin clip sampling for pallid sturgeon iridovirus.**

**Please read instructions through Post Sampling before beginning.**

Materials Needed:

☐Gloves- multiple pairs

☐Sterile packs of scissors and forceps

☐Plastic rack of tubes containing buffer

☐Small cooler with gel packs or ice

☐2 Plastic containers and Ziplock bags–one labeled "Sterile Only" and the other "Used – Do Not Reuse"

Prior to Sampling:

- Keep the rack of tubes in the cooler with the gel pack. Only bring as many out in the field as you believe you will need. Keep the sterile packs clean and dry in their container until ready to use.

Sampling:

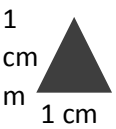
- **Wear new gloves and use a new sterile pack of scissors and forceps for each fish**.
    - Non-sterile instruments carry virus particles over from one sample to the next.

- Wearing new gloves and using sterile scissors, carefully remove a wedge-like clip of pectoral fin. Either pectoral fin is fine.
    - The wedge should be approximately 1cm or very slightly less in size.
    - Bigger is not better.

- Using the sterile forceps, place the fin clip into a tube containing buffer.

- Cap and shake the tube so the fin clip is covered with buffer.

- Label the tube with the identifying number of the fish and return it to the rack in the cooler.



- Dispose of the gloves and place the instruments in the plastic “Used” container and “Used” Ziplock for return to the Fish Health Lab. Do not reuse instruments as DNA can be carried over and

- Keep samples at a cool temperature while in the field.

Post Sampling:

- When you get back to the office place samples in the -20°C freezer until enough are collected and they can be shipped. DO NOT place in refrigerator.
    - Once samples are frozen, keep frozen. Avoid refreezing after a thaw.

- Ship samples overnight on frozen gel packs to:
  Bozeman Fish Health Center

  U.S. Fish and Wildlife Service

  1805 South 22 Avenue, Suite #1

  Bozeman, MT 59718

  406-582-8656

    - Please make sure you notify Lacey Hopper or Renee Martin first that you will be shipping samples and…
    - Send them early in the week.

- If you have any questions, please call Lacey Hopper or Renee Martin (Bozeman Fish Health Center) – (406) 582-8656

**Appendix C. Protocol for Determination of Spawning Readiness in Pallid Sturgeon**

- At capture, collect ovarian follicles (n=20) for calculation of oocyte polarization index (PI). Ovarian follicles for calculation of PI must be placed in Ringers solution.

- Once the fish is in the hatchery, oocyte PI and the oocyte maturation assay need to be conducted to assess spawning readiness. A total of 90 ovarian follicles are needed from each female.

Ooctye Polarization Index

Place approximately 20 ovarian follicles from an individual female into a pre-labeled 30 ml beaker or flask containing Ringer solution (15-20 ml). The beaker or flask must remain in a cooler with ice packs or wet ice covered by towels until they can be boiled and fixed. Gently boil the follicles for 5 minutes in the beaker or flask with a piece of aluminum foil covering the top. A boiling stone may also be added to each beaker or flask. After boiling, chill the follicles by placing the beaker or flask directly on crushed ice for 30 minutes. The follicles can be cut after chilling with a razor blade to evaluate PI, however, storing them in 10% buffered formalin overnight will make cutting easier.

Section the follicles along the animal-vegetal axis (animal pole can often be recognized by the white spot or rings). Turn both halves section side up, and they should be mirror images of each other if sectioned properly. If they are not mirror images, the cut was not made directly midline and the follicle should not be used to calculate PI. Measure the distance of the germinal vesicle to the inner border of the oocyte chorion (A) and the oocyte diameter (B). The oocyte PI = A/B. These measurements are made several ways and include the use of image analysis or photographs.

The average of 15 bisected oocytes should be used to calculate the PI of a female.

Oocyte Maturation Assay

The PI alone provides a good indication of female readiness, but it does not directly measure the capacity of the oocyte to mature in response to hormonal stimulation which may be affected by stress, overripeness or the physiological state of the female. During the expected month of spawning, oocytes should be analyzed for PI and their capacity to undergo germinal vesicle breakdown (GVBD) in the presence of a maturation-inducing steroid (progesterone). The *in vitro* oocyte maturation assays are conducted in Ringer solution at 16ºC for 16 hours. Note: turn on the incubator and set at 16ºC one week prior to the time of sampling; the temperature should be 16$\pm$0.5ºC.

1) Label 2 Petri dishes, incubation plate wells or flasks with Control (C) + Female ID (Ringer solution only) and 2 with Progesterone (P) + Female ID. You will have four containers for each female (2 controls and 2 progesterone).

2) Add 15 ml of Ringer solution to each Petri dish, well, or flask.

3) Transfer 15 follicles into a 30 ml beaker, using a clean, disposable pipet with the tip cut off. Carefully remove all of the Ringer solution used to transfer the follicles to the beaker

by pipeting it out with a pipet without the tip cut off. Pipet into the beaker about 5 ml of Ringer solution for the Petri dish, well or flask the follicles will go into, gently swirl the beaker and pour the oocytes and media back into that Petri dish, well or flask. This is an important step to maintain the correct volume of Ringer solution in each Petri dish, well or flask. Do this for each of the 4 containers for a female.

4) Add 75 µl (0.075 ml) of progesterone stock solution (1 mg/ml) with a micropipette or a 1 cc syringe to the progesterone labeled dishes. The control wells should receive 75 µl (0.075 ml) of 95% ethanol (the vehicle or carrier for the progesterone). To avoid any cross-contamination, use separate pipet tips or syringes for the progesterone and the control (ethanol). Gently swirl the dish or flask to mix the solution. Place the control dishes, plates or flasks on the top shelf of the temperature-controlled incubator and the progesterone dishes, plates or flasks on the bottom to prevent any potential spills that may cross-contaminant the assay and affect the results.

5) Record the time and incubate for 16 hours. (If the assays are set up at 4 PM, they will be finished at 8 AM.)

6) When the incubation is complete, use one pipet labeled control and one pipet labeled progesterone to transfer the oocytes to individual 30 ml beakers labeled with C (control) or P (progesterone) and the female ID. Add Ringer solution to reach 15 ml and boil gently for 5 minutes. Cover each with aluminum foil to ensure that the follicles to do "pop" out and to avoid Ringer with progesterone from spilling or getting on to you.

7) Place the beakers directly on crushed ice for 30 minutes.

8) At this time the oocytes could be cut with a razor blade and evaluated. However, they can be placed in a vial of 10% buffered formalin overnight to make cutting easier.

9) Section each oocyte along the animal-vegetal axis as described for PI determination. Turn both halves section side up, and observe the presence or absence of the germinal vesicle by focusing a light beam on the section surface. Record GVBD or intact germinal vesicle for each oocyte. Oocytes from both control and progesterone solutions should be examined. Make notes on germinal vesicle shape, size, and whether it migrated to the very top of the animal pole. If you are not sure that you properly sectioned the oocyte, take the two halves and cut them again into quarters. This will undoubtedly reveal any germinal vesicle that may have been missed in the first cut.

10) Evaluate the results by calculating the percentage of oocytes that underwent GVBD in the control and progesterone treatments. Record the average percent of GVBD of the two replicates (% pooled).

11) Wash the Petri dish, incubation plate or flask well with hot water and soap and rinse several times to be able to use them again for the next oocyte maturation assay.

<u>Decision to Spawn or Not to Spawn and Timing</u>

The best spawning success has been found with females that have a PI of 0.06-0.08 and 100% GVBD response in the progesterone treatment. However, 75% of your predictive power comes from the oocyte PI.

A 100% response in the progesterone and some response in the controls with a PI of 0.06-0.08 indicate that you should spawn the female within the week.

A 100% response in the progesterone and no response in the controls with a PI of 0.06-0.08 indicate that you should spawn the fish in 1 week.

*These time to spawn estimates assume that you are holding the females at 16-18ºC. At warmer temperatures, these time periods should be reduced.

**Appendix D. Minimum reported ultraviolet dosages for inactivating fish pathogens.**

**Minimum Reported Ultraviolet Dosage**
**For Inactivating Fish Pathogens**
(micro-watt seconds per square centimeter @ 254 nm)

| **Pathogen** | **Dosage (μws/cm$^2$)** | **Reference** |
|---|---|---|
| IHNV (CHAB) | 20,000 | Yoshimizu, Takizawa, Kimura |
| IHNV (RTTO) | 30,000 | Yoshimizu, Takizawa, Kimura |
| IPNV (Buhl) | 150,000 | Yoshimizu, Takizawa, Kimura |
| CSV | 100,000 | Yoshimizu, Takizawa, Kimura |
| CCV | 20,000 | Yoshimizu, Takizawa, Kimura |
| OMV (00-7812) | 20,000 | Yoshimizu, Takizawa, Kimura |
| *Aeromonas salmonicida* | 3,620 | Normandeau |
| *Bacilus subtilus* spores | 22,000 | Nagy |
| *Sarcina lutea* | 26,400 | Nagy |
| *Saprolegnia hyphae* | 10,000 | Normandeau |
| *Saprolegnia* zoospores | 39,600 | Normandeau |
| *Costia necatrix* | 318,000 | Vlasenko |
| *Myxosoma cerebralis* | 35,000 | Hoffman |
| *Ceratomyxa shasta* | 30,000 | Bedell |
| *Trichodina sp.* | 35,000 | Hoffman |
| *Trichodina nigra* | 159,000 | Vlasenko |
| *Ichthyophthirius tomites* | 100,000 | Hoffman |

From “Considerations for the Use of Ultraviolet in Fish Culture”, WDECO Ideal Horizons.

## Appendix E. Protocol for collection of biological samples from moribund pallid sturgeon.

**Collection Kit for Sturgeon Samples:**

Scissors – 1 pair
Scalpels – size 10 and 22
Scalpel blades – 5 for 10 and 22 each
PCR collection tubes – 12 vials with 180 ul of ATL buffer*
Histology collection containers – 12, 28X57 mm vials and 3 large specimen jars
Labels for collection containers – 12 plus extras
Forceps – 1 pair
Sharpie container
Disinfection containers - 2 vials
Sharpie
Collection form

**Additional Materials Needed:**

Davidson's fixative (obtain from Fish Health Centers)
Household bleach
Nitrile gloves
70% ETOH
Pencil

**Procedures:**

**Non-Lethal Sampling for Iridovirus**

**PCR**

- Label vials for PCR with sharpie using consecutive numbers starting at 1 and type of sample (i.e. 1- pectoral)
- Take a fin clip of the pectoral fin the size of a pencil eraser.
- Cut the fin clip into equal halves and add ½ to PCR labeled vial. It is very important that the tissue sample for PCR is covered completely in the ATL buffer*.
- Record information on the back of the collection sheet.
- Change gloves, sanitize and rinse tools in between each fish.

**Histology**

- Label white tags with chemical resistant marker or pencil and affix to histology vials with corresponding numbers used for PCR.
- Add second half of pectoral sample to histology vial with corresponding number for PCR sample. It is very important that the tissue sample for histology is at a 1:10 ratio of tissue to Davidson's fixative.

**Lethal Sampling**

**PCR**

- Label vials for PCR with sharpie using consecutive numbers starting at 1 and type of sample (i.e. 1- pectoral)
- Remove one whole pectoral fin. Take base of the fin that includes cartilage and epithelial tissue for PCR (size = ½ a pencil eraser in diameter). The remaining tissue is for

histology. It is very important that the tissues for PCR and histology are covered completely in the preservative.

**Histology**

- Label white tags with chemical resistant marker or pencil and affix to histology vials with corresponding numbers used for PCR.
- Add remaining pectoral sample after PCR sample is collected to histology vial. It is very important that the tissue samples for histology are at a 1:10 ratio of tissue to Davidson's fixative.
- Dissect out internal organs i.e. Gills, spleen, GI tract, liver and kidney for histological examination and place into sample jar with pectoral fin.
- Record information on the back of the collection sheet.
- Change gloves, sanitize and rinse tools in between each fish.

PCR samples should be kept on ice or frozen until received at the Bozeman Fish Health Center. If samples are frozen keep frozen to maintain sample integrity.

Histological samples are to be preserved for 48 hrs in Davidson's fixative and then transferred to 70% ETOH. **Do not send samples in Davidson's fixative through the mail.**

Contact the Bozeman Fish Health Center or Montana Fish, Wildlife & Parks State Fish Health Laboratory for needed supplies.

* If precipitation forms, buffer is still good. Shake vial until ATL buffer is back in solution.

**Appendix F. Disposition of surplus artificially propagated pallid sturgeon.**

Captive propagation can be a major element in recovery programs for threatened and endangered fish species. There are eight listed species in Region 6 (Colorado squawfish, bony tail, humpback chubs, razorback sucker, pallid sturgeon, Neosho madtom, greenback cutthroat trout, and Kendall Warm Springs dace). According to propagation and genetics management plans, the principle areas of emphasis at this time are development of (1) refuge populations, (2) back-up refuge populations, and (3) production broodstocks. These activities are required to: (a) avoid immediate population extinction; (b) preserve unique genetic resources; and (c) maintain and establish self-sustaining populations of target species in suitable historic habitat. In addition, research and development studies and public education are dependent, to a substantial degree, on fish produced within the captive breeding program.

The number of fish produced in a propagation program is defined by (1) propagation goals and objectives, (2) propagation techniques, (3) fish fecundity, (4) fish mortality under fish culture conditions, (5) uncertainty of production at various operational steps, and (6) available facilities. It follows, therefore, that fish in excess of program needs and program goals may be incidentally produced. By definition, hatchery fish exceeding needs explicitly defined in the recovery program are "surplus".

Surplus fish are a frequent by-product of any propagation program. They do not contribute to recovery of the species. Indeed, in some cases, surplus endangered fish become a liability to the program and compromise recovery. It costs just as much to take care of surplus fish as non-surplus fish. Surplus fish eat just as much and require just as much attention, facilities, and water to maintain as broodstock, production fish, and research animals. They are a potential source of disease and genetic contamination to wild and captive stocks. When resources are limited, caring for surplus fish affects the care required to ensure the health and well-being of high priority fish produced specifically for essential recovery activities.

One factor exacerbating the production of surplus fishes is their fecundity. Many offspring are often produced from a single spawn. All fish produced may not be needed in the recovery program and some will have to be disposed of. The disposition process is further complicated when endangered fish species are involved, principally because of the legal requirements associated with their endangered status. Disposition must be approved in the permits required by Federal and State laws. The specifics of disposition may be included within the permitting requirements.

Disposing of Surplus Fish:
There are ways to reduce the numbers, costs, and risks associated with surplus fish:

1. Planned production minimizes excess fish and cost of their maintenance and disposal. Production efforts must be identified in the propagation plan prepared for the species and implemented through recovery activities approved and funded in the recovery process. Planned production has the following characteristics:

a. Minimizes production of surplus fish. Production targets are based on an approved stocking plan (Guidelines for Preparation of a Stocking Plan for Threatened and Endangered Species) which includes numbers of fish required for specific research projects, stocking efforts, refuge populations, and broodstocks. Further, production numbers are based on formal timely fish/egg requests submitted by requesting entities to the appropriate production and permitting entities. If the eggs or fish are to be provided by the Service through its National Fish Hatchery system, fish and egg request forms are available from the Propagation Coordinator (Fisheries/Federal Aid) in the Service's Regional Offices. A duplicate form is submitted to the appropriate U.S. Fish and Wildlife Service, Ecological Service Field Office for permitting. Planned production not only assures fish are available to meet fish needs, but also helps limit the production of surplus fish.

b. Efficient planning and use of funding, personnel, and facilities, which precludes maintenance of surplus fish.

c. Identifies fish to be disposed of as well as protocols and methods of disposition.

d. Disposal of surplus fish occurs as early in the production cycle as practical.

e. Report all fish disposition on a semi-annual bases to the Recovery Coordinator.

f. Discourage incidental spawning of endangered fishes outside planned and approved recovery projects.

g. Humane and effective euthanasia must be used during the disposal process. All disposal methods must be consistent with the rationale behind recommendations of the American Veterinary Medical Association Panel on Euthanasia and the Royal Society.

2. Those individuals or agencies desiring possession of surplus fish must bear the cost of specimen preparation, shipment, and subsequent maintenance of specimens. The Recovery Program should minimize funding for maintaining and disposing of surplus fish. Therefore, the cost burden, including the necessary permitting and reporting responsibilities, should be born entirely by the recipients of surplus fish.

3. Disposition of surplus hatchery produced fish will follow modified recommendations in "Guidelines for Use of Fishes in Field Research". These recommendations were developed by American Society of Ichthyologists and Herpetologists; the American Fisheries Society; and the American Institute of Fisheries Research Biologists.

*In both the field and laboratory, the investigator must be careful to ensure that animals subjected to an euthanasia procedure are dead before disposal. In those rare instances where specimens are unacceptable for disposition as vouchers or teaching purposes, disposal of carcasses must be in accordance with acceptable practices as required by applicable regulations. Animals containing toxic substances or drugs (including euthanasia agents like T-61) must not be disposed of in areas where they may become part of the food web.*

Surplus fish will be euthanized using an appropriate anesthetic such as tricaine methane sulfonate (MS-222). Carcasses will be disposed of in a legitimate and ecologically sound manner.

4. Each facility engaged in propagation of endangered fishes must have a current, approved fish disposition plan for all species propagated at the facility. Fish must be spawned, reared, and maintained in a manner designed to conserve unique genetic resources at reduced risk to captive and wild populations. Breeding strategies, spawning techniques, and rearing methods will often result in offspring in excess of program needs. Upon request, samples of surplus fish will be preserved and retained for future study and reference, if the cost of preservation and storage is born by the properly permitted requestor. Upon request, surplus fish will be provided alive, if available, when the requesting party is properly permitted, when facilities are available, and when the requestor is prepared to pay the cost of maintaining the fish, preparing the fish for shipment, and shipment of the fish.

Surplus fish will not be released into the wild. Only wild fish released following capture and fish produced specifically for approved stocking projects should be released into the wild.

Endangered fish produced in excess of program needs become property and responsibility of the U.S. Fish and Wildlife Service.

## Appendix G. Guidance for the use of chemotherapeutants, spawning agents, and chemicals in pallid sturgeon.

| Drug | Indication | Dosage Regimen | Limitations/Comments |
|---|---|---|---|
| Formalin | Control protozoa (*Chilodonella, Costia, Epistylis, Ichthyopthirius, Trichodina spp*.) and monogenetic trematodes (*Gyrodactylus spp.*) | 75 ppm not to exceed 1 hour flow-through treatment. Recommended two treatments at 48 hr. intervals | Exceeding 75 ppm may cause direct mortality in sturgeon. |
| Oxytetracycline* | Broad spectrum antimicrobial- effective against both gram positive and gram negative bacteria, rickettsias, and chlamydias.; Bacteriostatic agent: | 0.045 cc/lb body weight (0.10 ml/kg) | Administer intramuscular injection (dorsal musculature, split between two sites if greater than 1cc.) 16-18 gauge needle. Treat adults when staging eggs (prophylactic) and after spawning. Treatments should be two weeks apart. |
| Florfenicol (Nuflor, Aquaflor) | Bacteriostatic agent; broad spectrum-effective against both gram positive and gram negative bacteria, rickettsias, and chlamydias. Extended release. | 0.03 cc/lb body weight (0.07 ml/kg) | Administer intramuscular injection (dorsal musculature, split between two sites if greater than 1cc.) 16-18 gauge needle. Treat adults when staging eggs (prophylactic) and after spawning. Treatments should be two weeks apart. |
| Sodium Chloride | Osmoregulatory aid for relief of stress and prevention of shock; and as a parasitide. | 1% for 30 minutes | Pallid sturgeon are susceptible to salt treatments. 1.5% concentration has produced direct mortalities in cultured sturgeon. |
| Amoxicillin | Broad spectrum antimicrobial- effective for both gram positive and gram negative bacteria; Bactericidal agent | 10 mg/kg intermuscular injection in dorsal sinus with no more than 2-3 mls per site. | Avoid major swimming muscles to reduce pain. May be used to treat known, or suspected, bacterial septicemia. Not for preventative treatment on adults. |
| Leuteinizing Hormone-Releasing Hormone (LHRH) | Chemical Induction of Ovulation and Spermiation in Broodfish | Total dosage is 0.05-0.1 mg/kg. Females use 10% primer dose, 90% resolving dose administered 12-16 hrs. after initial dose. Males single dose of 0.01-0.02 mg/kg. | Ovulation should start 10 hrs after resolving dose in 65 F. Spermiation occurs approximately 10 hrs. after injection. It is recommended that males be injected at least 12 hrs before females. |
| Argentyne (buffered iodophore) | Egg Disinfection | 100-200 ppm for 60 minutes for water-hardening. 100 ppm for 15 min. when receiving eggs. | |

* Both oxytetracycline and Florfenicol may be effective as preventative treatments for adults. Either one may be used depending upon availability, personal preference and efficacy of previous treatment