Range-wide
Pallid Sturgeon
Propagation Plan

Prepared by the
Range-Wide Pallid Sturgeon Propagation Committee
Final May 30, 2019

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For

Mountain-Prairie Region
U.S. Fish and Wildlife Service
Denver, CO

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Regional Director Date

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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 largest fish species found in the Mississippi and Missouri rivers, attaining a length of 183 cm and weight of 36 kg. Pallid Sturgeon are genetically similar to the more common Shovelnose Sturgeon (*Scaphirhyncus platorynchus*), and hybrids have been documented in wild populations. Hybridization is rare in the Great Plains Management Unit (GPMU), probably occurs in the Central Lowlands Management Unit (CLMU), and has been occurring and may be natural in the Interior Highlands Management Unit (IHMU) and Coastal Plains Management Unit (CPMU) (Schrey et al. 2011).

While limited specific knowledge exists about its life history, the Pallid Sturgeon is known to be long-lived, late-maturing, and is an 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 in the upper basin of the Missouri River.

Age at first sexual maturity was determined for wild 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 was determined to be 9-12 y of age with first spawning at age 15. However, this method may be inaccurate as other factors such as growth and health may influence annuli formation. Hatchery-origin male and female Pallid Sturgeon released in the lower Missouri River were first documented in spawning condition in 2006 at 9-14 y old (DeLonay et al. 2009). In the upper Missouri River, the youngest released hatchery-origin Pallid Sturgeon male to reach reproductive maturity was 10 y of age at 1.8 kg (Webb and Halvorson 2017). This fish was from the 2001 year-class and was captured in the fall of 2011. All other mature hatchery-origin males have been 14+ years of age (7.0 kg ± 0.4; range 5.5-8.8 kg) (Webb et al. 2016a). Two 1997 hatchery-origin female Pallid Sturgeon were ripe in 2015 in the upper Missouri River indicating that Pallid Sturgeon females can reach sexual maturity at 18 years of age in the northern portion of their range. In captivity at the Bozeman Fish Technology Center, Montana, Pallid Sturgeon females maintained at 16-20°C matured at 6 years old. These fish were on average 1.7 kg and 76 cm fork length (FL) (Jordan et al. 2016). At Gavins Point National Fish Hatchery, South Dakota, where the captive Pallid Sturgeon brood population for the upper Missouri River resides, the youngest females to be spawned were 9 years of age (3.3 ± -0.4 kg; range was 2.0-3.7 kg) and the youngest males were 4 years of age (1.2 ± -0.3 kg; range 0.7-2.1 kg) (Webb et al. 2016b). The fastest spawning periodicity has been observed to be annual for males and biennial 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, creates 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), and 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 wild 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 recovery can occur to allow natural spawning and recruitment.

Objective

The objective of this *Range-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 Stocking Plan* (USFWS 2018a).

This plan can be revised at any time by the Range-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 (Miles City State Fish Hatchery, Garrison Dam National Fish Hatchery, Gavins Point National Fish Hatchery) that promoted successful spawning and produced healthy Pallid Sturgeon. This current manual expands its coverage to include all hatcheries currently spawning and/or rearing Pallid Sturgeon. 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 (MDC)
Neosho National Fish Hatchery (USFWS)

Iridovirus originally identified as the Pallid Sturgeon iridovirus has been found to be endemic in wild populations of Shovelnose and Pallid Sturgeon in the Missouri River 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, Pallid Sturgeon propagation has advanced as a result of greater knowledge and improved spawning protocols. This has in turn yielded more consistent identification of adult spawning readiness and the production of viable gametes. Through the process of hatchery technical reviews, 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.

Gametogenesis

Like teleosts, sturgeons have a biological clock controlled by endogenous and environmental factors (Doroshov et al. 1997; Webb et al. 2001; Papoulias et al. 2011). 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.

There are multiple guides or published papers describing stages of gonadal maturity in sturgeon (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; Webb et al. 2017). 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 conservation aquaculture as the only option to perpetuate the species through the existing reproduction/recruitment bottleneck. Conservation aquaculture will be used to increase the current abundance and genetic diversity of Pallid Sturgeon within the species' historic range until river function and habitat changes allow sufficient natural reproduction and recruitment to maintain the species. Even then, the

broodstock and stocking programs will have to continue beyond until the reestablished hatchery-origin populations are proven to provide the necessary recruitment in those areas where recovery is possible. In areas where Pallid Sturgeon will not naturally recruit, 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 called 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 housed at Gavins Point NFH 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 because of decreasing numbers and, ultimately, 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, the captive broodstock, currently held at Gavins Point NFH will 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 because of the impacts of low body condition on gamete production. Wild fish captured for use as broodstock often require conditioning due to low body condition prior to reproductive cycling to promote gamete production (Steffensen and Mestl 2016). It is not the intent of this program to permanently remove these wild donors from their environment. Captured wild fish will be reconditioned to initiate reproductive cycling, spawned, and returned to the river reaches where they were captured.

Wild Broodstock Program

Donor Populations

Only individuals genetically identified as Pallid Sturgeon will be used for establishing the captive broodstock and for restoration stocking. It is recommended that only fish stocks that are genetically appropriate for the management unit may be used as donors when supplementing a population with hatchery-origin 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 CLMU and IHMU will be used to produce offspring for conservation stocking in these two management units. The fish will be maintained at Gavins Point NFH and Neosho NFH until spawning and released back into the wild. There is currently no captive broodstock population for the Middle Basin. There are no current plans for conservation stocking in the 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 render an individual prone to reproductive failure, injury, and/or 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 *Biological Procedures and Protocols for Handling Pallid Sturgeon* (USFWS 2018b) will guide the capture, handling, transportation, holding, spawning and release of wild adult Pallid Sturgeon used as broodstock.

Collection of Adults

It is extremely important that the existing wild Pallid Sturgeon genome be captured and preserved within the captive broodstock population and be represented in the reestablished populations within each management unit. 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 absent in the captive broodstock population).

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

4) Recaptured females that have previously spawned and are not un- or under-represented, but are the only female available to spawn with a previously not-spawned or an underrepresented male, and the female-male pairing represented a unique cross.

All broodfish that are adequately represented as recruited progeny (to one year) in the wild or in the case of males represented in the cryopreservation repository will be released immediately. In the Great Plains Management Unit, in addition to the above requirements, all broodfish should be adequately represented in the captive broodstock population and in each of the three Recovery Priority Management Areas prior to release.

The USFWS will maintain the database for captured Pallid Sturgeon. Copies of the 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.

The collection of wild adult Pallid Sturgeon for use as broodstock occurs in the GPMU in the spring. The adults collected are spawned that summer. The CLMU and IHMU 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 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 Handling Pallid Sturgeon* (USFWS 2018b) and in compliance with specific state or federal permit conditions. Stress during capture can be reduced by minimizing drift time, 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 1 minute 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 easily accommodate the length of the captured fish and be 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. 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 *Biological Procedures and Protocols for Handling Pallid Sturgeon* (USFWS 2018b). Two 1 cm^2 samples of caudal fin from each "new" fish will be collected for genetic analysis. The fin clips will be placed in properly labeled vials containing 95% denatured ethanol according to the protocol described in Appendix A. A fin clip collected for iridovirus screening should be collected 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 Handling Pallid Sturgeon* (USFWS 2018b).

To provide protection against stress-induced bacterial infections, trained personnel can administer a prophylactic intramuscular antibiotic injection to Pallid Sturgeon prior to their transport to the spawning facility if deemed necessary. Fish that will be released into the river will not be injected unless conditions, such as ongoing infections, recent telemetry tag implantation, urogenital catheterization, or gonadal biopsy, warrant it. 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, and/or ultrasound may be performed by trained personnel while the fish is held in a holding tank in the capture boat or on the transport 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. Females in the GPMU with immature ovarian follicles that will not reproduce that season will be released immediately. Non-reproductive females captured in the CLMU or IHMU 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 cannot be visually determined. 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 identified from gonadal biopsies with macroscopic and histological descriptions in sturgeons and paddlefish from Webb et al. (2017).

Developmental Stage		Description
Differentiation	1	Ovarian groove starts to develop into the small and very thin ovigerous "ribbon". Histology reveals clusters of oogonia and potentially a few very small oocytes just beginning the endogenous growth phase.
Pre-vitellogenic	2	Obvious ovigerous folds with small translucent oocytes. Histology reveals perinucleolar oocytes in the endogenous growth phase. The follicular epithelium (granulosa) in the larger oocytes begins mitotic proliferation, and the outer follicular layer (theca) has some vascularization (~ 0.2-0.4 mm).
Early to mid-vitellogenic	3	Ovigerous folds contain small white oocytes (0.5-1.0 mm) to larger oocytes that are white, cream, or yellowish in color (1.0-1.5 mm). The distinguishing histological features are the differentiation of the zona radiata and the presence of yolk platelets in the cytoplasm. The granulosa cells increase in thickness and become cuboidal. As ovarian follicle size increases, the density and size of yolk platelets also increases. The nucleus (germinal vesicle) is centrally located.
Late vitellogenic	4	The ovarian follicles darken in color as melanin pigment is deposited under the oolemma. Grey to black ovarian follicles are visible. Ovarian follicle size continues to increase, but maximum size has not been reached (1.5-3.0 mm) The follicular layers (theca, basal lamina, and granulosa) and three layers of the chorion (zona radiata interna, zona radiata externa, and

		gelatinous coat) are fully differentiated. The nucleus begins to move off-center toward the animal pole.
Post-vitellogenic/Ripe	5	Fully grown ovarian follicles are usually black (but can vary from olive brown, greyish to very light-golden color; >3.0 mm). Cytoarchitectural changes within the oocyte have occurred. The nucleus is displaced to the animal pole. The oocyte has a polarized structure with the animal hemisphere containing the bulk of the cytoplasm and small, round yolk platelets and lipid inclusions, while the vegetal hemisphere contains large, oval-shaped yolk platelets and numerous large lipid inclusions.
Oocyte maturation and ovulation	6	Ovarian follicles have undergone the final stages of maturation (i.e., germinal vesicle breakdown) and are ovulated. Eggs are freely flowing from the vent when captured in the wild or from a hormonally induced captive fish.
Post-ovulatory	7	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	8	Oocytes in the ovary are soft, crush easily, and have a marbled appearance. Histology reveals atretic vitellogenic follicles containing residual yolk or atretic mature follicles containing residual yolk and 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 ovarian follicles approach ovulation, the germinal vesicle migrates toward the animal pole. The distance of the germinal vesicle to the animal pole relative to the ovarian follicle 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 ovary. 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 ovarian follicle 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.

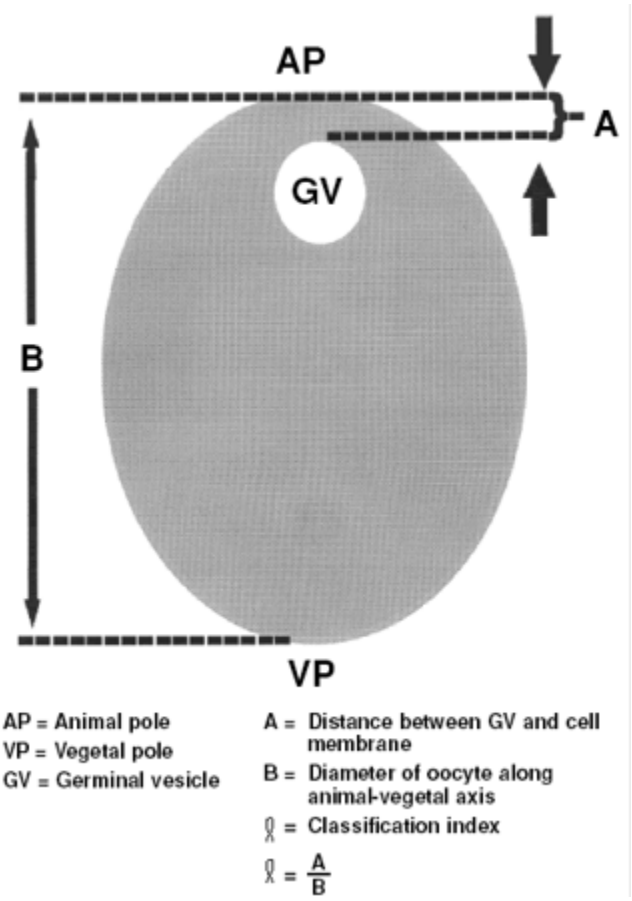

Figure 1. Calculation of oocyte polarization index (from Mims, S.D., A. Lazur, W.L. Shelton, B. Gomelsky and F. Chapman. 2002).

Post-capture Transportation to Hatchery

Round tanks are best for transportation of adult Pallid Sturgeon greater than 7 kg body weight. Rectangular tanks may be used for smaller adults. Pallid Sturgeon to be utilized for spawning activities should not be transported when ambient water temperatures are greater than 60°F (15.6°C). Pallid Sturgeon should be transported in river water obtained near the capture site. During transportation, tank water temperature should be maintained within ± 3ºF (1.6ºC) of ambient river water temperature. Gas supersaturation causes gill emboli 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 pathogens, 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 Adults 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 historical and emerging 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. The numbers of Pallid Sturgeon kept in tanks should be scaled according to tank size ($\leq$150 kg/20 ft circular). 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 and from a certified disease-free source. If on-site forage is unavailable, live forage should be obtained from a "certified disease free" source. Live forage should be administered "by eye." If possible, live forage fish should mimic what Pallid Sturgeon would naturally encounter (i.e., Chub species). 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 5 kg of 2-3" rainbow trout every two weeks for 20 adults (5% body weight).

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

Experience indicates that a suitable temperature range for spawning Pallid Sturgeon is 61-68°F (16-20°C) in the GPMU and 54-68°F (12-20°C) in the CLMU and IHMU. 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 GPMU and 59-64°F (15-18°C) in the CLMU and IHMU. Immediately after spawning is completed, water temperatures should be gradually reduced by 5-7°F (3-4° C) to slow pathogen reproduction and minimize stress in the fish or matched to river temperatures if fish are to be released immediately.

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 because of 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 until notified by authorities.

Returning Adults to the Wild

Post-spawn adults should be returned as soon as they are determined to be healthy enough and the receiving waters' temperatures are adequate. 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 fish, their holding water temperature should be gradually reduced by 5-7ºF (3-4ºC) to slow pathogen reproduction and reduce stress. Adults can be released at any available site within the Management Unit from which they were collected generally in close proximity to their capture location.

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 telemetry tags immediately post-spawn may be sufficient to cause mortality. 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 tag adult pallid at other opportunities such as when adult sturgeon are captured during either the spring or fall broodstock collections but not held for spawning.

Captive Broodstock Program

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 used to establish a captive broodstock that would preserve the maximum amount of remaining genetic variability.

The only captive broodstock ever established is the one at the Gavins Point NFH, which holds offspring of adults representing the genome of Pallid Sturgeon from the GPMU. No captive broodstock have been developed from any other Management Unit. 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, only the GPMU utilizes a captive broodstock as a genetic reserve of the existing genome and as a source of genotypes not represented or under-represented in the stocked population. 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.

There will be a great amount of effort incorporated into the rearing regime to avoid traits introduced due to culture methods or domestication influences. Captive broodstock retention must be done in a random fashion to avoid any type of selection. Once families have been established through the spawning and rearing process, there are multiple 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 20-25 individuals until these fish are mature in 10 to 15 years. If yearling fish (>12 months old) are chosen for broodstock, then 20-25 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 fish food, live forage, experimental diet, or any combination of foods to maximize survival, preserve a disease-free status, and provide for a

healthy fish. Healthy broodstock 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.

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 to reduce stress in the hatchery environment.

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. Ovulation can occur 10-24 hours after either the priming or the resolving dose is administered and is temperature dependent. 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. Behavioral changes often follow spermiation or ovulation with a pronounced swimming activity.

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

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. There is some speculation that 'clumped' eggs may block the flow of ovulated eggs from entering the urogenital ducts or that a blockage develops in the urogenital duct where the two ducts converge preventing a flow of eggs in some instances. If in the rare case that a female is not able to release eggs naturally following ovulation using the palpation method, catheterization or a C-section may be performed. These methods should be used only as a last resort as both increase the risk of mortality to the fish.

Eggs are collected in a dry pan. After confirming motility, milt from a single male is diluted 1:200 by adding 5 mls of milt to 1,000 mls of ambient temperature hatchery water and quickly, but gently, pouring into the bowl containing the eggs. In cases where sperm motility is low, the amount of milt may be increased proportionally. 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. Typically, 1:3 matings are used by the GPMU and 1:2 matings are used by the CLMU and IHMU. The egg/milt mixture is stirred for 3 minutes. Next the fertilized eggs are drained of the excess milt/water mixture and a supersaturated 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 fertilized eggs are continuously and gently stirred with a feather until deadhesion is complete, usually 20 minutes. The bowl is then decanted through a wire mesh strainer held in a tank at water level to gently rinse the fertilized eggs. Embryos are transferred to a beaker for enumeration prior to their transfer into hatching jars. Egg stickiness can be variable, and there are times when it may take an hour or longer in Fullers Earth solution to deadhease the fertilized eggs. Also, if clumping occurs immediately after placing embryos in jars, remove the embryos, and treat them with Fullers earth again. Experience has

shown that embryos with a tendency to clump after performing the deadhesion process are generally of poor quality and will need to be monitored closely for *Saprolegnia* during the incubation process. In severe cases, individual embryos that have matured past neurulation may be moved to a clean jar to prevent *Saprolegnia* related mortality.

Embryo Care

Embryo Disinfection

Pallid Sturgeon embryos can be disinfected in a 100-200 ppm buffered iodophore for 30-60 minutes. A 30-minute treatment using 100 ppm iodophore is standard. Iodophores have limited potential to eliminate viral pathogens within a Pallid Sturgeon embryo as iodophores are more effective bactericides than viricides.

Embryo Enumeration

Embryos are initially enumerated by using volume displacement methods or Von Bayer egg counts prior to being placed into incubation jars. Typical counts for Pallid Sturgeon embryos are 47,000 to 51,000 embryos per quart. The average number of embryos per milliliter is 45 with a range of 37 to 62 in the GPMU.

It is recommended to assess embryo survival at late gastrulation when there is an obvious black and white demarcation present in viable embryos. Since handling of the embryos is not recommended at this stage, a 50-100 embryos subsample should be removed via a pipette attached to a siphon hose so as not to disturb the remainder of the developing embryos. As some point after neurulation has occurred, water flow to the hatching jar can be shut down, the tube removed, and the embryo volume marked on the side of the jar as a reference to the total volume of embryos surviving pre-hatch. At this time, a subsample of embryos should be measured to determine the number of embryos in a 5 ml sample. This value will be used to determine the total count of embryos based on the volume mark left on the jar. This volume count is not a true displacement measurement but will provide a reasonable estimate without causing harm to the developing embryos.

Incubation

Pallid Sturgeon embryos are incubated in hatching jars similar in design to McDonald jars. Flow through each jar is initially adjusted so that the incubating embryos are suspended and mildly rolling. After neurulation occurs (approximately 48 hours but is temperature dependent) flows can be increased to vigorously roll the embryos pre-hatch. 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 the GPMU appears to be 55-65°F (13-18°C). The CLMU and IHMU embryos 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. Based on recent research, the estimated optimal temperature for successful incubation of Pallid Sturgeon embryos is 17-18°C (Kappenman et al. 2013).

Fungal infections - primarily *Saprolegnia* - can be a serious problem, killing embryos either by invasive damage to the embryo 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. Dead embryos are lighter than live ones during the later developmental stages and will float to the top of the jar. To prevent the spread of *Saprolegnia*, it is advantageous to siphon off dead eggs throughout the day during the course of incubation. 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 Shipping

The shipping of embryos is done to maximize the genetic variability (i.e., the number of families) represented at each facility. Because of the short period of time between fertilization and hatching, embryo shipping logistics need to be scheduled immediately after spawning so that facilities can get fertilized eggs when they are available. Handling needs to be minimized during embryo 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 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.

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, sturgeon seem to prefer dark tank interiors over light-colored tanks. Pallid Sturgeon have evolved as a bottom dwelling fish in a turbid environment. Intuitively then, the use of dark tank interiors and minimal lighting would likely be preferred by the fish.

Pallid Sturgeon, 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. Monitor growth rates and adjust feeding at regular intervals to avoid overfeeding and fouling the water. During the first three months of life, weekly adjustments are necessary as growth is very rapid. 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 historical and emerging 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. Water temperature and dissolved oxygen recommendations are discussed in specific sections throughout the document.

Rearing Densities

Newly hatched Pallid Sturgeon fry initially distribute themselves throughout a tank's water column. As Pallid Sturgeon larvae transition to feed (7-10 days post hatch), 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 in rectangular tanks, rather than being flushed toward drains or tail screens. 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. These issues can be negated when using circular

tanks due to their feed dispersing and self-cleaning design. Tanks used for rearing Pallid Sturgeon should be sized to provide densities below 0.5 pounds/square foot.

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 because of runoff.

Because of 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 and remain oriented into the flow 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 tank's 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.

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 45-70°F (6-21°C). The water temperature profile should mimic the natural thermograph.

After hatching, the water temperature for larval Pallid Sturgeon should be gradually increased from the temperatures recommended for incubation and hatching of 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). As a result of 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 1.02-2.16 mm per day.

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. The use of knotless nets and transfer buckets 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 21-30 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.

Fish that must be removed from hatchery inventories to meet hatchery carrying capacity will be stocked as described within stocking plans or properly disposed. 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 (e.g., Kappenman et al. 2011; Kittel and Small 2014). Sturgeon respond to external food stimuli before their mouths and digestive tracts are completely developed. The results from a study by Webb et al. (2007) suggest that Pallid Sturgeon larvae should be fed 5 days prior to yolk absorption (equivalent to day 8 post-hatch at 16°C) for optimal long-term 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 larval diets from Otohime and salmon and trout formulations from Nelson's Silver Cup (Kappenman et al. 2011). 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 day (see Table 2). 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 at a specific % body weight for their corresponding size for optimal growth (see Table 2). Table 2 shows 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.

Table 2. Feed type and rates for Pallid Sturgeon at various sizes by hatchery.

Pallid Sturgeon Feed Calculator Chart								
Weight (grams/fish)	Length (inches)	% Body Weight						
Temperature (°F)		46-50	50-54	54-57	57-61	61-64	64-68	68-72
1	2.5	9.5	10.2	10.6	11.3	11.4	11.7	12
3	3.6	5.8	6.2	6.5	6.7	7	7.2	7.4
5	4.3	4.6	4.9	5.1	5.3	5.5	5.7	5.9
10	5.4	2.9	3	3.2	3.3	3.4	3.5	3.6
15	6.2	2.6	2.8	2.9	3	3.1	3.2	3.3
20	6.8	2.2	2.4	2.5	2.6	2.7	2.7	2.8
25	7.3	2	2.2	2.3	2.4	2.5	2.6	2.7
30	7.8	1.9	2.1	2.2	2.3	2.4	2.5	2.6
35	8.2	1.8	2	2.1	2.2	2.3	2.4	2.5
40	8.6	1.7	1.9	1.9	2	2	2.2	2.2
60	9.8	1.5	1.7	1.8	1.9	2	2.1	2.2
80	10.8	1.4	1.5	1.5	1.6	1.7	1.7	1.8
100	11.6	1.2	1.4	1.5	1.6	1.7	1.8	1.9
150	13.3	1	1.2	1.2	1.3	1.3	1.3	1.4
200	14.7	1	1.2	1.2	1.3	1.3	1.3	1.4
250	15.8	0.9	1.1	1.1	1.2	1.2	1.3	1.3
300	16.8	0.9	0.9	1	1	1	1.1	1.1
350	17.7	0.8	0.9	0.9	1	1	1.1	1.1

Gavins Point NFH

Feed Type	Apx. 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	Apx. Start Size	% Body Weight Feed
Otohime B2	Larvae - 1.4"	15
Otohime B2 & C1	1.5" - 1.7"	15
Otohime C1	1.8" - 2.5"	14
Otohime C1&C2	2.6" - 2.7"	12
Otohime C2	2.8" - 2.9"	10
Otohime C2 & Silver Cup Salmon #2	3.0" - 3.3"	10
Silver Cup Salmon #2 crumbles	4"	6
Silver Cup Salmon #2 crumbles	5"	4
Silver Cup Salmon #2 crumbles	6"	3
Silver Cup Salmon #2 crumbles	7"	2.8
Silver Cup Salmon #2 crumbles	8"	2.5
Silver Cup Salmon #2 crumbles	9"	2.2

Miles City SFH

Feed Type	Apx. 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

Neosho NFH

Feed Type	Apx. 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

Blind Pony SFH

Feed Type	Apx. 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

Selection of Fish Destined for Captive Broodstock Program or Release into Management Units

The numbers of fish from each mating to be stocked into a Management Unit or incorporated into the captive broodstock population will be determined by the population genetics management plan or Captive Broodstock Management Plan. 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 prevent phenotypic bias. 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. 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.

Transportation

The transport of Pallid Sturgeon is discussed in the *Pallid Sturgeon Range-wide Stocking Plan* (USFWS 2018a). 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 or rectangular tanks may be used 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). When tempering water temperature, do not exceed 2ºF (1ºC) per hour. Gas supersaturation causes gill emboli in Pallid Sturgeon, therefore oxygen levels should be kept below 105% if transported for an extended period of time but above 5 ppm. Electric agitators can help reduce oxygen supersaturation. The use of an oxygen meter is also required when hauling Pallid Sturgeon to monitor gas saturation levels.

Salt has traditionally been used as a stress reliever when transporting fish, reducing the need for osmoregulation. To reduce the osmotic potential of the hauling water, 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 unloaded from transportation trucks by hand netting or through a discharge pipe. Pallid Sturgeon are not as easily flushed as other species, consequently the bulk of the fish should be unloaded with a net. If using a discharge pipe to release the remaining fish, extra water should be available to flush them from the tanks. Any unnecessary handling at the time of release, such as boating, should be avoided. If boats are necessary for the distribution of Pallid Sturgeon, they should use covered holding tanks fitted with oxygen induction systems.

Coordination

The timing and location of stocking will be determined by the Basin Workgroups responsible for managing Pallid Sturgeon in that Management Unit. 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 Management Unit are documented in the *Pallid Sturgeon Range-wide Stocking Plan* (USFWS 2018a). The stocking events need to be coordinated with the State Game and Fish Departments to ensure the proper permits are in place. In addition to import permits, some states require export permits.

The time and location of stocking and any assistance with stocking will be coordinated well before the day of stocking occurs. Water temperatures in the distribution tanks should be adjusted to meet the receiving water temperatures to prevent extended tempering times and additional stressors at the stock site. Each transport truck is required to have a cell phone on board during transportation of Pallid Sturgeon in the event of a breakdown or other emergency. Contact phone numbers will be made available to all persons participating in the stocking event.

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-origin 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 within six weeks prior to the anticipated date of stocking. All hatcheries rearing Pallid Sturgeon must make arrangements for health testing with the appropriate fish health lab.

During an annual inspection, 60 Pallid Sturgeon representing that year's production are randomly collected. This random sampling should come from all family groups, unless numbers preclude the loss to sampling. 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 and bacteria using kidney tissue. Fin clips are used to detect specific viruses (MRSIV; Appendix B) that have caused epizootic outbreaks in Pallid Sturgeon using 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.

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 tested by a qualified fish health laboratory and kept isolated from other fish on station to reduce the risk of potential infection.

Health Indices

Indices of health have been developed and used, with the goal of being able to stock Pallid Sturgeon from lots asymptomatic for a disease (primarily iridovirus) or possessing a deformity such as 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 showing no clinical signs of infection, external lesions or deformities may be stocked.

Fish Health Testing and Monitoring

No fish health screening is currently required prior to bringing adult Pallid Sturgeon into a hatchery facility. Isolation or quarantine of these adults is recommended. Fin clips are collected from adults and preserved in buffer for MRIV PCR testing in the GPMU. 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, live samples of moribund fish, if small enough, should be sent to fish health specialists as this aids in the early detection of fish pathogens and the determination of specific fish health problems. Observations of the fish within their environment and information about the progression and suspected causes of the disease are helpful in diagnosing and treating a fish health problem. These include changes in 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 necessary for importation permitting, random, representative samples from each production lot should be tested.

PCR diagnostic tools for the MRSIV and *Ranavirus* have been developed. However, care must be employed when evaluating pathogen detections by PCR because of the high sensitivities of these tests. The Bozeman Fish Health Center will provide fin clip sampling supplies as needed hatcheries or will collect fin samples for virus evaluations. The Center performs all fish health

assays on site. Suspected *Ranavirus* samples are confirmed by quantitative PCR (qPCR) at the Center or 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. For instance, Chloramine-T has been used successfully to treat bacterial gill disease. The recommended dose is 10-20 mg/L. Additionally, Florfenicol, a broad-spectrum antibiotic 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 CLMU and IHMU 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. Both Florfenicol and oxytetracycline are viscous, and a 16-18 gauge needle, 1-1.5" long is recommended.

Severe Pallid Sturgeon Pathogens and Conditions

The best method to control disease and reduce mortality is to proactively avoid disease. Reducing or eliminating all sources of stress reduces the risk of disease. Providing favorable rearing conditions such as low light levels, minimized handling, low densities, adequate water flows, and acceptable water temperatures and quality 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 (tank brushes, mort pickers) 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 can be 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.

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 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, and have been demonstrated to affect post-stocking survival (Rotella 2015). These diseases usually require careful management of infected lots, and disrupt the stocking of Pallid Sturgeon for conservation purposes.

MRSIV

MRSIV has caused high mortality in Pallid Sturgeon reared in Upper Basin hatcheries. In the early years of propagation, most lots of infected and exposed fish were destroyed. 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 the GPMU. 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 outbreaks of the disease. Interestingly, MRSIV has not yet been detected in adult fish in the Gavins Point NFH broodstock that had been exposed to MRSIV as young fish (Jeff Powell, pers. comm.). MRSIV has been detected in the wild and is being successfully managed by the hatcheries and rarely affects 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 should therefore 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. Studies are

currently being conducted to better understand the effect of water temperature on infectivity in Pallid Sturgeon.

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.

Post-stocking Hatchery Hygiene

After Pallid Sturgeon are removed, all tanks, water supply and drain lines, 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 and an export permit to transport fish to the state line. 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.

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 viremia of carp virus (SVCV).

Genetics

The GPMU 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 CLMU and IHMU do not have a similar guiding document, the principles outlined in this plan are also used by the program in these management units.

Geneticists with the USFWS Northeast Fisheries Center and Southern Illinois University assist the propagation programs. 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 program at Gavins Point NFH;
- Maintain a list of known wild Pallid Sturgeon to be targeted for capture during GPMU 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 NFH broodstock; and
- Assist with the management of the Gavins Point NFH 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.

During the spring broodstock collection in the GPMU, 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 parent's genotype or whose post-stocking survival results in fewer than desired surviving in the wild) adults in stocked populations.

A record of the matings used during the history of Pallid Sturgeon propagation is maintained. The Middle Basin keeps a detail record of spawning and stocking in Huenemann (2018). 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.

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. Tagging occurs under the guidance of *Range-wide Pallid Sturgeon Stocking Plan* (USFWS 2019) and the *Range-wide Pallid Sturgeon Tagging and Marking Plan* (2019). Fish 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.

Family Lot History and Health Assessment Database

A Pallid Sturgeon family lot history and health assessment database will be utilized to house data from each facility producing Pallid Sturgeon for release into the wild. The database will have the following data for each spawning year and family lot.

Broodstock Data: Origin (hatchery, hybrid, or wild), Management Area, PIT Tag, Iridovirus Status, Transmitter Present (yes/no), Sex, Reproductive Status, Oocyte PI with dates, Capture Date, Broodstock Weight and Length (at capture, at assessment(s), at release), Fate of Fish (returned to river, mortality; reason for mortality

Spawning Data: Spawn Date, Spawn Temperature, Polypodium (yes/no), Ovulatory Latency (time from priming injection), Time of First Milt Collection, Total Number of Eggs at Ovulation (calculated volumetrically; meniscus), Milt Motility (percent dilution, percent motile at activation, percent motile at 1 minute, density of sperm (low=clear in color, less than 25% of field of view contains sperm; medium=white in color and thin, 25-75% of field of view contains sperm; high=white in color and thick, >75% of field of view contains sperm),

Temperature Profile of Facility:

pH of Facility:

Embryo Quality (data as percentage): Neurulation, Hatch

Larval Quality (data as percentage): Survival at 1 Week (make a note in the comments to describe if/when the early mortality peak occurred), Survival at Initiation to Feed

Total Weight of Sample by Family Lot (data expressed in grams; 2 months, 4 months, 6 months, at stocking):

Number of Fish in Sample (2 months, 4 months, 6 months, at stocking):

Growth Rate (expressed as a percentage increase from previous sample; 4 months, 6 months, at stocking):

Condition Factor (at release; quartile 1, 2, 3):

Health Certification Prior to Release: (scaled evaluation for fin curl and presence of external lesions; sample number based on an assumed pathogen prevalence level (APPL) in the population of 5%; testing dependent on Regional Fish Health Center Director's suggested pathogen list and/or state requirements)*: Minimum health testing and reporting will include the following - MRSIV, Ranavirus, VHSV, Fin Curl, and Lesions with prevalence and severity of the specific pathogens or conditions noted

Number of Stocked Fish by Family: Location and Date

Comments about Transportation to Stocking Location (e.g. water temperature differences between hatchery and river):

Comments about Family Lot (e.g. lethargic, internal/external abnormalities, protozoans/parasites, etc…):

*Pallid sturgeon are regularly tested for MRSIV, Ranavirus, and VHSV in both the upper and middle basins of the Missouri River by standardized methods. Fin curl and lesions are reported using standardized scaled evaluations in both the upper and middle basins.

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 biologist responsible for the Management Unit into which the fish are released, to the Recovery Coordinator, and the manager of the Pallid Sturgeon Database (currently housed with the Missouri River FWMAO). 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. More information about data and evaluation can be found in the Range-wide Pallid Sturgeon Stocking Plan currently being finalized.

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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 Missouri River Sturgeon Iridovirus (MRSIV). 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.

- 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 Bozeman Fish Health Center staff prior to shipping samples.
    - Send them early in the week.

- If you have any questions, please call 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 minimum of 80 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±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.

Decision to Spawn or Not to Spawn and Timing

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 – 60 vials with aliquoted 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 or Dietrich'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.

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

- Dissect out gill tissue and internal organs (i.e., spleen, GI tract, liver, kidney, and lesions) from a freshly euthanized (dead less than 20 minutes) fish and place into Davidson's or Dietrich's fixatives.
- Small fish may be placed whole into fixative.
- Any bottles that have tight screw top or snap-style lids may be used.
- It is very important that the tissue sample for histology is at a 1:10 ratio of tissue:fixative. Samples in Dietrich's fixative can be shipped via FedEx directly to the Bozeman Fish Health Center. **Do not send samples in Davidson's fixative through the mail.**

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.

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 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. According to propagation and genetics management plans hatchery propagation is 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".

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 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.

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