Pallid Sturgeon Propagation Plan

Prepared by the

Pallid Sturgeon Propagation Committee

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Executive Summary

Only genetically pure pallid sturgeon will be used for establishing the captive broodstock and for restoration stocking. All propagation of pallid sturgeon will occur under the authority of an Endangered Species Act Section 10(a)1(A) permit or sub-permit. The Propagation Committee recommends that fall collection of wild adult pallid sturgeon be tried again in addition to spring collection.

Captured adult pallid sturgeon will be selected based on the following prioritization:

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

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

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

4. Should wild pallid sturgeon numbers decrease to the point where no or few new fish are captured, but it is determined by the Propagation Committee that it is necessary or desirable to reuse previously-spawned wild pallid sturgeon to create new, unique matings, recaptured fish may then be brought into a spawning facility.

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

The need to minimize the stress of capture cannot be over-emphasized. It is important to eliminate as many sources of stress and reduce the incidence of stress as much as possible. Streamside spawning is not currently considered to be practical for the large numbers of fish collected in RPMA 2. Because of the small numbers of fish typically collected in RPMA 1 and because it is not now recommended to move fish from RPMA 1 into hatchery facilities in RPMA 2, streamside spawning will continue in RPMA 1.

Spawning facilities will have filtered, disinfected (UV or ozonated) water supplies to improve water quality and reduce pathogen loads. 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. Signs of disease, behavioral changes, changes in feeding patterns or extreme loss of body weight will initiate contact with fish health personnel. Experience indicates that the recommended temperature range for spawning pallid sturgeon is 62-68° F (17-20° C). Immediately after spawning is completed, water temperatures should be gradually reduced 5-7° F (3-4° C). When an adult pallid sturgeon becomes moribund, or upon the death of an adult, the Recovery Team Leader and a regional fish health biologist must be notified. While 1 ♀ x 4 ♂ crosses are recommended in the Upper Basin Stocking Plan, the actual number of families created to produce four families per female may exceed four. Determination of the number of crosses created is made by the hatchery manager at each spawning facility and

will be based on the situation at the time of spawning. Sub-basin stocking plans will indicate how many progeny will be stocked per female. Microsatellite analysis is used to determine the suitability of captured pallid sturgeon as potential donors. Monitoring Polarity Index, performing Progesterone Assays, and observation of the color differentiation of the animal and vegetal poles of the oocyte as it approaches ovulation have all been used to determine the proper time to induce final ovulation. Although a suitable temperature range for spawning pallid sturgeon is 62-68° F (17-20° C), it is preferred that water temperatures throughout the spawning process should be maintained at approximately 63° F (17° C).

Eggs are expressed from the females by hand-stripping. The use of catheterization to express the eggs from a female is not condoned as a standard practice. Post-spawn adults will be returned as soon as they are determined to be healthy enough and the receiving waters' temperatures are adequate. It is in the best interest of the fish to release them as soon as possible and all efforts will be made to expedite 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. The final protocols for the Gavins Point NFH broodstock will be determined once fish have become sexually mature.

The acceptable temperature range for incubating pallid sturgeon eggs from adults collected in Montana and North Dakota appears to be 55-65°F (13-18°C). It is recommended that 65°F be considered an upper bound for the successful incubation of pallid sturgeon eggs.

The Propagation Committee has established maximum rearing densities of .5 lbs./ft.^{2} for fingerling pallid sturgeon and 0.7 lbs/ft^2 for yearling pallid sturgeon. The preferred temperature range for intensively cultured pallid sturgeon should be considered to be 43-70°F (6- 21°C). It is recommended that fish be kept on feed year round. Over-wintered pallid sturgeon should be kept at or above the temperature at which they are observed to stop feeding (40-45°F (4 - 7°C)). If a facility cannot keep its over-wintered pallid sturgeon above the minimum recommended temperature, it is recommended the fish be stocked in the fall. It is unwise to significantly retard or accelerate the growth rate of pallid sturgeon by manipulating rearing water temperatures to or beyond the limits of the preferred temperature range. All operations involving the handling or manipulation of young pallid sturgeon should be performed to minimize stress as much as practical.

The numbers of fish to be stocked into an RPMA or incorporated into the captive broodstock population will be determined by sub-basin stocking plans. Hatchery-to-hatchery transfers of fish are not recommended due to the risk of spreading disease to the receiving facility. The management authority responsible for the hatchery receiving fish and the appropriate state agency in which the receiving hatchery resides must be notified and approve of all fish transfers. The timing and location of stocking in any RPMA will be determined by the biologist responsible for managing pallid sturgeon in that RPMA. Stocking timing and location, the numbers and size of fish to be stocked, and the stocking goals for each RPMA will be addressed in sub-basin stocking plans. Prior to their release, individual lengths and weights will be collected and recorded for each PIT-tagged hatchery-reared pallid sturgeon. Each hatchery's manager is responsible for sending the data collected from his/her hatchery's fish to the

biologists responsible for the RPMA into which the fish are released and to the Recovery Team Leader. Management biologists will verify the accuracy of the data. The Recovery Team Leader is responsible for maintaining the database.

All permits and permission from state agencies must be obtained before the collection, possession, transport or importation occurs. The final decision to receive fish from any facility resides with the individual states.

Fish health reports will be sent to all appropriate sub-basin workgroup committee members. The Recovery Team Leader will be responsible for dissemination of this information. Acceptable Pre-release Health Assessment scores for virus severity should not exceed an average of 3.0 and liver conditions should at least average between 3 and 4. Certification for stocking will also consider a lot's health history, mortality and signs of clinical disease.

The Propagation Committee identified propagation issues that need further research, identified other program needs, and made recommendations for improving the current program.

Introduction

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

While limited specific knowledge exists about its life history, the pallid sturgeon is known to be a long-lived, late-maturing iteroparous species. Although the life expectancy of the pallid sturgeon is unknown, individuals older than 40 years have been documented and the maximum age is estimated to be between 50 and 60 years old. Age at sexual maturity is estimated to be 7 to 9 years for males and 15 to 20 years for females. The spawning periodicity of the pallid sturgeon is estimated to be 2 to 3 years for males and 3 to 10 for females.

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

Very little is known about specific environmental factors or cues pallid sturgeon require to develop viable gametes and successfully spawn. 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 of affecting gamete production, gamete quality and spawning success.

Because pallid sturgeon populations are limited in number and declining, with rare natural spawning and no documented recruitment, the Pallid Sturgeon Recovery Plan relies on a hatchery program as a short-term recovery objective to perpetuate the species until habitat modifications can occur to allow natural spawning and recruitment. Implementation of the pallid hatchery program began in 1992 when pallid sturgeon were artificially spawned at Blind Pony State Fish Hatchery (SFH), Sweet Springs, Missouri. The captive broodstock population that will serve as the source for gametes and fish when wild populations are no longer able to provide viable fertilized eggs was started in 1992 at Gavins Point NFH, Yankton , South Dakota, from fry from the matings created at Blind Pony SFH.

A lack of information about the distribution, movement, and timing and location of spawning, together with ineffective capture techniques and gear, hampered initial attempts to obtain wild pallid sturgeon brood fish. The first attempt to spawn streamside took place in 1993, but high flows that year created problems with sampling efficiencies and no female pallid sturgeon were captured. Captured pallid sturgeon brood fish were brought into Miles City SFH, Miles City, Montana, in April, 1995. The inability to produce fertilized eggs from this effort was attributed, in part, to the reliance on the spawning protocol developed for the white sturgeon. The next successful spawn of wild pallid sturgeon occurred during 1997. Age classes currently represented in the captive broodstock at Gavins Point NFH include progeny from matings in 1992, 1997, 1998, 1999, 2001, 2002, and 2003.

The initial plan for the artificial propagation of the pallid sturgeon, *Pallid Sturgeon Propagation/Genetics Plan,* was written in 1993 by Herb Bollig, manager of the Gavins Point NFH when the USFWS identified that the recovery of the endangered pallid sturgeon would depend on artificial propagation. This plan served as an excellent guide for pallid sturgeon propagation through the first ten years of the pallid sturgeon propagation program.

An ad hoc group within the Upper Basin Pallid Sturgeon Workgroup comprised of individuals with expertise in fish culture and fish health met in June 2002 to discuss pallid sturgeon propagation. The Propagation Committee was created at this meeting "to bring together fish health and hatchery experts involved with pallid sturgeon propagation and recovery to discuss, develop, and refine pallid sturgeon health testing protocols, propagation protocols, and state import recommendations and requirements." This Committee was given the task of rewriting the 1993 Pallid Sturgeon Propagation/Genetics Plan, incorporating the knowledge gained from eleven years of additional experience and new information. The Propagation Committee will also review research proposals to prevent the repetition of previous work, assure research methods are effective and appropriate, and assure requests for fish or gametes are within hatchery production capabilities.

Objective

The objective of this Pallid Sturgeon Propagation Plan is to describe and document the fish culture methods that will be used to propagate this species. 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.

The methods established in this Plan were derived from 11 years of pallid sturgeon culture experience. This Plan can be revised at any time by the Pallid Sturgeon 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 or in determining the suitability of a facility for the culture of pallid sturgeon.

Broodstock Management

Although pallid sturgeon recovery ultimately requires natural spawning and recruitment within all or a portion of the species' range, the Recovery Plan identifies artificial propagation as the only option to perpetuate the species through the existing reproduction/recruitment bottleneck. Artificial propagation will be used to increase the current abundance of pallid sturgeon within the species' historic range until river function and habitat changes allow sufficient natural reproduction and recruitment to maintain the species. The broodstock and stocking programs will have to continue beyond that time when suitable flows and habitats are made available until the reestablished hatchery-based populations are proven to provide the necessary recruitment in those areas where recovery is possible. In recovery areas where the pallid sturgeon is not recoverable due to insurmountable problems with habitat or flows, 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 Pallid Sturgeon Recovery Plan was written in 1993. Based on the assumption that there were only a few surviving wild pallid sturgeon, the Recovery Plan calls for the establishment of three separate broodstocks, each composed of ten to fourteen captive wild fish. These captive broodstocks would then serve as a source of gametes and fish for recovery stocking efforts. The discovery of larger-than-expected numbers of surviving wild fish and the limited available hatchery space made this strategy impractical. Fortunately, the development of relatively effective capture and spawning techniques permitted an optional strategy of capturing, spawning and releasing wild pallid sturgeon to obtain fertilized eggs for the creation of a broodstock population and for production of fish for restoration stocking.

The current pallid sturgeon propagation program includes a dual strategy:

1) Use the offspring of artificially-spawned captured wild pallid sturgeon to create a broodstock and to provide fish for recovery stocking and research.

2) Use a captive broodstock to produce gametes, eggs and fish for recovery 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 (>40 years old) fish. Eventually, the wild pallid sturgeon populations will cease being reliable sources of gametes due to senescence, their

reduced availability due to decreasing numbers, or their extinction. Exactly when this will occur is currently unknown, but is expected to be within the next decade. It is not the intent of this program to permanently remove these wild donors from the wild. These fish will be kept alive and returned to the river reaches where they were captured.

With the demise of wild pallid sturgeon as a source of fertilized eggs, 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 until habitat and environmental issues are addressed and natural reproduction and recruitment are proven to occur in the wild. The current expectation is that the wild pallid sturgeon populations currently used as sources of gametes will be extinct before the captive broodstock is fully functioning.

Wild Broodstock Program

Donor populations

Only genetically pure pallid sturgeon will be used for establishing the captive broodstock and for restoration stocking. As the incidence of hybridization is greater in lower basin populations, wild donor fish captured from Recovery Priority Management Areas (RPMAs) 1 and 2 will be used to produce progeny for the establishment of the captive broodstock population and for restoration stocking. Pallid sturgeon within RPMA 3 have been excluded as donors due to unacceptably low capture rates due to low population numbers. Fish from RPMA 3 may be used as donors if there is ever a need to use the hatchery-released pallid sturgeon in this RPMA as a source of progeny.

Collection of adults

The collection of wild adult pallid sturgeon for use as broodstock has occurred during fall (August-November, but mainly in October) and spring (April and May). Fall collection was used during 1996, 1997, and 1998 as a method of capturing pallid sturgeon to avoid the reduced capture efficiencies and safety issues associated with high spring flows. Spring collection also occurred during these years, to provide an alternative source of donors should the fall-captured fish not produce viable gametes. Due to the hatchery's water temperature profiles, the fall-captured fish were brought into Gavins Point National Fish Hatchery (NFH), while the spring collected fish were brought into Garrison NFH. A total of six fall-captured females were spawned. Although one female was lost due to a gill injury that occurred during capture, there was no mortality attributed to either the fall collection or the subsequent spawning process. The quality of fertilized eggs from fall-captured fish was better than the quality of eggs from spring-captured females. An iridovirus was identified in shovelnose sturgeon held at Gavins Point NFH in December 1998. Fall collection of adult pallid sturgeon was suspended because the virus issues prevented fish from Gavins Point NFH from being imported and released into Montana waters and Garrison Dam NFH could not over-winter adults. The sole dependence on the early spring capture of adult pallid sturgeon began in 2000 and spring capture has occurred annually since then.

The Propagation Committee recommends that fall collection be tried again in addition to spring collection. The Committee believes that the effects of the stressors of capture, transportation, exposure to a hatchery environment and spawning can be minimized if distributed over a few months time rather than all occurring sequentially in the spring. Fall collection of adults also permits better monitoring and control of egg development as water temperatures, water flows, and light periodicity can be manipulated throughout the winter and pre-spawning periods.

Sexing and selection of potential donors

Initial determination of the sex of a captured pallid sturgeon occurs at the time of capture. The appearance of the fish is typically used for initial sexing (females typically have a greater relative girth than males, but variability is high). Fish suspected to be females are catheterized to determine if they have mature eggs. Catheterization is performed by trained personnel while the fish are held in holding tanks in the capture boat or on the transportation truck. The presence of immature eggs indicates a female that will not produce eggs that season. These females will be immediately released, or, if mistakenly misidentified and brought into a spawning facility, released as soon as possible. Occasionally, the catheterization of testes material confirms the fish is a male. Newly captured fish suspected to be females are brought into a spawning facility for further identification at spawning time. The use of incisions for sexing is not recommended due to concerns about infection, the formation of adhesions due to scarring and the extended healing time due to the cold ambient water temperatures.

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

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

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

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

4) Should wild pallid sturgeon numbers decrease to the point where no or few new fish are captured, but it is determined by the Propagation Committee that it is necessary or desirable to reuse previously-spawned wild pallid sturgeon to create new, unique matings, recaptured fish may then be brought into a spawning facility.

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

The Pallid Sturgeon Recovery Team leader will maintain the database for captured pallid sturgeon. Copies of the fish database will be distributed to each capture boat and transport truck

to facilitate a quick determination of whether a fish should be released or held as a donor. In order to facilitate the selection of captured pallid sturgeon for spawning, this database will be sorted by PIT tag number and by second PIT tag, if any. Entries for previously captured fish will be color-coded to indicate where the fish ranks within the selection priorities.

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

Handling & stress

Capture, handling, transportation and spawning are all stressful to pallid sturgeon. The need to minimize the stress of capture cannot be over-emphasized. Stress reduces the probability of survival of the fish, compromises the fish's immune system and can precipitate fish health problems and disease. It is important to eliminate as many sources of stress and reduce the incidence of stress as much as possible. All propagation procedures will be periodically reviewed by the Propagation Committee to identify and mitigate sources of stress.

The current version of the "Protocol on Collecting, Tagging, Holding, Transporting and Data Recording for Researchers and Managers Handling Pallid Sturgeon" will guide the capture, handling, transportation, holding, spawning and release of wild adult pallid sturgeon used as broodstock. (Appendix A). The current version of this protocol can be found on the USFWS's Missouri River Fish and Wildlife Management Assistance Office's website at http://www.r6.fws.gov/moriver/pls%20handling%20protocols.pdf. This protocol will be annually reviewed and updated when necessary.

The white sturgeon recovery program has observed that the accumulated stresses present at one of their facilities significantly decreased the ovulation rate of the female brood and increased the variability in embryo survival to hatch. These same difficulties are observed in spawning wild pallid sturgeon. Further work is needed to identify and reduce the sources of stress in the captive broodstock.

Capture of wild adults

Stress during capture can be reduced by minimizing handling and the time a fish is kept out of water, maintaining adequate water quality in holding tanks, and keeping transportation times as short as possible. Individual trammel net drifts should be no longer than 20 minutes. Fish must not be held out of water for longer than 2 minutes, unless the gills are irrigated. If a pallid sturgeon is extremely tangled in the trammel net, the net must be cut to minimize handling stress and the time the fish is held out of water. Holding tanks in the capture boats should be a minimum of 6 feet in length and made of plastic or other non-abrasive material (if metal, tanks must be lined with a spray-on bed-liner compound or a similar substance). Holding tanks must be covered when transporting fish (although a tarp is preferred, a raincoat works in an emergency). Holding tank water should be exchanged at least every 15 minutes using, an electric aerator, a

bilge pump or a bucket. A non-abrasive cradle, preferably with a hood, should be used to move fish.

All capture boats must have a PIT tag reader and a coded wire tag reader. The use of heavy-duty alkaline batteries in all PIT tag readers is required, as weak batteries in PIT tag readers can cause problems with the detection of PIT tags. Therefore, all boats should carry fresh additional batteries. Fish should be inspected immediately upon capture for internal and external tags. Extreme diligence is needed when searching for PIT tags. Tag location and depth within the fish, reader orientation, and false readings as the result of conflicting signals when there are two PIT tags can affect the detection and reading of PIT tags. Fish that have not been previously PIT-tagged will be immediately PIT-tagged. A 1cm.^{2} sample of a pectoral fin from each "new" fish will be collected at this time. Fin samples will be placed in a properly labeled envelope and allowed to air dry. Physical measurements and collection information will also be taken and recorded at this time. To reduce stress, the captured adult should be kept in water, rather than on a stretcher, while physical data are collected. A data sheet and measurement diagram can be found within the "Protocol on Collecting, Tagging, Holding, Transporting and Data Recording for Researchers and Managers Handling Pallid Sturgeon" (Appendix A). During the inspection for tags, tagging, or the taking of physical measurements, fish must not be held out of water for longer than 2 minutes unless the gills are irrigated.

To provide protection against stress-induced bacterial infections, trained personnel will administer a prophylactic intramuscular antibiotic injection to each pallid sturgeon prior to their transport to the spawning facility. Fish that will be released will not be injected. Injection will be administered into the dorsal musculature. See "Use of Injectable Drugs" in Fish Health Section.

Post-capture transportation to hatchery

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

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

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).

Streamside (or remote site) spawning

The Propagation Committee recognizes that streamside spawning may potentially reduce many of the stresses and resultant fish health problems associated with transporting wild pallid sturgeon to a hatchery for subsequent spawning. Streamside spawning reduces the stresses of handling and transportation and eliminates the fish's need to adapt to a water supply with different chemical, turbidity and temperature properties than it is used to. Additionally, the "industry standard" for sturgeon culture is to "take the hatchery to the fish, rather than the fish to the hatchery".

However, streamside spawning is not without problems. Streamside spawning requires the purchase, set up, and maintenance of small hatchery facilities in remote sites. Water is usually pumped, requiring constant vigilance. Fish are still required to adapt to an artificial environment. Security is often compromised and it is difficult to have fish culturists present on site throughout the capture and spawning period to monitor the equipment and fish's condition. Due to the limited water clarity and inexperienced staff, infections and other fish health problems have not been detected or treated in a timely manner. Streamside spawning also requires that brood fish be captured and held immediately prior to spawning which often coincides with the spring runoff. Water quality is poorer and the pathogen load is higher than in a hatchery, as there is no realistic way to filter and disinfect the water at a remote site.

For these, and other reasons, streamside spawning is not currently considered to be practical for the large numbers of fish collected in RPMA 2. Because of the small numbers of fish typically collected in RPMA 1 and because it is not now recommended to move fish from RPMA 1 into hatchery facilities in RPMA 2 due to concerns about the Pallid Sturgeon Iridovirus, streamside spawning will continue to be used in RPMA 1.

When streamside spawning is used, the practices established for handling, holding, and spawning will be followed. Because of the reduced security at remote site facilities, special emphasis must be placed on minimizing the handling and disturbance of the fish. Temptations to "show-off" fish to visitors that frequent these sites must be avoided.

Holding wild adult fish in hatcheries

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

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

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

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

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

Experience indicates that a suitable temperature range for spawning pallid sturgeon is 62-68° F (17-20° C). Because warm water temperatures can cause undesirable rapid final development and ovulation of oocytes, optimally, water temperatures throughout the spawning process should be maintained at approximately 63° F (17° C). Immediately after spawning is completed, water temperatures should be gradually reduced 5-7° F (3-4° C) to slow pathogen reproduction and minimize stress in the fish.

Captured pallid sturgeon occasionally die when held in captivity. These fish offer unique opportunities to improve the program. Pallid sturgeon have difficulty tolerating handling and the accumulated stresses 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. Contact information is located in Appendix C.

If a pallid sturgeon dies while in captivity, the hatchery manager will document the death of each adult fish. 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. Every effort should be made to collect tissue samples immediately. As a last resort, if tissue samples aren't collected, fish should be kept whole and placed on ice or frozen. Tissue samples collected from fish that expire can further an understanding of causes of death. Fish health or trained hatchery personnel can take tissue samples following the established Pallid Sturgeon Sampling Protocol (Appendix D). Because tissue samples deteriorate rapidly due to post-mortem changes, tissue samples must be collected within 20 minutes of death to be of value.

Mating design

Factorial mating (where multiple males are used to fertilize each female) is typically used to generate half-sibling "family groups". The number of males used per females determines the number of families created. The total number of families created is limited by the carrying capacity of the hatchery facilities. The variability of egg and sperm quality and the difficulty of accurately sexing pallid sturgeon require the creation of more crosses than may eventually be realized. Although 1 ♀ x 4 ♂ crosses are recommended in the Upper Basin Stocking Plan, the actual number of families created to produce four families per female may exceed four. Determination of the number of crosses created is made by the hatchery manager at each spawning facility and will be based on the situation at the time of spawning. Sub-basin stocking plans will indicate how many progeny will be stocked.

Sperm cryopreservation

In RPMAs 1 and 2, a disproportionate number of male pallid sturgeon (approximately 4 males to 1 female) are being captured during broodstock collection efforts. Given the few remaining wild pallid sturgeon, it is important that the genetics of all of these males be captured for representation in hatchery-released fish or in the captive broodstock. It is not possible to incorporate all of the males captured in a given year into the propagation program as available hatchery space limits the number of crosses (family groups) that can be held. Milt cryopreservation can be used to capture and store the genetic contribution of male pallid sturgeon that are not used to fertilize eggs during the annual spawning program.

In 2000 a major breakthrough for sturgeon cryopreservation was achieved when Warm Springs Fish Technology Center (FTC) personnel fertilized and successfully hatched pallid sturgeon eggs using cryopreserved milt at the Garrison Dam NFH. As a result, the development of pallid sturgeon milt storage capabilities and additional testing of cryopreservation techniques were initiated. Storage and cryopreservation equipment was purchased for the Garrison Dam NFH to complement what was already in place at the Warm Springs FTC. Additional storage capability was developed at Gavins Point NFH. All three facilities are sent samples of milt from all males collected. Having redundant milt storage repositories at the three facilities should prevent the catastrophic loss of the genetic contribution of any male.

Current cryopreservation technology has provided very good post-freeze motility with some fish, while in others, motility is less than desired. Refinements in techniques may provide higher quality milt from all fish sampled. The USFWS has funded a ten-year cryopreservation research and development program at all three sites beginning in FY2004.

Currently, the milt repository is directed at providing small lot production with the use of 0.5 ml straws. The original intent of the program was to be able to infuse new genetic material from wild pallid sturgeon into the captive broodstock program when wild fish are no longer available. The repository may also serve as a secondary source in the current propagation program in years when there are insufficient males available during the spawning process to meet mating design goals. There are currently straws from over 40 males in the repository (2003), including males that are already represented by stocked progeny

The cryopreservation of sperm, although not especially difficult, incorporates many variables that can affect the outcome. Such valuable sperm should not be cryopreserved by untrained individuals. In order to maintain quality control of the process and product, the cryopreservation of pallid sturgeon sperm will be performed by personnel from the Warm Springs FTC or by personnel trained by Warm Springs FTC staff. The protocol for the collection and storage of pallid sturgeon milt appears in Appendix E. A thorough discussion of sturgeon sperm cryopreservation by William Wayman can be found at http://etd.lsu.edu:8085/docs/available/etd-0710103-135135/.

Mating strategy based on microsatellite analysis

Without immediate environmental changes the pallid sturgeon population in the upper Missouri basin will likely be extirpated within the next decade. The Recovery Plan identifies stocking as a measure to prolong their existence in the wild until conditions can be met that will allow for natural recruitment. Consequently, the species' recovery depends on an augmentation program that maximizes the available genetic variability by employing the largest effective population possible within the confines of the program and the remaining wild population.

A higher diversity of alleles enables a species to respond better to change and is characteristic of a genetically healthy population. Because the augmentation program can capture only a small sample of the existing and historic wild pallid sturgeon populations, the few donors collected probably do not represent the range and distribution of alleles that once existed in the pallid sturgeon genome. Enhancing the genetic fitness of stocked pallid sturgeon will provide the species with the best chance of recovery. The pallid sturgeon augmentation program maximizes the genetic variability of hatchery-produced pallid sturgeon by creating as many unique matings as possible, equalizing their genetic contribution within the fish stocked, and mating the most genetically distant parents. This mating strategy increases the abundance of uncommon alleles in the hatchery-produced progeny, allowing natural selection to determine the most suitable genotypes in the stocked fish

To determine the relative genetic distance of potential parents, air-dried $1 cm.^2$ fin tissue samples from each spawning adult pallid sturgeon are analyzed to determine allele frequencies at specific locations on the DNA strand. This analysis is performed at the Genomic Variation Laboratory,

University of California, Davis under the direction of Dr. Bernie May. The results are provided in table format with both Nei and Rogers Genetic Distance values given for all broodstock sampled. These values are then used to determine which crosses would best maximize genetic diversity. The fin samples are archived at UC Davis for future reference.

Microsatellite analysis was initiated in the pallid sturgeon augmentation program in April 2000. In addition to assisting with mating design, this process can also:

1) Identify suspect hybrid sturgeon so they are excluded from matings.

2) Provide a genetic profile of each adult that will be used to determine the parentage of unmarked progeny (provided all parents have been sampled and genotyped).

3) Measure the genetic distances between pallid sturgeon populations.

Character index

Using the methodology described in the USFWS Technical Note "Character Index for Pallid and Shovelnose Sturgeon", a character index for each adult pallid sturgeon can be calculated (Appendix F). Although character indices are not constant across the pallid sturgeon's range, it is currently recommended that only pallid sturgeon from Montana and North and South Dakota having Character Indices higher than 425 be used as broodstock. This will reduce the risk that pallid/shovelnose sturgeon hybrids will be used in pallid sturgeon propagation. Relying instead on genetic mapping, the Upper Basin's pallid sturgeon propagation program does not currently use a Character Index to determine the suitability of captured pallid sturgeon as potential donors. Character Indices have been developed and are used by the Lower Basin pallid sturgeon recovery program.

Staging

Determining the best time to induce ovulation is an imprecise process involving a certain amount of art. General indicators of a female's ripeness include a softening of the abdomen, coloration of eggs (coloration difference of animal and vegetal poles, with clear demarcation between the two poles), and nuclear (germinal vesicle) breakdown. Egg samples are collected to monitor egg development (staging) by catheterizing the female. During catheterization, a 1/8" ID, semi-rigid translucent tube (ice machine tubing works well), disinfected with Nolvasan, is inserted through the urogenital pore into the egg mass. A few eggs are then aspirated into the tube for inspection. The use of an incision is not used or recommended at this time due to concerns about infection and development of internal adhesions. The Propagation Committee believes that catheterization is the less dangerous process. Eggs are aspirated and boiled in a saline solution (isotonic Ringer's solution or contact lens solution), cooled in ice water and later bisected along the animal-vegetal pole axis. Keeping the eggs in 10% formalin for 24 hours before bisection toughens the egg, making sectioning easier. These reference samples from each egg sample collected during catheterization will be preserved in 10% buffered formalin, labeled and stored at the facility where they were collected. Additionally, a photographic record showing the final

Polarity Index of the last egg sample from each female will be maintained in order to refine egg staging methodology.

Determining the proper time to initiate final ovulation

Monitoring Polarity Index, performing Progesterone Assays and observation of the color differentiation of the animal and vegetal poles of the oocyte as it approaches ovulation have all been used to determine the proper time to induce final ovulation by injecting luteinizing hormone-releasing hormone analog (LHRHa), a hormone analog that promotes oocyte maturation and ovulation.

As a pallid sturgeon egg approaches ovulation, the germinal vesicle migrates towards the cortex at the animal pole of the egg. The distance of the germinal vesicle to the cortex relative to the diameter of the egg is the Polarity Index (PI) (Figure 1). A Polarity Index less than 0.1 is considered to be acceptable, while a lower PI is preferred. Sturgeon culture literature relies heavily on egg Polarity Index and Progesterone Assay to determine the optimum time to induce ovulation. However, the use of PI and Progesterone Assay have given mixed results in the pallid sturgeon program.

Progesterone assays have been used to monitor the egg development of pallid sturgeon in order to determine the appropriate time to initiate hormone injections. The complexity of the process and the lack of consistent results raise questions about its value. The use of progesterone assay is not recommended as a stand-alone technique for accurately staging pallid sturgeon eggs.

Close observation of the progress of germinal vesicle polarization and of the demarcation of the polar bodies has also been effectively used to determine the proper time for the hormonal injection.

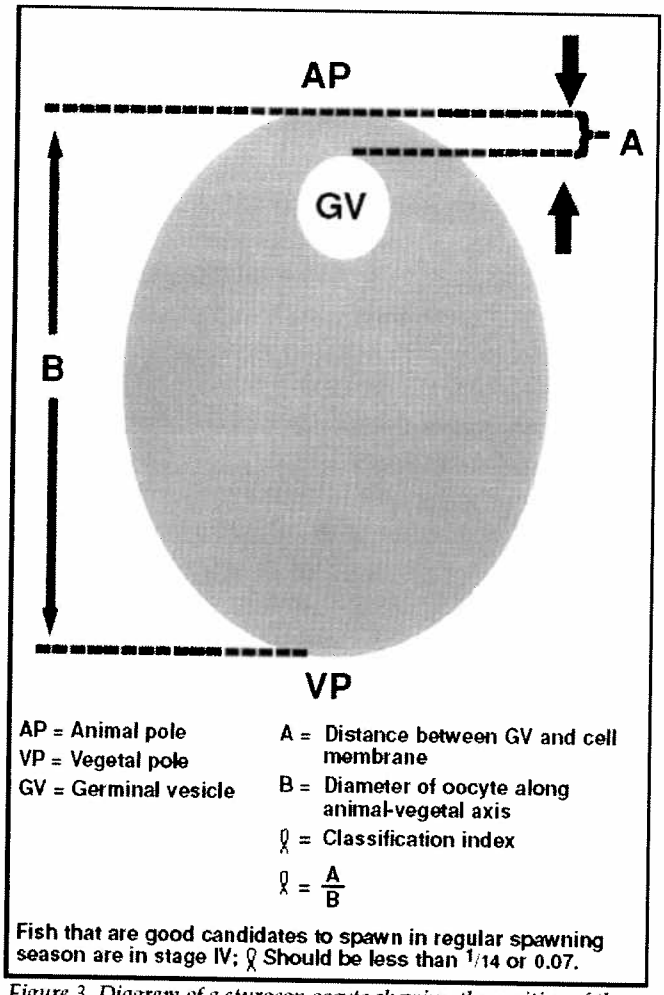

Figure 3. Diagram of a sturgeon oocyte showing the position of the germinal vesicle (GV) at the animal pole and the formula for determining the oocyte polarization index (PI) (modified from Detlaf et al., 1981, and Conte et al., 1988).

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

Spawning

Egg development can proceed rapidly as the egg completes germinal vesicle polarization and proceeds to germinal vesicle breakdown. This rapid development can be exacerbated by high water temperatures, causing fish culturists to miss the peak of ovulation. It is recommended that water temperatures throughout the spawning process be kept at approximately 63° F (17° C).

Injections of LHRHa are used to stimulate final ovulation and spermiation. Ovaprim has been tried on a limited, experimental basis, however, initial results indicated that it may not be suitable for use with pallid sturgeon. The Propagation Committee recommends that LHRHa be used until further research into the appropriateness and effectiveness of other hormones is completed. LHRHa is administered via injection into the dorsal musculature of the fish. All administrations

of LHRHa will comply with the LHRHa INAD program including the collection and reporting of data.

The total dosage of LHRHa administered to females is .05-.1 mg/kg of fish weight. LHRHa is given to females in two injections: a primer dose equal to 10% of the total dosage, and a resolving dose equal to 90% of the total dosage that is administered 12 to16 hours after the primer dose. At 65°F (18° C), ovulation usually begins approximately 10 hours after the resolving dose is administered.

Males are given a single LHRHa injection at a dosage of .01-.02 mg/kg. Spermiation occurs approximately 10 hours after injection. It is recommended that males be injected at least 12 hours before females to allow time for determining the viability of milt before ovulation occurs.

Sperm is typically expressed into a glass container and then transferred in to zip lock bags for storage. A sperm sample from each male will be evaluated for potential fertility by checking motility. As there is some evidence that the fertility and motility of sperm are directly correlated, sperm samples with zero motility will not be used.

Collected sperm should be kept between 35 and 40° F (2-4° C). Sperm can be refrigerated or placed in a cooler with wet towels or cardboard separating the zip lock freezer bags from ice placed in the coolers bottom. Sperm has been refrigerated for up to two weeks, however a loss of motility and viability occurs over time. Sperm should not be frozen for short-term use. Immediately prior to its use, zip lock bags containing sperm should be tempered to the ambient water temperature of the tank holding the adult fish to avoid thermal shock.

Sperm can be transported for use at other facilities. Sperm should be shipped in double (bag within a bag) zip lock freezer bags. Bags should be partially inflated with oxygen to allow for expansion in unpressurized airplane compartments. Zip lock bags containing sperm should be oriented to maximize the sperm's exposure to oxygen. Various soft-plastic containers with snap on lids (Tupperware, Zip-loc, etc.) have also been used to ship fish sperm. Their use will be further evaluated. It is recommended that the temperature of the sperm be held between 35 and 40° F (2-4° C) during shipment. Maintaining a constant temperature during sperm sample storage is crucial. Large, sudden temperature fluctuations can result in water condensing on the internal surface of the bags, which can initiate sperm activation, rendering the samples unusable.

Pallid sturgeon are spawned using a dry spawning technique, in which eggs are stripped into a dry pan, milt is added and then activated by the addition of hatchery water. Eggs are expressed from the females by hand-stripping. The egg mass within a female ripens over time, with eggs posterior in the egg mass ripening first. This requires that eggs from a female be collected periodically, typically at 2 hour intervals, throughout the spawning period. The use of an incision to release the eggs from female pallid sturgeon is not condoned at this time due to concerns about creating a site for the formation of internal adhesions, the undesirable stress of surgery, the increased risk of infection, and the extended recovery time required for incisions to heal.

The use of catheterization to express the eggs from a female should not be conducted as a standard practice. Instead, patience is encouraged in those responsible for spawning. Occasionally, a female does not respond normally to the LHRHa resolving dose and it may be difficult or impossible to express her eggs by hand-stripping during the expected ovulation period. If it is determined that the eggs from this female are important to the propagation program, a small sample of eggs should be collected utilizing a catheter, fertilized and observed for development. If the egg sample shows signs of development in 4-6 hours, and the female does not respond within this period, expressing the eggs via catheterization can be attempted. This procedure should not be attempted without peer consultation and approval if at all possible. Abnormal responses by females to LHRHa injections can be reduced by inducing ovulation only at the optimal time. This is best determined by closely monitoring the Polarity Index and egg appearance while staging females.

Eggs are expressed into a dry pan. Sperm from a single male is added, gently mixed throughout the eggs and then activated by the addition of ambient temperature hatchery water. The egg/sperm mixture is stirred for 5 minutes at which time excess sperm is rinsed from the eggs. The fertilized eggs are then drained of excess water. When pallid sturgeon eggs come in contact with water a gelatinous layer is activated. This gelatinous layer causes the eggs to adhere to one another or to any surface with which they come into contact. Unless this layer is compromised or removed, the eggs will clump and suffocate. A de-adhesion solution using a supersaturated aqueous solution of Fullers Earth is added at the rate of approximately 2-4 times the egg volume. The eggs are continuously and gently stirred with a feather until de-adhesion has been completed, usually 20 minutes.

Once de-adhesion is completed, the eggs are water-hardened for 60 minutes in a 100 ppm iodophore solution to reduce the incidence of bacteria, and then placed into incubation jars, where they are enumerated by displacement.

Immediately after spawning is completed, a prophylactic antibiotic is administered to all brood fish via an intramuscular injection. As with the post-capture prophylactic treatment, the injection will be administered into the fish's dorsal musculature. See "Use of Injectable Drugs" in the Fish Health Section.

Returning adults to the wild

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

Tagging adult pallid sturgeon using Combined Acoustic/Radio Transmitters (CART tags) is necessary for movement and habitat use research. While captured pallid sturgeon broodstock offer a readily accessible source of adult fish for tagging, the additional stress of holding them until they can be tagged and the implantation of CART tags immediately post-spawn may be sufficient to cause mortality in brood fish. If an individual fish's post-spawn health allows, adults can be implanted before their release. The manager of the hatchery holding the post-spawn adults will make the final determination of whether the adults can withstand the additional stress of transmitter implantation.

Instead of tagging post-spawn pallid sturgeon adults, it is preferable to CART tag adult pallid at other opportunities. Adult sturgeon that are captured during the spring brood fish collection but not held for spawning can be CART tagged before their release. An intensive fall collection effort, similar to the spring collection of brood fish, could also be initiated. Adult pallid sturgeon would be collected with trammel nets, CART-tagged and released. The use of external CART tags is being investigated. If appropriate, these external tags could be applied without the trauma of implantation to adult pallid sturgeon at any time the fish are available.

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 (year-classes and families) used to establish a captive broodstock that will preserve the maximum amount of remaining genetic variability. Once the captive broodstock had reached maturity within the hatchery environment, they could be crossed according to strict spawning protocols to obtain progeny for future recovery efforts.

The only captive broodstock ever established is the one at the Gavins Point NFH, which holds fish representing the genetics of pallid sturgeon from the Missouri River below Fort Peck Dam to the Gavins Point Dam including the Yellowstone River. More specifically, the only broodstock now existing at the Gavins Point NFH have come from RPMA 2 and a few from RPMA 4. No captive broodstock have been developed from any other RPMA. Gavins Point NFH was selected as the site for the pallid sturgeon broodstock program because it had previously been designated as the lead facility for culturing declining fish species within the Missouri River system, the facility has optimum water quality and quantity parameters, the hatchery has excellent sturgeon culture facilities, and, since there is no need to heat or chill water to obtain optimum growth, the culture of pallid sturgeon at this facility is very efficient.

Captive broodstock management

The exact spawning protocols for the captive broodstock program at Gavins Point will not be determined until the fish have become sexually mature. The protocols adopted for the captive broodstock program will be based on information gained through the handling, holding, and spawning of wild adult pallid sturgeon. It is anticipated that many of the processes and procedures described in this Plan for the wild pallid sturgeon spawning will be used in the captive broodstock program.

The hatchery-reared broodstock will be developed using the latest genetic procedures, guidelines, and recommendations outlined within this document and other sturgeon publications. This includes relocating wild broodstock into a hatchery for spawning purposes, PIT tagging each fish for later identification, providing food or forage to maintain adult body and egg condition, and checking all adults for spawning condition and oocyte maturation. The mating design incorporated into the program provides for, as much as possible, the equalization of the contribution of parents to the next breeding generation. If more than one female and more than one male spawn simultaneously, they should be injected with the appropriate hormone, spawned, and the green eggs divided into the same number of sublots (cells) as there are spawning males (di-allele mating). There should be matings between all possible parents. Gametes from different individuals will not be mixed prior to fertilization. This type of activity will be continued each year until the desired number of year-classes has been developed. A goal of the pallid sturgeon recovery program was to have a minimum of 5-10 pairs of adults contributing to each year-class. However, when working with very low numbers of individuals in an endangered species population located within a large river system where adults may spawn every 3-5 years, these numbers may be unattainable. Another goal is to have a minimum of 25-50 mated pairs contributing to the future broodstock at Gavins Point NFH in order to maximize the effective population size. Experimental, reintroduction, or augmentation stocking will be accomplished once progeny have been produced that are genetically representative of the wild population.

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

Once families from each year-class have been established, there are a couple of options for choosing the broodstock numbers needed for the captive program. If advanced young-of-the-year fish (< 6 months old) will be used for future broodstock, then approximately 50-100 fish will be randomly selected from each family for year-class participation. A year later this number

can be randomly reduced to 30-40 individuals until these fish are mature in 10 to 15 years. If yearling fish (>12 months old) are chosen for broodstock, then 30-40 fish can be randomly selected at that time from each family. Thus, no matter when future broodstock are selected, there should be a sizeable group of fish set aside so that a genetically representative number of fish will be available for spawning when sexual maturation occurs. When reducing numbers from year to year, any fish surplus to the captive program can be stocked or used for other approved purposes.

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

Matings of hatchery-reared broodstock should generally be done using individuals from different year classes. This crossing will convert genetic differences between the year classes, which undoubtedly exist because of the relatively small number of fish spawned each year, into genetic variation within the broodstock. Matings should be random to avoid genetic changes due to inadvertent selection. In order to prevent selection for early or late maturation, fish should contribute progeny to future broodstock only after most of the individuals of both sexes within a year class have become sexually mature.

Once a captive broodstock has been established, it is desirable to periodically introduce genes from wild fish into it. This introduction of new genes will increase the number of founders and can potentially prevent establishing a highly domesticated broodstock. Genes from wild fish can be introduced into the broodstock by crossing wild fish with hatchery fish or by raising progeny from wild fish to maturity and then spawning them with hatchery fish. Either method is acceptable and the choice should be based simply on which is the most feasible. Every effort should be made to maintain the genetic diversity of wild populations in broodstocks and in those fish produced for reintroduction or augmentation.

Continuous genetic evaluation and monitoring of the captive broodstock should be conducted and is a central feature of a well-designed 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 eyeup, percent hatch, fry size, stocking densities, and survival will be noted. Any other important incubation and rearing characteristics will be documented, also. Rearing parameters will, most likely, follow that used for production or stocking fish.

Egg Incubation

Egg disinfection

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

Egg enumeration

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

The extreme variability of both eye-up and initial fry mortality in current pallid sturgeon culture makes estimating the number of progeny produced from an initial egg inventory impractical. It is more effective to make the estimates of expected progeny 7-10 days post-hatch, when mortality usually stabilizes.

Incubation

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

Fungus infections - primarily Saprolegnia - can be a serious problem, killing eggs either by invasive damage to the egg structure or by causing the eggs to form clumps that lead to suffocation. Rolling the eggs during incubation is an effective technique to keep eggs from clumping. Incubating eggs at temperatures near the upper limit of the accepted range of incubation temperatures reduces egg incubation time and, therefore, egg exposure to fungus. When necessary, dead eggs should be siphoned from incubation jars to prevent them from becoming a medium for fungal growth. Based on the results of its use on paddlefish eggs at

Gavins Point NFH, the use of formalin to control fungus during pallid sturgeon egg incubation is not recommended.

Egg allocation, egg shipping, and the use of eggs for stocking

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

The shipping of eggs is the only safe and acceptable method to transfer pallid sturgeon between hatcheries. Because of the short period of time between fertilization and hatching, egg shipping logistics need to be scheduled immediately after spawning is concluded so that facilities can get eyed eggs when they are available. Handling and disturbance need to be minimized during egg shipment. Although it may be more convenient to ship eggs immediately after spawning, and pallid sturgeon eggs have been successfully shipped the day after fertilization, it is best to ship eggs after neurulation occurs, as the eggs are less sensitive to physical shock at this time. Eggs should be completely water-hardened prior to shipping. Eggs 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 eggs 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 eggs. Eggs should be disinfected with a 100 ppm iodophore solution for 10 minutes prior to being brought into the production area of the receiving hatchery.

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

Rearing

Rearing environment

Every effort should be made to minimize stress in hatchery-reared pallid sturgeon. A chart describing the rearing environments and selected fish culture parameters of hatcheries raising pallid sturgeon in the upper Missouri basin appears in Appendix G.

Although round tanks are preferred for rearing pallid sturgeon, rectangular tanks have been successfully used to rear smaller fish. Round tanks have several advantages over rectangular tanks in the culture of pallid sturgeon. Round tanks with center drains are somewhat self-cleaning, improving tank hygiene and reducing the amount of disturbance (stress) the fish have to endure during tank cleaning. Water velocities in round tanks can be easily adjusted to provide

the velocities that are appropriate for or preferred by the size of the fish. Tanks should have smooth (gel coated or lined) bottoms and sides to minimize abrasion of the fish and to improve ease of cleaning.

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

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

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

Water quality

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

Rearing densities

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

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

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

Pallid sturgeon reared at low densities in large (20 feet diameter) circular fiberglass tanks at Gavins Point NFH grew faster than counterparts in smaller tanks. These fish, given the relatively large amounts of space (area) available for swimming, did not always orient themselves into the current, but frequently exhibited large, cross-tank swimming patterns. This seems to indicate that it is best to rear pallid sturgeon in the largest tanks that a facility can manage. Although the same number of fish at the same density index may be held in many small tanks or a single large tank, experience suggests the single large tank provides a less stressful (healthier) environment. Larger tanks offer fish more mobility, less confinement, less apparent stress, and a rearing environment that appears to be more conducive to overall better fish heath.

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

Due to the potential risks of holding fish near their maximum density threshold, the various fish health issues complicating the pallid sturgeon propagation program, 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.

Flow

The distribution of pallid sturgeon reared in round tanks is partially determined by the water velocity within the tank. Pallid sturgeon prefer not to have to continually fight high water velocities. The fish typically spread out when velocities are low, but move towards the center drainpipe when velocities are high. Experimental work at Garrison Dam NFH demonstrates that fry in 30" diameter tanks use the entire bottom surface area of the tanks when water velocities at the tanks' circumference are between 0.1 to 0.2 ft/sec. When circumference water velocities

reached 0.3 ft/sec, the fry would begin to move towards the center drain where water velocities were lower. Fingerlings and advanced fingerlings (3-9") held in tanks with 4, 5 and 8 feet diameters used the entire bottom surface area of the tank when water velocities at the tank circumference are between 0.3 to 0.6 ft/sec. When circumference water velocities reached 0.7 ft/sec, the fish would begin to move towards the center drain where water velocities were lower.

For tanks with diameters less than 10 feet, exchange rates should be approximately .3-1 exchanges per hour. Large tanks can have lower exchange rates, as fish are not required to continually orient themselves into the current.

Rearing temperatures

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

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

It is recommended that fish be kept on feed year round. Pallid sturgeon appear to go off feed at or slightly below 45°F (7°C). Over-wintered pallid sturgeon should be kept at or above the temperature at which they are observed to stop feeding. This is expected to be 40-45°F (4 - 7°C). If a facility cannot keep its over-wintered pallid sturgeon above the minimum recommended temperature, it is recommended the fish be stocked in the fall.

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 have been successfully grown at growth rates of .040-.085" per day. Feeding the current commercial diets available and typically used for pallid sturgeon culture at the high feed rates associated with rapid growth rates can cause potentially irreversible and fatal liver damage. Barrow's has concluded that the currently available commercial diets do not permit rapid growth with lean livers (Rick Barrows, personal communication). Further research relating diet, growth rates and liver condition needs to be continued. While a range of optimum growth rates for pallid sturgeon has not been developed, it is unwise to significantly retard or

accelerate the growth rate of pallid sturgeon by manipulating rearing water temperatures to or beyond the limits of the preferred temperature range.

Handling, enumeration, sorting

All operations involving the handling or manipulation of young pallid sturgeon should be minimized, and when necessary, should be performed in ways to minimize stress as much as practical. It cannot be over-emphasized that pallid sturgeon should be handled or disturbed as little as possible. It has been demonstrated that prolonged periods of low stress can be worse on pallid sturgeon than intense stress over a short period of time, therefore culture operations such as handling, measuring, counting and cleaning should be performed as quickly as possible. The use of knotless nets for handling all young pallid sturgeon too small to require a stretcher is encouraged.

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

The first enumeration of fry usually occurs approximately 45 days after hatch and is scheduled to coincide with other needs for handling, such as splitting or their transfer into larger tanks. This eliminates the need for extra handling and, since the fish are usually 2 inches or longer, minimizes handling stress and reduces the physical damage to the fish from netting. Neosho NFH is experimenting with the use of photography in enumerating pallid sturgeon without handling the fish.

Selection of fish destined for captive broodstock program and for release into recovery areas

The numbers of fish from each mating to be stocked into an RPMA or incorporated into the captive broodstock population will be determined by sub-basin stocking plans. As these fish will be the basis for the future wild and captive broodstocks, they must represent as much of the original pallid sturgeon genome and available genetic variability as possible. Fish should be chosen randomly to include as much genetic variability as possible. While the culling of fish with obvious physical deformities or health problems is permissible, selecting fish for size, disease resistance, or any other attribute is prohibited. The number of fish needed should be randomly removed. All tanks holding the progeny of any single mating should be included in the sample of that mating. If a group of fish to be stocked contains fish that are too small to be tagged, the small fish should be stocked along with the lot. Small fish should also be included in the random, representative samples destined to be included in the captive broodstock program and kept until it is determined that they are unsuitable for this purpose.

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

Anesthetic use

Because juvenile pallid sturgeon exhibit a limited thrashing response when handled and adults can be easily restrained, the use of anesthetics during the shipping or handling of pallid sturgeon is not generally considered to be necessary. Limited experimentation at Garrison Dam NFH and Natchitoches NFH has demonstrated that MS-222 at 50 mg/l can be a safe and effective anesthetic for pallid sturgeon. Further research of the use of anesthetics to reduce stress and injury during handling, spawning, shipping and tagging is needed.

Feeding and Nutrition

Natural and prepared feeds are being used in the culture of pallid sturgeon. There is some evidence that pallid sturgeon initially fed live feeds have difficulty subsequently adapting to commercial diets. Sturgeon respond to external food stimuli before their mouths and digestive tracts are completely developed. Early familiarization with food scent has been found to improve the acceptance of feeds. Therefore, pallid sturgeon should be initially exposed to feed before their yolk sacs are absorbed.

Commercial feeds successfully used in pallid sturgeon culture are primarily salmon and trout formulations of Bio-Oregon's Bio-Diet and Nelson's Silver Cup. These feeds are used separately or in combination. Feed size is normally increased gradually as the length of the fish increases. To help fish transition to new feed or diet changes, feed sizes and feed types are blended for 7-10 days. Vibrator and mechanical feeders are typically employed to present feed to the fish 24 hours a day. Feed levels are calculated to feed the fish to satiation while minimizing waste. Juvenile pallid sturgeon must be kept on feed all year long, with feed levels adjusted to meet the fish's intake.

Table 1 shows the feeding regimes used at the four upper Missouri basin 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. Pallid sturgeon at this facility were found to waste considerable amounts of the commercial feeds used. Pallid sturgeon initially fed bloodworms lost quality when their diet was changed to krill, but their condition improved when they were put back on bloodworms. Fish on bloodworms are fed to satiation three times per day. Although there is little waste when using bloodworms, their use can be problematic. Frozen bloodworms are expensive (US$6-7 per "slab" in 2003) and the water in which they are frozen may carry unidentified and undesirable fish pathogens. It is important to use reputable sources for any natural feeds.

The use of forage fish for general production of pallid sturgeon is cost prohibitive. There are also concerns over importing fish pathogens with forage fish. Any use of forage fish in pallid

sturgeon culture will follow the recommendations established for their use with adult pallid sturgeon.

Feed conversion

Little definitive work has been completed to establish feed conversions for pallid sturgeon fed a variety of diets. Feed conversion ratios of 1:1.2 to 1:1.5 have been measured for fingerling and advanced fingerling pallid sturgeon production. Conversion ratios for juvenile brood fish have been measured in the range of 1:5 to 1:7.

Condition factor and relative weight

Length-weight relationships for fish can be expressed as condition factor or relative weight. Measured pallid sturgeon condition factors have been highly variable. Pallid sturgeon released from Miles City SFH had an average condition factor of .000132. Pallid sturgeon stocked from Garrison Dam NFH had an average condition factor of .00012 and Gavins Point NFH had an average condition factor of .000144. More work is needed to identify what are normal and acceptable ranges of condition factor for hatchery-reared juvenile pallid sturgeon. A relative weight curve based on data from hatchery reared pallid sturgeon appears in Figure 2.

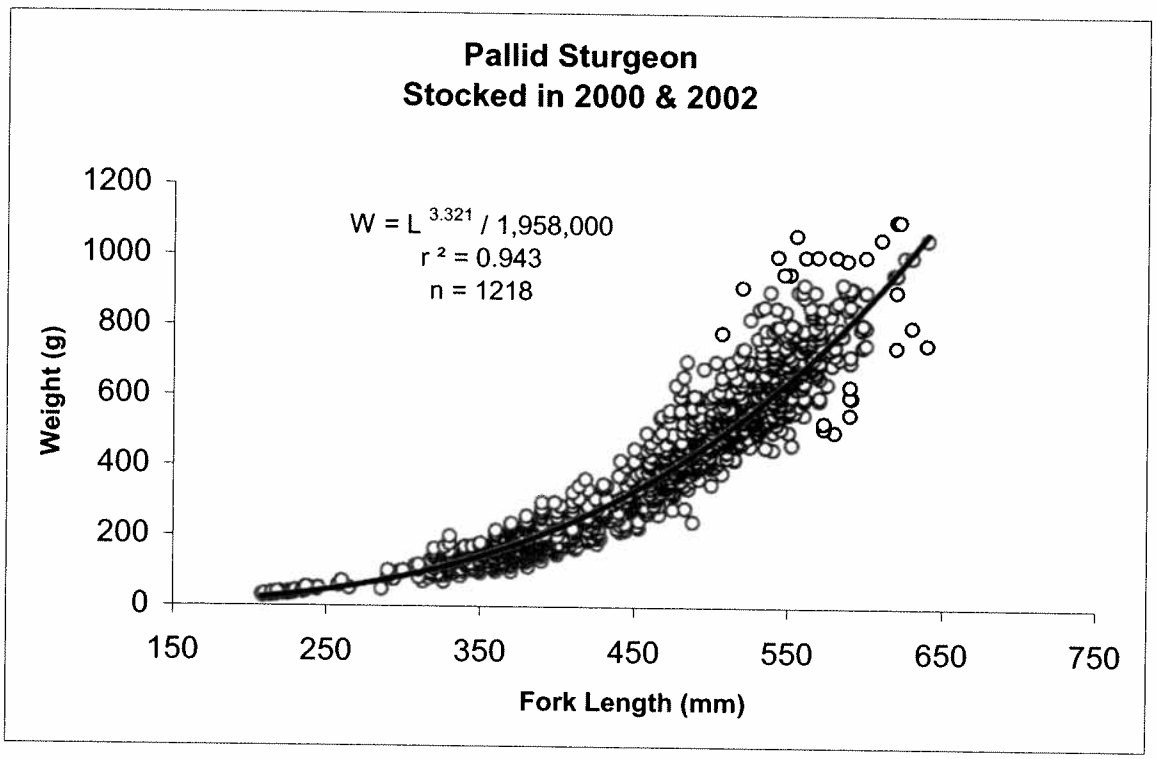

Figure 2. Length-weight relationship of hatchery reared pallid sturgeon

Gavins Point NFH			Garrison Dam NFH			Bozeman FTC			Miles City SFH		
Feed Type	**Approx. start size**	**% Body weight feed**	**Feed Type**	**Approx. start size**	**% Body weight feed**	**Feed Type**	**Approx. start size**	**% Body weight feed**	**Feed Type**	**Approx. start size**	**% Body weight feed**
BioDiet Starter #1	fry	10+	BioDiet Starter #1	fry	Satiation	BioDiet Starter #1 x cyclopeze	Fry	Satiation	BioDiet Starter #3	fry	1.5 conversion
BioDiet Starter #2	1"	10	BioDiet Starter #2	advanced fry	Satiation	Experimental diet equal to Grower #1	1.5"	5			
BioDiet Starter #3	2"	8-10	BioDiet Starter #3	1.97"	Satiation						
BioDiet Grower #4	3"	5-8	BioDiet Grower #4	3.5"	10	Experimental diet equal to Grower #2	3.5"	3.5	Silver Cup Trout #2	3"	1.5 conversion
			Silver Cup Salmon #2	3.94"	7						
Biodiet 2.0 mm x Silver Cup Salmon #2	6"	3-5	Silver Cup Salmon #3	5.9"	5	Experimental diet equal to Grower #3 - #4	5"	3	Silver Cup Trout #3	5"	1.5 conversion
			Silver Cup Salmon #3	6.9"	3						
Biodiet 3.0 mm x Silver Cup Salmon #3	8"	2-3	Silver Cup Salmon #3	7.5"	2	Experimental diet equal to Grower #4 - ⅛"	8"	2.5			

Table 1. Feeding regimes at four upper Missouri basin pallid sturgeon facilities

Excess hatchery production and the disposition of surplus eggs and fish

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

Inventory Adjustment	When	Size of fish
Post-spawn	late June, early July	Eyed eggs
Initial inventory	mid-July	Advanced fry
Late fall	September-October	Fingerlings (4-7")
Early spring	April-May	Juveniles (5-9")

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

Stocking

Pre-release health assessment

Prior to stocking, all lots of fish will be tested using the Pre-release Fish Health Assessment (Appendix I).

Hatchery-to-hatchery transfers of fish are not recommended due to the risk of spreading disease to the receiving facility. Hatcheries with closed water systems should never take in live fish. Alternative means utilizing egg transfers must be developed as soon as possible. Where fish transfers cannot be avoided, transfers are best accomplished with fish that are as small as possible. All lots of fish will be tested using the pre-release assessment prior to transfer. The management authority responsible for the hatchery receiving fish and the appropriate state agency in which the receiving hatchery resides must be notified and approve of all fish transfers.

Transportation

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

Round tanks are best for transportation of large pallid sturgeon. To maintain good tank water quality during transportation, taking fish off of feed 24 hours prior to transportation can minimize fecal waste and released ammonia. Loading rates during transportation should be kept

as light as possible, with 0.5 pounds of fish per gallon of water as a suggested maximum loading rate.

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

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

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

Coordination

The timing and location of stocking in any RPMA will be determined by the biologist responsible for managing pallid sturgeon in that RPMA. Hatchery managers will strive to meet these stocking requests. Stocking timing and location, the numbers and size of fish to be stocked, and the stocking goals for each RPMA will be addressed in sub-basin stocking plans.

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

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

Tagging

Hatchery propagated pallid sturgeon do not need to be marked or tagged prior to their release, although it is preferable if they are marked. Those fish that are marked will preferably use two marking methods. Pallid sturgeon that average nine inches in length or longer can be PIT tagged and elastomer tagged. When an appropriate wire-coding system is designed and accepted, fish less than 9 inches can be marked using differentially placed coded wire tags and elastomer tags.

The method of tagging, tag numbers and identifying elastomer colors used will be recorded for each fish tagged.

The current methods of tagging hatchery pallid sturgeon are unreliable. There are problems with PIT tag retention, consistently locating and reading PIT tags, and accurately identifying the colors of the elastomer marks in recaptured fish. The current tagging methodology used in the pallid sturgeon program needs review.

Data and database management

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

Post-stocking hatchery hygiene

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

Permitting

The propagation of pallid sturgeon will only occur under authority of an Endangered Species Act Section 10(a)1(A) permit or sub-permit. All permits and permission from state 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 eggs 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 (FWP). Montana requires an importation permit for the importation of live fish or eggs into Montana. Montana also requires that all releases must be reviewed by FWP's Fish Health Committee, 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 live pallid sturgeon into that state. A courtesy call is required if eggs are shipped into South Dakota.

North Dakota currently requires that its Chief of Fisheries be notified prior to the importation of pallid sturgeon. 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.

Fish Health

The USFWS's "DRAFT Pallid Sturgeon Fish Health Management Plan" describes personnel responsibilities, issues and procedures associated with pallid sturgeon artificial propagation. This plan will be updated to assist federal hatchery managers and administrators make decisions regarding pallid sturgeon health issues.

Fish health testing and monitoring

No fish health screening is currently required prior to bringing adult pallid sturgeon into a hatchery facility. Fin clips will be collected to assess the presence of Pallid Sturgeon Iridovirus in each of the captured adult pallid sturgeon. These fin clips are usually taken at the same time fin clips are collected for genetics monitoring and archiving. 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.

Initially, routine monitoring of hatchery production populations of pallid sturgeon consisted of histological screening of three fish per family every other month. This has been abandoned in preference of a fish health program that employs diagnostic testing of fish when a fish health problem occurs or is suspected, and a pre-release fish health assessment prior to stocking.

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

For fish health evaluations ("Are my fish healthy?"), random, representative samples from each production lot should be used. See "Pre-release Health Assessment" below.

Although there has been some work to develop a Polymerase Chain Reaction (PCR) diagnostic tool for the Pallid Sturgeon Iridovirus, there is currently no effective PCR test for this pathogen. Accurate diagnosis depends upon histology. If a validated Pallid Sturgeon Iridovirus PCR test is developed, it will be incorporated into the fish health monitoring program.

Accurate diagnoses of disease and effective treatment programs depend on open and effective communication between fish culturists and fish health staff. Fish health reports will be sent to all appropriate sub-basin workgroup committee members. The Recovery Team Leader will be responsible for dissemination of this information.

Pre-release Health Assessment

The Pre-release Health Assessment (Health Assessment) was developed to measure and evaluate the general health of hatchery-produced pallid sturgeon (Appendix I). The Health Assessment will be performed on a total of 60 randomly collected fish from each cultural unit representing each female, preferably 6 weeks prior to stocking. Acceptable scores for virus severity should not exceed an average of 3.0 and liver conditions should average less than 4. Certification for stocking will also consider a lot's health history, mortality and signs of clinical disease.

Use of injectable drugs

It is currently believed that the use of injectable drugs is necessary to maintain the health of captured adult pallid sturgeon. Injectable drugs are typically administered both prophylactically, to prevent or minimize potential bacterial infections, and therapeutically, to stop or slow the development of disease by a diagnosed pathogen. More research is needed into the role of injectable drugs in pallid sturgeon management. The efficacy of injectable drugs on specific pallid sturgeon pathogens, acceptable dosages, tissue retention times, and the physiological response of pallid sturgeon to injectable drugs need further research.

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 FTC.

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

Acceptable injectable drugs for pallid sturgeon are Nuflor and oxytetracycline (OTC). Oxytetracycline is preferred over Nuflor as there is more experience with the use of OTC in controlling fish pathogens. The recommended dosage for oxytetracycline (LA200) is 0.10 ml/kg of fish body weight. OTC is known to depress the immune systems of some fish species. It is unknown if this undesirable effect occurs in pallid sturgeon. Almost nothing is known about appropriate dosages for Nuflor, its efficacy against fish pathogens, or pallid sturgeon responses to the drug. Nuflor, with florfenicol as the active ingredient, has extended release action and it is effective against a broad spectrum of pathogens. The recommended dosage for pallid sturgeon is 0.07 ml/kg of fish body weight. This is based upon the recommended dosages for cattle and swine of 40 mg/kg of body weight.

Blood sampling

The blood chemistry of captured pallid sturgeon may give insight into the fish's response to stress and the health of the fish. Currently there is little information about pallid sturgeon blood chemistry, especially for establishing what normal blood chemistry is. The Propagation Committee will develop a blood sampling program that includes a protocol, sampling program, and database. When it is finalized, the blood sampling protocol will be added as an appendix to this plan. Once the blood sample program is established, blood samples will be taken from all handled adult wild pallid sturgeon. The blood sampling program may also be expanded to include shovelnose sturgeon. The database will be maintained at the Bozeman Fish Health Center and at the Missouri River Fish and Wildlife Management Office.

Hatchery pathogen histories and identified remedies

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

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

Gavins Point NFH

Fish health problems at Gavins Point NFH seem to arise during the fall, winter and spring. The first fall of propagation can be especially trying. Gavins Point NFH has installed UV disinfection units and drum filters to improve water quality at that facility.

Invasive fungal infections have been observed in adult pallid sturgeon. Formalin doesn't seem to control it and there currently seems to be no effective cure. Disinfecting the incoming water supply with UV may help to reduce Saprolegnia spores in the incoming water.

External Aeromonad and Pseudomonad infections in adults are treated using OTC injections. Infections on small fish has been effectively treated with an OTC bath (100 ppm for 60 minutes, for as many as 5 days in a row).

Costia has been effectively treated with a flow through 75 ppm hydrogen peroxide treatment for 30-60 minutes.

Post-spawn adult pallid sturgeon broodstock are treated with a 2% salt bath to stimulate mucous production.

Miles City State Fish Hatchery

A major portion of the water used for pallid sturgeon culture at Miles City SFH comes from open river sources. Miles City SFH has experienced problems with damaging silt loads and high pathogen loads during run-off events, so UV disinfection units and drum filters have been installed to improve water quality at that facility.

Bacterial Gill Disease has been effectively treated with a 2 ppm Hyamine 3500 drip for 1 hour.

External Aeromonads infections on adult pallid surgeon broodstock can be caused by handling or abrasion. These infections are treated with topical iodophore swabs after the fish are handled. It is important to keep the iodine away from the fish's gills.

External parasites have been effectively controlled with formalin at concentrations of 75-100 ppm for 1 hour.

Captured adult pallid sturgeon brood are periodically given standing salt baths at a concentration of 2% to stimulate the replacement of mucus removed by handling.

Garrison Dam National Fish Hatchery

Garrison Dam NFH has installed an automatic backwash filter with 15 micron screen and UV disinfection units to improve its incoming water supply.

Nodular gill disease (caused by a gill amoeba) has been effectively controlled with 1 hour treatments of 100 ppm formalin in addition to .5% static salt baths.

Bacterial gill disease has been controlled with 1 hour, 8-15 ppm Chloromine-T flow-through treatments.

External parasites have been effectively controlled with formalin at concentrations of 75-100 ppm for 1 hour. Treatments can be repeated if the initial treatment is ineffective.

Captured adult pallid sturgeon brood are periodically given salt baths at a concentration of 2% to stimulate the replacement of mucus removed by handling.

Bozeman Fish Technology Center

Bozeman FTC experiences problems with curling of the pectoral fins in the pallid sturgeon raised at this facility. Fin curling has been observed in the culture of other sturgeon species at other facilities. Bozeman FTC staff believes that fin curling is caused by an undetermined environmental deficiency in their well water supply. Currently, a fluoride deficiency is suspected to cause the curling and experimental trials of fluoride-enhanced diets are being performed at the Bozeman FTC.

Research Needed

During the development of the Pallid Sturgeon Propagation Plan, the Propagation Committee identified the following areas of research needed to further the efficient and effective culture of pallid sturgeon.

- At what stage of egg development is it safe to ship eggs?
- Is water-hardening eggs in iodine effective? What is the effect of iodine on various pathogens inside the egg? What is the effect of iodine on pathogens in various locations within an egg?
- Development of appropriate diets and feeds for pallid sturgeon.
- Carbohydrates can be converted to fat in fish. Will a low carbohydrate diet reduce lipid accumulations in livers of pallid sturgeon?
- What is the cause of fin curling? How can it be prevented?
- What is the "best" hormone to initiate ovulation in pallid sturgeon? What are appropriate dosages? How do pallid sturgeon respond to hormones (physiological responses, ovulation, stress)? The committee suggests initial investigations be done on shovelnose sturgeon.
- Perform Health Assessment on fish held through their spring/summer growing season and after their release to investigate the regeneration of damaged epithelial cells and liver tissues.
- Does exposure to the Missouri River Pallid Sturgeon Iridovirus affect the condition, acclimation and survival of stocked fish?
- More information is needed about the efficacy, tissue accumulation and retention times of antibiotics in pallid sturgeon.
- Research the use of Beta-Glucans as immune system enhancers.
- Develop PCR to detect MRPSIV.
- Investigate the progression of disease in fish. What are the causes of epizootics in pallid sturgeon? What are the best controls for pallid sturgeon epizootics?
- Investigate the use of and pallid sturgeon responses to available anesthetics.
- Investigate the use of topical medications to be used as healing agents.
- Investigate medications for the treatment of internal fungus.
- Investigate the availability and efficacy of viricides.
- What are normal and acceptable ranges of condition factors and relative weights for hatchery-reared pallid sturgeon?

Other Needs and Recommendations

- Develop a blood sampling program as a potential fish health diagnostic tool.
- Tagging
  - Investigate various tagging methods and determine tagging requirements for pallid sturgeon.
  - Develop a tagging protocol with experts within the Upper Basin Workgroup.
  - Develop a long-term elastomer marking scheme.
  - Action Item: "Elastomer mark" (colors used & orientation) needs to be added as a column in database spreadsheet.
- The Handling Protocol needs a thorough review and update.
- An adequate broodstock facility at Gavins Point NFH must be completed as soon as possible. The highest priority of the broodstock program must be on developing a secure, genetically diverse broodstock program at Gavins Point NFH.
- Develop an AUSUM-type evaluation system for hatchery-reared pallid sturgeon.
- Hatchery managers will keep logs on their pallid sturgeon production.
- There is a need to discuss the issue of access to the pallid sturgeon database. Who has access to the data? How can the data be used and by whom? Should it be available via the Internet?
- The USFWS should update and adopt the Draft Pallid Sturgeon Fish Health Management Plan.

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A - Protocol on Collecting, Tagging, Holding, Transporting and Data Recording for Researchers and Managers Handling Pallid Sturgeon

B - Minimum Reported Ultraviolet Dosage For Inactivating Fish Pathogens

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APPENDIX A

Biological Procedures and Protocol for Collecting, Tagging, Sampling, Holding, Culture, Transporting, and Data Recording for Researchers and Managers Handling Pallid Sturgeon

12/17/2002

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

Prior to performing any work with pallid sturgeon, researchers and managers are required to obtain a Federal endangered species permit or sub-permit. For Louisiana, Mississippi, Arkansas, Tennessee and Kentucky: contact 404-679-4176. For Missouri, Illinois and Iowa: contact 612-713-5343. For Nebraska, South Dakota, North Dakota, and Montana: contact 303-236-7400 ext. 227. Questions, comments or suggested changes to the protocol should be directed to Steve Krentz, Pallid Sturgeon Recovery Team Leader, at U.S. Fish and Wildlife Service, 3425 Miriam Ave, Bismarck, ND 58501 or at 701-250-4419. Proposed activities should also be coordinated with appropriate State agencies where a State permit may also be required.

Deviations from the protocol may be requested during the application or renewal process. Researchers and managers should use their best judgement in cases where guidelines are not directly applicable, or if in question, contact Steve Krentz, Bismarck, ND.

The following guidelines will be followed to ensure that modern and peer accepted techniques are used regarding collecting, tagging, sampling, holding, culture, transporting, and data recording of pallid sturgeon.

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

Record Keeping

All permittees will maintain a copy of the Endangered Species Act permit and this protocol during all field operations as well as on file. Tagging and sampling records shall contain specific information on each individually tagged pallid sturgeon such as that listed on the data sheet provided (Appendix 1).

Personnel and training requirements

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

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

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

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

Trainees: Those individuals not meeting minimum qualifications will be considered to be trainees and will not be allowed to independently work with pallid sturgeon. They will be trained in protocols and procedures under the direct supervision of a qualified biologist.

Collection Methods: Two weeks prior to actual field work, all field personnel, the Regional Fish Health Center, and hatchery personnel will be notified. All pallid sturgeon are to collected non-lethally. A fish holding container on the boat shall be at least six feet in length and deep enough to cover the fish completely.

Gill Nets/Trammel Nets - Monofilament and multi filament mesh nets may be used to collect pallid sturgeon. There are no mesh size restrictions for gill and trammel nets. Drifting sets should be monitored continuously. Time and position of net sets are recorded using a stopwatch and global positioning system (GPS). This will provide positional data and time for each set. Total numbers of each species is then noted and recorded with the GPS way points to apply to a Geographic Information System (GIS). Drift distance starts and stops with the clock. Indicate net length, mesh size, and mesh type in reports. Stationary sets may be used for pallid sturgeon, but must not be left unattended for more than 3 hours. If water temperatures are less than 55 F, then

overnight sets may be used cautiously, but for no more than 24 hours. Weather conditions must be watched to insure that nets can be picked up as soon as possible the next day. Calculate CPUE as fish per-net-hour for stationary sets. For drifting sets, CPUE shall be reported as fish per-net-hour and number of fish per-meter of the drifted area.

Trot Lines/Angling - Larger hooks are more desirable to guard against pallid sturgeon swallowing them. Mustad Tuna Circle Hooks in sizes up to 14/0 have proven successful in capturing larger pallid sturgeon in Montana. Trot lines must be checked at least once every 18 hours. Calculate CPUE as fish-hook- hour. Indicate line length and number of hooks per set in reports.

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

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

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

Data collected - The Pallid Sturgeon Data Sheet (Appendix 1), dated May, 2000 lists the physical data to be recorded from each specimen, as well as general data about the collection. While collecting morphometric data, pallid sturgeon should be kept moist and held out of the water for no longer than 2 minutes, unless the gills are irrigated. It is preferred to hold the fish in the water in a stretcher or in a "stock" tank large enough to accommodate the fish. For procedures on taking measurements refer to: Bailey, R.M., and F.B. Cross. 1954. River sturgeons of the American genus Scaphirhynchus: Characters, distribution, and synonymy. Michigan Academy of Science, Arts and Letters, Vol XXXIX.

Copies of completed data sheets must be mailed to the Recovery Team Leader (Steve

Krentz, U.S. Fish and Wildlife Service, 3425 Miriam Ave., Bismarck, ND 58501) during or at the end of each field season for recording into the Range-wide Pallid Sturgeon Catch Record Database. Copies of the Catch Record Database can be obtained from the above address.

Tagging, sampling methodologies and sampling protocols

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

1) Identification Tags

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

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

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

b) External Tags - External tags have met with little success when applied to sturgeon and are therefore no longer permitted on adult pallid sturgeon until further field evaluation and laboratory studies can recommend an acceptable tagging method. Various external tag types (dangler, cinch, dart, disc) have been used on shovelnose sturgeon and juvenile pallid sturgeon with limited success. Disc tags have had higher long-term retention on sturgeon than other external tags. However, the majority of recaptured adult pallid sturgeon that had previously been externally tagged exhibit tissue inflammation severe enough to be concerned about infection. In some cases, severe inflammation was still evident 2 years after the fish had been tagged. External tags can still be used on shovelnose sturgeon, as well as on pallid sturgeon stocked for research purposes.

2) Radio/Sonic Transmitters

a) Internal Transmitters - Internal transmitters are preferred over external transmitters; however, implanting should be performed only by individuals with experience in surgical procedures. Transmitters with external antennas protruding from the body cavity are generally not permitted and will be evaluated on a case by case basis. During surgery, the head either should be placed in water or the gills flushed with water. Transmitters should have a biologically inert coating to prevent expulsion. An incision, only slightly larger than the tag to be used, should be made in the ventral body wall, one to one and a half inch off the midline and anterior to the pelvic fins. Care should be taken to prevent severing blood vessels and damaging organs while making the incision. The incision should be closed with individually knotted sutures. Before and after surgery, the incision site should be wiped with an antiseptic to prevent infection. This same small incision should be used for sexing the fish. For additional information and guidance on surgical procedures refer to: Conte et al. 1988. Hatchery manual for the white sturgeon. University of California, Division of Natural Resources, Cooperative Extension Publication 3322. The duration of surgical procedures should be limited to a maximum of 15 minutes per fish.

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

Handling and fish transportation

Truck transport: When the objectives of field work are to capture pallid sturgeon broodstock, a hauling truck and tank should be on site for immediate transport. Use a circular hauling tank for larger specimens (>10 pounds), that is equipped with oxygen and a fresh-flow aerator system. Transportation times should not exceed 12 hours and may need to be less depending upon number of fish and water/air temperature. Maintain temperature of hauling-tank water within $\pm$ 3°F ($\pm$ 1.6°C) of ambient water temperature of origin. Temper the fish when moving them between bodies of water. Pallid sturgeon should not be transported when ambient water temperatures are greater than 60°F (15.6°C). To reduce stress during transport, non-iodized salt should be added to water in the hauling tank to provide a 0.25 percent salt solution for juveniles and 0.5 percent solution for adults.

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

Box and bag shipping equipment: Shipping of fish or eggs in boxes containing plastic bags is recommended for larval and juvenile sturgeon, exceeding 5 inches total length. Industry standard boxes and square bottomed shipping bags should be used. If possible, withhold food for 24 hours prior to shipment. Use two bags in the box. The box should be cardboard with a Styrofoam box insert with fit lid. Check the bags for leaks prior to use. Fill the inside bag with about 2 gallons of water, water additives, and fish. Deflate the bag of air and inflate the bag with oxygen. Twist the top of the bag to put pressure in the bag. Fold over the twisted top and seal with a docking ring (preferred) or two heavy duty rubber bands. Separately, twist the top of the outer bag and double it over prior to sealing with a docking ring or two rubber bands. Place the styrofoam lid on the styrofoam box and seal with shipping tape. Then seal the cardboard box with two complete rounds of shipping tape. Load and ship with the 'up' arrows pointing up at the lid. If needed, temperature can be maintained by placing cold packs on the sides of the bags. Smaller plastic bags such as ziplock heavy duty freezer bags can be used but care must be taken to inflate and pack these in such a manner that the fish cannot be crushed or sharp edges are exposed to create a puncture. Bags used for shipping must not have corners that could trap and crush the fish. The water temperature should be similar to or slightly lower than that used to rear the fish and the bag temperature should be lowered to less than 60° F (15.6° C) prior to shipping. The hauling density should not exceed 0.5 pounds of fish per gallon of water.

Fish acclimatization and therapeutants:

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

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

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

tetracycline hydrochloride - Fry and fingerling pallid sturgeon can be treated with tetracycline hydrochloride soluble powder at a rate of 10 ppm and up to 60 ppm for up to four hours per day. This can be done daily for up to five consecutive days with no major problems when holding conditions or stress may be induce a systemic infection.

Following transport, stress reduction techniques will include adding non-iodized salt at 0.5% (18.9 grams per gallon) levels to holding water for at least two days following transfer. Water temperatures will be similar to that at the location and time of capture. Water turnover rates will be between 2 and 4 times per hour in all culture tanks. If parasites have been found in the water supply, the supply will be filtered (15-20 micron) and disinfected using UV irradiation with a minimum of 100,000 microwatts per square centimeter of ultraviolet light intensity. Photo period will approximate levels similar to environmental conditions. Variations in photoperiod should be submitted in the permit application. Oxygen levels will be maintained at > 6.0 mg/L or saturation as measured with an oxygen meter. pH will range from > 6.5 to <7.5. Ammonia levels will be maintained at less than 0.0125 parts per million (ppm) and nitrite levels will be kept below 0.1 ppm for soft water and 0.2 ppm for hard water. Nitrogen supersaturation levels will be maintained below 100 - 102%.

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

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

Fish Culture/Holding procedures:

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

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

   b) Modified Hoop Nets/Underwater Keeps - Modified hoop nets/underwater keeps can be used as a temporary holding facility, but for no more than 16 hours. Holding pallid sturgeon in hoop nets or keeps might be necessary for a short period if one or more pallid sturgeon are incidentally captured and field crews are not set up with a holding tank. Commercial fishermen, who are previously authorized by permit, may keep incidentally captured pallid sturgeon in hoop nets until personnel who are previously authorized by permit to obtain the pallid sturgeon arrive. Commercial fishermen must notify their contact within 2 hours of capturing a pallid sturgeon. Mesh size must be 1½-inch (3.81-cm) bar measure

or smaller to prevent gilling and keeps should be circular. Hoop nets or keeps should be located such that adequate temperature and oxygen conditions vary little from ambient conditions at the capture location. Flow-through is very important if conditions permit and the structure will not be jeopardized. Hoop nets or keeps must be checked every eight hours and posted with a sign or float cautioning against disturbance.

2) Long-term Holding Facility Requirements and Rearing Facilities

a) Hatchery or Aquarium - Pallid sturgeon have been held for more than 8 years in circular tanks with water circulation. Tanks should be covered and located in an area free from disturbances. An automatic standby power and water supply must be provided to maintain the fish in the event of a failure. These facilities must have a "contaminant-free" water supply. Fish health must be regularly monitored. If signs of disease are noted or if a 20 percent loss of body weight occurs during holding, fish health personnel at the Service's Fish Disease Control Center in Bozeman, Montana (406-582-8656) should be contacted for treatment recommendations. Long-term holding facilities must be within the historical range of pallid sturgeon or be designed to prevent escapement. Water temperatures should be maintained between 40 and 70 degrees Fahrenheit. Densities for adults should not exceed 1.0 pound per square foot of surface area. Densities for juveniles should be maintained at less than 0.5 pounds per square foot of surface area.

Propagation and Stocking

Prior to any spawning activities, propagation plans must be prepared and activities coordinated with the Pallid Sturgeon Recovery Team and the U.S. Fish and Wildlife Service. Before any release of pallid sturgeon to the wild, a comprehensive reintroduction plan must first be developed and then approved by the Pallid Sturgeon Recovery Team and the U.S. Fish and Wildlife Service. Guidelines of propagation and stocking plans are available by contacting the Recovery Team Leader.

Disposal of incidental take

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

Fish Health Samples - Refer to Fish Health Protocols (Appendix 2) for proper procedures and data sheet. These samples are only to be taken if part of another study evaluating fish health. All samples shall be labeled with the PIT tag number. Please notify before shipping and forward all samples labeled with the PIT tag number to:

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

Contaminants Samples - Refer to Standard Operating Procedures for Collection, Storage, and Shipment of Pallid Sturgeon Tissue Samples for Analysis of Organic and Trace Element Contaminants (Appendix 3). These samples should only be collected if on a mortality and part of a study evaluating contaminant levels. All samples shall be labeled with the PIT tag number and sent to:

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

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

Genetic Analysis - Collect all morphological data before collecting the sample and: 1) Wash hands well with soap.2) Dip the "clipper" (knife, razor blade or whatever is used to cut the tissue with) in alcohol and then flame it, clip off one centimeter fin punch of the trailing edge of a fin, place in a container for drying or place tissue in 95% ethyl alcohol (do not handle with bare hands). Photos should be taken of both the side and ventral views of the head.

Equipment & Solutions

Screw-cap microfuge tubes (2.0ml) filled to the midline with desiccant (Silica Gel beads) and razor blades.

Procedure

1) Assign an ID# (PIT tag number) for each animal from which a sample is collected. With permanent ink, record this identifier on the plastic bag (and tube) along with all other pertinent information about the animal (e.g. a copy of the data sheet; location is very important!, and date).

2) Excise tissue from each animal by ear notch/ear punch or remove a small bit of skeletal muscle from mortalities. When necessary, reduce tissue sample to <0.4cm length x 0.4cm width x 0.4cm thickness. A small piece of soft fin tissue from the trailing edge of the caudal or anal fin will be suitable from those specimens destined to be released back to the wild. **To avoid contamination, use a new razor blade for each sample!**

3) Place tissue section in tube and cover with desiccant by shaking. **DO NOT** overfill tube or the sample will decay. Return the tube to its appropriate bag.

4) Maintain samples at a cool temperature (ambient temperature) and out of direct sunlight.

5) Transport inside a padded envelope, Federal Express Pak or cardboard container along with a copy of the data sheets for each fish and a list of the log entries for each sample.

Forward all genetic samples to:

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

Disposal of surplus pallid sturgeon produced by controlled propagation - refer to Disposal of Surplus Artificially Propagated Fishes. Appendix VI of the Propagation / Genetics Plan. USFWS, Gavins Point NFH, RR1, P.O. Box 293, Yankton, SD 57078.

Pallid Sturgeon Data Sheet (12/2002)

Appendix 1

Capture Location _____

Latitude _____ Decimal degrees Longitude _____ Decimal degrees

River _____ River Mile _____

State _____ Method: Gill/Trammel/Hoop Net/Trotline/Fishing/Other _____

Duration of Set/Drift: _____ hrs/min Mesh Size _____

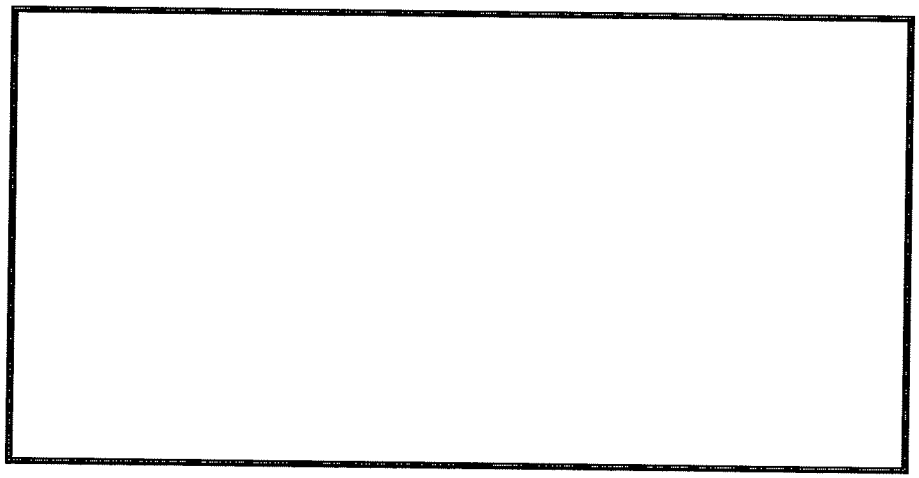
Habitat Data:

Water Depth: _____ m/ft Turbidity: _____ Secchi/NTU

Water Velocity: _____ mps/fps Substrate: _____

Water Temperature: _____ °F/°C Date ____/____/____

Schematics of Sturgeon Capture Location



(Sketch river channel, main current, sandbars, capture location, and indicate north in the box above)

Physical Data:

Interrostral Length _____ mm

Mouth - Inner Barbel _____ mm

Outside Barbel _____ mm

Inside Barbel _____ mm

Head Length _____ mm

Fork Length _____ mm

Dorsal fin rays (optional) _____

Anal fin rays (optional) _____

Weight _____ lbs/kg

Sex: M / F / U: Ripe / Green / U

PIT Tag #: _____

_____

Other Tag Information: _____

_____

Recapture: Yes / NO

Comments: _____

_____

_____

_____

_____

Genetics Sample # _____

(same as PIT tag number, include photos head w/ side and ventral views)

Captured by: _____

_____

_____

Appendix 2

FISH HEALTH PROTOCOLS

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

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

Collection of fin punches, barbel clips:

General:

* Label and track each fish individually with unique numbers (i.e. PIT #) for easy reference.

* Utilize only sterilized dissection equipment for collecting samples.

* Disinfect dissecting tools and DNA sampling tools between fish samples.

* Make sure fish are well oxygenated during fin punch collection.

Collection for histology:

* Individual fin punches will be collected from pectoral and caudal fins using a small paper hole puncher. Fins can also be clipped or notched using scissors or pig ear notcher. Refer to sturgeon anatomy picture for proper location of fin samples.

* Barbel clips may be collected by clipping the distal end of the barbel with sharp scissors.

* Both fin punches and barbel clips will be immediately placed into Davidson's fixative for a minimum of 48 hours, followed by immediate transfer to 70% ethanol.

* Place fish tissues into the Davidson's fixative at a ratio of 1 part tissue to 5 parts fixative.

* All histology samples should be collected in chemically resistant plastic containers or glass collection jars for transportation and storage. Seal jars tightly before transport.

Collection for Viral DNA analysis:

* Collect fin punches from the caudal and pectoral fins using a paper hole punch. Scissors may be used to clip the edge of the fins.

* Collect a portion of barbels with sharp scissors.

* Place each tissue type from individual fish in small 1 ml plastic tubes.

* These samples should be immediately frozen for transportation and then maintained at -70 F ultra-cold temperature for DNA analysis.

* Change gloves between each fish to be sampled.

* Disinfect sample collection instruments between fish.

* Refer to sturgeon diagram for sample locations.

Collection of Virology Cell Culture Samples:

* Collect both fin punches and barbel clips aseptically with sterilized dissection tools. Sample collectors should wear protective examination gloves.

* Refer to sturgeon diagram for sample location.

* Sample collection for virology may be as individual fish or pooled not to exceed a five fish pool.

* Samples will immediately be placed in small whirlpak sample bags. These bags should be chilled, not frozen. They can be kept in the refrigerator before transportation and should be transported chilled, insulated from ice packs. At no time should samples be allowed to become warm.

* These samples must be forwarded to receiving laboratory within 48 hours from collection.

* It is very important to sterilize dissecting tools between fish samples. An appropriate virucidal agent should be used.

LETHAL SAMPLING TECHNIQUES (Only on mortalities):

Collection of complete internal and external fish tissue samples.

General:

* Label all containers, showing species, and date collected.

* Maintain fish sample collection report with:
  ** fish source
  ** fish condition
  ** water temperature
  ** fish handling
  ** fish culture information
  ** mortality records

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

* Sample collectors should wear protective gloves during collection procedure.

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

Collection of Histology Samples:

* Fish should be dead no longer than 15 minutes for good histological sample collection.

* Fish smaller than 60mm can be preserved as whole fish. Slit fish ventrally along the belly, from the vent to the gills. Pull viscera away from the kidney area and puncture the air bladder to facilitate fixation of the kidney.

*Fish larger than 100mm will require thin sections of each organ for fixation. Tissues for histology: gill, heart, liver, spleen, kidney, muscle, ceca, digestive tract, fins, barbels, nares, rostrum, mouth parts, any lesions that are visible.

* The tissue pieces may be as large as 25 mm (1 inch square), but no thicker than 5 mm (about 1/4 inch).

* Histology tissues should be immediately placed in Davidson's fixative. One fish per collection jar. Do not combine tissues from other fish.

* Sample tissues should be placed in fixative at a ratio of 1 part fish to 10 parts fixative.

* After specimens have been in fixative for 48 hours, transfer to 70% ethyl alcohol.

* Samples can be transported in ethyl alcohol and stored for histology processing.

* Sample containers can be glass or chemical resistant plastic.

Collecting Tissues For DNA Analysis:

* Please refer to previous protocols on taking of genetic samples.

Collecting Virology Cell Culture Samples:

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

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

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

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

* Transport samples to receiving laboratory within 48 hours.

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

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

2. Rinse fish clean of any debris.

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

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

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

6. Complete a Fish Health Examination Sheet (attached)

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

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

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

10. Complete a Chain of Custody Record (attached)

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

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

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

List for Field Collection

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

List for Genetic Samples

- ☐ Tissue Forceps
- ☐ Scissors
- ☐ Ethyl alcohol or 2 ml Screw-cap microfuge tubes filled to the midline with desiccant (Silica Gel beads)
- ☐ Permanent marker
- ☐ Data sheets
- ☐ Butane lighter
- ☐ Latex gloves
- ☐ Single use razor blades

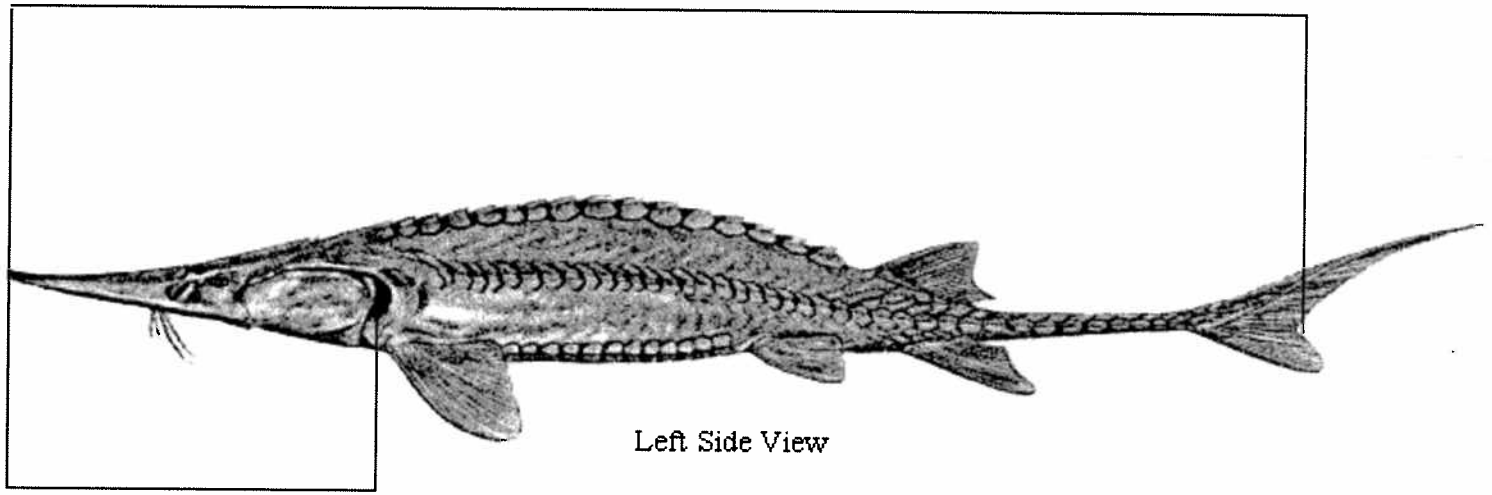
Hauling truck check list

- ☐ crew trained in hauling procedures
- ☐ loading crew trained in best handling procedures
- ☐ drivers know the route and maps available
- ☐ personnel at receiving point are expecting shipment
- ☐ cellular phone
- ☐ tanks properly mounted
- ☐ adequate fuel
- ☐ adequate tires and emergency equipment
- ☐ oil and other fluid levels checked
- ☐ tank filled to proper level with water
- ☐ water temperature in tank similar to host water (within 3 degrees Fahrenheit)
- ☐ water additives in tank water (salt)
- ☐ stretchers and nets in place
- ☐ oxygen/temperature meter calibrated, in place, and operating
- ☐ primary aeration system functioning
- ☐ oxygen bottles full - adequate supply for trip
- ☐ emergency aeration system in place and workable
- ☐ filling pump present and functioning
- ☐ receiving facility/tanks ready and filled
- ☐ two large buckets available
- ☐ salt bucket pre-marked for non-iodized NaCl
- ☐ pit tag reader, injectors and tags
- ☐ waterproof field notebooks and data sheets

Required Morphological Measurements for Pallid Sturgeon

01/03/2003

A



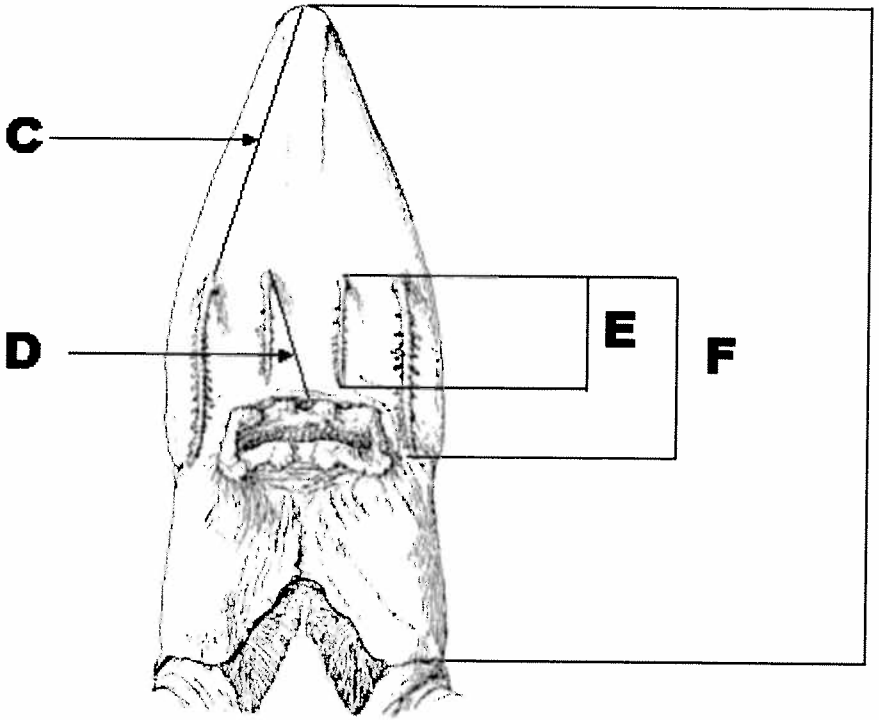
Left Side View

B

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

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

01/03/2003



Line drawing taken from:

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

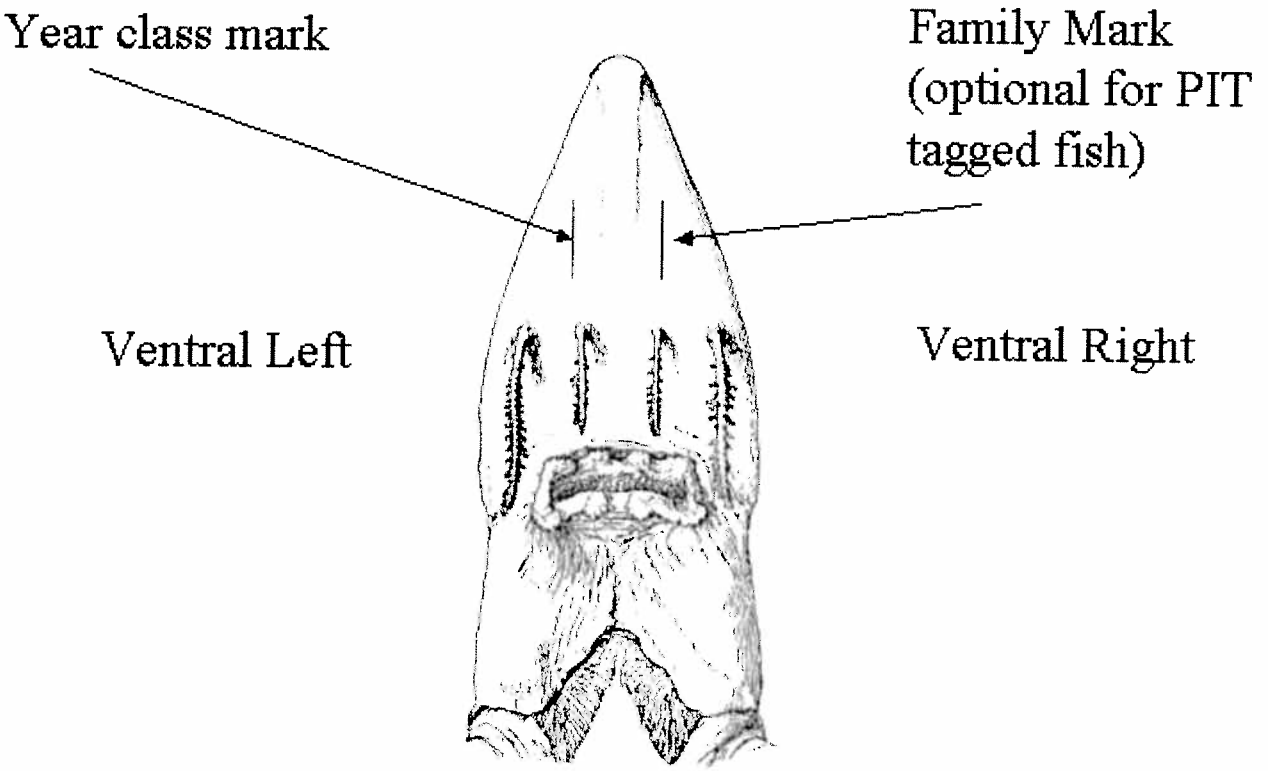
B – Head Length (see previous page)

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

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

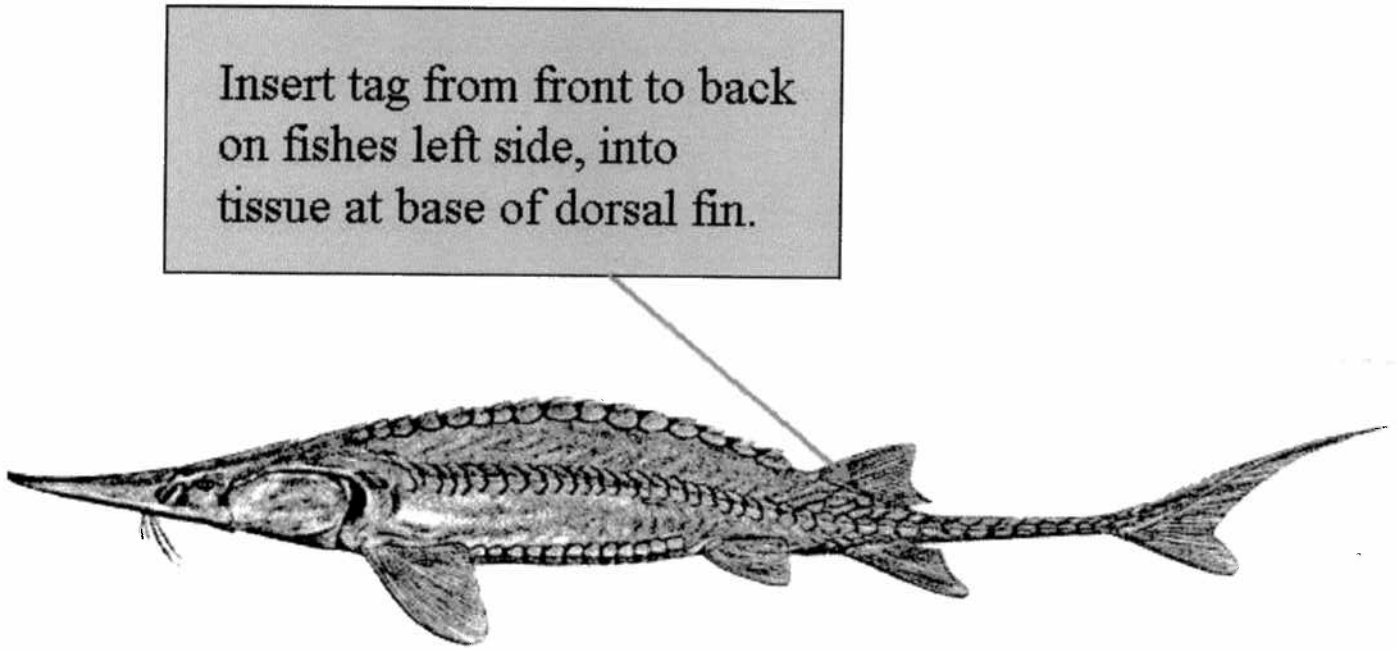
E – Inner Barbel Length – Front leading edge of inner barbel to it's tip.

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


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

01/03/2003

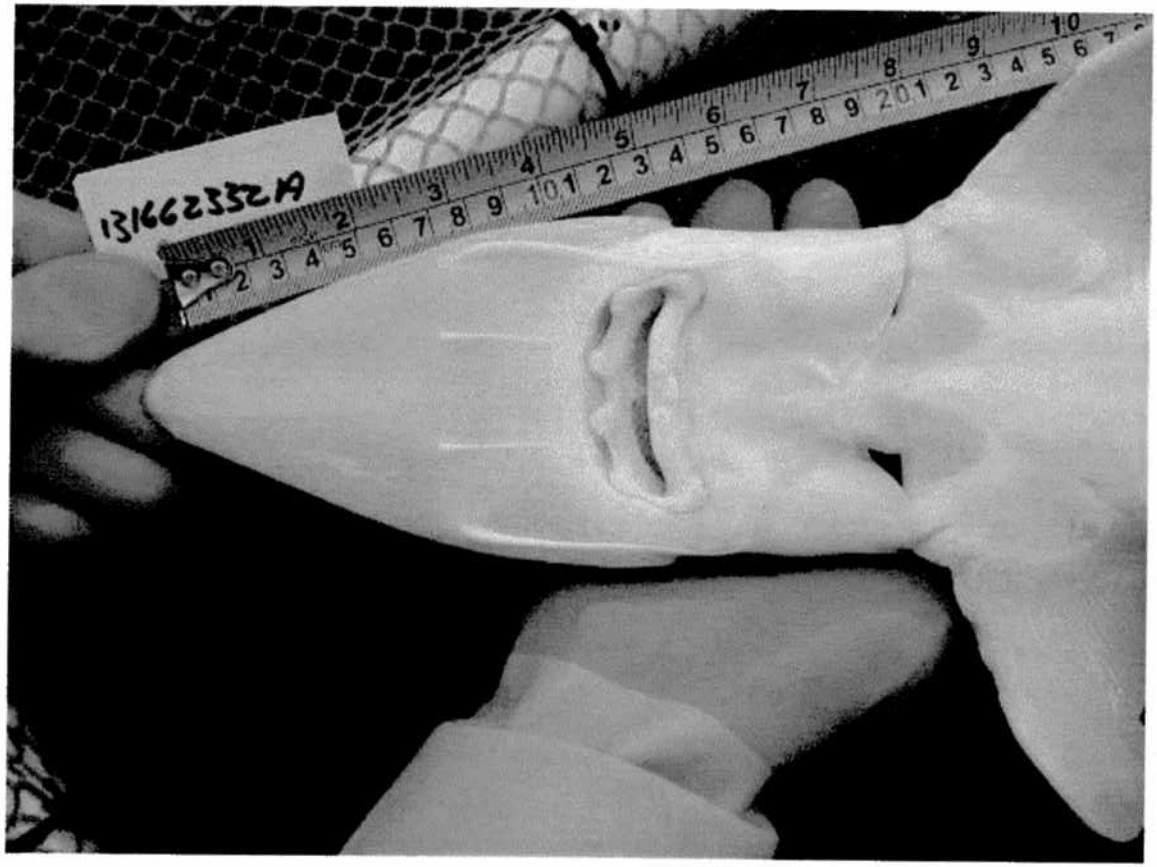
Required Tagging Location for Passive Integrated Transponder (PIT) for Pallid Sturgeon



Left Side View

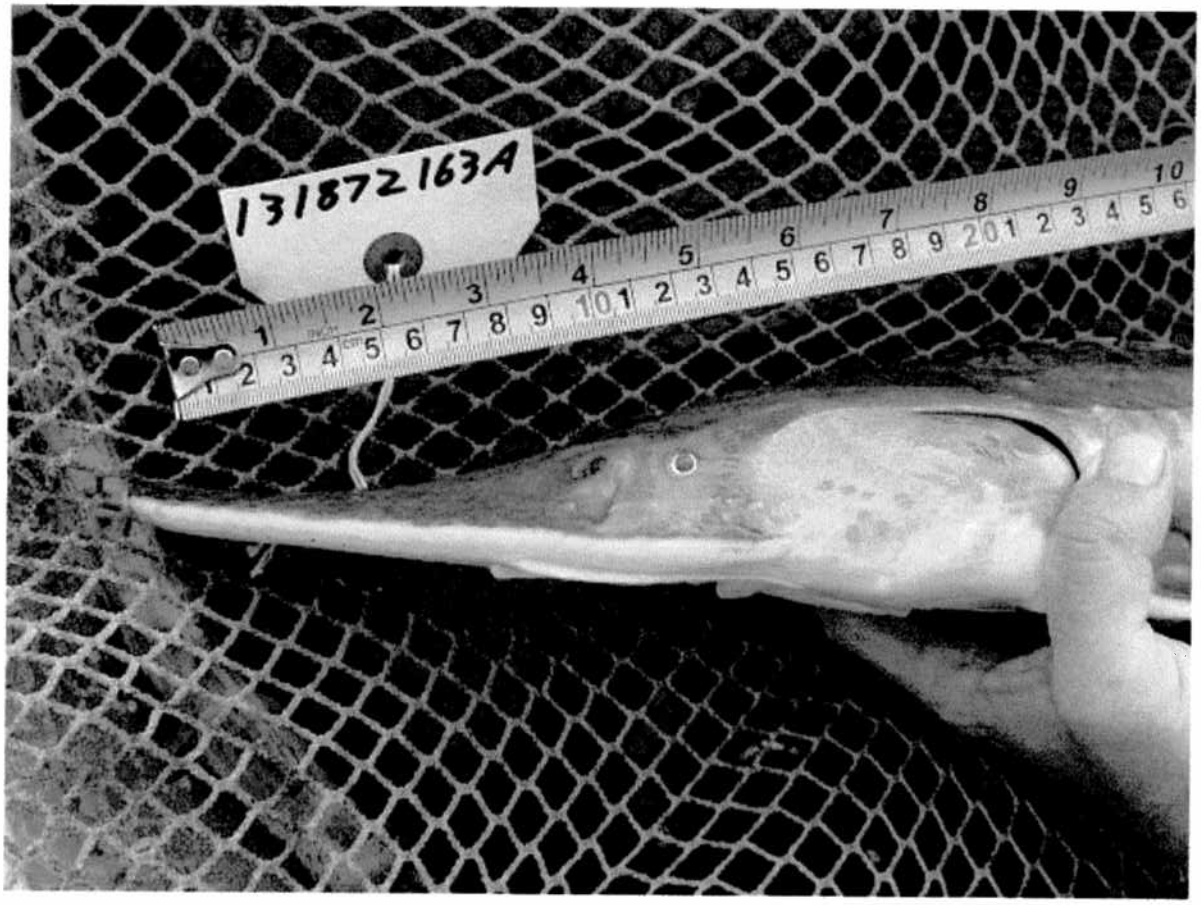
01/03/2003

Ventral view of pallid sturgeon photo for genetic sample.



01/03/2003

Side view of pallid sturgeon photo for genetic sample.



APPENDIX B

Minimum Reported Ultraviolet Dosage For Inactivating Fish Pathogens

(micro-watt seconds per square centimeter @ 254 nm)

Pathogen	Dosage ($\mu ws/cm^2$)	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 C

Fish Health Contact Information

Recovery Team Leader
Steve Krentz
phone: 701-250-4419
fax: 701-355-8550
Steven_Krentz@fws.gov
cell: 701-471-6605

Region 3 - Fish Health (MO, IA, IL)
Rick Nelson
phone: 608-783-8441
fax: 608-783-8450
cell phone: 608-769-3241
Rick_Nelson@fws.gov

Region 4 - Fish Health (LA, AR, MS, TN, KY)
Norm Heil
phone: 706-655-3382
fax:
Norm_Heil@fws.gov
cell phone:

Region 6 - Fish Health (MT, ND, SD, NE, KS)
Crystal Hudson
phone: 406-582-8656
fax: 406-587-3998
Crystal_Hudson@fws.gov
cell phone:

APPENDIX D

Protocol for Sturgeon Sampling

Collection Kit for Sturgeon Samples

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

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

Procedures:

Non-Lethal Sampling

PCR

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

Histology

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

Lethal Sampling

PCR

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

Histology

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

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

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

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

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

APPENDIX E

Protocol

for
Pallid Sturgeon Sperm Cryopreservation
Looney & Wayman
June 24, 2003

- Estimate, and record, the initial motility of the unextended sperm.

- The mixture will be one part sperm to four parts of the extender (HBSS)/cryoprotectant solution. In other words, the sperm will make up 20% of the total mixture to be frozen. The cryoprotectant will be methanol(MeOH) at 5% of the total mixture.

As an example for fifteen 0.5 ml straws:
- 15 X 0.5 = 7.5 ml. Add an additional 2.5 ml for motility testing and filling ease. The total volume will be **10.0 ml.**
- The sperm will make up 20% (1:4) of the total mixture, or **2.0 ml** (=2000*u*l).
- The MeOH will be 5% of the total mixture, or **0.50 ml** (=500 *u*l).
- The extender will make up the remaining volume, or **7.5 ml** (=7500 *u*l).

- The procedure is:
1. Mix all of the cryoprotectant (MeOH) with the total volume of the extender. In the example above, this would be 0.5 ml and 7.5 ml for a volume of 8.0 ml. Invert or swirl the container to obtain a homogenous solution.
2. Add the sperm to the extender/cryoprotectant solution. In the example above, this would be 2.0 ml to the 8.0 ml for a total volume of 10.0 ml. Invert or swirl the container to obtain a homogenous solution.
3. As soon as the sperm is added to the extender/cryoprotectant solution, start the timer and begin filling straws.

- An alternative to the above procedure is to:
1. Mix an equal portion of extender with the cryoprotectant. In the example above, this would be 0.5 ml of MeOH + 0.5 ml of HBSS to obtain a volume of 1.0 ml.
2. Mix the volume of sperm with the remaining volume of extender. In the example above, this would be 2.0 ml of sperm + 7.0 ml of HBSS to obtain a volume of 9.0 ml.
3. Combine the two solutions to obtain the total 10.0 ml of sperm, cryoprotectant, and extender.
4. As soon as the sperm/extender solution is added to the cryoprotectant/extender solution, start the timer and begin filling straws.

- Fill the straws either by the use of single straw fillers (syringe with rubber tubing adaptor) or 15 straws at a time by the use of the vacuum pump with manifold attachment.

- Once the straws are filled and the upper end is sealed, remove the straw(s) from the filler and tap the open end of the straw in sealing power.

- Place the straws, powder end down, in a 100 ml beaker, or other container, containing a small quantity (approximately ½ inch) of extender.

- Wait approximately 20 seconds for the powder to absorb extender and seal.

- Remove the straws from the beaker and clean off the excess powder from the end and sides of the straw(s). This can be accomplished by pushing a paper towel against the end of the straw to cut off the excess on the sides. Now wipe the outside of the straw to remove the excess wet powder. Failure to clean and dry the outside of the straw will cause the straws to freeze together and make removal of a single straw difficult at a later time.

- Place a maximum of 5 straws in each goblet. Be sure that the goblet is properly labeled using a cryo marker prior to initiating the freezing process.

Label the goblets with the following information:
1. Species (pallid sturgeon)
2. PIT number
3. Date
4. Freezing location
5. Capture location
6. Extender used (HBSS)
7. Cryoprotectant used and concentration (5%)
8. Sperm concentration (1:4)
9. Initials of the individual(s) conducting the procedure

- Place the goblet at the bottom of the aluminum cane. The cane should be labeled on the top to aid in identification of the samples when searching the storage dewar.

    1. Species and year (ex. Pallid 2003)
    2. Last 4 digits of the PIT

- Place the cane with goblet and straws in a dry shipping dewar to freeze. Do not have liquid in the shipping dewar. There should be liquid nitrogen vapor only. This is accomplished by filling the dry shipper with liquid nitrogen, waiting 30 minutes or longer, and then pouring out all of the liquid that will come out.

- Record the time from the timer. This should be recorded for future reference.

- Estimate the motility of the remaining sperm/extender/cryoprotectant solution and record.

- After a minimum of 15 minutes, the canes can be removed from the shipping dewar to liquid nitrogen in the storage dewar. The goblets can be combined on canes at this time to reduce the space used in the storage dewar.

APPENDIX F

Character Index for Pallid and Shovelnose Sturgeon

Number: 1:96
Date: February 1996
Revised: June 2000

TECHNICAL NOTES

From

Missouri River Fish & Wildlife Management Assistance Office
U.S. Fish and Wildlife Service
3425 Miriam Avenue
Bismarck, ND 58501
(701) 250-4419
FAX: (701) 250-4400

Title: Character Index for Pallid and Shovelnose Sturgeon.

Resource Objective: To provide a means to differentiate pallid sturgeon and shovelnose sturgeon using six morphological measurements. This would be used to quantitatively identify pallid and shovelnose sturgeon with distinctive species characteristics for culturing.

Methodology: Data was collected from a database of pallid sturgeon captures throughout the pallid sturgeon's range being maintained by the U.S. Fish and Wildlife Service. Data used for this index was supplied by researchers in Montana, North Dakota, and South Dakota. This character index was modified from work done by Keenlyne etal (1994) and Carlson (1981). Six measurements were used; fork length (FKL), head length (HDL), mouth to inner barbel. Distance (MTB), interostral distance (INT), outer barbel length (OTB), and inner barbel length (INB).

The index is set up so sturgeon will score between 0 - 700. Pallid Sturgeon will score on the upper end of the scale and shovelnose sturgeon will score on the lower end of the scale. Five morphological measurements were converted to a percentage of the fork length (PFLM) so that differences of individual fish sizes will be standardized and comparisons can be made regardless of size. Mouth to inner barbel and interrostral measurement ration and the inner barbel to outer barbel ratio are also used in the calculation of the character index. The reciprocal value was used for mouth - inner barbel to fork length, inner barbel to fork length, mouth - inner barbel distance to interrostral distance, and inner to outer barbel ratios. This was done to insure that the lower value of a pallid characteristic would score higher.

The minimum and maximum PFLM's and morphometric ratios were calculated from a sample of 167 pallid sturgeon and 95 shovelnose sturgeon. The pallid sturgeons were all from Montana and North Dakota. The data for shovelnose sturgeon was collected from North Dakota from below Garrison Dam on the Missouri River and above Lake Sakakawea on the Missouri River near the confluence of the Yellowstone River.

					PFLM value	
					Min	Max
HDL/FKL	X	100	=	PHDFK	21.30	33.97
INT/FKL	X	100	=	PINFK	6.22	17.86
MTB/FKL	X	100	=	PMTFK	3.36	8.35
OTB/FKL	X	100	=	POBFK	6.51	12.99
INB/FKL	X	100	=	PIBFK	2.08	7.45
MTB/INT	X	100	=	PMTIN	22.04	94.34
INB/OTB	X	100	=	PIBOB	21.36	95.65

Figure 1. PFLM's and morphometric ratios are used in the calculation of the character index.

To calculate the character index, two calculations must be made for each of the morphological measurements. First convert morphological measurements to a percentage of the fork length (PFLM), then use those values to calculate the sturgeon character index (CI) using the formula in Figure 2. If any of the PFLM's fall outside those listed in Figure 1, the CI formula will need to be updated with the new min/max values.

Using the PHDFK as an example, character indexes are calculated with the following formula:

$$\text{CHFK} = \frac{\text{Observed PHDFK} - \text{min PHDFK}}{\text{(max PFLM - PFLM)}} \times 100$$

[((PHDFK)	-	21.)/12.66	X	100	=	CHFK +
[((PINFK)	-	6.22)/11.63	X	100	=	CIFK +
100 – [(((PMTFK	-	3.36)4.98)	X	100]	=	CMFK +
[((POBFK	-	6.51)/6.47]	X	100	=	COFK +
100 – [(((PIBFK)	-	2.08)/5.36)	X	100]	=	CNFK +
100 – [(((PMTIN)	-	22.04)/72.29)	X	100]	=	CMIN +
100 – [(((PIBOB)	-	21.36)/74.28)	X	100]	=	CIOB +
						Sturgeon CI

Table 1. Formulas using the PFLM's to calculate the character index.

Example:

A sturgeon with the following morphometric measurements would have the Character Index calculated as follows:

FKL	=	1240		INT	=	179 mm	
HDL	=	405		OTB	=	121 mm	
MTB	=	72		INB	=	46 mm	

PHDFK	=	405/120	X	100 =	32.66	CHFK	= 32.66	- 21.3/12.66x100	=	89.73
PINFK	=	179/1240	X	100 =	14.44	CIFK	= 14.44	- 6.22/11/63X100	=	70.68
PMTFK	=	72/1240	X	100 =	5.81	CMFK	= 100	- (5.81-3/36/4.98x100)	=	50.80
POBFK	=	121/1240	X	100 =	9.76	COFK	= 9.76	- 6.51/6.47x100	=	50.23
PIBFK	=	46/1240	X	100 =	3.71	CNFK	= 100	-(3.71-2.08/5.36x100)	=	69.59
PMTIN	=	72/179	X	100 =	40.22	CMIN	= 100	-(40.22-22.04/72.29x100	=	74.85
PIBOB	=	46/121	X	100 =	38.02	CIOB	= 100	-(38.02-21.36/74.28x100)	=	77.57
								CHARACTER INDEX	=	483.5

Findings: Sturgeon from the current database that were assumed to be pallid sturgeon, scored on the character index CI) from a low of 358 to a high of 636 with a mean of 509 and a median of 514. From the shovelnose sturgeon data, character indexes were calculated and ranged from 154 to 293 with a mean and median of 230 (Figure 3).

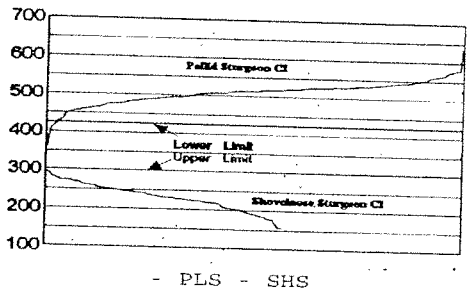


Figure 1. Character index values for pallid and shovelnose sturgeon from Montana, North Dakota, and South Dakota.

Recommendations: This character index is meant to identify individuals that fulfill the characteristics of a pallid sturgeon or a shovelnose sturgeon to be used as broodstock. As more data is collected this index should be updated with current information. As hybrids are cultured for research purposes, morphological data should be calculated to determine the range of the character indexes.

Any pallid sturgeon or shovelnose sturgeon that are used for propagation purposes should be tested against this index to determine level of pallid characteristics before spawning. I am recommending that only pallid sturgeon that score above 425 and shovelnose sturgeon that score below 300 should be used for propagation purposes in North and South Dakota and Montana.

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APPENDIX G

Rearing Environments At Specific Pallid Sturgeon Culture Facilities

	Gavins Point NFH	Garrison Dam NFH[2]	Bozeman FTC[5]	Mile City SFH	Neosho NFH
WATER SUPPLY TRAETMENT	17 micron rotating drum filter UV disinfection (>100,000 microwatts/sec/cm^2	15 micron screen UV disinfection 35,000 microwatts/ sec/ cm^2	Recirculating system with UV disinfection, bead & sand filtration and packed columns.	35 micron 1st stage, ~27 micron second stage UV disinfection 80,000 microwatts/sec/cm^2	each tank has UV system. dosage unknown
WATER TEMPERATURE					
Spawning	60s	mid 60s	NA	62-63	NA
Post-spawn	57-59 to reduce stress	Reduce to 60°F	NA	59-61	NA
Egg incubation	55 well	Natural hydrograph	62	63	54 spring
Hatch	63-65	Natural hydrograph	65-67	63	54 spring
Rearing	43-45 winter	35-39 winter 70-75 summer		">68 fish showed signs of stress"	52-56 winter 65-70 summer "keep below 70"
Once on feed	68-70	Natural hydrograph	63-68	63	
Over-winter	45 lake/well mix "Got gain down to 52. <60 fish reduce feeding. Below 50 they wasted feed, 45 they go off feed"	Natural hydrograph "<50 fish wasted feed"	63-68 "Never < 60"	40-44 "Fish fed all winter. Over-wintered fish maintain, but show no growth" "Never below 40"	NA

MAXIMUM REARING DENSITIES					
Fry (initial stocking density)	177/cu. ft. "3000/28.3 cu. ft."	3000/30" dia. Tank	Light stocking rates due to low numbers	2000/ 6' dia. tank	
Fingerling	.04 -.22 lbs./ft^2	.5 lbs./ft^2	.5 lbs./ ft^2	.5 lbs./ft^2	
Yearlings	.54 lbs./ft^2	.75 lbs./ft^2	.75 lbs./ft^2	.7lbs. /ft^2	
Brood	.5 lbs./ft^2				
GROWTH RATE (ΔL)	.067-.085 2.0-2.5"/ month	.055 1.65"/month		.040 1.2"/month	>.03 >1"/month
CONDITION FACTOR	.000144	.00012		.000132	

APPENDIX H

Disposition of Surplus Artificially Propagated Endangered Fishes
March 24, 1994

Background

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

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

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

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

Disposing of Surplus Fish

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

References

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APPENDIX I

Sturgeon Pre-release Fish Health Assessment Protocol

Lethally sample YOY sturgeon twice at a rate of 60 fish/female for histological evaluation. Fish will be randomly collected and representative of family groups and/or rearing tanks. Collections will take place 6 weeks prior to stocking.

Information regarding rearing conditions (e.g. water temperature, feeding rate, diet, mortality, etc) for the 30 days prior to sample collection will be included with samples.

1. **Gross Necropsy** – will be conducted on all fish collected
   a. Length
   b. Weight
   c. Body condition
   d. Lesions

2. **Virus Status** – one pectoral fin per fish will be examined for presence of iridovirus.
   a. Positive samples will be further evaluated for severity and scored.

   Scoring of iridovirus was done by assigning a numeric value on a 0-5 scale.

   1 = minimal, one or two infected cell(s) present in the entire section of pectoral fin.
   2 = mild, 3-8 infected cells widely scattered throughout the section.
   3 = moderate, 9 -20 infected cells widely scattered throughout the section and/or focal areas containing 3 or more + cells.
   4 = moderately severe, numerous (>20) infected cells in section of pectoral fin.
   5 = severe, too many infected cells to count in an entire section of pectoral fin.

3. **Liver condition** - 3 areas of each liver will be evaluated from each fish
   a. Fat will be scored.

   Scoring of liver fat was done by assigning a numeric value on a 0-5 scale.

   0 = no fat present
   1 = minimal, fat vacuoles present but few in number.
   2 = mild, fat vacuoles widely scattered throughout section, <50% hepatocytes contain fat.
   3 = moderate, most hepatocytes contain fat vacuole but cells retain normal shape/structure.
   4 = moderately severe, hepatocytes are enlarged due to size of fat vacuole, normal cytoplasm is displaced.
   5 = severe, hepatocytes membranes have ruptured due to fat accumulation in the cell resulting in loss of normal liver architecture.

   A score of 4 is considered borderline pathological; 5 is pathological. Fat utilization and accumulation is an active process often resulting in observable zones of fat storage

(particularly around vessels). To accommodate this occurrence, three sections of liver (obtained from 3 different areas) are examined and zones are scored separately for each section, then averaged to obtain a score for that individual liver.

4. **Skin Condition** - Pectoral fin and barbell will be examined
   a. Numbers of mucus cells per 10 fields at 10x will be counted
   b. Sensory epithelia (taste buds, sensory pits) – numbers per 10 fields at 10x will be counted and condition (normal, degenerate, necrotic) noted.

*Note: due to their small size, barbel sections had variable number of fields so results are reported as an average per field.

APPENDIX J

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:	.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.
Nuflor (Florfenicol)	Bacteriostatic agent; broad spectrum-effective against both gram positive and gram negative bacteria, rickettsias, and chlamydias. Extended release.	.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.
Leuteinizing Hormone-Releasing Hormone (LHRH)	Chemical Induction of Ovulation and Spermiation in Broodfish	Total dosage is .05-.1 mg/kg. Females use 10% primer dose, 90% resolving dose administered 12-16 hrs. after initial dose. Males single dose of .01-02 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.	
Baytril (enrofloxacin: fluoroquinolone)	Bactericidal; Effective against primarily gram negative bacteria and some gram positive bacteria.	5 mg/kg 10 days orally administered. Injectable: 5 mg/kg one injection (small animals). (Dosage recommendations unknown for sturgeon).	Extra label use of Baytril is monitored closely by FDA due to human health concerns. These concerns include the increased prevalence of certain pathogens with zoonotic potential and possible development of antibiotic resistant organisms.