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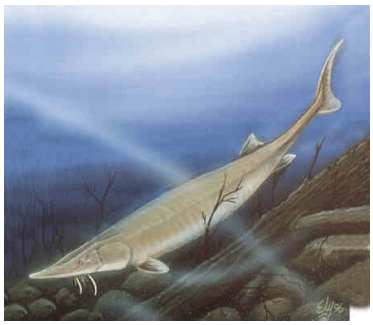
PALLID STURGEON RECOVERY UPDATE

- the latest research and management actions for recovery -



Louisiana Pallid Sturgeon Activities
1999-2000

The Louisiana Department of Wildlife and Fisheries (LDWF) in cooperation with the U.S. Fish and Wildlife Service at Natchitoches National Fish Hatchery (NNFH) continue to sample and PIT tag pallid sturgeon at the Old River Control Structure (ORCS) at the junction of the Mississippi and Atchafalaya Rivers in Concordia Parish. Sampling is done during the cool fall, winter, and spring months when sturgeon concentrations are highest. In addition to standard samples of measuring, tissue removal, and tagging, many suitable broodstock candidates are returned to the NNFH for propagation purposes.

During the fall/winter/spring 1999/2000, a total of 46 pallids and 21 hybrid types were collected at ORCS and were either tagged and released immediately or returned to the hatchery for potential breeding. All were returned to the wild at ORCS when spawning activities were terminated. While biologists were optimistic during the spring of 1999, there was no successful artificial breeding of pallids.

Thus far in the fall of 2000, a total of 98 sturgeon have been collected in two net nights of fishing and returned to NNFH for propagation purposes. The fall collections are centering on mature males for cryopreservation studies in conjunction with the Louisiana State University Aquaculture Center. Biologists have selected what are believed to be mature males and a few select females and are returning the remainder of the fish to the river after morphometrics PIT tagging. Only those fish that are "certified" (high index scores) are being used in propagation and cryopreservation trials. All other "hybrid types" are being returned to the river. Tissue samples are being sent to the repository in Alabama for storage.

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Nebraska Game and Parks Commission 2000 Sturgeon Report

Note on lake sturgeon. The Missouri Department of Conservation is augmenting lake sturgeon in the lower Missouri and Mississippi Rivers. According to Kim Graham, lake sturgeon were stocked in 1984, 86, 88, 90, 91, 93, 94, 95, and 96. In 84 and 85, fin clips were used, and beginning in 1994, lake sturgeon were marked and stocked with coded wire tags.

Between January 1 to May 19, 2000, Daryl Feit at Nebraska's Aksarben Aquarium reported 6 lake and 6 pallid sturgeon caught and released either in the Missouri, the Platte (tributary to the Missouri), or the Elkhorn (tributary to the Platte). One lake sturgeon tagged at Herman, MO (RM 97.7), on September 29, 1994, in the Missouri River (Kim Graham, personal communication) was caught and released on March 13, 2000, at RM 590, just south of Plattsmouth, Nebraska, which is close to the mouth of the Platte River. This sturgeon was 27.5 inches in length when it was caught and had traveled 492.7 miles upstream from the original tagging site. Another lake sturgeon died in a July 2000 Platte River fish kill south of Columbus, which is 100 miles above the mouth of the Platte River. It was approximately 48 inches long and weighed 22 pounds. Aging should help ascertain whether or not this particular lake was wild or if it could have come from one of Missouri's stocking. The lake sturgeon is a Nebraska threatened species, which has not been augmented within the State to date.

Two 24-inch pallid sturgeon were reported caught and released in the Platte River approximately one mile downstream of the Aksarben Aquarium on April 18, 2000. This would have been about 5 miles above the site where 412, 10 to 12-inch pallid sturgeon were stocked in October 1997 (see March 1998 Nebraskaland, pg 24-27). Since these were angler caught and released fish, there was no opportunity to wand the pallids for

coded wire tags. The angler was knowledgeable and reported catching both fish on nightcrawlers.

The pallid sturgeon is one of four threatened and endangered species being addressed in a Department of Interior and three state (CO, WY, NE) Cooperative Agreement for the central and lower Platte River. The key question being asked in regard to pallids in the lower Platte is - will flows released for the central reach of the Platte have a beneficial impact on the pallid sturgeon and/or its habitat in the lower Platte? Simple to ask, but much harder to quantitatively determine. Much information is needed to answer basic life history questions. Vaughn Snook, M.S. graduate student of Dr. Ed Peters, will be completing his thesis sometime this fall in regards to movement of pallids in the lower Platte. Microhabitat parameters of depth, velocity, temperature, D.O., conductivity, etc., were measured.

Because of water right issues on the Platte, a number of parties representing Natural Resource Districts, Public Power Districts, Irrigation Districts, Reclamation Districts, and the Nebraska Game and Parks Commission forged an interlocal cooperative agreement (different than the three state and Interior Department cooperative agreement) to help fund an additional 5-year pallid sturgeon and sturgeon chub study with Dr. Peters starting in 2000. The objectives of this peer reviewed study are as follows:

➤ Document habitat use, relative habitat preference, and species assemblage associated with adult and juvenile pallid sturgeon and sturgeon chub in the lower Platte River.

➤ Document the phenology and relative abundance of larvae for pallid sturgeon, sturgeon chub, and associated species in the lower Platte River.

➤ Determine if changes in ambient river habitat conditions influence habitat use by pallid sturgeon and sturgeon chub life stages in the lower Platte River.

➤ Document the catch of sturgeon in the lower Platte River.

➤ Develop management recommendations and educational materials to facilitate appropriate recovery efforts for pallid sturgeon and sturgeon chub in the lower Platte River.

Gerald Mestl and staff participated in the MICRA pallid sturgeon study by sampling two sections of the upper unchannelized Missouri River above Lewis and Clark Lake in May, June, and September 1999. Although no pallids were sampled, shovelnose were. Using a Kolmogorov-Smirnov test (SAS Institute 1989), the length distribution of shovelnose sturgeon captured in this reach in 1999 was found to be significantly different ($P<0.0001$) than the length distribution of shovelnose sturgeon sampled in the unchannelized reach below Gavins Point Dam in 1998. The question has to be asked as to why shovelnose sturgeon in two reaches of unchannelized river 45 miles apart exhibit such differences in population structure.

While sampling Missouri River mitigation sites, Kirk Steffensen, Mark Staab, Jason Skold, and David Isoodle of Gerald Mestl's crew sampled a 49-inch pallid sturgeon using a benthic trawl on a river bend just south of Plattsmouth in May 2000. This fish, which was not tagged, is pictured on page 6 of the July 2000 issue of Nebraskaland magazine.

Biological comments were also offered to support pallid sturgeon reasonable and prudent alternative recommendations in the July 2000 U.S. Fish and Wildlife Service Biological Opinion to the Corps of Engineers on their 2000-2001 Annual Operations Plan for the Missouri River. Comments were generated for the Nebraska Game and Parks Commission, as well as the Missouri River Natural Resources Committee.

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Share what you are doing for conservation of pallid sturgeon and/or other elements of its ecosystem. Submissions of articles and associated materials are welcome. Please send a hard copy, disc copy or email (preferred) to Steve Krentz, U.S. Fish & Wildlife Service, Bismarck, North Dakota, [Steven_Krentz@fws.gov].

Big Muddy National Fish and Wildlife Refuge Monitoring

Lisbon Bottoms and Jameson Island are the first complete units of the Big Muddy National Fish and Wildlife Refuge. Lisbon Bottoms is approximately 2,300

acres and Jameson Island is approximately 2,200 acres. Primary objectives of the Refuge are to create and restore a diversity of riverine aquatic habitats and reconnect the Missouri River to its floodplain where feasible. Management seeks to accomplish this through encouraging natural processes of erosion, deposition and succession to the greatest extent possible.

The predominant aquatic feature of the Lisbon Bottoms Unit is a newly created 2-mile long free-flowing chute or side channel. This chute began forming as a levee breech scour hole during the 1993 flood. The chute continued to develop during the 1995 flood and finally cut through to a flowing side channel during the 1996 flood. Extensive erosion and bank sluffing continued during 1997-99 due to sustained high flows, which occurred throughout most of the year. The chute became progressively wider and deeper with a developing meander pattern and formation of channel and point bars.

The Columbia Fishery Resources Office (CMFRO) initiated fishery survey and monitoring work on the chute in 1997. Thirteen sampling stations were established in the chute and adjacent Missouri River from River Mile 219 to 213. Field surveys are conducted 4-6 times per year with seine, mini fyke nets, benthic trawl, and hoopnets. Depth, velocity, substrate and some water quality parameters were also measured at sampling stations.

Over 8,000 fish have been collected in Lisbon Chute and the adjacent river monitoring stations to date. Sixty-four species have been collected in the Chute, 26 from adjacent river stations, 34 from nearby river side channels, 13 from Lisbon floodplain wetlands, and 32 species from a channel border sandbar complex at Jameson Island. Federal listing candidate species sicklefin and sturgeon chub were collected, as were species of concern, plains minnow and blue sucker. Larval sturgeon were collected in Lisbon Chute by benthic trawl in August 1998 and 1999. Three of these have been confirmed as pallid sturgeon and seven others identified as probable pallid sturgeon. No pallids were found in 2000, but a small number of larval and juvenile shovelnose sturgeon were collected.

The Kansas City District Corps of Engineers (Corps) completed construction of grade and flow control structures in the chute this spring to maintain the integrity of the navigation channel. The notch on the flow control inlet appears to be too small and sometimes plugs with debris. It currently allows about 6 percent of the river to flow down the chute at 170 kcfs discharge and about 3 percent of the flow at 70 kcfs. This has not been sufficient to keep suspended sediment moving and resulting sedimentation has filled the upper half of the chute with fine sediment and severely degraded aquatic habitat over the past 4 months.

Preliminary review of this year's data indicates species diversity and overall abundance have declined dramatically from previous years. Overall abundance of larval and juvenile fish has been much lower at all sampling stations this year, indicating that a suitable spring hydrograph, which did not occur this year due to drought, is necessary to provide usable habitat in this area. We are currently negotiating with the Corps to get the inlet notch widened and deepened. CMFRO will continue monitoring of this area to evaluate impacts of the Corps' project on habitat and fishes of the Lisbon Bottoms Unit of the Big Muddy National Fish and Wildlife Refuge.

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Pallid Sturgeon Activities from South Dakota

Study Area

The riverine reach of Lewis and Clark Reservoir extends approximately 72 km from below Fort Randall Dam to near Springfield, South Dakota, were its features become more like a reservoir. To aid in sampling, the riverine reach was divided into four sample areas of approximately equal length. The upper site extends from Fort Randall Dam (river mile (RM) 880) to downstream of Greenwood, South Dakota (RM 865). The upper-middle site extends from RM 865 to near Verdel, Nebraska (RM 856), the lower-middle site extends from RM 856 to Running Water, South Dakota (RM 845). The lower site comprises the remainder of the river (RM 845 to near Springfield, SD). The fifth sample site is Lewis and Clark Reservoir from downstream of Springfield, SD, to Gavins Point Dam. If all fish are found to remain in the riverine reach, this section will be excluded as a sample site.

Methods

Six adult and 50 juvenile pallid sturgeon, *Scaphirhynchus albus*,

were surgically implanted with a sonic transmitter and a PIT tag at Gavins Point National Fish Hatchery. Each transmitter emits a unique code specific to an individual fish, and has a life expectancy of 36 months. These fish were held several weeks, following implantation, to determine tag retention and survival rates. Following this holding period, surviving fish were transported and released near Verdel, NE, in the riverine portion of Lewis and Clark Reservoir.

Two tracking methods are employed during each sample period; extensive and intensive. Extensive tracking involves the location of as many fish as possible per zone, and intensive tracking is the following of a few fish for the entire tracking period. During each sample period, at least two zones were tracked extensively, and at least one fish intensively. Tracking began immediately post stocking and continued bi-monthly, i.e., every other week, until weather conditions prohibited tracking during the winter months. Tracking will resume as early as feasible in the spring. All sample zones and sample periods are selected at random to reduce bias. An ultrasonic receiver and directional hydrophone were used to determine fish locations. A location was recorded when the coded impulses from the sonic transmitter became equally audible with a 360° rotation of the hydrophone. Once a fish location was determined, latitude and longitude coordinates were recorded with a PLGR+96 Global Positioning System (GPS) receiver and habitat types were assigned. The habitat types were designated as: main channel, side channel, backwater, island, reservoir, tributary mouths, and tailrace. Combinations of these descriptors may be necessary to get specific habitat types, like side channel island or main channel island. Tracking in tributaries will be conducted if deemed necessary, and will be recorded as a separate habitat type. Along with the habitat types, surface water temperature, bottom flow, water depth, turbidity, and percent maximum depth were collected at fish relocation sites. Percent maximum depth is the ratio of the fish depth relative to the maximum cross sectional depth where the fish is located.

Diel movement patterns were determined by dividing a 24 h period into four sub-periods; dawn (1 h before to 1 h after sunrise), day (2 h after sunrise to 2 h before sunset), dusk (1 h before sunset to 1 h after sunset), and night (2 h after sunset to 2 h before sunrise). The tracking periods were determined randomly, and as many fish as possible were followed during that time. Water level fluctuations and poor visibility made night tracking difficult due to decreased flows and safety. To minimize risk, night time tracking was conducted in conjunction with dusk and dawn periods. This allowed biologists to begin tracking during daylight hours and finish tracking during daylight hours. The GPS coordinates and habitat types for each relocated fish were recorded approximately every hour. Tracking is planned to continue through 2002, or until transmitter batteries fail.

Results

Twenty-two of the fifty juveniles and the six adults survived the tagging operation and holding period. These fish were stocked into the riverine portion of Lewis and Clark Reservoir in July of 1999. Following that time, 16 of the 22 juveniles and 4 of the adults

Pallid Sturgeon Recovery Team Members

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have been relocated at least once. Currently, too few relocations on multiple fish have occurred for statistical analysis. The following data merely summarize what has been found to date. Both juveniles and adult data are combined, at this time, but will be separated as samples increase.

For all relocated fish, the mean depth of location was 3.6 m (N=29, SE=0.231), turbidity ranged from 3.3-36.8 NTU's (mean 6.2 NTU's, SE=1.9), and bottom flows ranged from 5.3-42.1 cm/sec (mean 17.6 cm/sec, SE=2.02).

All fish were relocated in main channel habitats. No fish have been located in the reservoir proper or the river marsh area near Springfield, SD; in fact, the most downstream relocation was approximately 100 m upstream of Running Water, SD.

During intensive tracking, most fish stayed within a few hundred meters of their original location. However, one fish moved steadily in a downstream fashion, traveling approximately 11 km over a 12 h period. This was the largest single directional movement observed.

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Reporting of Pallid Sturgeon Telemetry Study Results

A series of three reports are in progress describing the methods developed and discussing the results of 4 years of pallid sturgeon telemetry data. These reports are scheduled to be released beginning the Winter 2000-2001.

Development of Side Scan Sonar Capabilities

The Columbia Environmental Research Center (CERC) has initiated the development of side scan sonar capabilities to complement existing biological remote sensing and physical habitat mapping capabilities. The Center has acquired a 900 MHz high-resolution side scan sonar system manufactured by Marine Sonic Technology, Ltd. of White Marsh, VA. This system provides high-resolution, underwater images of river beds and lake bottoms. These images reveal fine-scale details and features not detectable with other technologies. This sonar has the unique ability to image soft tissue and has been deployed successfully in the Columbia River to detect sub-adult sturgeon and has been successfully used in the Missouri River to detect biological targets, including benthic fish. Imagery data collected is geospatially referenced and can be mosiaced to create complete habitat coverages for habitat mapping and visualization. CERC plans to complete protocols for system deployment and imagery data processing by the spring of 2001. This system will be deployed in the Missouri River to collect continuous imagery over the bottom of the river to determine substrate, detect sturgeon presence and abundance, and identify unique habitat features, such as woody debris and other structural features. This system will be deployed in conjunction with multiple field studies to: 1) describe, characterize, and identify important habitat elements and variables during telemetry studies with sturgeon and other native fish species; 2) identify and evaluate areas where sturgeon may be congregating during staging or spawning; and, 3) complement the deployment of traditional fisheries sampling gear to increase the safety of open river gear deployment, to evaluate gear performance and efficiency, and to verify the area of habitat sampled.

Pallid and Shovelnose Sturgeon Behavior, Reproductive Physiology, and Population Modeling

Four proposals for work with pallid and shovelnose sturgeon were submitted by CERC for funding consideration in the internal USGS Species At Risk (SAR) program. Work includes: 1) documentation of the reproductive physiology of *Scaphirhynchus* species and the development of a suite of measurements to rapidly assess sturgeon reproductive status; 2) work towards the development of a population viability analysis (PVA) model to guide management and recovery efforts of *Scaphirhynchus* species; 3) laboratory behavioral studies examining the physical habitat preferences of *Scaphirhynchus* species; and, 4) intensive telemetry studies with shovelnose and pallid sturgeon on the lower Missouri River. While funding through the Species At Risk (SAR) program is highly uncertain, CERC will continue forward and will pursue these efforts within a more limited scope until reliable funding opportunities can be identified and secured.

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Columbia Fishery Resources Office Monitors Lower Missouri River Bridge Replacement Site for Pallid Sturgeon Use

The Missouri Department of Transportation (MDOT) has proposed replacing the Route 19 Missouri River Bridge in Hermann, Missouri, at River Mile 98. During project consultation, concerns were raised that bridge replacement activities may affect pallid sturgeon habitat. Columbia Fishery Resources Office (FRO) had located pallid sturgeon in similar habitat within one river mile of the bridge alignment in spring and summer 1999. MODOT is providing funds for Columbia FRO to conduct field surveys, analyze data, and provide advice and recommendations to avoid and/or minimize potential impacts to pallid sturgeon. This work is being done in conjunction with the USGS-Columbia Environmental Research Center (CERC).

CERC staff is mapping the physical habitat characteristics (substrate, bottom contours, water velocity) of the project site. Columbia FRO will correlate pallid sturgeon presence/absence with CERC's habitat data. Field work began in January 2000 and will be conducted monthly until January 2001. As of September 2000, 293 nets fishing 6,132 hours have collected 1,190 shovelnose sturgeon, 14 pallid sturgeon hybrids, and 2 pallid sturgeon. The first pallid (701 mm FL, 1333 g) was collected in March in 0.6-10.4 m of water, with a surface temperature of 11.3 C, turbidity of 45 NTU, and bottom velocity of 0.08 m/s. The second pallid (785 mm FL, 1733 g) was collected in April in 0.92-3.05 m of water with a surface temperature of 15.0 C, tubidity of 48 NTU, and bottom velocity of 0.58 m/s. Both sturgeon were collected in stationary gill nets fished behind wing dikes. Barbel samples from all pallids, pallid hybrids, and a subsample of shovelnose sturgeon have been sent to Southern Illinois University (SIU) to be stored for genetic analysis.

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Cryopreservation of Sperm of Pallid Sturgeon *(Scaphirhynchus albus)*

We sought to develop sperm cryopreservation methods for the pallid sturgeon, a federally listed endangered species. Four males were injected with synthetic luetenizing-hormone releasing hormone at 0.05 mg/kg of body weight in June 1999, at the Garrison Dam National Fish Hatchery (GDNFH). After 24 hrs, sperm were collected by use of a small diameter tube attached to a 60-ml syringe inserted into the urogenital opening. The sperm were diluted at a ratio of 1:4 (sperm:extender) with a commercially prepared Hanks' balanced salt solution (HBSS) (H4385, Sigma, St. Louis, MO) at 100 mOsmol/kg and kept refrigerated until use. Methanol (MeOH) and dimethyl sulfoxide (DMSO) were used as cryoprotectants, each at 5 percent, 10 percent and 15 percent (v:v). Each cryoprotectant was mixed 1:1 with HBSS prior to the experiment to reduce toxicity effects. Sperm were mixed with the cryoprotectants, loaded into 0.5-ml straws, packed into goblets (5 straws per goblet) and placed on the lower portion of aluminum canes. Motility was estimated before freezing to determine the effects of cryoprotectant toxicity. After a 2-minute equilibration period, the canes were lowered into a nitrogen vapor shipping dewar. Samples exposed to 15 percent DMSO retained significantly lower motility (P = 0.0113) than did samples diluted in the other cryoprotectants. The cooling rate (-22 °C/min) was recorded by thermocouples inserted into 0.5-ml straws filled with extender and cryoprotectant. After freezing, the samples were shipped to the Warm Springs Fish Technology Center and stored in liquid nitrogen.

After 1 year, the samples were shipped back to Garrison Dam National Fish Hatchery (GDNFH) for use in fertilization trials. Straws were thawed in a 40 °C water bath for 9 sec, and motility was estimated. There was no significant difference (P = 0.06) in post-thaw motility among the cryoprotectants tested. Each sample was used to fertilize ~200 eggs, which were incubated at 21 °C. The eggs began hatching after 3.5 days and hatch rates were determined after 5 days. Eggs fertilized with sperm cryopreserved with 5 percent or 10 percent MeOH had significantly higher fertilization rates ($P < 0.0001$) and hatch rates ($P < 0.0001$) than did eggs fertilized

with 15 percent MeOH or DMSO at any concentration. Eggs fertilized with sperm cryopreserved with 5 percent or 10 percent MeOH had similar fertilization and hatch rates as control sperm. Cryopreserved sperm can be used for the conservation of genetic diversity and the creation of a germ plasm repository to aid in the recovery of this species.

Other studies included: 1) addition of antibiotics to extenders to increase refrigerated storage time and reduce the risk of disease transmission; 2) ionic analysis of seminal plasma composition; 3) preservation of blood and sperm for genome size analysis by flow cytometry; and, 4) cryopreservation of sperm in 5-ml straws, which may allow more efficient hatchery usage of cryopreserved sperm. These studies are still being analyzed.

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University of Alabama Activities

The USFWS produced specimens of pallid, shovelnose, and pallid x shovelnose hybrids at Miles City State Fish Hatchery, Montana, and Gavins Point National Fish Hatchery, South Dakota. All of the breeding stock was captured in the upper Missouri River Drainage in extreme western North Dakota and eastern Montana. Samples were preserved in formalin at various times from early in development up to small adults. Here at the University of Alabama, Rick Mayden and I have been examining a total of 61 of these specimens (14 pallid, 13 shovelnose, and 34 hybrids) ranging in size from 78 to 600 mm standard length (SL) (85 to 641 mm fork length (FKL)). Specimens appeared normal, except for the lack of spines on the snout and a reduction of spicules laterally and dorso-laterally. Snout spines are present in almost all wild-caught pallid and shovelnose sturgeon.

We have used these specimens to test the various character indices used by fisheries biologists to identify between these two sturgeon species and potential hybrids. Some indices rely solely on head and body measurements (Keenlyne et al. 1994, Technical Notes from Missouri River Fish and Wildlife Management Assistance Office 2000), while others have formulas for either measurements only or for both measurements and fin-ray counts (Carlson and Pflieger 1981, Sheehan et al. 1997). When all 61 specimens were evaluated together, pallid and hybrid specimens were indistinguishable, while most, but not all, shovelnose specimens were identified correctly. The Sheehan et al. (1997) index using both morphometrics and meristics did identify most specimens correctly, but numerous hybrid specimens had values for pallids and numerous shovelnose specimens had hybrid values. This same pattern was observed for small specimens 78 to 227 mm SL (85 to 245 mm FKL). Unfortunately, our sample of larger sturgeon (> 250 mm SL) was limited; we only had 5 and 2 specimens of shovelnose and pallid sturgeon. All indices correctly identified large shovelnose sturgeon, but specimens of pallid and hybrid sturgeon were difficult to distinguish. One specimen of pallid sturgeon was identified as a hybrid and several hybrid specimens were identified as pallids in indices by Carlson and Pflieger (1981) and Keenlyne et al. (1994). The Missouri River Fish and Wildlife index correctly identified the pallids, but had some hybrids with scores very close to these. Both the morphometric index and the morphometric and meristic index of Sheehan et al. (1997) graphically separated pallids and hybrids, but several hybrid specimens had values outside of the given range for hybrids and within the range of pallids. These results indicate that current character indices do not correctly identify between small specimens of pallid, shovelnose, and hybrid sturgeon. All indices work well identifying adult shovelnose sturgeon, but even the latest indices can incorrectly identify hybrids as pallids. Additional analyses by us may reveal other measurements useful in distinguishing pallid sturgeon from hybrids.

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Larval Pallid Sturgeon Collected in the Lower Missouri River

Columbia FRO staff collected three larval pallid sturgeon in a monitoring study on the lower Missouri River. A total of 44 larval sturgeon were collected from 1997-2000 in a 7-mile stretch (RM 213-219) of the Missouri River. Three larval sturgeon were identified as pallid sturgeon, 3 as shovelnose sturgeon, 31 as *Scaphirhynchus* sp., and 7 were tentatively identified as pallid sturgeon. The three recently confirmed pallid sturgeon were caught approximately 9 miles south of Glasgow, MO, in Lisbon Chute (RM217), a 2-mile naturally formed side channel of the lower Missouri River. The side channel is located in the Lisbon Bottoms Unit of the Big Muddy National Fish and Wildlife Refuge. The Lisbon side channel possesses a diversity of habitat, including point mid-channel sandbars.

Two of the confirmed larval pallid sturgeon were collected August 13, 1998, in the side channel trough, over a soft detritus substrate. The third larval pallid sturgeon was collected August 20, 1999, also in the side channel trough, over a sand/gravel substrate. The three pallid sturgeon were 26.2, 25.9, and 25.0 mm in length. This is the first documented evidence of natural reproduction of the species in the lower Missouri River.

The sturgeon were collected by a benthic trawl equipped with a roller rock lead line. The trawl was 2 m wide, 0.5 m high, 5.5 m long and had an inner mesh size of 0.32 cm and outer mesh size of 3.81 cm. The pallid sturgeon were caught at depths ranging from 0.92 m-3.97 m. Surface velocities recorded ranged from 0.40-0.55 m/s and bottom velocities ranged from 0.30-0 .43 m/s. Water temperature on August 13, 1998, averaged 26.6 ºC and turbidity averaged 112 NTU. Water temperature on August 20, 1999, was 25.6 ºC and turbidity was 94.0 NTU. River stage at the Glasgow gauging station during pallid sturgeon collection was similar for both years at 4.9 m.

Verification of the larval specimens was made by Darrel Snyder, Curator for the Larval Fish Laboratory, Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, Colorado.

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Lower Missouri/Yellowstone River Pallid Sturgeon Study-2000

Thirteen adult pallid sturgeon were captured in the Yellowstone River confluence area during April 2000. Seven of these fish were "new fish" and the others were recaptures from previous years capture and tagging efforts. Several were transported to the Garrison National Fish Hatchery to determine state of gonad maturation and a few were later used for spawning at the hatchery. One additional adult was captured in August, also in the confluence area. This fish was a new fish, but was not PIT tagged due to lack of tags. Three hatchery-reared juvenile pallid sturgeon were captured by drift netting trammel nets (one-inch bar, 75-feet long) in the Fairview bridge area of the Yellowstone River during August. These fish had been released August 11, 1998, upstream of the bridge area, and were from three different families of pallid crosses. These fish appeared to be in excellent condition. Original lengths and weights are unknown, so growth information is not available.

Plans to release hatchery-reared pallid sturgeon progeny, produced in 1999 at the Garrison hatchery, in Montana waters were again postponed because of possible viral problems which surfaced in 2000 at the Garrison hatchery. However, the Gavins Point hatchery in South Dakota also has hatchery-reared pallids from 1998 and 1999 and has not experienced any pallid-associated disease problems. The Montana Fish Health Committee decided these fish would be acceptable for release in the lower Yellowstone and Missouri Rivers this year. As a result, 200 juvenile pallids raised in 1998, and 480 from 1999, were released at two sites in the Yellowstone River (Sidney and Fairview areas) and two sites in the Missouri River (Culbertson and Wolf Point areas) during October. All were PIT tagged and weighed and measured at the hatchery so movements and growth information will be available in the future.

Young-of-the-year (Y-O-Y) shovelnose sturgeon were again captured during August by bottom trawling in the lower Missouri River (below Highway 58 Bridge, ND). A total of 144 Y-O-Y

sturgeon were captured and FED-Ex'd live to the University of California, Davis, for iridovirus testing experimentation. A similar effort last year failed to detect any evidence of an iridovirus. In addition, 86 wild juvenile shovelnose sturgeon were captured in the lower Yellowstone River during August and tissue samples extracted from fins and a barbel for viral testing at the Bozeman Fish Tech Center. Finding evidence of this virus in the wild would show it to be endemic and alleviate some of the concern relative to its presence in hatchery conditions. It would also help to accelerate the recovery efforts of pallid sturgeon in Montana waters, particularly in Recovery Priority Area 1. Other species of interest captured by trawling included 997 channel catfish, 44 sturgeon chub, 63 sicklefin chub, 23 stonecat, 20 freshwater drum, and lesser numbers of carp, smallmouth buffalo, longnose dace, flathead chub, sauger, fathead minnow, goldeye, and sauger/walleye.

Trawling was done in the Intake area of the Yellowstone River during July to help Fish and Wildlife Service personnel collect sturgeon chub for transplanting in the Little Missouri River recovery effort. A total of 516 sturgeon chub, as well as 430 stonecat, 102 longnose dace, and lesser numbers of flathead chub, sicklefin chub, burbot, carp, sauger, blue sucker, channel catfish, plains/silvery minnow, and shovelnose sturgeon were captured.

Nine sites of the Missouri River below Fort Peck Dam were monitored during June and July by netting, trawling, seining, larval fish sampling, hoop netting, and electrofishing. The purpose of the monitoring is to collect and establish baseline information relative to fish populations, water temperatures, and physical habitat of the river. This information will be used to evaluate the possible benefits to fish populations, particularly pallid sturgeon, by increased flows and water temperatures associated with releasing reservoir water via the Fort Peck spillway. There has been no documented evidence of successful spawning or recruitment of pallid sturgeon in Montana waters for at least the past decade. Releases from the spillway will be an attempt to somewhat mimic the natural hydrograph of the river during the spring prior to the construction of Fort Peck Dam. Even if pallid sturgeon do not respond to the increased flows and warmer water temperatures by migrating upriver and spawning, other native fishes such as sauger, shovelnose sturgeon, paddlefish, goldeye, freshwater drum, sturgeon chub, sicklefin chub, flathead chub, and others should definitely benefit. A "mini-release" is scheduled for 2001, primarily to check the physical integrity of the spillway, with a sustained release scheduled for late May and June 2002. The present low reservoir level may negate or delay spillway discharges, particularly during 2001. A high mountain snowpack and above average winter/spring moisture is needed to recharge the reservoir in order for spillway releases to occur.

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Shovelnose Sturgeon in the Lower Missouri and Middle Mississippi Rivers

(Excerpted from: Grady, J., J. Milligan, C. Gemming, D. Herzog, G. Mestl, L. Miller, and B. Sheehan. in prep. Pallid and shovelnose sturgeons in the Lower Missouri and Middle Mississippi Rivers. Final Report Prepared for MICRA.)

Recent concerns about sturgeon caviar harvest have led to an examination of long-term trends in shovelnose sturgeon numbers. Excessive exploitation of shovelnose sturgeon may be evident in reduced numbers of large reproductive fish. Although large historic datasets for shovelnose sturgeon are not available for statistical testing, average length of shovelnose sturgeon can be compared over time. Evermann (1902) reported females in Ohio River commercial harvest averaged 645 mm and 1.5 kg, while males averaged 551 mm and 0.9 kg. Barnickol and Starrett (1951) collected shovelnose sturgeon in the Mississippi River that averaged 589-mm in length and 0.6-kg in weight.

The minimum reproductive size for females and males appear to vary by study location. In the Missouri River in South Dakota, the minimum sizes were 48 cm for females and 44 cm for males. In the Lower Missouri, Carlson and Pfleiger (1981) found minimum sizes of 51 and 45 cm, respectively. In the Upper Mississippi River, the smallest sexually mature females were 60-64 cm, while males were 47-56 cm (Monson and Greenback 1947, Helms 1974).

Carlson and Pfleiger (1981) reported average lengths, percent of catch over 50 cm and percent of catch over 60 cm for shovelnose sturgeon collected in the Lower Missouri and Middle Mississippi River from 1978-1979. These values were compared with recent statistics. With the exception of the St. Louis site, values for mean lengths, percent over 50 cm (minimum male reproductive size), and percent over 60 cm (minimum female reproductive size) increased for shovelnose sturgeon in most locations in the Lower Missouri and Middle Mississippi Rivers. The largest increase in mean size was found near Brownsville, Nebraska. While Carlson and Pflieger (1981) found shovelnose sturgeon increased in size as moving downstream in the Missouri River, this trend no longer appears evident.

Work currently being completed on Ohio River shovelnose sturgeon indicates a significant decline in shovelnose sturgeon lengths between 1999 and 2000, and has raised concerns about shovelnose sturgeon exploitation rates (Chad Stinson, FWS, pers. comm.). Shovelnose sturgeon lengths were compared at Hermann, Missouri, between March 1999 and March 2000. No significant differences were found in length distributions or relative weights between the two sample years.

In summary, over-exploitation of shovelnose sturgeon stocks does not seem to be a problem yet in the Lower Missouri and Middle Mississippi Rivers. However, sturgeon populations are sensitive to fishing mortality and the 2000 Russian sturgeon harvest was 60 percent lower than in 1999 (Boreman 1997, The Russian Environmental Digest 2000). Sturgeon populations in the Missouri and Mississippi Rivers should continue to be monitored for potential overharvest.

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Pallid Sturgeon in the Lower Missouri and Middle Mississippi Rivers

Columbia Fishery Resources Office (CFRO), FWS, is coordinating report preparation for the MICRA pallid sturgeon project. The cooperative project covered sections of 1,000 river miles in the Lower Missouri and Middle Mississippi Rivers, and included sampling efforts by the Nebraska Game & Parks Commission, Iowa Department of Natural Resources, CFRO, Southern Illinois University, and Missouri Department of Conservation's Long Term Resource Monitoring Program station. Field sampling began in November 1997 and was completed in April 2000. In total, 5,400 fish of 39 species were collected in 1,033 nets and lines. The report is scheduled for completion by the end of the calendar year.

Four presumed wild origin pallid sturgeon and two recaptured hatchery pallid sturgeon were collected in the Lower Missouri River by CFRO staff. Seven hatchery origin pallid sturgeon were collected by MDC-LTRM staff (see Peterson 1999). The ratio of wild pallid sturgeon to all river sturgeon collected dropped from 1 in 396 (0.2 percent) collected by Carlson et al. (1985) to 1 in 724 (0.14 percent). All but one of the pallid sturgeon were collected in deep holes associated with wing dikes. The remaining pallid was collected in a side channel border habitat.

Six pallid-shovelnose sturgeon hybrids were collected in the Middle Mississippi River, while 14 were collected in the Lower Missouri River. The rate of hybridization increased from 1 in 361 (0.3 percent) river sturgeons in the late 1970s to 1 in 133 (0.8 percent) in the 1990s (Carlson et al. 1985).

The shovelnose sturgeon data (2,861 fish) were also examined to determine if the long-feared increases in exploitation caused by the decline of Russian sturgeon stocks was impacting the shovelnose population. There was no apparent decline in mean shovelnose lengths, percent of fish greater than 50 cm (minimum male reproductive size) or percent of fish greater than 60 cm (minimum female reproductive size) from late 1970s values (Carlson et al. 1981). In addition, the average length of shovelnose sturgeon collected in the Middle Mississippi River was the exact same number reported by Barnickol and Starrett in 1951.

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Nebraska FWS Pallid Sturgeon Activities

Much of the activity involving pallid sturgeon issues in the Grand Island Ecological Services office has revolved around the Platte

River Cooperative Agreement. Pallid sturgeon utilize the lower Platte River, and as such, is one of four target species to be addressed under the agreement. Due to the need for more time to develop a viable program and provide the necessary program details for analysis under the Endangered Species Act, the term of the Cooperative Agreement is being extended. While the details of the extension are still being worked out, the individual parties to the agreement have settled on another 2 years as the working figure. The intent of the effort is to develop a program that can serve as a reasonable and prudent alternative for a number of depletion projects that have been developed over the years throughout the North Platte, South Platte, and Platte drainages. These specific projects have been found to jeopardize the continued existence of the whooping crane, interior least tern, piping plover and pallid sturgeon. The program will provide specific land and water benefits to endangered species in the central Platte, and test the assumption that it is possible to improve habitat for the pallid sturgeon in the lower Platte River by managing flows in the central Platte River. In addition, the program will provide a monitoring and research component that should provide substantial information on the use of the Platte River by pallid sturgeon.

A workshop was held on September 26 and 27th to develop species recovery goals for the Platte River for those four target species. The effort involved species experts from Federal and State agencies, as well as the academic community and private sector. The pallid sturgeon workshop participants determined that at this point in time, measurable goals cannot be defined for pallid recovery in the Platte. The group outlined the research questions that first must be answered in order to define such goals in the future. They then developed a recommendation to "preserve, or restore where appropriate, the hydro-geomorphic processes and functions that are responsible for creating or maintaining the physical habitat template characteristic of the lower Platte River: a dynamic, sandy, shallow, free-flowing, braided river."

Funding was provided to Ed Peters at the University of Nebraska – Lincoln to monitor river temperature, turbidity, and total suspended solids in the lower Platte River and some of its larger tributaries beginning this year. This should begin to answer some of the questions about water quality in the lower Platte River, which can hopefully dovetail into his ongoing pallid sturgeon studies.

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Evaluation of the Pallid Sturgeon Release in Missouri River Upriver of Fort Peck Reservoir, Montana

A total of 758 hatchery-reared yearling (HRJ) pallid sturgeon (1997 year class) were released into the Upper Missouri River, at 3 locations, during the summer 1998. The average weight of these yearling fish were 0.17 lbs. and all were PIT-tagged. No other pallid releases have subsequently occurred because of the concern about the Missouri River Sturgeon Iridovirus, discovered in the pallid sturgeon hatcheries since 1999. Therefore, only this initial pallid sturgeon release has been evaluated over the past 3 years.

Attempts were made to capture the HRJ pallid sturgeon drifting small mesh gill nets and using a benthic trawl. A total of three HRJ pallids were recaptured during 1998, all using the trawl. Their average sizes were 11.5 inches (10.4-13.2) and 0.18 lbs. (0.12-0.27). Two of these were recaptured in the nearby site where they were released, while the third pallid moved 135 miles downriver. All three pallids were captured in the lower reach of the study area.

A total of three HRJ pallid sturgeon were recaptured during 1999; all in the lower reach of the study area. One was captured with the trawl and two while drifting gill and trammel nets. Their average sizes were 15.3 inches (13.3-17.8) and 0.42 lbs. (0.26-0.68). Only one of the pallids was recaptured near its release site; the other two both moved about 65 miles downriver since the 1 year they were at large.

A total of five HRJ pallid sturgeon were recaptured during 2000; three pallids were captured in the lower reach of the study area, while the remaining two were captured in the upper reach of the 168-mile study area, all while drifting trammel nets. They have now been at large in the wild for over 2 years. Their average sizes were 18.2 inches (16.3-20.4) and 0.70 lbs. (0.46-1.02). Four of the five pallids were checked for tags and only three pallids were detected with a PIT tag. Of these 3 fish, 2 moved

downriver about 140 miles. The third pallid moved upriver 41 miles. It is still too early to state with any certainty about how successful the pallid release was in the Upper Missouri River. However, there are a few items that are worth noting. The pallids are growing at a rate of 3.4 inches per year after 2 years in the wild. The recaptured fish originated mostly from the upriver release sites (Loma and Judith Landing). Six of the nine pallids with known release site locations (as determined from detected PIT tag readings) were from the upper and mid-release sites.

It an attempt to capture and preserve a portion of the Upper Missouri River pallid sturgeon gene pool, a pilot effort was initiated to test the feasibility of collecting sperm from wild male pallids in this area and ship the fresh milt to Garrison Dam National Fish Hatchery (GDNFH) for use in their pallid sturgeon propagation program. River flow conditions during June 2000, were exceptionally low and allowed for effective sampling for mature adult pallids. A total of 11 individual adult pallids were netted, 6 of which produced milt samples. These samples were sent to GDNFH, which took 2 days to arrive at GDNFH from MT. Four of the six sperm samples arrived in good shape and contained viable sperm, that was subsequently used in the 2000 propagation program.

These results were promising and I plan to continue with collecting sperm samples when conditions allow. Future Upper Missouri River pallid sturgeon sperm samples will also be cryopreserved.

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Bighorn River Shovelnose Sturgeon Restoration

Shovelnose sturgeon were reintroduced into the Bighorn River in Wyoming during 1998. These were fish produced from Yellowstone River broodstock and reared at Gavins Point NFH. Iridovirus concerns have prevented any further stockings, however, recaptures are being observed. The stocked fish appear to have phenomenal growth (up to 27" length) in the 3 years and are distributing widely throughout the upper Bighorn River

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Gavins Point National Fish Hatchery Activities

1. The pallid sturgeon series, sampled for thyroxine (imprinting) analysis, were shipped to Dr. Allan Scholz, Eastern Washington University, Cheney, WA. The tissue from these fish was analyzed for thyroxine concentrations to determine whether they display a tendency for imprinting or returning to their natal areas when they spawn.

2. We were informed by Dr. Ron Hedrick, University of California, Davis, CA, that the paddlefish advanced fry, that we shipped to his laboratory, were not able to contract the disease caused by the iridovirus. The decision was made to continue with stocking of paddlefish into the Missouri River (specifically, Lake Francis Case).

3. On September 14, 1999, a total of 15, 1999-year-class fingerling pallid sturgeon were shipped to the Bozeman Fish Health Center, MT, for iridovirus testing/screening. The results came back negative.

4. Dr. Bruce Barton, Department of Biology, University of South Dakota, Vermillion, SD, forwarded a copy of our most recent publication entitled "Juvenile Pallid (*Scaphirhynchus albus*) and Hybrid Pallid X Shovelnose Sturgeon (*S. Platorynchus*) Sturgeon Exhibit Low Physiological Responses to Acute Handling and Confinement," which was also presented at the Aquaculture Canada Conference '99 in British Columbia. This project was a cooperative effort/venture between the University of South Dakota and the Gavins Point NFH, Yankton, SD. This paper was published in the Journal of Comp. Biochem. Physiol.

5. On October 12, 1999, a total of 400 fingerling, young-of-the-year pallid sturgeon were shipped to Dr. Ron Hedrick, University of California, Davis, CA, for him to do research to learn more about the identification, susceptibility, and transmission of iridovirus within this group of fish.

6. Sixty live pallid sturgeon from the 1999 year-class were shipped to the Bozeman Fish Health Center to be inspected for iridovirus. None was detected.

7. Blood was drawn from 10 shovelnose sturgeon, and these samples were shipped (10/15/99)

(along with the carcasses of the 10 dead fish from which the blood was drawn) to Dr. Ron Hedrick, University of California, Davis, CA. He wanted the blood and carcasses in an effort to determine whether the disease (iridovirus) can be detected again, even though the fish were asymptomatic after having the disease.

8. On March 7, 2000, the Bozeman FHC sampled 84 pallid sturgeon juveniles to determine whether they might possibly have the iridovirus. Nothing was found.

9. The hatchery crew PIT-tagged all 125 of the 1997 year-class pallid sturgeon that will be used for future broodstock at our hatchery (4/20/00). These fish were derived from five male-female crosses, resulting in these five family groups (25 fish/family group).

10. A total of 397 pallid sturgeon juveniles (1997 year-class) were PIT-tagged and Dangler-tagged (5/31/00). A total of 50 pallid sturgeon juveniles from the 1997 year-class were sonic-tagged by surgical implantation within the abdomen of each fish (6/1/00). Six pallid sturgeon adults were, also, sonic-tagged on 6/1/00.

11. The entire hatchery staff participated in the stocking of the endangered pallid sturgeon in the Missouri River near Verdel, NE. A total of 397 pallids from the 1997 year-class weighing 582.8 lbs. (18.0" long). Six pallid sturgeon adults weighing 248.6 lbs. were stocked in areas from Verdel, NE, to Springfield, SD.

12. A total of 36 pallid sturgeon juveniles (1999 year-class) weighing 5.26 lbs. were shipped to Jan Hoover, Waterways Experiment Station (COE), Vicksburg, MS, for stamina tunnel research (endurance testing).

13. Twenty-two sonic-tagged pallid sturgeon juveniles (1997 year-class) weighing 44.4 lbs. were stocked in the Missouri River near Verdel, NE.

14. The 1998 year-class pallid sturgeon were PIT- and Dangler-tagged prior to them being stocked in the Missouri River near Verdel, NE.

15. We received a copy of Justin Sipiorski's Masters Thesis entitled "Neurotransmitter Activity in the Fore- and Hind-brain of the Pallid Sturgeon (*Scaphirhynchus albus*) Following Acute and Chronic Stress". This thesis project was completed as a cooperative venture between the Department of Biology, University of South Dakota, and the Gavins Point NFH. We encouraged Justin to do this project, provided him with the surplus pallid sturgeon, and allowed him to use our tanks and facilities for conducting the experiments within the Endangered Species Building.

16. On September 14, 2000, the Gavins Point NFH received an Import Permit from the Montana Fish, Wildlife, and Parks. This permit authorizes our hatchery to import up to 700 live pallid sturgeon juveniles into the State of Montana for stocking purposes below Fort Peck Reservoir in the Missouri and in the lower Yellowstone Rivers. No fish may be stocked above Fort Peck Dam at this time.

17. Three adult pallid sturgeon were released (9/20/00) into the Missouri River at the Verdel, NE, boat ramp. Two of these adults were implanted with sonic tags to monitor their movements after release. All three of the fish were previously PIT-tagged. Total weight of these fish was 121.8 lbs.

18. One hundred juvenile pallid sturgeon from the 1998 year-class were released (9/20/00) into the Missouri River at the Verdel, NE, boat ramp. Each of these fish had been PIT- and Dangler-tagged. Total weight of these fish was 83.8 lbs.

19. Fifty pallid sturgeon from the 1998 year-class were PIT-tagged for identification as future captive broodstock at the hatchery.

20. Herb Bollig attended a meeting (9/26-28/00) near Grand Island, NE, entitled "Workshop to Develop Species Recovery Objectives for Four Target Species in the Central Platte River (Whooping Cranes, Least Tern, Piping Plover, and Pallid Sturgeon)."

21. On September 28-29, 2000, 680 juvenile pallid sturgeon from the 1998 and 1999 year-classes were PIT-tagged prior to them being stocked during mid-October, 2000, below Fort Peck Reservoir in the Missouri River and lower Yellowstone River. Scheduled stocking dates will be October 11-12 and 17-18.

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Attempts were made this past year to capture shovelnose sturgeon to continue the virus studies.

Objectives were to spawn shovelnose sturgeon in order to document the presence of the recently found iridovirus in the wild populations of sturgeon. The first occurrence of the virus was originally found in shovelnose sturgeon that originated from the Yellowstone River in Montana. Unfortunately, all 27 fish captured from the Powder River, a tributary of the Yellowstone and suspected spawning habitat for the shovelnose sturgeon, were female. Due to the early spring arrival and time restraints, further efforts were not attempted.

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Comparison of Fish Habitat Availability in the Yellowstone and Upper Missouri Rivers

The purpose of our project is to compare the fish habitat characteristics for sites in the lowerYellowstone River and the Missouri River above and below Fort Peck Reservoir. To accomplish this purpose, we are using two-dimensional hydrodynamic simulation models in concert with Arc/Info GIS technology to prepare spatially explicit models of habitat distributions in all three segments, for a range of flows from low base flow (about 1,000 cfs) to high flood flows. We have completed the hydraulic simulation phase for the following sites on the Yellowstone and upper Missouri Rivers:

Intake (Yellowstone)
Elk Island (Yellowstone)
Fairview (Yellowstone)
Starve Out Flat (Missouri above Judith River confluence)
Culbertson (Missouri below Fort Peck)

Habitat map analysis has been completed for the Intake site, for discharges ranging from 1,000 cfs to 100,000 cfs. Habitat classes were defined by combinations of depths and velocities.

We used the FRAGSTATS spatial analysis program to generate 40 class-level and 46 landscape-level habitat metrics. Many of these measures contained redundant information, however, so we have reported only eight class statistics and six landscape statistics in our summary. These statistics and their definitions are as follow:

Class Statistics

Class Area – the sum of the areas of all patches of each class type, reported in hectares.
Percent Area – total class area divided by total landscape area (sum of all patches) expressed as percentage.
Patch Density – the average number of patches of each class type, per hectare of landscape area.
Mean Patch Size – the average area of patches in each class type, expressed in hectares.
Edge Density – the average perimeter of patches in each class type, per hectare of landscape area.
Mean Shape Index – the average ratio of patch perimeter to the square root of patch area. The larger the ratio, the more irregular the patch. This can be an important metric for edge-loving species.
Core Area – the sum of areas of all patches greater than 2.0 m diameter of each class type, in hectares. This metric is similar to class area, but does not count small patches in the sum.
Patch Interspersion Index – a measure of the degree of fragmentation of patches in each class type. Larger values indicate a higher degree of dispersion of a class type among other classes, i.e., higher fragmentation of the landscape.

Landscape Statistics

Shannon diversity – a measure of landscape composition, approximating the probability that two patches selected at random will be different types. A high value for Shannon diversity would represent a landscape containing all potential patch types, all of which are about the same size. Diversity indexes do not account for interspersion or other measures of landscape configuration.
Patch richness – the total number of class types represented in the landscape at a particular discharge. Richness is not affected by the relative abundance of patch types in the landscape.
Richness density – standardizes richness to a per area (of landscape) basis that facilitates comparison among landscapes of different size.
Shannon evenness – a measure of habitat diversity compared to maximum possible diversity. Evenness equals zero when there is only one patch type, and 1 when the distribution of area among patch types is perfectly even (proportional abundances are the same).
Contagion – measures the extent to which patch types are aggregated or clumped. Higher values may indicate landscapes with larger, contiguous patches, whereas low values generally characterize landscapes with smaller, more highly dispersed patches.
Interspersion/juxtaposition – an index of patch adjacency

throughout the landscape. The interspersion index measures the extent to which patch types are interspersed, but not necessarily dispersed. Higher values result in landscapes where patch types are equally adjacent to each other, whereas low values indicate a disproportionate distribution of patch adjacencies. In other words, the higher the score, the more random the adjacency of one patch type among all other patch types. This may be a measure of heteroscadasticity, the tendency to patch types to align longitudinally and grade consistently into other patch types (a natural characteristic of river habitats).

Over the next few months, our first priority will be to complete the map analysis for the remainder of the Yellowstone sites. We will then complete hydraulic simulations and maps for the Missouri River sites. We expect to write a draft journal article comparing the seasonal availability of class types in both rivers sometime this winter.

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Summary of Pallid Sturgeon Work—Southern Illinois University

During November 1995 through the fall of 1999, we implanted 26 pallid sturgeon with sonic transmitters, returned them to their capture points in the middle Mississippi River, and monitored their movements and locations. Study fish were located approximately 200 times. Pallid sturgeon were most often in the main channel (MCL) (43 percent) and main channel border (MCB) (33 percent). They were also frequently (15 percent) between wing dams (WDB). This habitat use pattern was consistent through much of the year, with the exception of spring. Pallid sturgeon appeared to seek lower velocity areas during spring flooding. They showed positive selection (Strauss's Linear Selectivity Index; L_i) for the MCB, downstream of island tips (ITD), the WDB, and wing-dam tip habitats. They showed negative selection for MCL, downstream of wing dams, and upstream of wing dams habitats. Comparison of L_i values for 4 temperature ranges and 3 daily mean discharge ranges revealed little change in habitat selection. Observed home ranges were 1.0 km (0.6 mi) to 97.0 km (60.3 mi), with an average of 34.1 km (21.2 mi). Pallid sturgeon tended to move upstream during the months of July through October, slowly downstream during the months of December through March, and had variable movements from March through July. Pallid sturgeon were active day and night, although movement was greater during the day than at night at 11-12°C (GLM, $F = 14.298$, $P < 0.0001$, $df = 44$).

We constructed two indices for identifying pallid sturgeon by applying multiple regression analysis to morphometric and meristic data from Carlson and Pflieger (1981). Consistency of identifications made by the two indices was examined using Discriminate Functions Analysis (DFA) on 257 *Scaphirhynchus* from throughout the pallid sturgeon range. Specimens identified as pallid sturgeon grouped distinctly from shovelnose sturgeon, and hybrids were intermediate. The majority (89.7 percent to 90 percent) of specimens identified as pallid sturgeon by the indices were classified as such by DFA. This morphological evidence supports the status quo of pallid sturgeon and shovelnose sturgeon as distinct species with some hybridization (Wills et al. *in press;* American Fisheries Society Special Publication). Our concurrent development of genetic methods that discriminate among *Scaphirhynchus* indicate these indices work well. A guide for use and interpretation of the indices is now available (Sheehan et al. 1999) from the Fisheries and Illinois Aquaculture Center, SIUC. Low levels of genetic variation at traditional molecular markers have hampered genetic research within the family Acipenseridae. In an effort to develop a large set of polymorphic genetic markers, a total of 172 clones were sequenced from three shovelnose sturgeon (*Scaphirhynchus platorynchus*) sub-genomic libraries enriched for two di- and one tetranucleotide microsatellite motifs (CA, GA, and TAGA) by researchers at SIUC and the University of Califorinia-Davis. Primers were designed for 113 of the sequences and tested against shovelnose sturgeon, pallid sturgeon (*S. albus*), white sturgeon (*Acipenser transmontanus*), lake sturgeon (*A. fulvescens*), and green sturgeon (*A. medirostris*). Of the 113 primer sets tested, 96 percent amplified in one or more species (58 dimeric and 50 tetrameric). In *Scaphirhynchus*, 93 percent of all loci amplified and 76 percent were polymorphic. Within the individual *Acipenser* species, 65-80 percent of loci amplified with 42-58 percent being polymorphic. Polymorphic systems for *Scaphirhynchus* predominately

displayed simple, disomic banding patterns, while those for *Acipenser* typically displayed banding patterns characteristic of tetraploid or higher polyploid levels. These new microsatellite loci provide a group of genetic markers that are detectable with non-invasive sampling and should prove useful in the preservation of threatened and endangered sturgeon species worldwide (McQuown et al. *in press*; Transactions of the American Fisheries Society). Initial investigations using some (20 percent) of these newly developed genetic markers suggest the following: 1) the pallid sturgeon is a good species; 2) population structure is apparent in both the pallid sturgeon and shovelnose sturgeon, based on specimens collected throughout the pallid sturgeon range; and, 3) interspecific *Scaphirhynchus* hybridization appears to be occurring.

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Scaphirhynchus Update

Since January 20, 2000, the USFWS Forensic Laboratory has been working on developing new diagnostic markers to distinguish between shovelnose, pallid, and Alabama sturgeon. With this purpose, four mitochondrial markers, eleven microsatellite markers, and eight nuclear markers were initially identified for examination. All 23 markers have been subjected to the majority of the methods described below, although work is still ongoing.

Methods

For the majority of the markers, PCR primers were designed based upon literature research. Because almost all of these primers were not originally designed for use on sturgeon, the initial step for each primer set was to alter PCR conditions to achieve optimal amplification. In many cases, primers produced multiple products of different sizes. In cases where manipulating PCR conditions could not diminish spurious bands, PCR products where separated by gel electrophoresis and physically cut out of the gel. The PCR product was then ligated into a plasmid vector and cloned using Invitrogen Original TA Cloning Kits. Multiple clones from each ligation were sequenced and verified by multi-species alignment.

Markers

Mitochondrial Markers

Primer sequences for three NADH dehydrogenase genes were designed by Cronin et al. (1993) for use on Chinook and Chum salmon. For ND-1 and ND-3/4, the cloned sequence could not be verified as ND-1 and no further work was done on these markers. ND-5/6 clones were verified and three new PCR primer sets were designed with M-13 sequencing tails for direct sequencing. Two of the three primer sets produced sequences without difficulty, the third is in need of primer redesign. So far, 10 pallid sturgeon and 50 shovelnose sturgeon have been sequenced using the first two ND-5/6 primer pairs, however the sequences do not identify any unique substitutions for pallid, shovelnose or Alabama sturgeon.

The fourth mitochondrial marker is a 496 base pair region of a highly variable region of the mitochondrial control region using primers designed by Campton et al. in 2000 for use on the *Scaphirhynchus* species. Five pallid, 73 shovelnose, and 3 Alabama sturgeon have been sequenced so far; the remaining 30 pallid and 64 shovelnose are currently being sequenced.

Microsatellite Markers

All eleven of these primer pairs were designed by May et al. in 1996. Conditions described by May et al. are designed to be analyzed using agarose gel electrophoresis. For our purposes, the conditions for some of these loci must be altered and/or primers redesigned to optimize conditions for use in the ABI 377 sequencer. Three of the eleven microsatellite markers have been cloned and sequenced, while the others are still in progress. New primers have been designed for these three loci, and sequencing of the database reference samples is in progress.

Nuclear Markers

One of the eight nuclear markers is known as ITS-1. This marker is the first internal transcribed spacer region of the ribosomal RNA gene cluster and was amplified using primers designed by Booton et al. (1999) for use on Cichlid fishes in Lake Victoria. The expected size range was 550 base pairs (bp), while observed PCR product size ranges were between 200-600 bp. These products were cloned, and the resulting sequences were positively identified as 18s rDNA

sequence. Further primer design is in the works for large-scale databank sequencing in *Scaphirhynchus.*

C-*mos* is another nuclear marker that we have been working on. C-*mos* is a proto-oncogene that encodes a serine/threonine kinase expressed in germ cells. Amplification was just recently optimized using primers described by Saint et al. in 1998, and cloning and subsequence sequencing is in progress.

The Von Willebrand Factor gene codes for a plasma glycoprotein that plays two major roles in haemostasis. We used primers described in Huchon et al. in 1999. The expected size is 1300 bp and the resulting amplification from shovelnose and pallid sturgeon produced multiple products ranging in size from 250 bp to 100 bp. Products were cloned into plasmid vectors; however, the resulting sequences failed to be positively identified as vWF.

A very promising gene is the Major Histocompatibility Complex E²2 gene, class II. Primers were designed by S. Fain based upon bovine, mouse and human sequences. The expected size is approximately 1,000 bp, and resulting amplification from pallid and shovelnose sturgeon was approximately 700 bp. The sequences from these clones are currently being analyzed.

P53 primers designed by Park et al. in 1996 amplified products in the size range between 75 bp to 350 bp, while expected length is 1082 bp. Although clones of these products could not be varied as P53 products, primers were designed for two clones that produced fragments of 170 bp and 280 bp that appeared to be promising markers. Ten pallid and 40 shovelnose individuals were sequenced, however these markers failed to provide variants.

Primers described by Oakely and Phillips in 1999 for Growth Hormone 1-Intron C are currently in the process of being optimized, while primers described by Colgan in 1999 for Phosphoglycerate Kinase (PGK) have given poor and inconsistent amplification products.

The eighth nuclear marker is Calmodulin gene (CaM-1) intron 3. Primers described by Côrte-Real et al. in 1994 have an expected length of 500 bp in *Mus musulus.* Amplification resulted in a fragment of approximately 120 bp in *Scaphirhynchus* and was verified as calmodulin genes, the intron of which is only 75 bp in length. Sequence primers have been designed for *Scaphirhynchus,* although there appears to be an extremely rich GC region in the intron that seems to be interfering with direct sequencing. Solutions to this problem are currently in progress.

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Evaluation of Thyroxine Content in Egg and Larval Pallid Sturgeon (*Scaphirhynchus albus*) as a Potential Indicator of Imprint Timing. (Draft abstract)

Thyroid hormone peaks are indicators of critical periods for metamorphosis and imprinting in a variety of vertebrates. Whole body thyroxine [T_4] concentrations were measured in pallid sturgeon, *Scaphirhynchus albus* (Forbes and Richardson 1905), to indicate potential periods for imprinting. A total of 600 F_1 progeny derived from one female and three male parents collected near the confluence of the Missouri and Yellowstone Rivers, Montana, were sampled. Eggs were incubated and larvae reared at Gavins Point National Fish Hatchery in Yankton, South Dakota. Eggs and larvae were collected at daily intervals from day 0-17 post-fertilization and 3-4 day intervals thereafter until day 76 post-fertilization. Mean whole body T_4 content of eggs between days 0 and 10 post-fertilization ranged from 1.1 to 2.1 ng/g body weight and peaked on the day of hatch (day 11 post-fertilization) at 3.2 ng/g body weight. After receding to 1.5 ng/g body weight on day 15 post-fertilization, T_4 content peaked a second time at 4.8 ng/g body weight on day 17 post-fertilization (day 6 post-hatch). This coincided with the period of yolk reabsorption. After receding to 3.0 ng/g body weight by day 30 post-fertilization, T_4 content peaked a third time between days 34 and 44 post-fertilization (day 23-33 post-hatch) at 6.0-6.7 ng/g body weight. This period coincided with the developmental transition from protolarvae to metalarvae life stage. Thereafter, T_4 content steadily declined until day 76 post-fertilization, when it was 0.7 ng/g body weight. The first T_4 peak

occurred when the fish rapidly lost body weight at the time of hatch. The peak probably does not represent a thyroid surge so much as shedding of fluids and egg coat membrane that did not contain T_4. Thyroxine was selectively retained because it was bound to target cells that were not shed. This T_4 was probably maternal T_4, already present in the egg before fertilization occurred. Maternal T_4 content of unfertilized eggs was 2.7 ng/g body weight. This maternal T_4 appeared to fluctuate, first becoming reduced in concentration as the egg gained weight by becoming water hardened; then increasing in concentration as the hatchlings lost weight at the time of hatch. The latter peaks represented the time when the larval thyroid gland became functional and began producing T_4. These data suggest that if pallid sturgeon experience thyroxine mediated imprinting to sites where their parent's spawned, they likely do so before the protolarvae/ metalarvae transition, because all the T_4 peaks occurred prior to or during this transition. Larval pallid sturgeon begin drifting passively downstream on water currents soon after hatching and begin to stop drifting between days 8-13 post-hatch. Depending upon the water current velocity, the larvae may be carried 40 to 400 miles downstream from the spawning site during this time. The timing of the first T_4 peak would potentially allow spawning site imprinting to occur before larvae begin to drift downstream into nursery areas. The second T_4 peak may also allow spawning site imprinting to take place if some (small) percentage of the hatchlings remain in contact with the bottom for several days before starting to drift.

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Missouri River Fish and Wildlife Assistance Office Activities

A pallid sturgeon recovery team meeting was held this August in St. Louis, Missouri, in conjunction with the National AFS meeting. The primary discussion is the process of updating the Recovery Plan for the pallid sturgeon. Since it was finalized in 1993, considerable information has been learned about this species. Enough that an update on the life history is warranted and needed. We'll attempt to have a draft available for the team sometime in January.

This office was again involved with collecting broodstock pallid sturgeon for propagation efforts this past spring at Garrison Dam NFH. Due to virus concerns, quarantine limitations and space limitations, it was decided in the fall of 1999, that propagation attempts would not take place at Gavins Point NFH in 2000. The facility had 2 year classes that had not been stocked out and had never tested positive for the virus and the Upper Basin Pallid Sturgeon Recovery Workgroup decided that it would be prudent to hold off until further information was obtained on this virus (Missouri River Sturgeon Iridovirus, MRSIV). Since pallid sturgeon progeny that had been cultured at Garrison Dam NFH in 1999 hadn't tested positive and had not had any connection with previous outbreaks, these would be held over winter for potential stocking in 2000. Unfortunately, following propagation efforts this last spring, two families began exhibiting symptoms of the virus and were subsequently tested positive for the virus. This put the remaining families from 1999, as well as the progeny from 2000, under quarantine and postponed any stocking effort. Meetings with pallid sturgeon researchers and the fish health and fishery personnel from Montana, North and South Dakota resulted in a decision to destroy the remaining 1999 progeny and continue holding the negative testing progeny from 2000 until additional research results were presented. The difficultly lies in that the virus does cause mortality, however, if left to run its course, survival is possible and likely and currently fish health policies do not adequately address these circumstances, especially with warm water diseases. The meetings did result in a decision that the adults used in the propagation were returned to the wild following testing and the progeny from Gavins Point NFH ('98 and '99) were stocked into the Missouri and Yellowstone Rivers below Fort Peck Dam.

The Service and the Corps have initiated a study that will identify post and pre-spawn behavior of pallid sturgeon broodstock, as well as hopefully identify spawning behavior and habitats. Over the next few years, adult pallid sturgeon of known sex and stage will be implanted with sonic/radio transmitters. This year, two females and eight males were implanted, released, and subsequently relocated before winter. These fish are located in the Yellowstone Confluence area above Lake Sakakawea, ND. For additional information on this project, contact Wade King,

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Endangered Species Act Consultation on Missouri River Operations

The Fish and Wildlife Service and the Corps of Engineers have completed section 7 consultation on the Corps of Engineers' Missouri River operations. These operations include the operation of six main stem dams and reservoirs, the Bank Stabilization and Navigation Project, and the Kansas River projects.

The endangered pallid sturgeon, the endangered least tern, and the threatened piping plover are the primary species covered by the consultation.

The Service's biological opinion finds that the Corps' current operations are likely to jeopardize the continued existence of the pallid sturgeon, least tern, and piping plover primarily due to modifications to habitat and modifications to the flows, temperature regime, and water quality of the system. The biological opinion includes a Reasonable and Prudent Alternative that the Service believes would avoid jeopardizing the listed species.

Flow modifications below Gavins Point – higher spring flows are needed to provide spawning cues to the sturgeon, to build and maintain sandbars for tern and plover nesting, and to reconnect floodplain habitats to the main channel; lower summer flows are needed to provide secure sandbar habitats for the birds and shallow water habitat for juvenile sturgeon.

Flow modifications below Gavins will not be implemented before 2003, because they will involve changes to the Missouri River Master Water Control Manual. The Corps will prepare an Environmental Impact Statement on these and other proposed revisions to the Master Manual.

Flow enhancement below Fort Peck – will provide spawning cues for pallid sturgeon and improve the temperature regime to increase the amount of warmwater habitat.

Unbalanced intrasystem regulation – alternating the distribution of stored water among the upper three reservoirs will increase the availability of tern and plover habitats within the reservoir reaches.

Habitat restoration – goal is to restore, enhance, or conserve 20-30 acres of in-channel shallow water habitat per stream mile.

Pallid sturgeon propagation and augmentation – ensure genetic integrity and prevent extinction of existing pallid sturgeon populations while other aspects of the RPA are developed and implemented.

Adaptive management – included to address areas of scientific uncertainty. The adaptive management approach, coupled with environmental monitoring and assessment, will allow for efficient modification of management actions in response to new information and changing environmental conditions.

The Corps has committed to developing a plan for implementing the components of the Reasonable and Prudent Alternative. The Corps' plan will be circulated for public comment in draft during the December/January time frame. The Corps' plan will be completed by February 2001. The Fish and Wildlife Service will continue to work with the Corps on implementation of that plan.

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Endangered Species Act Consultation on Mississippi River

The U.S. Fish and Wildlife Service (Service) and the U.S. Army Corps of Engineers (Corps) have completed a process under the Endangered Species Act known as formal Section 7 consultation to address threats to endangered species resulting from operation and maintenance of the 9-foot navigation channel and its associated locks and dams over the next 50 years.

The stretch of river covered by the biological opinion extends from Minneapolis, Minn., to Cairo, Ill. Within that area, the impacts of operating the lock and dam system, channel maintenance dredging and material disposal, and construction/maintenance of channel training structures on several endangered and threatened species were examined. This included the endangered pallid sturgeon.

The Service concluded that the operation and maintenance of the 9-foot navigation channel is likely to jeopardize the continued existence of the pallid sturgeon. The pallid sturgeon is jeopardized

because of the expected continued degradation of its habitat. The reasonable and prudent alternative for the pallid sturgeon includes: implementation of a long-term habitat/life history study and habitat restoration in the Middle Mississippi River.

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Genetic Distinction of Pallid, Shovelnose, and Alabama Sturgeon: Emerging Species and the U.S. Endangered Species Act (Abstract)

The sturgeon genus *Scaphirhynchus* consists of three recognized species. Pallid and shovelnose sturgeon (*S. albus* and *S. platorynchus*, respectively) are sympatric in the Missouri and lower Mississippi Rivers of the central United States. The Alabama sturgeon (*S. suttkusi*) is endemic to the nearby Mobile River drainage and is isolated geographically from the other two species. Pallid sturgeon and the extremely rare Alabama sturgeon are listed as endangered under the U.S. Endangered Species Act (ESA). In contrast, shovelnose sturgeon are relatively common and are not listed. Despite these taxonomies and morphological evidence, some biologists have questioned the genetic and taxonomic distinctions of the three species, thus raising doubts concerning the validity of protecting pallid and Alabama sturgeon under the ESA. To investigate these questions, we compared a 436 base-pair sequence of the mitochondrial DNA (mtDNA) control region among the three species. We observed 16 mtDNA haplotypes defined by 27 single base-pair substitutions (transitions) and one single base-pair insertion/deletion (indel) among 78 individuals examined. The maximum sequence divergence among those haplotypes (2.06 percent) was less than values usually observed between fish species. However, Alabama sturgeon (n = 3) were distinguished from the other two taxa (n = 75) by a unique base-pair substitution and haplotype, and pallid and shovelnose sturgeon at their northern range of natural sympatry (upper Missouri River) did not share any haplotypes. On the other hand, only frequency differences among shared haplotypes distinguished ($P < 0.01$) pallid and shovelnose sturgeon at their southern range of natural sympatry (Atchafalaya River), and genetic distances between northern and southern localities for each species were nearly as large as the distances between species. These latter results are consistent with several hypotheses, including reports (based on morphology) of putative natural hybrids in the Atchafalaya River, but not in the upper Missouri River. Overall, these mtDNA results indicate significant reproductive isolation between pallid and shovelnose sturgeon in areas of natural sympatry, and recent evolutionary divergence of Alabama sturgeon. These mtDNA results provide the first molecular genetic evidence for distinguishing the three *Scaphirhynchus* species and, coupled with morphological and biogeographic data, indicate that pallid and Alabama sturgeon should be evaluated as distinct species under the ESA.

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If you've ever had a chance to look into the eyes of a sturgeon, there are unfathomable depths there that take you back millennia; they take you back ages and ages ago. And having looked into the eyes of a sturgeon, you can fully understand that these animals swam practically unchanged from the way they are today when dinosaurs walked the earth.

Christopher Letts, Hudson River Educator

What I do you cannot do; but what you do, I cannot do. The needs are great, and none of us, including me, ever do great things. But we can all do small things, with great love, and together we can do something wonderful.

Mother Theresa

...conflict is the primary engine of creativity and innovation. People don't learn by staring into a mirror; people learn by encountering difference.

Ronald Heifetz

Note to Readers

This issue is also available on the following website under pallid sturgeon activities:

www.r6.fws.gov/moriver