

# PALLID STURGEON RECOVERY UPDATE

*- the latest research and management actions for recovery -*



## U.S. BUREAU OF RECLAMATION MONTANA AREA OFFICE PALLID STURGEON UPDATE

Montana Area Office (MTAO) of the U.S. Bureau of Reclamation entered formal consultation on the pallid sturgeon under Section 7 of the Endangered Species Act (ESA) concerning Intake Diversion Dam of the Lower Yellowstone Project on the Yellowstone River near Glendive, MT. Initially the consultation was for the current operations of the project. As studies progressed, two major issues emerged. First, the Diversion Dam may adversely affect pallid sturgeon by blocking migration to historical spawning habitat in the Yellowstone River. Second, large numbers of fish were found to be entrained into the main canal. MTAO decided to amend the consultation to include proposed modifications to provide passage and prevent entrainment. Concept designs for passage and entrainment prevention were developed in 1999 and 2000.

MTAO submitted the amended Biological Assessment, including

proposed modifications for fish passage and entrainment prevention, on August 3, 2001. The Fish and Wildlife Service (Service) began preparation of the Biological Opinion, and was scheduled to complete the consultation by mid-December. However, new information on sturgeon swimming ability will soon be available that will be essential to designing a fish passage structure. MTAO and the Service have agreed to suspend the consultation so this information can be incorporated by reference before completing the process.

During informal consultation, there were some concerns raised about designing a fish passage that would be used by large benthic species such as sturgeon. The U.S. Army Corps of Engineers (Corps) became involved at Intake through a budget appropriation, and have used that funding to develop more alternatives, run hydrologic modeling, and to sponsor the study to determine sturgeon swimming capabilities in relation to fish passage structures. Reclamation's Technical Service Center in Denver, CO, was awarded the study to look at parameters such as depth, substrate, velocity, and turbulence on the sturgeon's ability to move upstream. It was decided that wild shovelnose sturgeon from the Yellowstone River would be used as a surrogate species for pallid sturgeon in this study. The sturgeon swim meet has been completed, and preliminary results are encouraging that a fish passage structure can be designed that will be useful to sturgeon. They were able to move upstream through a

range of velocities up to 7 ft./sec. in laminar flows, but buffering the velocity with turbulence seemed to cause difficulties. Fish were also tested in prototypes of three different fishways including single, vertical slot fishway, dual vertical slot fishway, and rock fishway with boulder constrictions. This report should be available early in 2002.

Upon completion of that study, MTAO plans to conduct a "Value Planning Study," which will be completed in early 2002. This study will assemble experts in the project, engineering, and sturgeon biology to examine fish passage and entrainment prevention concepts and develop a recommended alternative for implementation. Construction timeline will depend upon availability of funding.

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## U.S. ARMY CORPS OF ENGINEERS UPDATE

The 2000 Missouri River Biological Opinion included the pallid sturgeon; the first formal consultation on the Missouri to include this species since it was listed in 1990. The Corps of Engineers has begun efforts to implement a Reasonable and Prudent Alternative that identified pallid sturgeon propagation/

augmentation and population assessment as the cornerstones for pallid recovery. We intend to develop a propagation plan with several Missouri River Basin state and Federal fish hatcheries to meet stocking goals annually. A comprehensive monitoring and evaluation plan is being prepared which will guide a standardized monitoring program for assessment of pallid sturgeon population status and trends throughout the Basin. Our intent is to partner with state and Federal agencies and academic institutions throughout the Missouri River Basin in the development and subsequent implementation of this plan. Several agencies are currently conducting population assessment activities on the Missouri River following guidelines established in the "Pallid Sturgeon Population and Habitat Monitoring Plan for the Missouri and Kansas Rivers" (Draft 2001) for sampling from Ft. Randall Dam to St. Louis, including the Kansas River. In 2002, with assistance from the Upper and Middle Basin Pallid Sturgeon Workgroups, this plan will be expanded to include the Missouri River from Ft. Peck Dam (Montana) to St. Louis, Missouri. The Corps of Engineers is committed to seeking new opportunities for pallid sturgeon recovery and population sustainability on the Missouri River.

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### **IMPLEMENTATION OF THE NOVEMBER 2000 MISSOURI RIVER OPERATIONS BIOLOGICAL OPINION AND STATUS OF THE MISSOURI RIVER MASTER MANUAL STUDY**

The completion of a Missouri River Biological Opinion (November 2000) and the pending selection of a preferred alternative for the Missouri River Master Manual represents a major milestone for change in management of the Missouri River system. The Service issued a jeopardy opinion on the endangered least tern, threatened piping plover, and endangered pallid sturgeon in November 2000 and a Reasonable and Prudent Alternative (RPA) to avoid jeopardy. The RPA included a Gavins Point spring rise and summer lows, Fort Peck flows, unbalanced intrasystem regulation, adaptive management, habitat restoration, and a monitoring and assessment program. In theory, the biological opinion provided the Corps with a road map to develop a Master Manual alternative that would likely avoid jeopardy.

To respond to the biological opinion, the Corps initially developed a draft "Implementation Plan" in early 2001. That plan was not finalized. The Corps decided that reference to a specific Gavins Point flow in the Plan would be premature in light of the ongoing Master Manual NEPA process. In lieu of the Plan, the Corps sent a letter to the Service in October 2001 documenting their current plan to respond to the biological opinion. In November 2001, the Corps and Service met and agreed that the Corps is currently making sufficient progress in meeting the milestones identified in the

biological opinion to implement elements of the RPA.

In August 2001, the Corps released the Master Manual RDEIS, but did not identify a preferred alternative. Four Gavins Point alternatives encompassing the range of the Gavins Point flow recommendation in the biological opinion were included in the RDEIS. The public comment period closes on February 28, 2002, and a Final EIS with a preferred alternative is scheduled for May 2002. Over the next few months, the Service will continue to provide assistance to the Corps and official comments on the RDEIS to help identify a Master Manual preferred alternative for the Gavins Point flow regime that likely will result in a non-jeopardy opinion.

In September 2001, 6 of 8 basin states' governors, led by ND Governor Hoeven, sent a letter to the President supporting the Missouri River Basin Association's compromise drought conservation plan to benefit upper basin reservoirs, several measures to help threatened and endangered species, and implementation of a change in river management without further delay. The Corps' Gavins Point flow alternatives provide varying degrees of benefits to endangered species, as well as providing drought conservation measures, higher reservoir levels and greater recreational benefits.

In January 2002, the National Academy of Sciences' National Research Council issued a report on the status of the Missouri River ecosystem which affirmed the direction of the Service's Missouri River Biological Opinion and calls for reestablishment of some degree

of natural hydrological processes. This report also generally echoes the position of river groups like the Missouri River Natural Resources Committee, and environmental groups like American Rivers.

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### LOWER MISSOURI AND YELLOWSTONE RIVERS PALLID STURGEON STUDY 2001 FIELD UPDATE

This past April we again combined efforts with the Service to capture adult pallid sturgeon for the hatchery augmentation program. Low flows (between 5,000 and 5,700 cfs) in both the Missouri and Yellowstone Rivers provided optimal netting conditions at the confluence area. Our crew captured 11 pallids in 24 drifts 6 of these were 'new' fish, the other 5 were recaptures. Two females and four males were transported to the state hatchery at Miles City and successfully spawned. Provided these fish stay healthy, they will be stocked back into the Missouri and Yellowstone Rivers during 2002 as yearlings. One of the highlights of the spring sampling was the capturing of a 1,092-mm FL, 5.3-kg sturgeon exhibiting all of the phenotypic characteristics of a pallid. However, the mid-range character index value calculated for this individual was indicative of a hybrid.

Summer field efforts focused on collecting additional baseline information on fish populations, water temperature, and physical

habitat at seven standardized sites on the Missouri River, located from immediately below Fort Peck Dam to Poplar. This data will complement information collected during 2000 and will be used to help evaluate proposed flow modifications from Fort Peck Reservoir as outlined in the Missouri River Biological Opinion. Each of the sites were sampled six times from May through August, with seven different gear types targeting all life stages of both native and introduced fish species. General results thus far indicate that the number of species and individuals sampled in the upper reaches near the dam was low, but increased downriver as water temperature warmed. A single hatchery-reared juvenile (HRJ) pallid sturgeon was captured in a drifted trammel net at the Wolf Point station in July. This is the first pallid we have sampled in our standardized sampling efforts. Considering the current elevation of Fort Peck and continuing drought, it is very unlikely proposed flow modifications will be implemented in 2003.

Our crew dedicated considerable time during May and August / September to follow-up on the HRJ pallids released into Recovery Priority Area 2 in 1998 and 2000. A total of 780 and 680 were stocked in 1998 and 2000, respectively. Sampling efforts during May were concentrated in the lower Yellowstone River, the confluence of the Yellowstone and Missouri Rivers, and the Missouri River above and below the confluence. A total of 62 drifts were completed with 1-inch mesh trammel nets. No HRJ pallids were sampled. August sampling was completed at several sites on the Missouri River between Wolf Point and the confluence area. We were

unable to access the Yellowstone River because of very low flows. A total of 179 drifts were completed among the sites on the Missouri; a single HRJ pallid (1999 year class) stocked in 2000 was sampled at Wolf Point. Thus, a total of two HRJ pallids were sampled (both at Wolf Point) in our netting efforts during 2001. In addition, six adult pallids (including two 'new' fish) were captured in our efforts to sample HRJ pallids.

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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

### FORT PECK FLOW MODIFICATION BIOLOGICAL DATA COLLECTION PLAN

The Fort Peck Flow Modification Biological Data Collection Plan is a 4-year study (2001-2004) designed to evaluate the influence of proposed flow modifications from Fort Peck Dam on physical habitat and biological response of pallid sturgeon *Scaphirhynchus albus* and other native fishes in a

300-km reach of the Missouri River below Fort Peck Dam. Modified dam operations (i.e., water released over the spillway) are proposed to increase discharge and enhance water temperature during late May and June to provide spawning cues and enhance environmental conditions for pallid sturgeon and other native fishes. The Data Collection Plan is comprised of five components: 1) monitoring water temperature and turbidity at several locations downstream from Fort Peck Dam, 2) examining movements of pallid sturgeon that historically have been found immediately below the dam, 3) examining movements by paddlefish *Polyodon spathula*, blue suckers *Cycleptus elongatus*, and shovelnose sturgeon *Scaphirhynchus platyrhynchus*, 4) quantifying larval fish abundance, and 5) examining food habits of piscivorous fishes. The Data Collection Plan is funded by the U.S. Army Corps of Engineers and cooperatively implemented by the Montana Department of Fish, Wildlife, and Parks and the U.S. Geological Survey – Columbia Environmental Research Center.

Lack of adequate precipitation and low water levels in Fort Peck Reservoir during 2001 precluded release of water over the Fort Peck spillway. As a consequence, data collected during 2001 was representative of low flow conditions in the river. Continuous-recording water temperature loggers were deployed in late April and May and collected in November. Water temperature data will be used to examine spatial and temporal variations in thermal regimes resulting from modified discharge releases. No pallid sturgeon were collected in the Fort Peck tailwater or implanted with radio transmitters in 2001. During

September 2001, 19 paddlefish, 17 blue suckers, and 28 shovelnose sturgeon were implanted with radio and acoustic transmitters (minimum 3-year life span). Intensive tracking of movements via boat and fixed data logging stations will be used to examine the influence of modified discharge and temperature regimes on spawning movements and locations of paddlefish, blue suckers, and shovelnose sturgeon in subsequent years. Larval fish were sampled at 3-4 day intervals from late May through July 2001. Six larval fish sampling sites were distributed throughout the 300-km reach of the Missouri River below Fort Peck Dam and in the Yellowstone River. Piscivore sampling during June and July 2001 resulted in the collection of 86 goldeye *Hiodon alosoides*, 78 channel catfish *Ictalurus punctatus*, 59 northern pike *Esox lucius*, 46 sauger *Stizostedion canadense*, 33 walleye *Stizostedion vitreum*, 29 shovelnose sturgeon, 10 freshwater drum *Aplodinotus grunniens*, and 5 burbot *Lota lota*. During the course of 2001 field activities, three hatchery-raised juvenile pallid sturgeon released in 2000 were also sampled.

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#### **Pallid Sturgeon Recovery Workgroup Chairpersons**

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## PALLID STURGEON POST SPAWN TELEMETRY STUDY

This past year the Service initiated a long-term telemetry study to monitor post spawn migrational behavior, possibly identify spawning habitat, and recapture female pallids to help answer questions concerning reproductive physiology. Another important aspect of our study is to monitor pallid sturgeon response to natural spring flows from the Yellowstone River and the proposed test flows conducted by the US Army Corps of Engineers (Corps) on the Missouri River from Fort Peck Dam.

This past field season and first year of tracking pallids served as a pilot phase for our study. Our main emphasis was to evaluate telemetry equipment and its effectiveness, relocate as many fish as possible to help develop statistical estimates, and most importantly, start collecting baseline data before the next spawning events and the proposed test flows.

We intensively tracked 10 pallid sturgeon by boat in the months of April, May, and June; as well as continuing monitoring every third week throughout the summer into October. Five additional tagged, post spawn pallids were added to the study in the end of June to bring the total to 15 tagged fish ( 3 females and 12 males ). We also utilized three fixed data logging stations strategically placed within the study area to monitor movements 24 hours a day and to aid us in relocating fish by method of elimination of zones fish were not in.

Overall, pallid tracking went fairly well this past summer, relocating 14 of 15 pallids, although we did lose

a couple of fish up the Yellowstone for a few months. As a result, we will be installing another fixed data logging station in the Sidney, MT, area, which will cut the 72-mile study zone in half. Funds for the additional station have been provided by the Bureau of Reclamation, who also has a mutual interest in pallid movements concerning fish passage issues on the Yellowstone River in Montana.

We saw considerably more movement than anticipated, and actually two of the females ranked in the top three fish, for miles of movement this summer. Most movement was logged in the lower Missouri River below the confluence and up the Yellowstone River throughout spring and early summer. We only saw four fish (three females and one male) select the Upper Missouri River above the confluence, all of which were brief visits lasting for a day or two, and then they would settle back down in the lower Missouri.

Future plans for the 2002 field season include continuing the same tracking regime as last year, tagging another 10 to 20 fish, and most importantly, try to start answering questions about the pallid sturgeon reproductive behavior this spring.

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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, SD, where 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, SD (RM 865). The upper-middle site extends from RM 865 to near Verdel, NE (RM 856), and the lower-middle site extends from RM 856 to Running Water, SD (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

In 2000, 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, flow at 0.2 and 0.8 times water depth and at bottom, 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 failure.

## RESULTS

Twenty-two of the fifty juveniles and the six adults survived the tagging operation and holding period. Following the 2000 sampling, 16 of the 22 juveniles and 4 of the 6 adults have been relocated at least once. This year, three additional juveniles were located for the first time, increasing the total number of relocated juveniles to 19. However, only 2 of the 4 adults were found this year. The following data summarize what has been found to date.

### Habitat

In 2000, all fish were relocated in main channel habitats. This year, there were two fish locations recorded at the confluence of the Missouri and Niobrara Rivers, as well as a few locations documented in side-channel habitats. No fish have been located in the reservoir proper or the river marsh area immediately around Springfield, SD. In fact, the downstream most relocation was approximately 5 km

below Running Water, SD. It was our hopes that side-scan sonar data would be collected at fish locations this year. This data was to be collected by US Geological Survey staff from Columbia, MO. As of this writing, arrangements have not been made for this year, but will try to be scheduled during the 2002 sampling period. This data will be useful to help quantify bottom substrate profiles and help determine if these fish are selecting for certain habitats that cannot be identified from the surface.

### Movement

Like last year, most fish stayed within a few hundred meters of their original location during intensive tracking. However, seasonally the fish moved about substantially. During March and April, fish were mainly found in the middle section (sample zones 2 and 3) of the study area. By May, fish were only located in the upper section (sample zone 1 and the upper portion of zone 2) of the study area where they remained through June. Beginning in July, the fish appeared to disperse from the upper reaches and were found in all zones by early September. By late September, fish were only found in the middle section (sample zones 2 and 3) of the study area. This pattern of moving upstream in the spring and dispersing throughout the system in the fall suggests some type of migratory behavior. Because these fish are from the 1997 year class, it may be assumed that they are immature and not moving for spawning purposes. More likely, the movements were associated with flows from dam operations. This behavior will be statistically analyzed when the study is completed in 2002, at which time a

sufficient sample size should have been obtained for analysis.

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**PALLID STURGEON  
RECOVERY UPDATE  
GARRISON DAM NFH  
RIVERDALE, NORTH  
DAKOTA**

The year 2001 was a bittersweet year for pallid sturgeon recovery efforts at Garrison Dam NFH. Both the 1999 and 2000 year classes of pallid sturgeon spawned and cultured at Garrison Dam NFH were diagnosed positive for an iridovirus and were destroyed in the spring of 2001, prior to any stocking. The two year classes were made up of a total of 25 family lots and included genetics from fish above Fort Peck Reservoir, a first. Also represented in the 2000 year class were five families created from cryopreserved milt from the 1999 fish, the first ever cryopreserved pallid progeny. The diagnosis and destruction of these fish early in the spring of 2001 was particularly devastating to the recovery program in the upper basin.

In spite of the setbacks, the recovery effort was determined to move ahead. With the three Federal hatcheries in the upper basin now under quarantine, plans for 2001 were directed at isolating the source for the iridovirus. In an attempt to rule out the water supply or hatchery transfer of the pathogen, spawning in the Upper

Missouri River was accomplished on site. A 20-foot circular tank and temporary hatching battery was assembled on the banks of the Missouri River in the C.M. Russell National Wildlife Refuge, MT. Five males and a single female were captured and successfully spawned. Eggs were shipped to Bozeman FTC for isolation and Garrison Dam as a 'backup.' In addition to the spawning effort, milt from these five fish and an additional four captured at the confluence of the Missouri and Yellowstone Rivers in North Dakota was cryopreserved and is held in a repository at three sites. Progeny from a successful spawn at Miles City SFH are also being held at Garrison Dam NFH. Periodic inspections were made for the presence of the virus at all three facilities, but to date there has been no occurrence in the pallid sturgeon.

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**MONTANA FISH HEALTH  
LAB PALLID STURGEON  
WORK SUMMARY**

Due to the presence of the Missouri River sturgeon iridovirus in pallid sturgeon at Garrison Dam NFH, no pallid sturgeon were allowed stocked from Garrison Dam NFH into Montana waters in 2001. In an effort to develop an alternative plan which would allow stocking of pallid sturgeon in Montana, fishery biologists, researchers and hatchery staff from North Dakota, South Dakota and Montana captured and spawned adult pallid sturgeon from

above and below Fort Peck Reservoir in Montana. Adults collected downstream of Fort Peck were taken to Miles City SFH. Eggs were collected at Miles City and the fingerlings are currently being held there. Sturgeon collected above Fort Peck were spawned streamside and eggs were taken to the Bozeman Fish Technology Center, where they hatched and fingerlings are being reared. Fish collected from Montana spawning operations are scheduled to be stocked in Montana in the summer of 2002. Fish from Bozeman will be stocked back into the Missouri River above Fort Peck, where they originally came from. Pallid sturgeon from Bozeman may also be stocked into the Missouri River below Fort Peck Dam. Pallids from Miles City are scheduled to be stocked downstream of Fort Peck Reservoir. Final stocking plans, including stocking locations, will depend on results of virus testing at Bozeman and Miles City.

In addition to the pallid sturgeon collected, shovelnose sturgeon eggs were also collected and taken to Bozeman and Miles City. The primary purpose for the shovelnose sturgeon was to conduct a series of tests in an effort to determine if the virus is present in young shovelnose sturgeon from eggs collected in Montana. Fingerlings being reared at both locations are being tested periodically for the presence of the virus. All samples have been sent to the Service's Bozeman Fish Health Center. PCR samples will be sent to UC Davis or held at the Bozeman Fish Health Center for testing.

Histology and PCR samples were also collected from adult shovelnose and pallid sturgeon

from both spawning groups. Histology was sent to the Bozeman Fish Health Center. PCR analysis was conducted at UC Davis. No virus was detected in adult sturgeon collected above Fort Peck. However, several of the adult shovelnose taken to Miles City SFH did test by PCR (UC Davis) for the Missouri River sturgeon iridovirus. Further validation of the PCR testing is currently being conducted to confirm these test results. Results of virus testing of the sturgeon collected in Montana will be used to determine future stocking plans for pallid sturgeon in Montana. The purpose of this work is to help ensure that the virus is not spread upstream with stocking sturgeon into waters in which it does not already occur. Previous efforts to test wild sturgeon in Montana for presence of this virus have failed to detect it.

This work involves a considerable coordinated effort by many state and Federal fisheries scientists in Montana, North Dakota and South Dakota.

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### **BOZEMAN FISH HEALTH CENTER PALLID STURGEON UPDATE**

On January 16, 2001, 15 pallid sturgeon were sampled at the Gavins Point NFH. Two year classes were represented: 1998 and 1999. All fish were screened for the iridoviral agent using histology. Two fish from each year class were confirmed positive for the virus by

histology. A sturgeon quarantine was placed on the facility and all cultured year classes that were retained at the facility.

March 23, 2001, 60 YOY pallid sturgeon from the Garrison Dam NFH were screened for the iridovirus with histology. Thirteen tanks were confirmed positive for iridovirus. The hatchery sturgeon quarantine remained in place until all sturgeon were removed and the sturgeon facility disinfected. The Garrison Dam facility was restocked with YOY sturgeon in spring 2001 and will be considered suspect for iridovirus until 1 year is complete with two negative inspections. Thirty-five pallid sturgeon were monitored from August to November 2001 for viral pathogens. To date, all testing has been negative.

Pallid and shovelnose YOY sturgeon cultured at the Miles City SFH were periodically monitored for iridovirus beginning in July 2001. Parasite problems were observed, but the iridovirus was not detected.

#### **PCR Validation Testing:**

The Fish Health Laboratory at the University of California at Davis received adult pallid and shovelnose sturgeon PCR samples collected in June from free-ranging fish. A total of five bags were received. The testing was conducted with newly designed primers for the polymerase chain reaction (PCR) to detect DNA from iridoviruses as previously identified in juvenile pallid and shovelnose sturgeon reared at Service facilities. The PCR is designed to detect the major capsid protein of the iridoviruses and both a single round and nested assay have been developed with the idea that the nested test will detect even lower

concentrations of virus DNA missed by histology or the single round assay. The results are summarized below.

#### **Conclusions:**

The PCR examinations found only one group of samples positive for iridovirus: Adult shovelnose from Miles City collected June 4, 2001. This detection was made from adult spawning shovelnose that were transported to Miles City SFH and maintained for a limited time before returning to the river. Previously, detection of the iridovirus was only accomplished when there was a high prevalence of infection in cultured sturgeon. These results suggest the use of PCR for iridovirus screening may prove to be a highly sensitive and valuable diagnostic method.

In addition, 700 samples were collected on known positive captive pallid sturgeon for validation of the PCR technique with comparison to the histology diagnostic method. This testing is currently ongoing, with the PCR testing performed at UC Davis and the histology at the Bozeman Fish Health Center.

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### **2001 NEBRASKA FWS PALLID STURGEON ACTIVITIES**

Development of a recovery implementation program for pallid sturgeon, least tern, piping plover and whooping crane continues under the Platte River Cooperative Agreement. The bulk of program

activities (e.g., retiming flows, acquisition and restoration of riparian habitat) are expected to take place within the central Platte River reach (RM 156-252), well above the pallid sturgeon habitat area (generally below about RM 32). Pallid sturgeon program development efforts are focused on determining the effects, if any, of flow manipulation through the central Platte reach on the magnitude and timing of flow within the pallid sturgeon habitat area. Currently, it appears this effort will utilize methods similar to those employed by the Nebraska Department of Natural Resources in tracking river flows for administration of storage water rights. The program will also incorporate a monitoring and research component, currently being developed to provide information on pallid sturgeon use of the Platte River.

In late 2000, the U.S. Fish and Wildlife Service consulted with the Corps of Engineers on impacts to pallid sturgeon from the proposed Western Sarpy and Clear Creek levee improvement project located along a 15-mile reach of the lower Platte River. Through close work with the project proponents, conservation measures were incorporated into the project design so as to avoid adverse impacts to the species.

Funding has continued for Dr. Ed Peters at the University of Nebraska – Lincoln to monitor water quality parameters in the lower Platte River and major tributaries. This information should bolster his continuing pallid sturgeon and sturgeon chub research in the lower Platte River.

In 2002, Service contaminants staff will begin a health risk assessment

for pallid sturgeon on the lower Platte River using shovelnose sturgeon as a surrogate. The research will evaluate environmental contaminant exposure and effects to pallid sturgeon that occupy the lower Platte River. Shovelnose sturgeon will function as a surrogate to compare measurements of chemical exposure to measurements of health status. Chemical exposure analysis will include measurements of water quality parameters (e.g., salinity, pH, and selected major ions) and biochemical or residue analysis for trace elements, triazine and organochlorine pesticides, and polyhalogenated hydrocarbons in water, fish tissues, and sturgeon food items. Shovelnose sturgeon health will be assessed by integrating measurements of general health with hepatic and reproductive system biomarkers. Differences in pallid and shovelnose sturgeon dietary exposure to environmental contaminants will be a key factor in evaluating contaminant-related risk to pallid sturgeon.

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### **NEBRASKA GAME AND PARKS COMMISSION PALLID STURGEON UPDATE**

On March 28, 2001, the Nebraska Game and Parks Commission collected 24 shovelnose sturgeon for virus testing. The sturgeon were collected using experimental mesh gill nets set off of the ends of wing dikes just below and across from the mouth of the Platte River. A pallid sturgeon was collected

during this effort (fork length - 922 mm, weight 2,766 g, fish was checked for pit tags, but was not tagged).

In late June, we started a 2-year pallid sturgeon project funded by the Corps of Engineers. The objective of the study is to evaluate the use of the 2 meter beam trawl as a sampling methodology for sturgeon and related species in various habitats of the channelized and unchannelized Missouri River in Nebraska. In addition, we sampled wing dike habitat on the channelized river during the fall, with the standard sturgeon gill that was adopted for the lower Missouri River (200 feet long with alternating panels of 1½", 2", 3" and 4" mesh). Sampling with the beam trawl and the sturgeon gill nets will continue during 2002.

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### **COLUMBIA FISHERY RESOURCES OFFICE PALLID STURGEON ACTIVITIES**

Columbia Fishery Resources Office (CFRO) completed the final report for the MICRA multi-state 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 Service, Southern Illinois Univ., and the fish & game agencies of Nebraska, Iowa and Missouri. From November 1997 to April 2000, a

total of 6,540 fish of 41 species were collected in 1,033 nets and lines. Seven presumed wild origin pallid sturgeon and two recaptured hatchery fish were collected in the Lower Missouri River. Two wild origin pallid sturgeon and 11 hatchery origin pallid sturgeon were collected in the Middle Mississippi River.

Columbia ES Office initiated informal Section 7 consultation under the ESA with Missouri DOT for the scheduled replacement of the Route 19 bridge over the Missouri River. Concerns were raised that bridge replacement activities may affect pallid sturgeon habitat. MODOT provided funds for CFRO to conduct field surveys, analyze data and provide advice and recommendations to avoid and/or minimize potential impacts to pallid sturgeon. The USGS-Columbia Environmental Research Center (CERC) mapped physical habitat characteristics (substrate, bottom contours, water velocity) of the project site. CFRO will correlate pallid sturgeon presence/absence with CERC's habitat data. CFRO staff collected 3 adult pallid sturgeon and 14 pallid x shovelnose sturgeon hybrids in the project area. Final report included recommendations to minimize project impact to sturgeon.

Monitoring continued at Lisbon Bottoms, restoration unit of the Big Muddy National Fish and Wildlife Refuge. Fish and habitat data have been collected in the chute and the adjacent river for the past 5 years. The chute serves as a nursery area for many native fish species. Fish community sampling at Lisbon Bottoms (RM 213-219) began in April and continued through September. Eleven YOY sturgeon were collected this year, 5 were

identified as shovelnose sturgeon and 6 have yet to be identified. The first YOY sturgeon collected at Lisbon was September 13 at a temperature of 25.1 °C. Depths ranged from 0.91 m to 4.97 m, with bottom velocities ranging from 0.33 m/s to 1.33 m/s.

The Kansas City District of the Corps of Engineers is funding a pallid sturgeon population and habitat monitoring project on the lower Missouri River. Sturgeon will be sampled across five, 10-mile reaches from Waverly to St. Charles, MO. Gears used will include trawling, gill netting, and hoop netting. Over 75 trawl hauls were conducted from April to September. No adult pallid sturgeon were collected and several YOY sturgeon have yet to be identified. Gill netting began in December and will continue through the winter.

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### **PALLID STURGEON CONTAMINANTS INVESTIGATION IN THE MIDDLE MISSISSIPPI RIVER**

A study will be launched in 2002 to investigate reproductive problems and larvae health that may be related to persistent contaminant body burdens in Middle Mississippi River sturgeon. The investigation is a collaborative effort between the Mark Twain National Wildlife Refuge, Middle Mississippi River National Wildlife Refuge, U.S. Geological Survey's Columbia

Environmental Contaminants Center (CERC), U.S. Fish and Wildlife Service's Rock Island Ecological Services Field Office (RIFO) and others. A preliminary investigation indicated that shovelnose sturgeon from the Middle Mississippi River were stressed by exposure to contaminants. The evidence of stress included somatic indices, endocrine disruption, intersex characteristics and elevated contaminant concentrations. An ecological risk assessment was completed to help rank the contaminants of potential ecological concern (COPECs) and identify problem areas. Now a series of egg microinjection assays will be completed at CERC in an attempt to determine the low-observed-adverse-affects-level in sturgeon eggs contaminated with the selected COPECs. The selected COPECs for the Middle Mississippi River include chlordane, PCBs and lead. A human health sturgeon consumption advisory for chlordane has been in place for the Middle Mississippi River since the 1980's. We hope to use the results of this study to evaluate if the fish tissue contamination in the Middle Mississippi River limits the recovery of the pallid sturgeon. Also, we hope to integrate the contaminants threshold information into the ongoing Endangered Species Act consultation related to river management and river habitat rehabilitation projects.

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### **PALLID STURGEON SPERM CRYOPRESERVATION UPDATE**

Pallid sturgeon were collected in the Missouri River above Fort Peck Reservoir in Montana. Due to a limited number of males captured during the spawning season, no experiments were carried out focusing on improving the current cryopreservation procedure for pallid sturgeon sperm, however, sperm were cryopreserved for repository purposes. Sperm from five males were frozen using the current protocol and transferred to Warm Springs, GA. Ten 0.5-ml straws were frozen for male 1F4A4B5973. Twenty 0.5-ml straws were frozen for male 17509415139. Sixty 0.5-ml straws and three 5-ml straws were frozen for males 41476A0462, 17509312265, and 411D0E2C57. In December, the cryopreserved straws were redistributed among the three repositories holding pallid sturgeon sperm (Warm Springs Fish Technology Center, Garrison Dam National Fish Hatchery, and Gavins Point National Fish Hatchery). Over the last 2 years, sperm from 12 males have been cryopreserved and deposited in these repositories.

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### **STURGEON SURVEY IN THE LOWER MISSISSIPPI RIVER**

In September 2001, the U.S. Fish and Wildlife Service and the Mississippi Museum of Natural

Science, with the assistance of the Lower Mississippi River Conservation Commission, initiated trawl surveys for sturgeon in the lower Mississippi River. The trawl was selected because of the success of the Missouri Department of Conservation trawling studies in the Lower Mississippi River.

We are using the trawl to identify sturgeon habitat use over a range of river stages and conditions in the vicinity of Vicksburg, MS (MRM 437). From September through November, we made 177 pulls for a total of 29 trawl-hours. Pull time ranged from 5-25 minutes, with an average of about 10 minutes. During this time, river stage fluctuated from 3-15 feet. We collected 11 pallids (203 -785 mm FL), 383 shovelnose (58-725 mm FL), and 3 intermediates. River bottom conditions and capture locations have changed with river stage fluctuations of only a few feet. However, all sturgeon captures have been associated with moderate to strong currents, depth (13-45 ft), sand or sand and gravel substratum, and structure (sand reefs, dunes, secondary channels). Pallid captures seem to be associated with greater depths (25-45 ft). We also captured several very small shovelnose (58-66 mm FL) this fall, with the last (61 mm FL) taken on November 19, 2001.

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### **LOUISIANA PALLID STURGEON ACTIVITIES 2001**

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 for measuring, tissue removal, and tagging, many suitable broodstock candidates are returned to the NNFH for propagation purposes.

During the fall/winter/spring 2000/2001, a total of 83 pallids and 109 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 2001, there was no successful artificial spawning of pallid or hybrid sturgeons.

A new 4-year pallid sturgeon study is now being implemented at ORCS and will begin in the fall of 2001 and extend thru 2005. Cooperators in the Lower Basin Workgroup will focus on river

sturgeon abundance, genetic make-up, morphometrics, and disease issues. Sturgeon samples will be collected monthly during the cool months (Nov. - April) of each year of the study. The sturgeon are being transferred to NNFH for study and tissue collection. Thus far in the fall of 2001, a total of 74 sturgeon have been collected in three net nights of fishing. Study samples to date have revealed 11 pallids, 20 pallid x shovelnose hybrids, and a number of shovelnose sturgeon. The sturgeon are returned to ORCS immediately after samples are taken. Artificial spawning of pallids will again be attempted in the spring of 2002. Only those fish that are "certified" (high index scores) are being used in propagation and cryopreservation trials.

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### NATCHITOCHES NATIONAL FISH HATCHERY UPDATE

Two spawning efforts were made in FY 2000: 1) fall capture with temperature regulation regime until spring spawning; and 2) spring capture with no temperature regulation regimes.

**1) Fall capture w/temperature regulation regime.** Fifty sturgeon were captured in late October/early November 1999 and brought to the station. Biopsies and morphometric measurements were taken. Testis and egg samples were shipped to Dr. Joel Van Eenennaam at the University of California, Davis, for histology tests. Of the 50 captured, 36 were pallids (9 females; 27 males). Of the 36

pallids, results from UC Davis indicated 7 females with 70-77 percent migration and 7 males that appeared to be good spawning candidates. Three more males were selected, for a total of 7 females and 10 males kept on station for spring spawning; the remaining fish were released to the Old River Control Complex (ORCC). The 17 selected fish were kept in recirculating systems on a temperature regime to mimic riverine conditions at the ORCC. These fish were maintained throughout the winter; on 3/27/00, two females and four males were injected with LHRHa. No sperm and no eggs were released. Egg samples were taken on 3/29/00 and actually showed a decrease in the percent GV migration.

On 4/5/00, four females and six males were injected with LHRHa. No eggs were obtained, but three of the six males produced viable sperm.

#### 2) Spring capture with no temperature regulation regimes.

On 5/4-5/00, 18 sturgeon were captured at the ORCC. Morphometric measurements determined that 11 of the 18 were pallids (3 female; 8 males). On 5/7/00, 6 pallids (2 known females, 1 known male, 3 unknown sex) and 2 hybrids were injected with LHRHa. Fertilized eggs were produced by 2 female pallids and 1 male hybrid. Fry hatched 5/12/01 and all fry were dead within 24 hours.

Three separate "events" characterized the FY01 sturgeon efforts for Natchitoches NFH: 1) cryopreservation/fall induced spawning; 2) spawning for culture; and 3) spawning for research (acoustic signatures, oocyte hydration).

**1) Cryopreservation/fall induced spawning.** Ninety-one sturgeon were captured at the Old River Control Structure late October/early November 2000. These fish were obtained in attempt to induce thermal regulated spawning in the fall of the year so that sperm could be cryopreserved for spring use. If this were successful, sperm could be stored for the spring and used in conjunction with fresh sperm or in lieu of fresh sperm in the event that there were no productive males during the spring season. Testes samples (49) were sent to the University of California/Davis for analysis. The remaining 42 fish were returned to the ORCC. UC/Davis reported 7 good and 2 questionable candidate males. The 9 fish identified by UC/Davis were combined with 7 more fish selected by size for a total of 16 fish. These 16 fish were injected 1/16/01 to induce spermiation. A few samples contained presumptive spermatocytes, but no mature, motile sperm were observed.

**2) Spawning for culture.** On 4/18-20/01, 53 sturgeon were collected for spawning and culture. Of these 53 fish, 11 were identified as pallids (2 females, 9 males). On 4/30 and 5/1, 3 females and 3 males were injected with LHRHa. Two of the males produced motile sperm prior to injection; all 3 males were given injections. All 3 females produced eggs (300ml, 470ml, 405ml for a total of 1175 ml). All 3 males produced sperm. Eggs were fertilized and began hatching 5/8/01. All fry died by 5/12/01 and samples were sent to Bernie Kuhajda at the University of Alabama for genetics research.

3) (a) **Spawning for research - acoustic signatures.** On 5/12/01, 3 shovelnose females and 3 hybrid males were injected with LHRHa to stimulate reproductive behavior and male sturgeon vocalizations as part of an acoustic signature study by Dr. Carol Johnston, Auburn University. Males were injected with twice the normal priming dose. Preliminary analysis suggested that there is potential that sturgeon are producing acoustic signatures.

3) (b) **Spawning for research - oocyte hydration.** On 5/13, Dr. Nigel Finn, University of Norway, arrived to do an oocyte hydration study on primitive fishes. One theory relating to poor fry survival is that perhaps hormonal injections of females with immature follicles (ovarian development) forces some of them to ovulate eggs which are lacking proper hormonal receptors and/or yolk. Oocyte hydration relates to this and so, on 5/13, 4 females (2 HSN, 2 PXH) and 1 male (pallid) were injected with LHRHa in an attempt to obtain eggs to investigate the hydration biochemistry of eggs as they mature. The three hybrid males used on 5/12/01 for the acoustic study were also used. The three hybrid males produced sperm; the pallid did not. Three of the 4 females produced eggs (2 shovelnose, 1 hybrid) and all eggs were fertilized with sperm from 2 male hybrids injected on 5/12/01. The fish hatched 5/19-20, but all fry were dead by 6/28/01. Results are still being analyzed. Lipid analysis has not been completed to date. Results from the amino acid analyses have been presented ("Yolk Proteins & Free Amino Acids in *Acipenseriformes*." RN Fish, M Leuhardt, A Ferrara, P Cakic, JJ Isely, J Dean, MS Evjen & HJ Fyhn. Poster at Tenth European Ichthyology Congress

(ECI-X), Prague, 3-7 September 2001.)

Two studies are currently being done at the Natchitoches NFH: (1) gathering several pieces of information to form a comprehensive database, and (2) taking blood samples to look at fish health and DNA integrity.

1) **Old River Control Complex Study.** The hatchery began a 6-month study in November 2001 in an effort to obtain comprehensive database information on 500-1000 sturgeon captured monthly at the Old River Control Structure in Concordia Parish, LA. Data gathered will help: (1) determine population size through mark/recapture percentages; (2) define hybrid:shovelnose:pallid ratios; (3) document the presence of fish disease occurrence in the wild; (4) define genetic differentiation between pallids, shovelnose, and hybrids; (5) provide documentation of physical anomalies in the wild; (6) provide voucher identification for species differentiation; (7) allow stomach content analysis via gastric lavage; and (8) refine development of character indices through morphometric measuring. Once transferred to the hatchery, staff sample the fish as follows: morphometric measurements; fin clips for genetic D-loop sequencing and microsatellite loci markers; fin clips for Shovelnose Sturgeon Iridovirus (SSIV) and other fish disease diagnostics; voucher identification of each individual for genetics confirmation and anomaly identification; PIT tagging for mark/recapture data; and gastric lavage to obtain stomach contents to determine feeding habits. Wild fish are not held on the station for longer than 10 days. The hatchery is working in conjunction with the University of Alabama

(genetics/morphometrics); Bozeman Fish Technology Center (fish health); Louisiana Department of Wildlife and Fisheries (collection); the USCOE Waterways Experiment Station (anomaly documentation); and the US Corps of Engineers (collection at ORCC).

To date, 132 (3 recaptures) sturgeon have been captured and analyzed. The most significant information from the study to date has been the verification of sturgeon iridovirus on a shovelnose captured in the wild during the December sampling period. Gastric lavage has revealed some food items in the stomach of gill-netted sturgeon. The quantity of lavaged material increased in the January versus earlier samples. The samples have not been analyzed, but preliminary observations include detritus, bloodworms (Chironomidae larvae) and dragonfly (Odonata:Anisoptera) nymphs. Voucher ID pictures are being used to evaluate and refine character indices based on morphometric measurements, as well as to document several physical anomalies.

2) **Blood Cell Study.** The study of blood cells from sturgeon was begun in November in conjunction with the Old River Control Complex Study. The study of blood cells can yield baseline hematology data. Differential staining and flow cytometry are being used as bioindicators of animal health status and of potential habitat disturbance. The project includes analyzing no more than 20 samples per month for the months November 2001 - April 2002 (6 months). Using fish caught during ORCC sampling (see above) blood samples are

obtained using sodium heparin for differential blood smears. One Wright Giemsa stain and one neutrophil stain are then done per fish. Samples of blood into acid citrate dextrose are then used for flow cytometry. Products will include peer reviewed publication and technical reports including results on: a) numbers and types of differentiated blood cell types; b) trends or abnormalities noted; and, c) blood cell DNA integrity. Reports to be completed by September 1, 2002. Results to date have revealed at least five sturgeon (sample size 57) with aneuploidy, i.e. an abnormal amount of chromosomal content. Analyses of blood samples for differential cell counts are pending.

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**MISSOURI DEPARTMENT OF  
CONSERVATION  
FISHERIES RESEARCH,  
ASSESSMENT, AND  
MONITORING SECTION**

Open River Field Station (ORFS) staff collected 40 larval sturgeon this April and May using the Missouri benthic trawl in the lower Missouri, middle Mississippi, and lower Mississippi Rivers. Water temperatures when we began capturing larval sturgeon and paddlefish were similar to last year (~22° C). Unfortunately, we have not had time to identify the larval sturgeon to species. We captured numerous larval paddlefish (>200). We also captured larval sauger and centrarchids (the centrarchids

probably were waifs from much warmer tributaries).

We captured nearly all larval sturgeons and paddlefish at downstream island bar tips. We surmise that sturgeons are being spawned at island heads or somewhere above and are being transported to eddy pools along island shores and at the tips. Large amounts of detritus were taken with the larval fish. Larval sturgeons captured in spring 2001 were more uniform in length compared to spring 2000 fish, in which the fish were smaller from the northern sites and larger in the southern sites (increase in length going from the Missouri River south to Wolf Island, KY, in lower Mississippi River).

Larval sturgeons were also captured in the Missouri River in September 2001, suggesting that spawning occurred twice in the Missouri River or perhaps shovelnose sturgeons are pluriparous. This phenomenon has not been observed on the middle Mississippi River (MMR), however.

In early April, we captured adult shovelnose sturgeon that were milting. We were unable to capture adult pallid sturgeon in the spring. We know that adult shovelnose sturgeon can be captured near tributary mouths in spring. We wonder if sturgeons are moving up tributaries to spawn in the MMR. The largest piece of the pallid sturgeon puzzle in the MMR has yet to be solved: where are the adults staging and then spawning in the spring? We felt we were close in spring 2001. In early April, we attempted to trawl the Wolf Island side channel (near Columbus, KY) below a closing structure and notch. Prior information suggested this might be the place to look for

gravid female pallids. Fishing directly on top of the very spot we wished to trawl was a part-time commercial fisherman from Columbus, KY. The commercial fisherman was “knifing” sturgeon to observe eggs. He had a boat full of sturgeons, including what appeared to be a hybrid pallid and a huge shovelnose. The fish went back into the water, wounds and all. The commercial fisherman explained to us that he is just a resource user trying to make a few bucks and that he doesn’t harm sturgeon populations because “ he only harvests the mummies”.

Similar to last year, we did not catch many juvenile sturgeons either by trawling or through routine Long Term Resource Monitoring Program (LTRMP) sampling, suggesting that sturgeons are reproducing in the MMR, but perhaps not recruiting. We will be further investigating this hypothesis.

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**LOWER BASIN WORK  
GROUP FORMED**

The Lower Basin Work Group was formed in March 2001 and includes 39 administrators and/or biologists from the states of Missouri, Arkansas, Louisiana, Mississippi, Tennessee, and Kentucky; the U.S. Army Corps of Engineers New Orleans, Vicksburg, Memphis, and St. Louis Districts, as well as the Waterways Experiment Station;

the U.S. Fish and Wildlife Service's divisions of Fisheries and Ecological Services; and three universities (University of Alabama; Auburn University; St. Louis University). The group has established priorities and has developed a funding package reflecting needs for the lower basin. The funding package is currently being prepared for printing.

Compared to the northern portions of its range, there has been very little effort to identify numbers, distribution, condition, and management needs of pallid sturgeon or its habitat within the lower mainstem Mississippi and Atchafalaya Rivers. The Lower Basin Work Group priorities are shown below:

- determine distribution, demographics, and density of pallid sturgeon in the Lower Basin;
- determine the relation of pallid sturgeon to natural and man-made features of the river channel;
- develop better methods to differentiate shovelnose, pallids, and hybrids;
- determine the presence or lack of iridoviruses in wild-caught fish, and fish health in general;
- provide digital voucher specimen identification of all pallid sturgeon caught;
- determine best management sampling gear and techniques;
- identify habitat, especially that essential for spawning and recruitment; and
- develop cooperative partnerships to manage and recover pallid sturgeon and its habitats in the Lower Basin.

## NATIONAL FISH AND WILDLIFE FORENSIC LABORATORY

### Introduction

Since January 20, 2000, the Service Forensic Laboratory has been working on developing new diagnostic markers to distinguish between shovelnose, pallid and Alabama sturgeon. Since our last update in February, we have discontinued work on markers that did not appear useful in our efforts to distinguish between the three species, but have begun work on new markers, as well as continued work on markers that still show promise. Last September, we submitted a manuscript (still in review) entitled "Forensic DNA Markers in *Scaphirhynchus*" to the Journal of Applied Ichthyology.

### Mitochondrial

A 496-base pair region of a highly variable region of the mitochondrial control region primers described by Campton et al in 2000 has been sequenced in 158 individuals: 33 pallid, 123 shovelnose and two Alabama sturgeon. We observed 51 variable sites, including 18 C-T transitions, 28 G-A transitions, 1 insertion and 4 deletions. Twenty-six haplotypes we observed had not been previously reported by Campton et al (2000). The two Alabama sturgeon we characterized also displayed the unique "I" haplotype described by Campton et al. (2000). We hope to have a manuscript for publication on this work sometime in the next few months.

### Microsatellite

Nine lake sturgeon (LS) microsatellite primers, originally designed for lake sturgeon (May et al. 1998), had been previously screened by May et al. (1998) for use in other species. All of these

primer pairs were designed by May et al. (1996). Conditions originally described by May et al. were designed for 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 on 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. We have focused on the single copy flanking sequence as a possible source of species specific variation. Direct sequencing of LS-34 on eight pallid and two shovelnose sturgeon, and subsequent alignment of these sequences with the appropriate lake sturgeon sequence (GenBank Accession # U72733), resulted in the observation of two polymorphic sites at positions 34 and 97. Both shovelnose sturgeon exhibit a G at position 34 and an A at position 97, while four pallid exhibit an A and a G at positions 37 and 97. The remaining four pallid sturgeon are heterozygous (i.e. demonstrated both an A and a G at both positions, Fig. 2). Alignment of sequences from 10 pallid and 4 shovelnose sturgeon with the lake sturgeon equivalent of LS-68 (GenBank Accession #U72739), revealed an insertion of eight base pairs between *Scaphirhynchus* and *Acipenser fulvescens* beginning at position 340. Position 341 in *Scaphirhynchus* is a polymorphic site, two pallid and two shovelnose exhibit a G.

### Other Nuclear Markers

The non-coding first internal transcribed spacer (ITS-1) region is located between the 18S rDNA and 5.8S rDNA coding regions and

has been found to be a source of informative variation at the species and population levels for several vertebrate species (Gerbi 1985, Domanico et al. 1996). We have used two primers designed for salmonid fishes to amplify a 480 bp segment of ITS-1 from 8 pallid and 8 shovelnose sturgeon. The ITS-1 sequence was verified in multiple species comparisons, and a *Scaphirhynchus* primer set was designed to encompass the ITS-1 region from conserved regions in the 3-prime end of the 18S rDNA and the 5-prime end of the 5.8S rDNA genes (ITS18F, ITS58R). Intrapopulation sequence variation was observed and consisted mainly of C-G transitions and insertions in the 5' end of the ITS-1 segment. One shovelnose sturgeon exhibited a 66 bp deletion. Although polymorphic in both species, none of the sequence differences discriminate between pallid and shovelnose sturgeon.

In February of last year, we obtained 22 primer sets from the National Marine Fisheries Service designed for use in salmonid species. Six of these produced PCR amplification products in *Scaphirhynchus*, however only one, Ikaros 3'-UTR, has been cloned and sequenced. BLAST comparisons of the Ikaros amplicon sequences revealed them to be of anomalous origin. A new primer set (IK-2) was designed for use on *Scaphirhynchus*. The new primers produced an amplification product of approximately 500 bp. We sequenced a 290 bp segment of the IK-2 amplicon in 11 pallid and 22 shovelnose sturgeon. The segment exhibited polymorphism at positions 103 and 187. At site 103, 5 pallid and 9 shovelnose display a C; 2 pallid display a T, while 4 pallid and 13 shovelnose clearly exhibit both a C and a T. Similarly, at site 187, 9 pallid and 17

shovelnose display a C; 1 shovelnose displays a T, and the remaining 2 pallid and 4 shovelnose were heterozygous.

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#### PHYLOGENETICS OF *SCAPHIRHYNCHUS* BASED ON MITOCHONDRIAL DNA SEQUENCES

Species delineation and taxonomy within the sturgeon genus *Scaphirhynchus* is controversial. This issue is made more complex by political issues regarding the Alabama sturgeon *S. suttkusi* and potential hybridization between sympatric shovelnose sturgeon *S. platyrhynchus* and pallid sturgeon *S. albus*. We investigated phylogenetic relationships among species of *Scaphirhynchus* based on nucleotide sequences for two mitochondrial loci, cytochrome *b* and the control region (D-loop). White sturgeon *Acipenser transmontanus* and green sturgeon *A. medirostris* were used as outgroups. Phylogenetic analyses did not recover monophyletic shovelnose or pallid sturgeon; however, some populations of pallid sturgeon were resolved as sister to the Alabama sturgeon, and

one specimen of shovelnose sturgeon was consistently resolved as the sister to all other ingroup taxa. The hierarchical pattern of relationships produced by analysis of mitochondrial DNA is not consistent with that produced by morphological data. It is consistent with the hypothesis of a low rate of evolution of these genes in *Scaphirhynchus* and reflects recent hybridization between shovelnose and pallid sturgeon, probably due to habitat degradation.

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***This issue will be available as soon as the U.S. Fish and Wildlife Service is back online on the following website under pallid sturgeon activities:***

***[www.r6.fws.gov/moriver](http://www.r6.fws.gov/moriver)***

