

Development of a Practical Methodology for Genetically Discriminating Pallid and Shovelnose Sturgeons

Scope of Work

Submitted by

Edward J. Heist and Roy Heidinger

To

U.S. Fish and Wildlife Service

December 2002

Background:

Discrimination between pallid (*Scaphirhynchus albus*) and shovelnose (*S. platorhynchus*) sturgeons is one of the most important and vexing problems in conservation of North American freshwater fishes (Campton et al. 2000). Shovelnose sturgeon is a common species native to the Mississippi, Missouri, and Ohio Rivers. Pallid sturgeon is a rare and endangered species with a distribution from the Missouri headwaters to the mouth of the Mississippi but not the upper Mississippi or Ohio Rivers. Adults of either species are distinguishable by subtle differences in the placement of barbels and by the larger size and more flattened head profile of pallid sturgeons. Distinguishing young is far more difficult. A recently published morphological index for distinguishing between the species (Wills et al. 2002) has been criticized on the basis that it does not work for smaller specimens (Kuhajda and Mayden 2002).

Previous attempts to genetically discriminate between the species based on allozymes (Phelps and Allendorf 1983) and mitochondrial DNA (Fain et al. 2000, Campton et al. 2000) failed, perhaps owing to a recent common ancestry and/or a slow rate of genetic evolution of the markers employed. Recently a study that used DNA microsatellite loci, the most rapidly evolving class of molecular markers yet discovered, showed that pallid and shovelnose sturgeons from the same geographic areas had significantly different allele frequencies (Tranah et al. 2001). This study was based on very limited geographic sampling, a small number of specimens, and only five loci. Thus it did not provide a means of genetically categorize individual fish to species. However, it did show that the two morphological forms are reproductively isolated to a significant degree and thus comprise distinct, albeit similar, species. Recently primer sequences for an additional 70 polymorphic microsatellite loci in *Scaphirhynchus* were published (McQuown et al. 2001). Using this larger suite of loci it will be possible to find a combination of loci that provides a high degree of certainty of species identification for any individual sturgeon. While we doubt that any single locus will provide a diagnostic difference between the two species, application of a statistical approach known as "assignment testing" (Paetku et al. 1995) applied over multiple loci may produce a very high (i.e. much greater than 99%) certainty in identifying individual fish to species.

Southern Illinois University Carbondale received a \$47,000 grant from the US Fish and Wildlife Service for a two-year project (August 2002 –July 2004) entitled “Microsatellite Tools for Genetic Identification of *Scaphirhynchus*”. The goals of this project are to score a large number of microsatellite loci from shovelnose and pallid sturgeons collected throughout the ranges of both species with the objective of developing the most reliable means of genetically distinguishing between the two sturgeons. We will also investigate the frequency and location of hybridization between shovelnose and pallid sturgeons as a tool for screening broodstock for pallid sturgeon recovery efforts.

While the results of this initial project will accomplish much towards developing tools for distinguishing between pallid, shovelnose, and hybrid sturgeons, additional funding will be necessary to make these tools applicable to the task of improving discrimination of all size classes of pallid and shovelnose sturgeons. Because our initial project has just begun we do not yet have enough data nor have we collected enough specimens to know exactly where we will need to concentrate additional efforts after July of 2004. However, it is clear that given the difficulty in obtaining the necessary permits and physically collecting pallid sturgeons from some locations we will need to do additional sampling following the end of the initial project. Besides increasing the numerical and geographic sampling, additional funding will allow us to pursue the following new objectives:

- 1) **Increased efficiency of genetic discrimination** – The initial two-year project will result in a suite of loci that provides the most reliable means of genetically discriminating between pallid and shovelnose sturgeons. However, it will require that each of the twelve or so loci chosen for the assignment test are amplified and scored independently. Through the use of multiplex PCR we can make this process far more efficient, allowing four or more loci to be amplified and scored simultaneously. Each locus will be scored in concert with several combinations of other loci to find combinations of loci that do not interfere with each other (e.g. prevent amplification of some loci or produce spurious products). The goal of this project is to streamline the process of obtaining the data for species discrimination and thus make it more likely that these methodologies are practical enough to ultimately be used by managers and by law enforcement.
- 2) **Genetic stock analysis of pallid sturgeon** – It is critically important to understand the stock structure of pallid sturgeon in the middle Mississippi and upper Missouri Rivers in order to assess the likely impact of stocking throughout the system with fish collected in the upper Missouri. A study of historic levels of gene flow using multiple microsatellite loci will be used to determine whether there has been sufficient gene flow among the upper Missouri and middle Mississippi to preclude the development of adaptive differences among pallid sturgeon stocks. The results from this study will indicate whether pallid sturgeons from the middle Mississippi are likely to possess adaptations to climate or river morphology that might be disrupted through outbreeding with fish originating in the upper Missouri.

- 3) **Refinement of morphological index** – By using genotypes of vouchered shovelnose and presumed hybrid specimens as well as measured pallid sturgeons of several discrete size classes we can refine the morphological index of Wills et al. (2002) to accommodate sturgeons of all size classes. We are currently collecting voucher specimens of shovelnose and “hybrid” sturgeon spanning a wide range of size classes as well as morphological and meristic data on all pallid sturgeons collected in the middle Mississippi. Vouchered specimens will be maintained in the SIUC fluid vertebrate collection to allow a re-examination of morphological traits following genetic analyses. Our goal is to produce a refined morphological index or perhaps several size-specific morphological indices for use in discriminating *Scaphyrinchus* in the middle Mississippi
- 4) **Application of DNA forensic methodology to caviar** – Application of genetic discrimination to caviar and to individual eggs in particular would be very useful. While mitochondrial DNA is routinely isolated and characterized from individual fish eggs owing to the presence of multiple copies, nuclear DNA (e.g. microsatellite DNA) is present as only one or two copies in an unfertilized egg. Thus attempts at isolating nuclear DNA from single fish eggs have apparently been unsuccessful as indicated by the lack of published material on this topic. It may be possible to isolate and characterize nuclear DNA from a number of loci by pre-amplifying the DNA using microsatellite primers and a single homogenized egg. To our knowledge this is a novel approach that has never been attempted. Conversely, characterization of DNA from a single egg may prove impossible in which case some minimum number of eggs may be required. We will investigate the problem of characterizing nuclear DNA in both fresh and processed caviar to determine the minimum number of eggs necessary to apply forensic genetic markers. Whether we are successful or not in characterizing nuclear DNA from single eggs we will publish our protocols and results as a means of furthering progress in the field of forensic genetics.

Literature Cited

- Campton, D.E., A.L. Bass, F.A. Chapman & B.W. Bowen. 2000. Genetic distinction of pallid, shovelnose, and Alabama sturgeon: emerging species and the US Endangered Species Act. *Conservation Genetics* 1: 17-32.
- Fain, S.R., B.C. Hamlin & D.S. Straugham. 2000. Genetic Variation in the River Sturgeon *Scaphyrhynchus* (Acipenseridae) as Inferred from Partial mtDNA Sequences of Cytochrome b. pp. 20, U.S. Fish and Wildlife Service.
- Kuhajda, B. R. and R. L. Mayden. 2002. Comparisons of procedures to differentiate hatchery-reared specimens of *Scaphirhynchus albus*, *S. platyrhynchus*, and *S. albus* x *S. platyrhynchus* hybrids. Unpublished abstract. American Society of Ichthyologists and Herpetologists annual meeting, Kansas City, MO.
- McQuown, E.C., B.L. Sloss, R.J. Sheehan, J. Rodzen, G.J. Tranah & B. May. 2001. Microsatellite analysis of genetic variation in sturgeon (Acipenseridae): new primer sequences for *Scaphirhynchus* and *Acipenser*.
- Paetkau, D., W. Calvert, I. Stirling & C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* 4: 347-354.
- Phelps, S.R. & F.W. Allendorf. 1983. Genetic identity of pallid and shovelnose sturgeon (*Scaphirhynchus albus* and *S. platyrhynchus*). *Copeia* 1983: 696-700.
- Tranah, G., H.L. Kincaid, C.C. Krueger, D.E. Campton & B. May. 2001. Reproductive isolation in sympatric populations of pallid and shovelnose sturgeon. *North American Journal of Fish Management* 21:367-373.
- Wills, P. S., R. J. Sheehan, R. Heidinger, and B. L. Sloss. 2002. Differentiation of pallid sturgeon and shovelnose sturgeon using an index based on meristics and morphometrics. *American fisheries Society Symposium* 28:249-258.

Budget

Graduate Student RA (12 Months)	\$15,323.00
Commodities	\$ 6,000.00
Travel (Recovery Team Meetings)	\$ 1,500.00
<hr/>	
Direct costs	\$22,823.00
Indirect (41%)	\$ 9357.00
<hr/>	
Total	\$32,180.00

Budget Justification

Personnel We are requesting \$15,323 to cover the Ph.D. research assistantship of Aaron Schrey who will be performing the genetic lab work and analyses and participating in field work collecting pallid and shovelnose sturgeons.

Commodities \$500 per month is requested for genetics supplies including PCR primers, gel reagents, buffers, and for museum supplies.

Travel We are requesting \$1500 per year for one of the PIs to attend pallid sturgeon recovery team meetings in the upper Missouri and Middle Mississippi regions

Indirect SIUC charges a standard rate of 41% indirect cost on personnel, commodities, and travel expenses.

034955