Gulf and Caribbean Research

Volume 20 | Issue 1

January 2008

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Brian R. Kreiser
University of Southern Mississippi, Brian.Kreiser@usm.edu

J. Berg
U.S. Geological Survey

M. Randall
U.S. Geological Survey

F. Parauka
U.S. Fish and Wildlife Service, Panama City, Florida

S. Floyd
U.S. Fish and Wildlife Service, Daphne, Alabama

et al.

DOI: 10.18785/gcr.2001.13

Gulf and Caribbean Research Vol 20, 91-95, 2008 Manuscript received October 29, 2007; accepted January 8, 2008

SHORT COMMUNICATION

DOCUMENTATION OF A GULF STURGEON SPAWNING SITE ON THE YELLOW RIVER, ALABAMA, USA

B. Kreiser[1*], J. Berg[2], M. Randall[2], F. Parauka[3], S. Floyd[4], B. Young[4], and K. Sulak[2]

[1]*Department of Biological Sciences, 118 College Dr. #5018, The University of Southern Mississippi, Hattiesburg, Mississippi 39406, Phone 601-266-6556, e-mail: Brian.Kreiser@usm.edu*

[2]*Center for Aquatic Resource Studies, United States Geological Survey, Gainesville, Florida 32653, USA*

[3]*United States Fish and Wildlife Service, Panama City, Florida 32405, USA*

[4]*United States Fish and Wildlife Service, Daphne, Alabama 36526,* USA

**Corresponding author*

INTRODUCTION

Gulf sturgeon (*Acipenser oxyrinchus desotoi*) have experienced population declines over the past century, primarily due to the effects of impoundments (e.g., barriers and altered flow regimes), water quality degradation, and overfishing (USFWS, GSMFC and NMFS 1995). These declines prompted the listing of Gulf sturgeon as a threatened species under the Endangered Species Act (U.S. Fish and Wildlife Service 1991). Effective management of Gulf sturgeon requires knowledge of its life history. Considerable effort has been expended in documenting the timing of the freshwater spawning migration and the location of habitats critical for successful spawning. To date, spawning sites have been identified and confirmed by the collection of fertilized eggs in the Apalachicola (Wooley and Crateau 1982, Wooley et al. 1982), Choctawhatchee (Fox et al. 2000), Escambia (Craft et al. 2001), Pascagoula (Heise et al. 2004) and Suwannee rivers (Marchant and Shutters 1996, Sulak and Clugston 1998, 1999). However, the timing and location of Gulf sturgeon spawning in other river systems is still unknown.

The Yellow River originates in southeast Alabama and flows southwest across northwest Florida into Blackwater Bay, near Milton, Florida. Craft et al. (2001) documented Gulf sturgeon movement in the Yellow River, and Berg et al. (2007) was able to provide a population estimate in 2003 of 911 fish (95% confidence interval, 550-1550). The fact that adults move up into the Yellow River and that three young-of- the-year sturgeon have been collected there (Berg 2004) suggests that spawning takes place, although this had never been verified by the collection of eggs. To afford Gulf sturgeon in the system some protection, the Yellow River from Alabama State Highway 55 downstream to its discharge in Blackwater Bay has been designated as Gulf sturgeon critical habitat (USFWS and NOAA 2003).

The Gulf Sturgeon Recovery Plan (USFWS, GSMFC and NMFS 1995) stressed the need to provide maximum protection to Gulf sturgeon spawning habitat. The approach employed by various Gulf sturgeon researchers, including ourselves, to document spawning has been to identify potential spawning habitat on the basis of physical characteristics and/or tracking data, collect eggs, and then raise the eggs in the laboratory until the point where the larval fish can be identified (e.g., Marchant and Shutters 1996, Sulak and Clugston 1998, 1999). However, collecting eggs in any appreciable number is usually difficult, and these eggs may not always be viable upon return to the laboratory. Molecular methods provide an alternative means of identifying the species represented by an egg. Notable examples related to sturgeon conservation include cases where molecular markers were used to verify the sources of commercially available caviar (DeSalle and Birstein 1996, Birstein et al. 1999).

Parauka and Giorgianni (2002) reported that potential Gulf sturgeon spawning habitat is present in the Yellow River; however, efforts to document spawning by the collection of eggs or larvae have been unsuccessful in the past. Herein, we report on the first successful collection of eggs from a potential spawning site on the Yellow River and the verification of their identity as Gulf sturgeon by using molecular methods.

MATERIALS AND METHODS

Field Methods

Egg samplers (Marchant and Shutters 1996, Sulak and Clugston 1998) consisted of a red circular floor-buffing pad (55.9 cm or 68.6 cm diameter) anchored to the bottom with a rebar grapnel hook. A 6.4 m length of braided polyrope (0.63 cm diameter) was attached to the pad, and a float was tied to the end of the line to mark the location of the pad and facilitate retrieval. Samplers were georeferenced at the time of deployment with a hand-held GPS. Sixty to seventy-seven pads were deployed from 11 April to 13 May 2005 at five potential Gulf sturgeon spawning sites in the Yellow River (60-65 pads at three sites between rkm 123-134; 10-12 pads at two sites between rkm 95-100, Figure 1). Parauka and Giorgianni (2002) identified these sites as having the limestone, cobble, and hard substrate composition characterized by other researchers as being associated with documented Gulf sturgeon spawning (Marchant and Shutters 1996, Sulak and Clugston 1998, 1999, Fox et al. 2000,

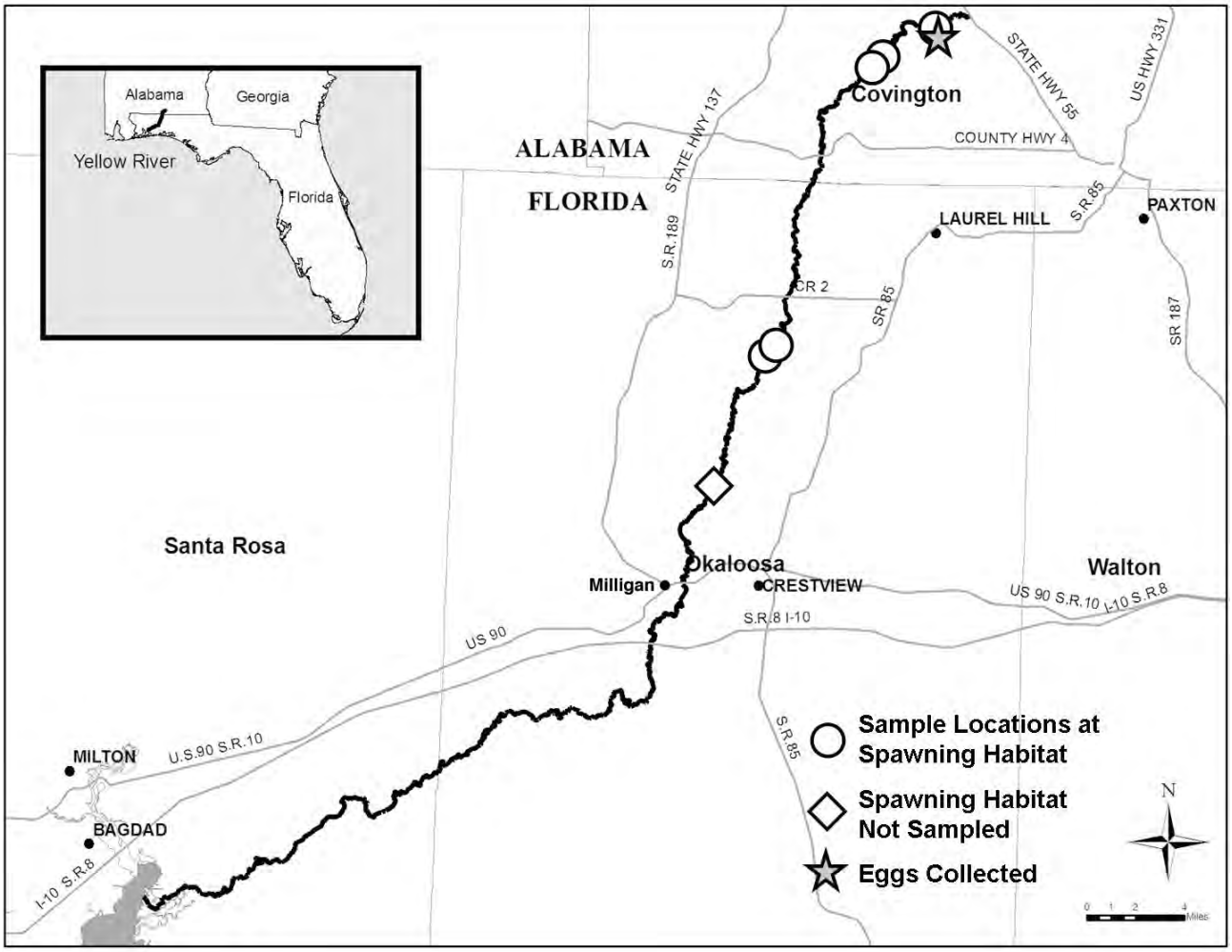

Figure 1.
Potential Gulf sturgeon spawning sites on the Yellow River, Alabama-Florida, USA, sampled during this study.

Craft et al. 2001). Samplers were retrieved and examined every 48-72 h for the presence of Gulf sturgeon eggs. Water temperature (°C), dissolved oxygen (mg/L) and conductivity (µS) measurements were taken 10 cm below the surface with a YSI-85 meter. River flow rates were obtained from the U.S. Geological Survey gauging station (02368000) located at rkm 64 on the Yellow River near Milligan, Florida. Any eggs collected from the pads were placed into water collected from the site and returned to the lab for rearing in covered, aerated holding containers. Eggs that failed to develop were placed into a tissue preservation buffer (Seutin et al. 1991).

The level of sampling effort and success in this study was compared to others by calculating catch per unit effort (CPUE). CPUE was quantified as the number of eggs collected divided by the number of sampling days (the number of sampling pads multiplied by the number of days the pads were deployed). Comparative data were gathered from the literature for the Pascagoula River (Heise et al. 2004) and from unpublished data for the Suwannee River (K. Sulak – United States Geological Survey). To account for the influence of prior knowledge of spawning locations, we calculated separate estimates of CPUE for the Pascagoula and Suwannee Rivers based on whether the sampling locations were known (targeted) or suspected (exploratory) spawning sites.

Molecular Methods

Each egg was rinsed with distilled water and blotted dry. Total genomic DNA was extracted from individual eggs with the DNeasy Tissue Kit, following the manufacturer's protocol (QIAGEN Inc., Valencia, CA). The quality of the extracted DNA was examined by gel electrophoresis on 1% agarose gels, stained with ethidium bromide (0.5 µg/ml) and then viewed under ultraviolet light. DNA concentrations were measured using a NanoDrop ND-100 spectrophotometer (NanoDrop Technologies Inc., Rockland, DE).

DNA from each egg was used in a polymerase chain reaction (PCR) to amplify a portion of the mitochondrial control region. We used the L15926 primer of Kocher et al. (1989) and another primer that we designed for Gulf sturgeon (GS-CRH1: 5'- GTGCCATTCACTGTTTGTCC). Amplifications were conducted in a total volume of 25 µl using 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.01% gelatin, 2 mM $MgCl_2$, 200 µM dNTPs, 0.75 units *Taq* polymerase,

0.3 µM of each primer, 22.5-32 ng template DNA and water to the final volume. PCR cycling conditions consisted of an initial 1 min denaturing step at 95 °C followed by 30 cycles of 1 min at 95 °C, 1 min at 50 °C and 1 min at 72 °C. A final elongation step of 7 min at 72 °C completed the cycle. Several precautions were taken to avoid amplifying potential contaminants. We used fresh reagents, including buffers, $MgCl_2$, *Taq* polymerase and primers for the egg amplifications, and negative controls were employed for each set of reactions. Multiple independent reactions were also conducted on each of the egg samples.

Successful amplifications were prepared for sequencing using the ExoSAP-IT system (USB Co., Cleveland, OH). Both the forward and reverse strands of the control region fragment were cycle sequenced using a BigDye Terminator cycle sequencing kit v. 1.1 (Applied Biosystems, Foster City, CA). Sequencing reactions were performed with the GS-CRH1 primer and an internal primer reported by Ong et al. (1996) in lieu of the L15926 primer. Gel runs were performed at the Iowa State University DNA Sequencing and Synthesis Facility. Sequence data were aligned and edited with Sequencher 4.1 (Gene Codes Co., Ann Arbor, MI).

Two approaches were employed to identify the species represented by the eggs. First, we obtained comparative control region sequences from 14 adult Gulf sturgeon collected from rivers across the Gulf of Mexico. These individuals are part of the large and growing collection of tissue samples accumulated through the efforts of the many researchers involved with the Gulf sturgeon recovery effort. Samples were from the Apalachicola (n = 1), Choctawhatchee (n = 3), Yellow (n = 3), Escambia (n = 1), Pascagoula (n = 2) and Pearl rivers (n = 4). Second, we compared the egg sequences to all available sequences on GenBank (http://www.ncbi.nlm.nih.gov/) by nucleotide BLAST (Basic Local Alignment and Search Tool, Altschul et al. 1997, accessed June 6, 2005). The BLAST search returns a list of sequences matching the query sequence along with an *E*-value that measures the strength of the match. The *E*-value indicates how many other similar matches in the database would be expected by chance alone; thus the smaller the *E*-value the more likely the match between the query sequence represents real similarity.

DNA from each egg was also used to amplify the eight microsatellite loci used by Dugo et al. (2004) in their survey of population genetic structure in Gulf sturgeon. Precautions to avoid amplifying potential contaminants were made as previously described. All other methods and PCR reaction conditions followed Dugo et al. (2004) except for the use of 45-100 ng of DNA as template. Genotypes for each locus were compared to those obtained from adult Gulf sturgeon from across their range.

Results and Discussion

Spawning Site Features and Egg Collection

TABLE 1. *The numbers of each mitochondrial control region haplotype identified in adult Gulf sturgeon (labeled by the river of capture) and the one egg from the Yellow River that was successfully amplified and sequenced. Each haplotype is identified by an arbitrary letter code.*

	A	B	C	D	E
Apalachicola			1		
Choctawhatchee			2	1	
Yellow	1	2			
Escambia		1			
Pascagoula					2
Pearl		1	1		2
Egg	1				

Three eggs were collected at rkm 134 (known locally as "Dripping Rock") on 4 May 2005 (31°5'23.9"N, 86°27'31.9"W, Figure 1). The eggs were collected on two samplers about 100 m apart in water depths of 2.1 and 3.0 m, respectively, with an ambient water temperature of 18.1 °C, dissolved oxygen concentration of 7.06 mg/L, and conductivity of 40.2 µS. The river discharge was 105 m^3/s. The environmental parameters (especially temperature) and a rising hydrograph for the Yellow River during this period in 2005 matched the spawning conditions reported in other rivers (Chapman and Carr 1995, Sulak and Clugston 1998, Fox et al. 2000, Heise et al. 2004). Lunar cycles did not seem to influence spawning activity for Gulf sturgeon in the Yellow River, which was 5-6 d before the new moon and similar to what Fox et al. (2000) reported on the Choctawhatchee River.

The eggs were placed in water collected at the site and taken to the laboratory to allow development and hatch. Within 36 h, fungus appeared on the eggs, and they were removed from the receptacle and placed in the tissue preservation solution.

The CPUE for the five sites surveyed in this study averaged 0.001 eggs/sampling day. Exploratory sampling at four sites in the Pascagoula and three in the Suwannee produced an average CPUE of 0.003 and 0.011, respectively (Heise et al. 2004, K. Sulak – United States Geological Survey). Once spawning sites were confirmed, targeted egg sampling produced a higher CPUE for both the Pascagoula (0.119) and Suwannee (0.085).

Molecular Results

High molecular weight DNA was recovered from each of the three eggs with concentrations ranging from 15.0-21.4 ng/µL. Despite the presence of DNA in all three egg extractions, only one egg was successfully amplified in any of the PCR attempts. All adult samples were successfully amplified.

The amplified control region fragment was about 710 base pairs (bp) in length. Fourteen adults and two independent amplifications of the one egg were sequenced, producing 595 bp for each individual after editing. Five unique haplotypes

(A-E) were represented by the 14 adult Gulf sturgeon control region sequences (Table 1, GenBank accession numbers DQ088959-DQ088963). The number of nucleotide substitutions between haplotypes ranged from 1 to 12 and uncorrected p distances ranged from 0.0017 to 0.0202 (Table 2). The sequences from the egg were a perfect match with haplotype A, which was found in one adult Gulf sturgeon from the Yellow River. Haplotype B was only one base pair different and was found in the other two adults from the Yellow River, as well as one individual each from the Escambia and Pearl rivers.

Results of the BLAST query also supported the identification of the egg as belonging to Gulf sturgeon. Of the top 100 sequences producing significant alignments, the first 44 were from *A. oxyrinchus* (*A. o. oxyrinchus* and *A. o. desotoi*) with the remaining representing other *Acipenser* and *Huso* species. In all cases, the *E*-values were extremely small ($< 2.0 \times 10^{-64}$), signifying a strong match between the query and GenBank sequences.

Only four of eight microsatellite loci successfully amplified in the one egg sample. These loci included Aox-D32, Aox-D64, Aox-D242, and LS-68. The lack of a complete multilocus genotype for the egg precluded any formal analysis (e.g., assignment tests). However, the allele sizes for each locus successfully amplified matched those previously reported for Gulf sturgeon. Qualitative comparisons with the data of Dugo et al. (2004) revealed that the alleles present in the egg were also found in adult Gulf sturgeon collected from the Escambia and Yellow Rivers.

Implications

Our work verifies that Gulf sturgeon use the Yellow River for spawning and supports the importance of upper river hard bottom sites for successful reproduction (Sulak and Clugston 1998, 1999, Fox et al. 2000, Craft et al. 2001).

The documentation of spawning by Gulf sturgeon in the Yellow River highlights the need to protect the limited potential spawning habitat identified by Parauka and Giorgianni (2002). The eggs collected during this study, as well as three of the four young of the year Gulf sturgeon (Berg 2004, Ken Weathers, Alabama Department of Conservation and Natural Resources, pers comm. 2003) in previous studies, were from locations above a proposed dam site on the Yellow River. Additional studies are required to better characterize the habitats actually used by Gulf sturgeon in the Yellow River so that these river reaches can be better managed in accordance with the recovery actions described in the Gulf Sturgeon Recovery/Management Plan (USFWS, GSMFC and NMFS 1995).

TABLE 2. *Number of pairwise base substitutions below the diagonal and uncorrected p distances above the diagonal between Gulf sturgeon mitochondrial control region haplotypes.*

	A	B	C	D	E
A	-	0.0017	0.0034	0.0034	0.0202
B	1	-	0.0017	0.0017	0.0185
C	2	1	-	0.0034	0.0168
D	2	1	2	-	0.0168
E	12	11	10	10	-

Acknowledgments

The baseline collections and research for this project would not have been possible without the assistance and cooperation of the Gulf Sturgeon research community. In particular, the USGS provided the fundamental field logistics and baseline data that made this investigation possible. We extend our thanks to the field assistants associated with this work: M. Friedman (University of Florida), J. Russell (Southampton College and Long Island University), W. Harden (USGS), G. Yeargin (USGS) and A. Quaid (USGS). The fieldwork for this study was aided by volunteers M. Duncan of NOAA and B. Sawyer of Alabama Electric Cooperative. Personnel of the Conecuh National Forest assisted by providing campground space to be used as a base of operations during the project. K. Herrington, U.S. Fish and Wildlife Service, was responsible for putting together Figure 1. Helpful comments on the manuscript were provided by J. Powell and T. Slack, and invaluable editorial advice was provided by D. Kreiser.

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