

Conservation and management implications of fine-scale genetic structure of Gulf sturgeon in the Pascagoula River, Mississippi

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Summary

The anadromous Gulf sturgeon occurs along the north central coast of the Gulf of Mexico and is federally listed as threatened. We analyzed fine-scale patterns of Gulf sturgeon population structure, focusing on the Pascagoula River drainage of Mississippi, in reference to movement patterns as determined via telemetry and capture data. We genotyped 361 Gulf sturgeon using eight microsatellite loci including samples from the Pascagoula, Pearl, Escambia, Yellow, Choctawhatchee, and Apalachicola river drainages. Pairwise F_{ST} estimates indicated that genetic structure occurs at least at the drainage level. The Pascagoula and Pearl rivers form a western group, demonstrating 100% bootstrap support for a division with drainages to the east. Assignment tests detected non-natal genotypes occurring in all drainages. According to assignment tests, the Pascagoula supports an admixture of individuals, containing minimal influence from drainages to the east (2%) and substantial interaction with the Pearl River (14.1%). The occurrence of Pascagoula River fish in the Pearl was non-reciprocal, observed at 1.1%. After accounting for non-natal genetic diversity within the Pascagoula, there remained a disparity between a pooled Pascagoula group and the only documented spawning site within the drainage located in the Bouie River. We interpret this as an indication of a second genetic stock within the Pascagoula River drainage. Radio telemetry data suggest that spawning likely occurs in the Chickasawhay River, in areas isolated from the Bouie River spawning site by about 350 river kilometers. We emphasize the utility of integrating field and molecular approaches when delineating fine-scale patterns of population structure in anadromous fishes.

Introduction

As basal members of the Actinopterygii, sturgeon (Order: Acipenseriformes; Family: Acipenseridae) are unique in their evolutionary and life histories. Regarded as ‘living fossils’, the sturgeon lineage extends more than 200 million years to the Jurassic (Grande and Bemis, 1996; Bemis et al., 1997) and represents a Laurasian faunal element (Bemis and Kynard, 1997). The Acipenseridae are among the highest priority taxa for conservation and management needs (Rochard et al., 1990). All 25 extant sturgeon species are listed by the IUCN, with species such as the beluga and Alabama sturgeon considered on the brink of extinction.

The imperiled status of sturgeon species is largely the result of a life history that is vulnerable to commercial harvest and the anthropogenic demands on coastal and riverine corridors in an urbanizing landscape. Many sturgeon species (e.g. *Acipenser* and *Huso*) are anadromous, migrating from salt water to fresh water for reproduction and all are considered ‘big river’ fishes that strongly select environmental parameters (e.g. temperature, substrate and flow) for successful spawning and survival of early life stages. The selection for these parameters thereby limits the spawning season spatially (e.g. limiting factors are substrate and flow) and temporally (e.g. limiting factors are temperature and flow). The ability to establish biologically meaningful recovery plans for the Acipenseridae has been inhibited by insufficient life history and population level data. From a conservation perspective, a primary objective for sturgeon recovery is to delineate the boundaries of populations across distributional ranges.

Molecular analyses on North American Atlantic sturgeon, *Acipenser oxyrinchus*, have been the most comprehensive for any sturgeon species to date (Stabile et al., 1996; Waldman et al., 1996; Wirgin et al., 1997, 2000; King et al., 2001). All published studies indicate that *Acipenser oxyrinchus* are genetically structured, hypothesized to be the result of philopatry to natal rivers (Stabile et al., 1996; Wirgin et al., 1997; Waldman and Wirgin, 1998). These studies have established range-wide trends of population structure and furthermore assisted in roughly outlining ‘management units’ for recovery efforts. However, as noted by King et al. (2001), a comprehensive understanding of fine-scale patterns of population structure requires more robust studies using highly variable markers such as microsatellites with larger sample sizes.

Herein we suggest that the complex life history strategy exhibited by sturgeons has inhibited the ability of traditional molecular approaches to fully capture the dynamics of their population biology. Our autecological research on *Acipenser oxyrinchus* suggests that delineating fine-scale patterns of population structure benefit from the coupling field and molecular methods. The incorporation of movement and habitat use data with molecular analyses using highly polymorphic markers (e.g. microsatellite loci) may provide the greatest potential for revealing the biological implications of life history, movement, and site fidelity on population structure, particularly for long-lived, slow maturing species such as sturgeon (Wirgin et al., 1997).

Acipenser oxyrinchus

The Atlantic sturgeon, *Acipenser oxyrinchus* (Mitchill 1814), is distributed in eastern and southeastern North America, in the coastal and inland waters of the Atlantic Ocean and the northern Gulf of Mexico. Based on morphological characters (Vladykov, 1955; Wooley, 1985), *Acipenser oxyrinchus* is currently divided into two subspecies, *A. o. oxyrinchus*, the Atlantic sturgeon and *A. o. desotoi*, the Gulf sturgeon.

Our research has focused on Gulf sturgeon of the Pascagoula River drainage in southeastern Mississippi. The Pascagoula River is the largest river drainage in the lower 48 states ($350 \text{ m}^3 \text{ s}^{-1}$) void of a dam or other major impediment on its main stem (Dynesius and Nilsson, 1994) that would alter the natural flow regime or impede movement of migratory species. As such, this drainage is ideal for studying population structure mediated through homing fidelity in an anadromous species. The Pascagoula River drainage encompasses an area of $15\,607 \text{ km}^2$ (United States Army Corps of Engineers, 1968). The main stem Pascagoula River is formed by the confluence of the Leaf River (5760 km^2) and the Chickasawhay River (4778 km^2), about 130 river kilometers (rkm) upstream from the drainage terminus (United States Army Corps of Engineers, 1968).

The Gulf sturgeon was listed as 'threatened' in 1991 under the Endangered Species Act of 1973, as amended (United States Fish and Wildlife Service, 1988). A major concern outlined in the 'Gulf Sturgeon Recovery/Management Plan' (United States Fish and Wildlife Service and Gulf States Marine Fisheries Commission, 1995) was the delineation of population genetic structure. Published molecular data on Gulf sturgeon using mtDNA RFLP and sequence data delineated five regional stocks (Stabile et al., 1996). These data were ambiguous in determining relationships between the Pearl and Pascagoula river drainages, which are the two most westerly drainages known to support populations of Gulf sturgeon and are separated from the other regional stocks by the Mobile River Basin. No delineation between the Pearl and Pascagoula rivers was observed with mtDNA RFLP data; however, slight levels of divergence were observed with sequence data of the mtDNA control region.

Integrating field and molecular approaches

Life history and movement data are important considerations when studying fine-scale patterns of genetic structure. Feeding in adult and subadult Gulf sturgeon predominately occurs in winter marine habitats (Fox et al., 2002; Edwards et al., 2003; Ross et al., 2003). The onset of migration into fresh water usually occurs in March (Heise et al., 2004). Ripe individuals make their way to upstream portions of the river where they remain as long as spawning conditions exist. By mid-May the spawning season is over and spent individuals generally migrate to and reside in the lower part of the river known as the 'holding area'. The holding area also serves as the summer habitat for immature sub-adults and non-ripe adults (sturgeon are non-annual spawners).

Since the onset of our research in 1997, 201 individuals have been captured within the Pascagoula River drainage (Ross et al., 2004). Autecological and telemetry data based on 146 individuals within the Pascagoula River drainage of Mississippi (Heise et al., 2004; Ross et al., 2004) have led to the description of migratory patterns, spawning areas, and habitat use. Although the population size of Gulf sturgeon from the Pascagoula River drainage is undoubtedly reduced relative to

historical records, population models suggest about 200 adult and sub-adult Gulf sturgeon utilize the river during summer resting periods (Heise, 2003).

Field components to our research are described in Heise et al., 2004. The following aspects of Gulf sturgeon movement in the Pascagoula River drainage suggest population genetic structure: (1) the documentation of homing fidelity to the Bouie River spawning site (ca. 250 rkm above the mouth of the Pascagoula River drainage) via capture or signal detection of radio-tagged individuals; (2) spawning migration into the upper Chickasawhay River system, geographically isolated from the Bouie River spawning area by about 350 rkm and; (3) the annual post spawning occupation of the lower Pascagoula River (30–50 rkm above the mouth of the drainage) as a 'summer resting area', or 'holding area' (Fig. 1).

Most recently, in contrast to documented natal philopatry, our attention has been brought to the potential lack of isolation across the range of Gulf sturgeon. Four individuals captured in the Pascagoula summer holding area were at another point captured in the summer holding area of other river drainages (two in the Pearl River, one in the Yellow River, and one in the Choctawhatchee River). Instances of interdrainage movements have been periodically field proofed throughout the range of Gulf sturgeon (Sulak and Clugston, 1999; Craft et al., 2001; Fox et al., 2002).

Herein we examine the interaction of Gulf sturgeon movement and location of capture with genetic population structure, with our primary focus on the Pascagoula River drainage. To address fine-scale patterns of population structure in Gulf sturgeon, we analyzed our data in a hierarchical progression. First, we referenced the genetic composition of Gulf sturgeon of the Pascagoula River relative to drainages across their geographic range. Secondly, we genetically

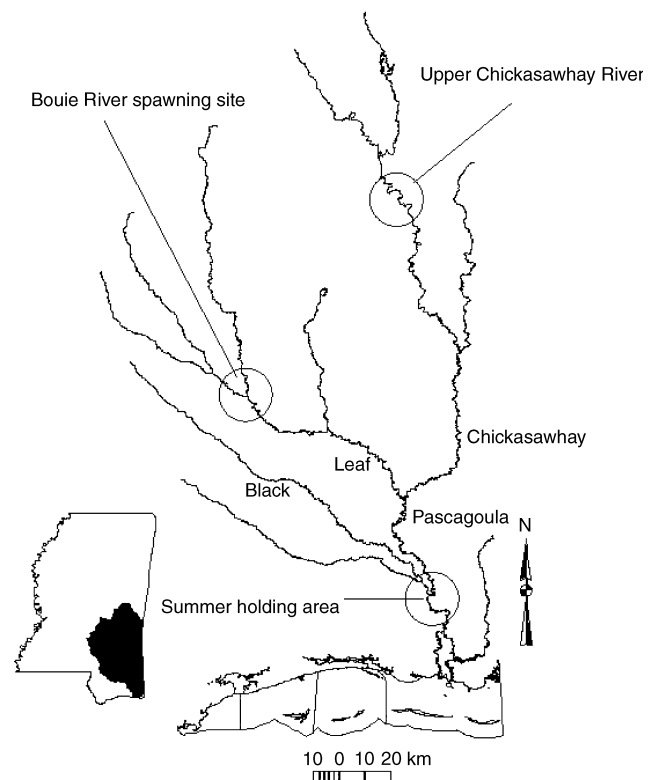


Fig. 1. The Pascagoula River drainage in SE Mississippi showing key water bodies and locations

identified non-natal individuals across all drainages assayed. In addition to detecting non-natal genetic diversity within the Pascagoula River drainage, the identification of non-natal samples in other drainages provides insight into the relative frequency and nature of interdrainage movement as a whole. Lastly, we tested for intradrainage population structure within the Pascagoula River drainage, which would indicate strong patterns of homing fidelity to natal spawning sites. Specifically, our final research objective was to assess whether the Bouie River spawning population could account for the total genetic diversity within the Pascagoula River drainage.

Materials and methods

Sample collections

Gulf sturgeon tissue samples (e.g. fin clips) were immediately placed into a preservation buffer (Seutin et al., 1991) or stored on ice for transport to the lab where they were then stored at -20°C . Tissue samples from areas outside of the Pascagoula River drainage include the Pearl River, LA; Escambia River, FL; Yellow River, FL; Choctawhatchee, FL, and the Apalachicola River, FL (Fig. 2). Drainages known to support spawning populations of Gulf sturgeon not represented in this study include the Ocklocknee River and Suwannee River drainages in the eastern panhandle of Florida.

Samples representing Gulf sturgeon from the Pascagoula River drainage were captured at the Bouie River, the Pascagoula holding area, and the West Pascagoula River at rkm 0 from 1997 to 2003. For all initial analyses, unless otherwise stated, samples ($n = 361$) were divided into eight separate units (Pascagoula, $n = 144$; Bouie, $n = 24$; Pearl, $n = 90$; Escambia, $n = 12$; Yellow, $n = 20$; Choctawhatchee, $n = 45$; Apalachicola, $n = 13$; unknowns, $n = 13$). The Pascagoula sampling unit represents individuals captured in the Pascagoula holding area or in the West Pascagoula at rkm 0 and then later relocated via telemetry at the Pascagoula holding area. The Bouie sampling unit consisted of individuals captured at the Bouie River spawning site, or individuals captured in the Pascagoula that were later relocated via telemetry at the Bouie River spawning site. Individuals from the other five sampling units were captured in the holding

areas of their respective drainages or in lower portions of rivers during the seaward autumn migration. This partitioning of samples was designed to have each individual represent single location captures.

A set of samples ($n = 13$) was separately designated as individuals of 'unknown origin' and represented three different categories. Four of these individuals were captured in multiple holding areas, including the Pascagoula holding area. Six individuals were captured at rkm 0 of the Pascagoula River drainage in the West Pascagoula River at the onset of the freshwater migration and fitted with sonic and radio tags, but were never relocated in the Pascagoula River drainage. However, these telemetered sturgeon were relocated in the Pearl River 1–2 months after their initial capture in the West Pascagoula River. The remaining three samples represent three juvenile Gulf sturgeon that were captured once at rkm 0 of the Pascagoula River drainage, in the West Pascagoula River, but not fitted with telemetry tags.

DNA extraction, amplification and visualization

Total genomic DNA was extracted with the DNeasy Tissue Kit (QIAGEN Inc., Valencia, CA). We examined 25 microsatellite loci from the literature and selected eight loci for use in this study based on their efficiency and quality of amplification (Table 1). All loci demonstrated disomic inheritance as published in the characterization summaries (May et al., 1997; Henderson-Arzapalo and King, 2002).

All amplifications were conducted in a total volume of $25\ \mu\text{l}$ using $50\ \text{mM}\ \text{KCl}$, $10\ \text{mM}\ \text{Tris-HCl}$ (pH 8.3), 0.01% gelatin, $2.5\ \text{mM}\ \text{MgCl}_2$, $200\ \mu\text{M}$ dNTPs, 0.75 units of *Taq* polymerase (Promega Co., Madison, WI, USA), $0.3\ \mu\text{M}$ of the M13 tailed forward primer (Boutin-Ganache et al., 2001), $0.3\ \mu\text{M}$ of the reverse primer, $0.1\ \mu\text{M}$ of the M13 labeled primer, 20 – $150\ \text{ng}$ of template DNA and water to the final volume. PCR cycling conditions consisted of an initial denaturing step of 94°C for 2 min followed by 35 cycles of 30 s at 94°C , 1 min at 53 – 60°C , and 1 min at 72°C . A final elongation step of 10 min at 72°C ended the cycle. Locus specific annealing temperatures are provided in Table 1. Microsatellite alleles were visualized on acrylamide gels using a LICOR 4200 DNA sequencer and scored using Gene Image IR version 3.55 (LICOR Co., Lincoln, NE, USA).

Data analysis

Summary statistics for microsatellite loci including observed heterozygosity were generated using MSANALYZER version 3.01 (Dieringer and Schlotterer, 2003). Genotypic frequencies were tested for predictions of Hardy–Weinberg equilibrium and tests of linkage disequilibrium were conducted using randomization tests implemented in GENEPOP version 3.3 (Raymond and Rousset, 1995). Statistical significance was adjusted using sequential Bonferroni procedures (Rice, 1989) for all multiple tests.

MSANALYZER version 3.01 (Dieringer and Schlotterer, 2003) was used to generate fixation indices and genetic distances as a measure of pairwise genetic divergence among sampling units using the F_{ST} estimate (Weir and Cockerham, 1984) and the Nei D_A distance measure (Nei et al., 1983). A neighbor-joining tree (Saitou and Nei, 1987) was generated using PAUP version 4.01 software (Swofford, 1998). Support for tree branching was obtained by bootstrapping using the program DISPAN (Ota, 1993).

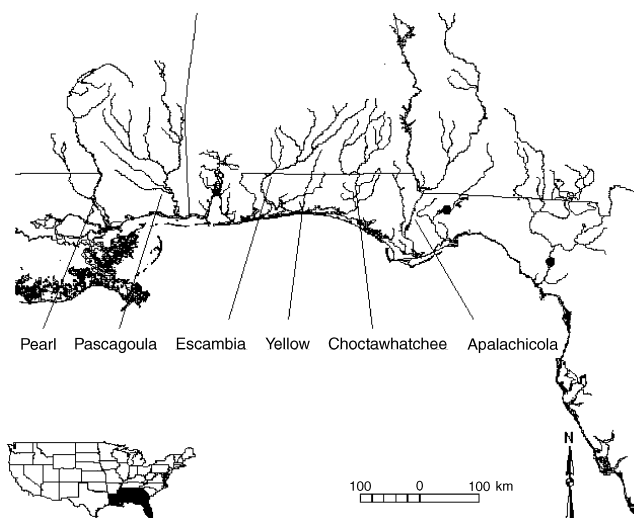


Fig. 2. The central coast of the northern Gulf of Mexico showing river drainages where tissue samples were obtained. The Ocklocknee and Suwannee Rivers, not included in this study, are indicated by closed circles

significant. Population level testing indicated that the Pascagoula sampling unit was the only group to exhibit linkage disequilibria, testing significant in the same 17 of 28 tests that were globally significant.

Drainage level patterns of population structure

All pairwise F_{ST} estimates were statistically significant ($P < 0.002$) at the drainage level (Table 3). Pairwise F_{ST} estimates among Florida sampling units (Escambia, Yellow, Choctawhatchee and Apalachicola) (0.063–0.101) were higher than those observed between the Pearl and Pascagoula (0.033). Across all sampling units, the highest F_{ST} estimates were observed in pairs that spanned the Mobile River Basin (0.131–0.224). A neighbor-joining tree of D_A distance (Fig. 3) indicates that each drainage level sampling unit formed a distinct group, although there was low bootstrap support for the Florida sampling units possibly due to the low sample sizes for some of these drainages. The split between the Pearl/Pascagoula and the Florida sampling units was strongly supported with a bootstrap value of 100%.

Assignment tests for single location captures

Overall ‘direct’ assignment rates to the drainage of capture (reported as the mean value across four test measures) ranged from 66.7% in the Escambia sampling unit to 95.6% in the Pearl sampling unit (Table 4). Out of 1269 individual assignments (324 fish \times four tests; frequency, Bayesian, D_C , and D_A), there were 196 total assignments outside of the drainage of capture and only 22 that resulted in an assignment to a drainage on the opposite side of the Mobile River Basin relative to drainage of capture. The observed assignment rates to drainages of capture are consistent with the degree of genetic structure observed in the statistically significant pairwise F_{ST} values. The lower rates of assignment observed in the Escambia and Apalachicola sampling groups may be due to limited sample sizes within those drainages.

Non-natal occupancy occurred in all river drainages according to concordant assignments across test measures. Among the Florida River drainages four individuals were identified as probable immigrants with support for natal drainage. There were no assignments among Florida drainages of samples from the Pearl and Pascagoula rivers. Among the Pearl and Pascagoula samples, three samples congruently assigned to Florida River drainages. Considerable interaction between the Pearl and Pascagoula was indicated, with interdrainage movement of genetically typed Pearl Gulf sturgeon in the Pascagoula holding area exceeding the occurrence of genetically typed Pascagoula Gulf sturgeon in the Pearl River. One sample in the Pearl sampling unit was concordantly identified as Pascagoula, while 21 samples in the Pascagoula group were concordantly identified as Pearl.

Genetic assignment of Pascagoula ‘unknowns’

Assignment analysis of the four Gulf sturgeon captured in multiple holding areas, including the Pascagoula (Fig. 4), indicated two fish were natal to the Pascagoula. All six Gulf sturgeon that were captured and tagged in the Pascagoula River drainage (rkm 0) and later captured or relocated within the Pearl River drainage were genetically assigned to the Pearl drainage (Table 5). The three juveniles captured at the mouth of the Pascagoula River drainage were concordantly assigned, two to the Pearl drainage and one to the Pascagoula.

Fine-scale population structure within the Pascagoula River drainage

Pairwise F_{ST} values did not differ between the Bouie and the Pascagoula sampling groups. To investigate this further, we included the Bouie sampling group in an assignment test using the four test methods, according to the simulation procedure.

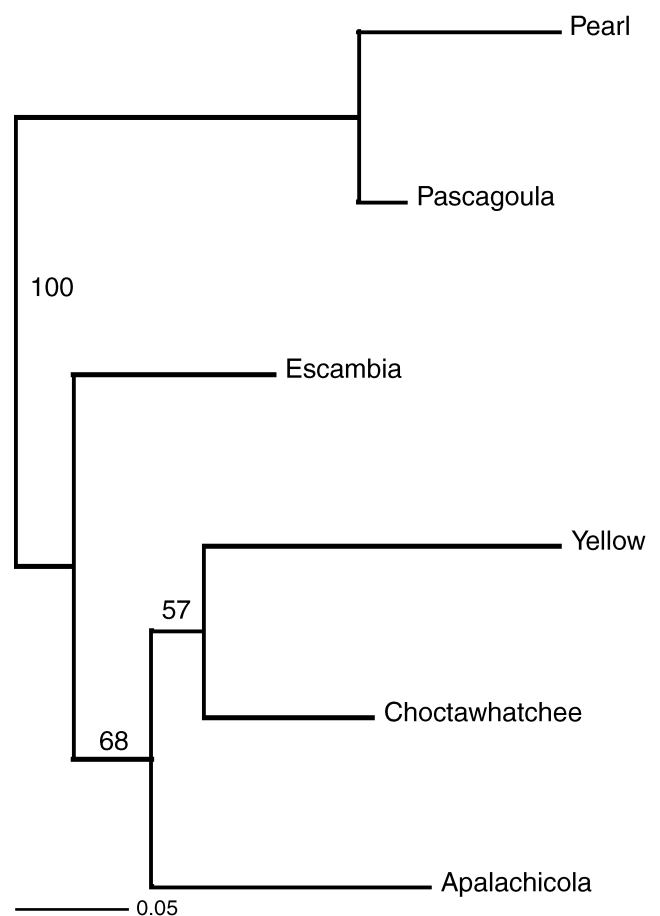


Fig. 3. Neighbor-joining tree (Saitou and Nei, 1987) for D_A genetic distance (Nei et al., 1983) of Gulf sturgeon using eight microsatellite loci. Numbers indicate bootstrap support, bar indicates level of divergence

Table 3
Pairwise F_{ST} (Weir and Cockerham, 1984) values below diagonal line and pairwise Nei D_A (Nei et al., 1983) genetic distance above diagonal line

	Pearl	Pascagoula	Escambia	Yellow	Choctawhatchee	Apalachicola
Pearl	–	0.112	0.348	0.452	0.479	0.410
Pascagoula	0.033*	–	0.305	0.388	0.378	0.339
Escambia	0.147*	0.133*	–	0.303	0.211	0.273
Yellow	0.176*	0.149*	0.070*	–	0.236	0.331
Choctawhatchee	0.224*	0.186*	0.063*	0.076*	–	0.204
Apalachicola	0.156*	0.131*	0.074*	0.101*	0.075*	–

*Adjacent to F_{ST} values indicates statistically significant after Bonferroni correction ($P < 0.002$).

Overall % correct (n = 324)	Bayesian	Frequency	D_C	Nei D_A	Mean by population
	83.3%	82.5%	80.6%	82.2%	
By population group (%)					
Pascagoula (n = 144)	80.6	80.6	75.0	77.1	78.3
Pearl (n = 90)	96.7	94.4	94.4	96.7	95.6
Escambia (n = 12)	66.7	66.7	66.7	66.7	66.7
Yellow (n = 20)	90.0	85.0	75.0	80.0	82.5
Choctawhatchee (n = 45)	88.9	91.1	95.6	95.6	92.8
Apalachicola (n = 13)	76.9	76.9	76.9	76.9	76.9

Sample sizes are in parentheses.

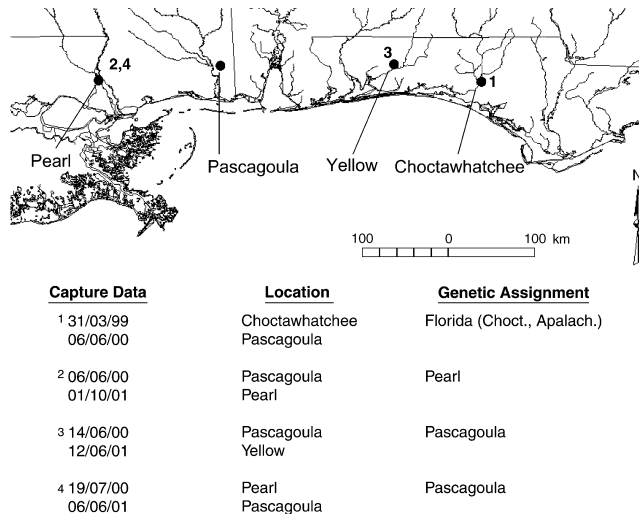


Fig. 4. Map indicating the interdrainage movement of Gulf sturgeon captured in the Pascagoula holding area that were also captured in other drainages. The table indicates the date of capture for each location and the genetic assignment according to the concordant results for the frequency, Bayesian, D_C , and D_A genetic distance test measures

Drainage level assignments scores were unchanged with the inclusion of the Bouie sampling group; however, 27 individuals within the Pascagoula were concordantly assigned into the Bouie group with 99% or greater likelihood. Most individuals in the Bouie assign into the Bouie or Pascagoula groups, although evidence of interdrainage gene flow is suggested by the genetic assignment of three individuals from the Bouie River spawning site to drainages outside of the Pascagoula River. One fish was genetically assigned as a Choctawhatchee fish and two fish were assigned as Pearl fish.

Capture data					
Id no.	Date (day/month/year)	Location	Fragment length (cm)	Weight (kg)	Genetic assignment
036-284-518	22/3/2001 19/4/2001	West Pascagoula Pearl-Rigolets	148 -	36.29 -	Pearl
035-808-114	21/2/2003 9/5/2003	West Pascagoula Pearl-Rigolets	127 -	21.32 -	Pearl
035-807-797	13/3/2003 28/3/2003	West Pascagoula Pearl-Bogue Chitto	156.8 -	45.36 -	Pearl
036-275-353	27/3/2003 10/6/2003	West Pascagoula Pearl-Bogue Chitto	125 125	19 16.3	Pearl
043-584-570	28/3/2003 20/5/2003	West Pascagoula Pearl-Bogue Chitto	113 -	14.5 -	Pearl
036-299-814	7/3/2003 20/6/2003	West Pascagoula Pearl-Bogue Chitto	190 -	58.5 -	Pearl

Table 4
Assignment rates to the drainage of capture using the 'direct' method implemented in GENECLASS version 1.0.02 (Cornuet et al., 1999), according to four test measures

We calculated *post hoc* pairwise F_{ST} estimates between the Pascagoula, Bouie, and Pearl sampling groups to refine our estimates of genetic structure within the Pascagoula River drainage. Using an approach similar to Vazquez-Dominguez et al. (2001), individuals were gleaned and relocated from the Pascagoula, Bouie, and Pearl sampling groups on the basis of their initial assignments. The three sampling groups were modified as follows: (A) the three samples from the Pearl and Pascagoula groups that assigned as Florida fish were excluded from the *post hoc* analysis; (B) the 21 samples in the Pascagoula that assigned as Pearl fish were removed from the Pascagoula and moved to the Pearl sampling group; (C) the one sample in the Pearl sampling group that classified as a Pascagoula fish was removed from the Pearl and added to the Pascagoula sampling unit; and (D) the three fish from the Bouie River sampling group that assigned outside of the Pascagoula drainage were excluded from the *post hoc* analysis. Twenty-seven fish originally in the Pascagoula sampling unit that concordantly assigned as a Bouie fish were moved from the Pascagoula to the Bouie sampling group. Sample sizes for the three *post hoc* groups were: Pascagoula (n = 95); Bouie (n = 48); Pearl (n = 109). *Post hoc* F_{ST} estimates were all statistically significant and suggested higher levels of divergence among groups than originally estimated (Table 6). Relationships of affinity remained with the Pearl that showed greater divergence from the Bouie group than from the Pascagoula sampling group. Slight levels of divergence were observed between the Bouie and Pascagoula sampling units (F_{ST} 0.017).

Discussion

Drainage level patterns of microsatellite variation in Gulf sturgeon

Observed patterns of nuclear microsatellite variation suggest that Gulf sturgeon exhibit population structure at the drainage

Table 5
Concordant assignments based on Bayesian, frequency, D_C and D_A test measures for six Gulf sturgeon initially captured at rkm 0 of the Pascagoula River drainage and later relocated in the Pearl River drainage

Table 6
Pairwise F_{ST} values including the Bouie River spawning site, the Pascagoula sampling group and the Pearl sampling group

	Bouie	Pascagoula	Pearl
Bouie	–	–0.0008	0.035*
Pascagoula	0.017*	–	0.033*
Pearl	0.055*	0.046*	–

Values below diagonal line are *post hoc* values.

*Indicates statistically significant.

level. The larger sample sizes and hypervariable nature of microsatellite markers used in our study were consistent with patterns of stock structure previously reported for Gulf sturgeon (Stabile et al., 1996; Waldman and Wirgin, 1998; T. King, pers. comm.) and further resolved drainage level differentiation across all drainages sampled, including differentiation between the Pearl and Pascagoula drainages. Although Gulf sturgeon populations are structured at the drainage level, results of our study reinforce regional groupings suggested by mtDNA. The Pearl and Pascagoula drainages form a western group, having much closer relationship among themselves than with any other drainages.

Non-natal occupancy of riverine habitat

The occurrence of interdrainage movement in Gulf sturgeon, and its implications for delineating population structure are important issues with potential conservation implications. However, the only field method that would detect natal drainage for captured Gulf sturgeon is the capture of juveniles in upstream riverine reaches nearest spawning sites or the capture of adults that were originally tagged as juveniles in these same upstream reaches. To date, this has rarely been accomplished so that when a Gulf sturgeon is captured there is usually no positive way to determine the natal drainage. Researchers generally assume that the natal drainage of captured sturgeon is the same as the drainage of capture, but when an individual fish is captured in multiple drainages across years, the obvious question of natal source arises. Intensive field surveys throughout the northern Gulf of Mexico have documented interdrainage movement between the Suwannee and Apalachicola drainages (Sulak and Clugston, 1999), the Choctawhatchee and Yellow (Fox et al., 2002), the Yellow and Choctawhatchee (Craft et al., 2001), the Pascagoula and Pearl (Ross et al., 2004), the Choctawhatchee and Pascagoula, and the Pascagoula and Yellow (Ross et al., 2004). Of all field-verified interdrainage movements, only two have spanned the Mobile Basin.

Our work demonstrates the utility of molecular techniques in supplementing field data for determining the natal origin of captured Gulf sturgeon. Assignment tests provided confident identification of four individuals captured in multiple holding areas, including the Pascagoula drainage. Furthermore, we demonstrated the ability of molecular analyses to identify likely non-natal individuals for individuals captured only once. Interestingly, we learned after this analysis that one fish genetically identified in this study as natal to the Choctawhatchee but captured only in the Apalachicola, was an individual later captured in the Choctawhatchee. This represents the only Gulf sturgeon field-verified to move between these two drainages (F. Parauka, 2003, pers. comm.). Trends in genetic assignment data mirror field-verified interdrainage move-

ments, primarily occurring within regions east and west of the Mobile Basin. Out of 361 tissue samples analyzed in this study, only six were identified as trans-Mobile Basin migrants, two field-verified individuals and four Gulf sturgeon captured once.

Fine-scale population structure within the Pascagoula River drainage

The majority of non-natal Gulf sturgeon documented within the Pascagoula River were genetically identified to the Pearl River, indicating unequal movement between the two drainages, with Pearl fish showing exceedingly higher rates of occupation in the Pascagoula holding area. In fact, the rates of assignment to the drainage of capture were highest for the Pearl group across the four tests methods.

The occurrence of two genetically identified Pearl River Gulf sturgeon at the Bouie River spawning ground suggests the potential for gene flow between the Pearl and Pascagoula drainages. It is unknown how many natal Pearl Gulf sturgeon captured in the Pascagoula holding area contribute to gene flow, but we believe the frequency of these individuals in the holding area exceeds actual genetic exchange. If 14% of the genetic source in the Pascagoula drainage originated from the Pearl drainage, we suspect that pairwise F_{ST} estimates between the two drainages would be lower than observed. Alternatively, we suggest that the majority of natal Pearl drainage Gulf sturgeon found within the Pascagoula drainage are using the river for summer staging and that initial estimates of F_{ST} are lower than actual, as noted in the *post hoc* test.

Site fidelity to the Bouie River spawning site has been verified in eight Gulf sturgeon via radio telemetry and captures. Because the Bouie River is the only verified spawning site within the Pascagoula River drainage (Heise et al., 2004), the statistically non-significant F_{ST} initially observed between the Pascagoula and Bouie sampling groups would be expected. The holding area is the portion of the river where Gulf sturgeon spend the majority of their time while in fresh water and is occupied in summer months by sub-adults, non-ripe adults and spent adults that spawned in the spring.

Using one round of iterations to filter genetically Bouie-typed fish from the Pascagoula holding area resulted in a statistically significant F_{ST} estimate in the *post hoc* test. We interpret this as evidence for additional genetic sources located within the Pascagoula River drainage. Field data indicate that spawning is likely occurring in the Chickasawhay River system of the Pascagoula River drainage. Out of 31 Gulf sturgeon tagged during the onset of the spring migration at the West Pascagoula (2000–2003), six individuals were relocated via radio signal in the upper Chickasawhay River (~320–355 rkm above the mouth of the Pascagoula River drainage) (Fig. 5) in reaches containing habitat characteristics of Gulf sturgeon spawning habitat in other regions (Sulak and Clugston, 1999; Fox et al., 2000; Craft et al., 2001; Heise et al., 2004). However, despite intensive field efforts in the upper segments of the Chickasawhay River system, positive documentation of spawning via egg collections is lacking.

Larger scale evolutionary picture and conservation implications

Data presented herein supports the previously reported hypothesis of population structure mediated through homing fidelity (Stabile et al., 1996; Waldman and Wirgin, 1998). We further advance the hypothesis that additional factors inclu-

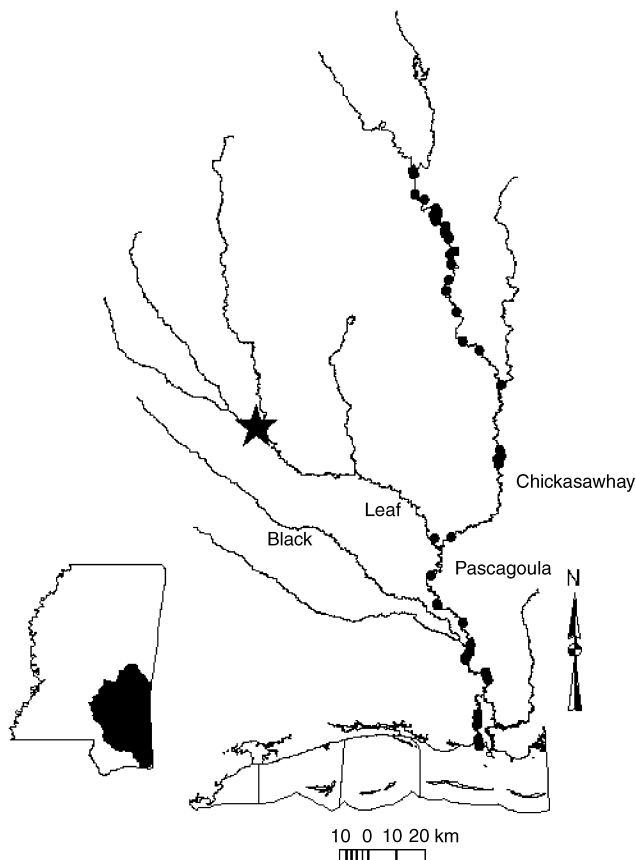


Fig. 5. Map of the Pascagoula River drainage illustrating the combined radio telemetry locations (black dots) for six Gulf sturgeon captured at the mouth of the Pascagoula River drainage and that migrated to the upper portions of the Chickasawhay River system during the spring spawning season. The star denotes Bouie River spawning location

ding coastal outline and marine habitat use may influence Gulf sturgeon genetic structure, notably at the regional level. The Pearl and Pascagoula are the only two drainages between the Mississippi and Mobile basins known to support populations of Gulf sturgeon. Sonic telemetry data indicate that Gulf sturgeon from the Pascagoula and Pearl rivers appear to equally occupy the barrier island passes of the Louisiana, Mississippi and Alabama Gulf coasts during wintertime marine habitat periods (Ross et al., 2003). The majority of this habitat is centrally located south of the Pascagoula River drainage. Gulf sturgeon natal to the Pearl River move westerly on their return migration, passing the Pascagoula River estuary. Consequently, the geographic locations of barrier island habitats may influence the rate to which Pearl River Gulf sturgeon encounter the Pascagoula River drainage during the onset of the spring migration. Preliminary support of this hypothesis can be found in the genetic assignment as natal Pearl fish for the six Gulf sturgeon that were originally captured at rkm 0 of the West Pascagoula and later relocated in the Pearl River. These fish represent the only fish tagged by our group that were never relocated in the Pascagoula River proper, post-capture. Additionally, the genetic assignment of three juvenile Gulf sturgeon captured in the Pascagoula estuary further suggests that the Pascagoula estuary is important for juvenile Pascagoula Gulf sturgeon as well as Pearl Gulf sturgeon. The habitat sharing of natal Pearl and natal Pascagoula Gulf sturgeon observed by the integration of

molecular and field data further indicates that the protection of these non-spawning areas benefits two genetically distinct entities.

Fine-scale patterns of population structure observed in our study indicate that Gulf sturgeon recovery requires conservation strategies minimally focused at the drainage level. Multilocus microsatellite genotypes for Gulf sturgeon are identifiable to drainages and possibly to the intradrainage level, as noted between the Bouie and potential Chickasawhay sources of the Pascagoula River. While we do not suggest that the Bouie and Chickasawhay rivers are completely isolated in terms of gene flow, our analysis suggests that the genetic composition of sturgeon genetically identified as Bouie fish cannot account for the total genetic diversity observed within the Pascagoula River drainage. Areas of upstream movement verified for Gulf sturgeon in the Chickasawhay are geographically isolated from the Bouie River spawning site by about 350 rkm. Fine-scale patterns of population structure apparent within the Pascagoula River drainage stress the need to protect riverine habitat in both the Bouie River and the Chickasawhay River. The resolution of intradrainage population structure is an unexplored concept among sturgeon studies but it is this scale of resolution that must be considered on a drainage-by-drainage basis before recovery plans can be completely comprehensive, particularly before stock enhancement programs are considered (Waldman and Wirgin, 1998).

To better facilitate the recovery of Gulf sturgeon throughout their range, we support additional fine-scale genetic studies using larger sample sizes and highly variable markers in concert with telemetry work. This would further delineate patterns of genetic population structure in other drainages known to support populations of Gulf sturgeon. It will be interesting to test the hypothesis of coastal outlines and marine habitat use as a secondary factor regulating regional associations thus far outlined through molecular analyses. Our data suggest that broad protection plans of habitats in western Florida will do little to benefit Gulf sturgeon of the Pascagoula and Pearl rivers, although they may benefit multiple genetic stocks in the eastern region.

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