

5-2007

Spawning Chronology of Native and Introduced Striped Bass (*Morone saxatilis*) in Two Gulf of Mexico Drainages

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SPAWNING CHRONOLOGY OF NATIVE AND INTRODUCED
STRIPED BASS (*Morone saxatilis*) IN TWO GULF OF
MEXICO DRAINAGES

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Fisheries and Wildlife Biology

by
Matthew Alexander Noad
May 2007

Accepted by:
Dr. J. Jeff Isely, Committee Chair
Dr. Arnold Eversole
Dr. Shawn Young

ABSTRACT

In this study, I investigated the potential for temperature to serve as a mechanism for maintenance of reproductive isolation in a sympatric population of Atlantic and Gulf striped bass in the Apalachicola and Ochlockonee rivers, Florida. Adult striped bass (n= 66, mean weight = 8.3 kg, mean total length = 780.6 mm) were collected from February to May below Jim Woodruff Lock and Dam, on the Apalachicola River, and below Jackson Bluff Lock on the Ochlockonee River, Florida. Ancestry (Gulf or Atlantic) of specimens was determined based upon the methods of Wirgin et al. (2005) using three diagnostic microsatellite loci: *SB 20*, *SB 1021* and *SB 111*. There was no difference between the mean date of arrival for female striped bass between strains within years. The mean date of first arrival for either strain of striped bass differed between 2003 and 2005. A comparison of the mean arrival temperature for Atlantic and Gulf striped bass also showed no difference within years. It is possible that the genetic introgression of Atlantic alleles has been so great that any difference in spawning temperature preference between strains that might have existed historically has been lost.

DEDICATION

I especially thank my mother, Jane. Without her everlasting love, support and encouragement, none of this would have been possible.

ACKNOWLEDGMENTS

To the people who truly made this possible, I thank you, sincerely, for your insight and friendship. I would like to thank Patrick Ely, Amy Garris and Tim Grabowski for their field assistance. I appreciate all of the efforts of the Georgia Department of Natural Resources, namely Ramon Martin, Travis Ingram and Craig Robbins. Karen Herrington of the U.S. Fish and Wildlife Service, and Rick Long of the Florida Fish and Wildlife Conservation Commission were instrumental in the successful execution of this project. I am grateful for the time and efforts of Bill Wayman and Krista Storey from U.S. Fish and Wildlife Service Warm Springs National Fish Hatchery, who assisted in the genetics portion of the study. A special thanks goes out to the great people of Chattahoochee, Florida—I am forever grateful for your insight and friendships. The South Carolina Cooperative Fish and Wildlife Research Unit provided important logistical support to this project, especially Carolyn Wakefield. I cannot forget my sisters, Melissa and Meaghan, as well as the rest of my immediate family—including pets—who have incessantly understood and embraced my nomadic lifestyle for the last several years. I thank all of my striped bass patients for allowing me to delve into their private cellular histories and gain knowledge that will hopefully preserve their existence in the southeast United States. Finally, I thank my committee members: Drs Arnold Eversole and Shawn Young for their assistance. Finally, I thank my advisor, Dr. Jeff Isely for his guidance and support.

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INTRODUCTION

Gulf populations of the striped bass *Morone saxatilis* once inhabited most of the rivers draining into the Gulf of Mexico from the Suwannee River, Florida, to Lake Pontchartrain, Louisiana (Raney and Woolcott 1955). They were thought to be separated from Atlantic populations by the emergence of the Florida peninsula approximately 14,000 years ago (Barkuloo 1970; Wooley 1985). In the face of widespread dam construction, fishing pressure and excessive use of agricultural chemicals, populations of Gulf striped bass were nearly extirpated by the mid 1960s (Wooley and Crateau 1983; Wirgin et al. 1991).

With the exception of a few remnant populations, the Apalachicola-Chattahoochee-Flint (ACF) River system supports the last naturally reproducing population (Wooley and Crateau 1983; Van Den Avyle and Evans 1990). Beginning in 1966, in an attempt to mitigate population declines, hatchery-reared Atlantic striped bass from the Santee-Cooper River system were introduced into the ACF (Wooley and Crateau, 1983; Fruge 2005). Since 1976, all fingerlings released in the system have been the progeny of ACF fish (Van Den Avyle and Evans 1990). By 1981, Wooley and Crateau (1983) estimated that the proportion of Atlantic striped bass in the Apalachicola River (> 381 mm TL) was 51%, Gulf striped bass 43% and intergrades 6%. Since the early 1980s, conservation focus has shifted from increasing striped bass biomass to the restoration of Gulf striped bass populations (Wirgin et al. 1991; Baker and Jennings 2005). The ACF has been used as a gene bank for recovery efforts, and although natural reproduction

may be low, the ability to artificially stock the ACF with hatchery-reared Gulf striped bass has been successful. Although it is unlikely that pure Gulf striped bass exist today, conservation agencies believe that introgression of Atlantic genes can be minimized by selecting broodfish with genes diagnostic of Gulf ancestry.

Empirical data from across multiple taxa demonstrate that northern populations of fishes spawn at cooler temperatures than southern ones (Isely et al. 1987; Coombs et al. 2006). Conover (1990), however, observed no difference in the peak spawning temperature of striped bass populations on the Atlantic coast. This trend has not been quantified in striped bass inhabiting Gulf of Mexico drainages. In this study, I investigated the potential for temperature to serve as a mechanism for maintenance of reproductive isolation in a sympatric population of Atlantic and Gulf striped bass. Specifically, I evaluated the effect of temperature and date on the arrival of female Atlantic and Gulf striped bass in the Apalachicola and Ochlockonee rivers, Florida.

STUDY AREA

This study was conducted in two locations. The first was below Jim Woodruff Lock and Dam (JWLD) on the Apalachicola River, Florida (Figure 1). This dam is situated at the confluence of the Flint and Chattahoochee rivers and forms Lake Seminole. The Apalachicola River then flows for approximately 172 km south into the Gulf of Mexico (Van Den Avyle and Evans 1990). JWLD is a hydroelectric facility that is operated by the U.S. Army Corps of Engineers (USACE).

The second location is below Jackson Bluff Dam (JBD) on the Ochlockonee River, Florida (Figure 1). The JBD restricts Gulf-bound flow of the Little River and upper portion of the Ochlochonee River and forms Lake Talquin (Turner et al. 1983). The JBD is a hydroelectric facility operated by the USACE. The outflow from the dam flows unobstructed into the Gulf of Mexico.

Both JWLD and JBD represent the first barriers to upstream migration. Adult striped bass migrate to these dams in the spring, making them susceptible to capture.



Figure 1. The study area consisting of the Apalachicola River, Florida, below Jim Woodruff Lock and Dam and the lower Ochlockonee River, Florida, below Jackson Bluff Dam.

METHODS

Adult striped bass ($n=66$, mean weight = 8.3 kg, mean total length = 780.6 mm) were collected from February to May below JWLD, on the Apalachicola River, and below JBL on the Ochlockonee River, Florida. Fish were sampled in the Ochlockonee River in 2005, because flooding in the Apalachicola River made collection difficult. Water temperature for the Aucilla River at Nutall Rise, Florida was substituted in 2005 as Ochlockonee River temperatures were unavailable. I captured most fish using a boat-mounted electrofisher; however, three fish were collected using hook and line in 2006. Prior to examination, fish were anesthetized using tricaine methanesulfonate (MS-222). Fish were then weighed (g), measured (mm total length) and identified to sex and reproductive stage. Determination of sex and reproductive stage was accomplished by direct observation of reproductive products. To accomplish this, a small hollow glass probe was inserted into the urogenital opening of the fish. Mild suction allowed a small sample of seminal fluid or eggs to be collected. Eggs were examined under 15-X magnification using a photographic slide magnifying eye-piece. Developmental stage of females was quantified using the methods described by Harrell et al. (1990). Females were categorized as being in either pre-spawning, spawning or post-spawning condition. A small (1 cm^2) sample of the caudal fin was collected, placed in a 2-mm centrifuge tube and preserved with 95% ethanol for later genetic analyses.

Ancestry (Gulf or Atlantic) of specimens was determined based upon the methods of Wirgin et al. (2005) using three diagnostic microsatellite loci: *SB 20*, *SB 1021* and *SB 111*. To accomplish this, DNA from preserved samples was isolated using a DNA isolation kit (Dneasy; Qaigen Science, Maryland, USA). Briefly, the procedure involves incubating samples in a buffer solution, digesting samples using a proteinase, and extracting and precipitating the DNA using a solvent and precipitant. A polymerase chain reaction (PCR) -based assay (Dneasy; Qaigen Science, Maryland, USA) was used to amplify diagnostic microsatellite loci using appropriately-labeled forward and reverse primers supplied by the test-kit manufacturer (GeneAmp PCR system 9700; Applied Biosystems, Foster City, California, USA). Each PCR product was compared against a size standard for genotyping using a genetic analyzer (Hitachi 3130 ABI PRISM 3130 Genetic Analyzer; Applied Biosystems; Foster City, California, USA). Output files were analyzed and genotypes for each individual striped bass were determined. Based upon the six possible genotypes, fish that had 4 or more Gulf alleles were considered striped bass of Gulf origin and fish that had 3 or fewer gulf alleles were considered to be of Atlantic origin.

All analyses were performed using SAS (SAS Institute, Cary, North Carolina). Differences in mean date of arrival and mean water temperature of arrival, and analysis of egg development stage with respect to water temperature for Atlantic and Gulf strain striped bass were evaluated using analysis of variance (ANOVA). Distribution of Gulf allele frequencies between sites was evaluated

using a Chi-square test. In order to compensate for a small sample size in each year group, Gulf allele scores of 0-2 and 5-6 were pooled.

RESULTS

The total number of actively reproductive females captured ranged among years from 17 to 32 (Table 1). No female striped bass were captured before water temperatures reached 17.0°C (Figure 2). There was no difference between the mean date of arrival for female ($F = 0.01$, $df = 1$, $P = 0.9076$) striped bass between strains. The mean date of first arrival for either strain of striped bass differed between 2003 and 2005 ($P = 0.0057$). A comparison of the mean arrival temperature for Atlantic and Gulf striped bass also showed no difference within years ($F = 2.15$, $df = 2$, $P = 0.1273$). Analysis of the relationship between egg developmental stage and water temperature revealed no difference between ($P = 0.3941$) strains, but was different within strains ($F = 6.46$, $df = 1$, $P = 0.0143$; Figure 3). The model:

$$\text{water temperature} = 18.823 + 0.625 (\text{egg stage})$$

accounted for 11% of the variance. The distribution of Gulf and Atlantic strain allele frequencies were not different ($\chi^2 = 5.90$, $df = 3$, $P = 0.1165$; Table 2). One fish was captured that did not exhibit any diagnostic Gulf alleles (Table 1).

Table 1. Total number of Gulf and Atlantic strain striped bass captured below Jim Woodruff Lock and Dam, Apalachicola River, Florida, and the Ochlockonee River, Florida.

Site	Year	<i>N</i>		Total
		Gulf Females	Atlantic Females	
Apalachicola River	2003	8	9	17
	2006	8	9a	17
Ochlockonee River	2005	14	18	32

^a one individual exhibited no Gulf alleles

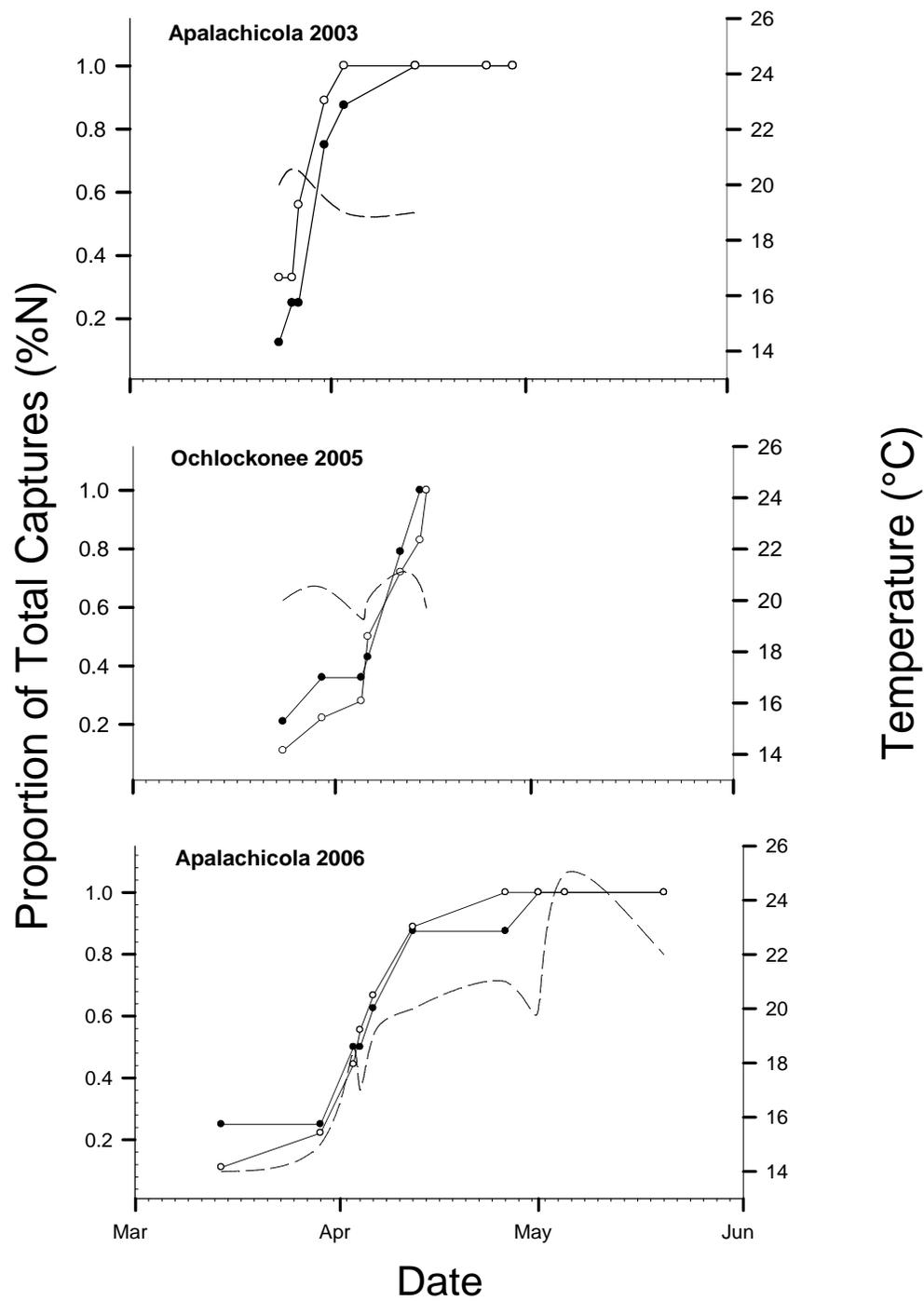


Figure 2. Proportion of total captures of Gulf (closed symbol) and Atlantic (open symbol) strain striped bass in the Apalachicola River, Florida and Ochlockonee River, Florida. Smooth dashed line denotes water temperatures during sampling

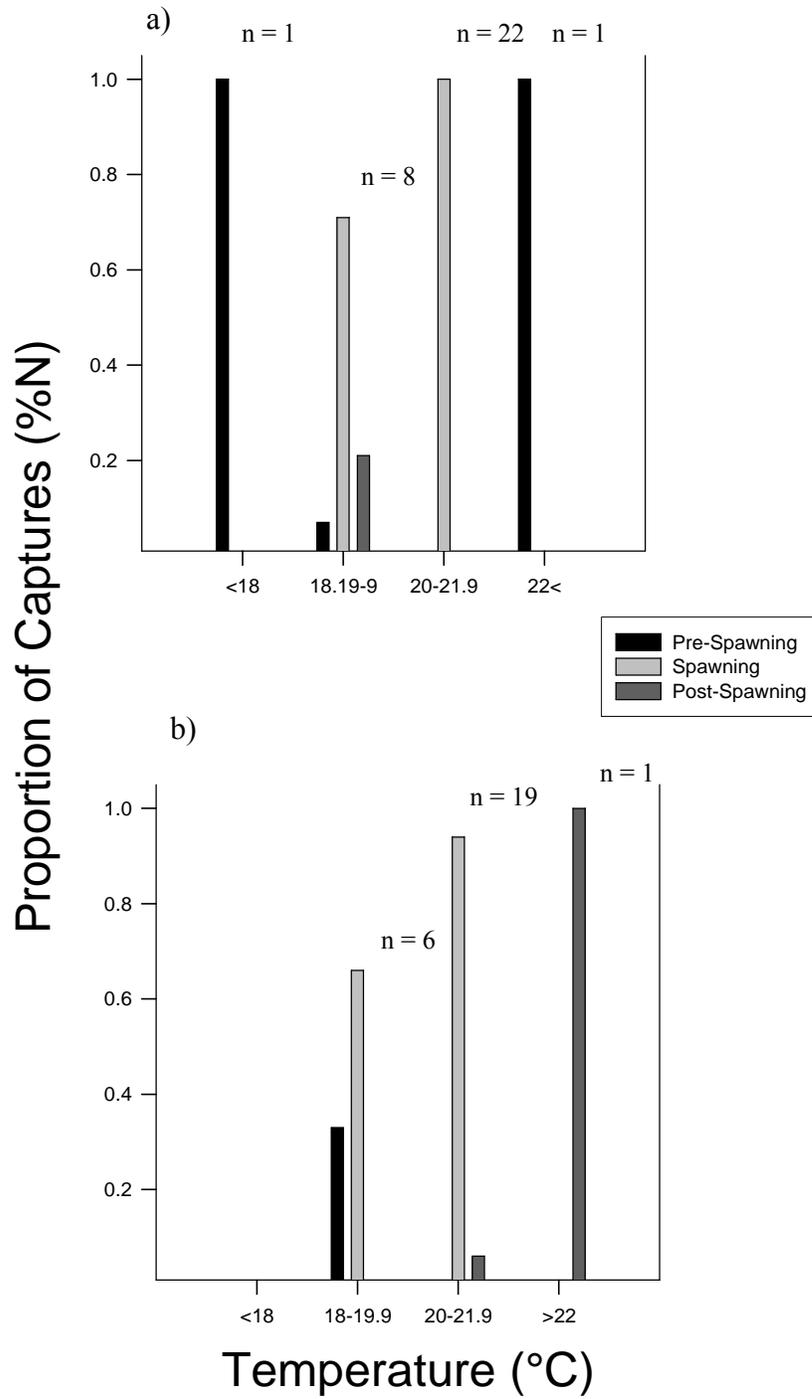


Figure 3. Proportion of female a) Atlantic strain and b) Gulf strain striped bass eggs from the Apalachicola River, Florida and Ochlockonee River, Florida at three developmental stages; pre-spawning (closed bars), spawning (light grey bars) or post-spawning (dark-grey bars), with respect to ambient water temperature.

Table 2. Frequency (%) of total number of Gulf Alleles in female broodfish captured on the Apalachicola River, Florida and Ochlockonee River, Florida.

River Site	Sample Year	Frequency of Gulf Alleles (%)						
		0	1	2	3	4	5	6
Apalachicola	2003	0	18	6	29	35	6	6
Ochlockonee	2005	0	3	9	44	22	19	3
Apalachicola	2006	7	7	18	24	32	12	0

DISCUSSION

The low catch of adult female striped bass per unit effort I observed is consistent with historic collections from the study areas (Van Den Avyle and Evans 1990). High harvest rates, low population size and inherent difficulties in capturing large fish all contributed (Mundy 1982; Collins et al. 2000).

Water temperatures during capture appeared similar to previous observations of striped bass preferences (Van Den Avyle and Evans 1990). Striped bass initiate their upstream spawning migration as water temperatures reach 15-19°C (Raney 1952; Coutant and Carroll 1980). Striped bass generally occupy a thermal niche approximately between 20-24°C. However, if these temperatures cannot be found during the spring, striped bass seek out the warmest possible waters (Coutant and Carroll 1980).

Studies by Isely et al. (1987) and Coombs et al. (2006) suggest that there may be differences in spawning chronologies of subspecies of fishes that evolved at different latitudes. Latitudinal differences in seasonal water temperature and day length were thought to promote earlier migration and subsequent spawning of fishes that evolved at northern latitudes. As Atlantic-strain striped bass evolved in environments where waters temperatures become favorable later and for a shorter period of time (Conover 1990), one might expect Atlantic strain striped bass to spawn at cooler temperatures where they occur in common with their Gulf-strain counterparts. However, this hypothesis was not supported by the present study. It is possible that the genetic introgression of Atlantic alleles has

been so great that any difference in spawning temperature preference between strains that might have existed historically has been lost. Consequences of stocking Atlantic strain striped bass has resulted in significant numbers of intermediate strains and has threatened the persistence of the native Gulf striped bass. This phenomenon has been reported in other fish species such as the Guadalupe bass *Micropterus guadalupe* of central Texas (Morizot et al. 1991) and cutthroat trout *Oncorhynchus clarki bouvieri* in Idaho (Henderson et al. 2000). In each case, the introduction of otherwise allopatric fish species has caused significant hybridization and diminished the genetic integrity of the native fishes.

The erection of Jim Woodruff Lock and Dam and Jackson Bluff Dam has inhibited natural spawning by striped bass in the Apalachicola and Ochlockonee rivers, respectively. Consequently, many of the striped bass inhabiting these waters are hatchery raised. Stocking programs, through their mandates to capture broodstock as early in the season as possible (Fruge 2005), may be artificially selecting for early spawning Atlantic-strain fish. Tipping and Busack (2005) showed that spawning timing is a heritable trait in coho salmon *O. kisutch* and that agencies who selected broodfish from the middle of the historical spawning period, led to a significant decrease in early and late spawning fish in subsequent years. In Gulf-strain striped bass, the homogenization of spawning stocks could be the reason that Atlantic-strain females no longer spawn at significantly earlier dates than Gulf-strain females.

The mean date of arrival for migrating fishes can vary within species (Brannon 1987; Beacham and Murray 1990). A study of striped bass in the

Roanoke River, North Carolina, observed similar inter-annual variability in mean spawning grounds arrival times. Studies indicate that arrival of females at spawning grounds is a function of a threshold related to the maturation of eggs (Carmichael et al. 1998). As spawning timing is heritable (Brannon 1987; Beacham and Murray 1990), variability in mean date of arrival is also a function of the time at which the parents of these fish spawned and differences in developmental temperatures.

Mean arrival temperature for Gulf and Atlantic-strain striped bass within years was not significantly different. These findings are similar to the homogeneity of peak spawning temperatures exhibited by striped bass and American shad *Alosa sapidissima* from various latitudes along the Atlantic coast (Conover 1990).

In each year, there were no differences in advancement of egg stage, within strains, with respect to increased water temperature. However, differences in development may not be significant until the fry stage or later. In several species of Pacific salmon *Oncorhynchus* spp., studies have demonstrated that fry emergence is based on life history and developmental temperatures. That is, salmon populations that are native to cool rivers, develop faster than salmon populations native to warmer rivers, when incubated at the same temperature. For example, fertilized eggs of chum salmon *O. keta* in Yukon, Canada, develop faster than comparable populations in lower British Columbia, Canada (Beacham et al. 1990). More experiments on the post-hatch rate of development should be conducted.

One sample containing undeveloped eggs was collected from a female striped bass in the Apalachicola River, on April 24, 2006 when water temperatures were $>22^{\circ}\text{C}$. This fish had only three diagnostically gulf alleles, so was considered to be of Atlantic origin. However, it was unlikely that this immature individual was preparing to spawn. In Atlantic striped bass it is thought that some immature females accompany adults upstream, but not onto the spawning grounds (Trent and Hassler 1968) As some populations of striped bass are riverine (Coutant 1985; Bjorgo et al. 2000), it is difficult to determine whether a fish has been a resident of the area or a recent immigrant. Future work should include the evaluation of the resident fish hypothesis.

The observation that the distribution of Gulf-allele frequencies did not differ between sites demonstrates that both the Ochlockonee and Apalachicola rivers are equally suitable areas for capturing broodfish for the purpose of protecting the genetic integrity of gulf-strain striped bass and maintaining population numbers.

The one individual that was captured without Gulf-alleles was probably not the offspring of two Atlantic parents, but more likely, the progeny of two intermediates and the result of random chance. This study demonstrates that maintaining the genetic integrity of the Gulf striped bass in these rivers will continue to be laborious, but nonetheless necessary to insure the persistence of the Gulf genotype in wild populations.

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