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ARTICLE

## Genetic Management of Black Sea Bass: Influence of Biogeographic Barriers on Population Structure

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### Abstract

The black sea bass *Centropomus striata* is a commercially important perciform fish with a general distribution along the U.S. Atlantic coast from Cape Cod, Massachusetts, to Cape Canaveral, Florida, and in the Gulf of Mexico from Mobile Bay, Alabama, to Tampa Bay, Florida. Currently, black sea bass are managed as three separate stocks: one in the Gulf of Mexico and two along the U.S. Atlantic coast. Fish from the Gulf of Mexico represent a separate subspecies, *C. striata melana*. The Atlantic subspecies, *C. striata striata*, is divided into two management units (separated at Cape Hatteras, North Carolina) based on the hypothesis that this subspecies comprises two distinct populations exhibiting life history and morphometric differences. To further investigate this differentiation, we employed mitochondrial sequence data to test whether genetic differences are observed among the three management units. The DNA sequence analysis revealed a significant amount of genetic variability partitioned among samples from the three management areas. Similar results were observed when the analyses were confined to the two Atlantic coast management units. These results support the designation of two distinct management units for black sea bass along the U.S. Atlantic coast.

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The black sea bass *Centropristis striata* is a commercially and recreationally important perciform fish that is distributed along the U.S. Atlantic coast and Gulf of Mexico coast (Miller 1959; Wenner et al. 1986; Vaughan et al. 1995). The largest concentrations of black sea bass are found from New Jersey to North Carolina, making this species a particularly important fishery resource in the mid-Atlantic region (Kendall and Mercer 1982). Black sea bass are demersal fish that prefer warm temperate waters and typically occupy structured benthic habitats, such as shellfish beds, rock and artificial reefs, and wrecks (Steimle et al. 1999). Black sea bass are important predators in live-bottom, shelf edge, and lower-shelf habitats, with prominent prey items including small fish, squid, and epibenthic invertebrates (e.g., crustaceans and mollusks; Steimle et al. 1999). Black sea bass are protogynous hermaphrodites, changing sex from female to male as they increase in size and age. As such, black sea bass stocks may be at particular risk to overfishing due to skews in sex ratio and fishing pressure size selectivity (Armsworth 2001; Alonzo and Mangel 2004).

There are three recognized stocks of black sea bass: one in the Gulf of Mexico and two along the U.S. Atlantic coast. Black sea bass from the Gulf of Mexico are considered to represent a separate subspecies, *C. striata melana*, and are managed independently from their Atlantic conspecifics, *C. striata striata* (Bowen and Avise 1990; Hood et al. 1994). Atlantic management units are split between the Middle Atlantic Bight (MAB; Cape Hatteras, North Carolina, to Cape Cod, Massachusetts) and the South Atlantic Bight (SAB; Cape Canaveral, Florida, to Cape Hatteras), with Cape Hatteras serving as the boundary between Atlantic stocks (Mercer 1978). This designation is supported by life history and morphometric studies and is based on differences in seasonal migrations, growth and maturity, and spawning times (Kendall 1972; Musick and Mercer 1977; Mercer 1978; Wenner et al. 1986; O'Brien et al. 1993; Collins et al. 1996; Steimle et al. 1999; McGovern et al. 2002).

Management of the Atlantic stocks is split between the Mid-Atlantic Fishery Management Council, which oversees the northern management unit, and the South Atlantic Fishery Management Council, which governs the southern stock. Separate fishery management plans have been developed for both stocks, and both councils conduct independent stock assessments. The most recent stock assessment for the northern unit suggests that the stock is not overfished, whereas the assessment for the southern stock indicates that overfishing is occurring (SAFMC 2006; Shepherd 2009). While genetic studies have been successful in documenting the differentiation between Gulf of Mexico and Atlantic coast conspecifics, their ability to support the differences in black sea bass north and south of Cape Hatteras has been less convincing (Bowen and Avise 1990; Chapman et al. 1999). Therefore, estimates of connectivity and exchange between northern and southern stocks are of particular importance and are critical for effective management.

In light of these data, we used a portion of the mitochondrial control region to test whether Cape Hatteras serves as

a geographic barrier to black sea bass along the U.S. Atlantic coast. Specifically, we examined whether genetic differentiation in samples collected north and south of Cape Hatteras showed results that are concordant with life history analyses and that support the two-stock hypothesis under which Atlantic coast black sea bass are currently managed. We examined gene flow and estimated individual exchange rates between northern and southern Atlantic stocks, and we included data from the Gulf of Mexico subspecies to examine within-region and between-region genetic differentiation.

## METHODS

**Sample acquisition, DNA extraction, polymerase chain reaction amplification, and sequencing.**—All samples were collected during fishing activities occurring within the 200-m isocline of the Atlantic Ocean and Gulf of Mexico along the coast of the United States. Heart tissue samples were collected during 1996 along the Atlantic coast (at  $\sim 27.7^\circ$ ,  $31.6^\circ$ ,  $32.3^\circ$  and  $39.5^\circ$ N) and from the Gulf of Mexico (at  $\sim 29.5^\circ$ N) and were preserved in sarkosyl-urea (Figure 1). Fin clip samples were collected during 2006 along the Atlantic coast (at  $\sim 27.7^\circ$ ,  $31.6^\circ$ ,  $32.3^\circ$ ,  $33.3^\circ$ ,  $34.3^\circ$ ,  $35.8^\circ$ ,  $36.9^\circ$ , and  $41.1^\circ$ N) and from the Gulf of Mexico (at  $\sim 29.5^\circ$ N) and were preserved in 100% ethanol (Figure 1). Heart tissue samples preserved in sarkosyl-urea were extracted using a standard phenol-chloroform-isoamyl protocol. Fifty microliters of preserved sample were added to 150  $\mu$ L of phenol-chloroform-isoamyl solution (Invitrogen Corp., Carlsbad, California), vortexed for 10 s, and centrifuged at  $15,000 \times$  gravity ( $g$ ) for 30 s. The aqueous top layer was transferred to a new microcentrifuge tube, 250  $\mu$ L of 100% ice-cold ethanol were added, and samples were incubated at  $-80^\circ$ C for 15 min. After incubation, the samples were centrifuged at  $15,000 \times g$  for 7.5 min and the supernatant was discarded. One milliliter of 70% ethanol was added in order to wash the pelleted DNA, and the samples were centrifuged a final time at  $15,000 \times g$  for 5 min. The supernatant was discarded, and samples were dried in a SpeedVac for 15 min. All centrifugation steps were performed at room temperature. Dried DNA pellets were reconstituted in 50  $\mu$ L of tris-EDTA buffer. The DNA from fin clip samples preserved in 100% ethanol was extracted using Qiagen DNeasy Tissue Kits (Qiagen, Inc., Valencia, California) in accordance with the instructions for animal tissue.

A portion of the mitochondrial control region was amplified from total DNA extracts using primers specifically designed for black sea bass (forward primer CstrCR-F2: 5'-GAACCAGATGCCAGGAATA-3'; reverse primer CstrCR-intR1: 5'-ATATCAGCATACATCTGTGTC-3'). Approximately 100 ng of DNA template were used in a 25- $\mu$ L reaction consisting of  $1 \times$  polymerase chain reaction (PCR) buffer, 2.5-mM  $MgCl_2$ , 0.2-mM deoxynucleotide triphosphate, 0.1 mM of each primer, double-distilled  $H_2O$ , and 0.25 units of *Taq* polymerase (Bio-Rad, Hercules, California). All PCRs were conducted using an Applied Biosystems 9700 GeneAmp PCR System

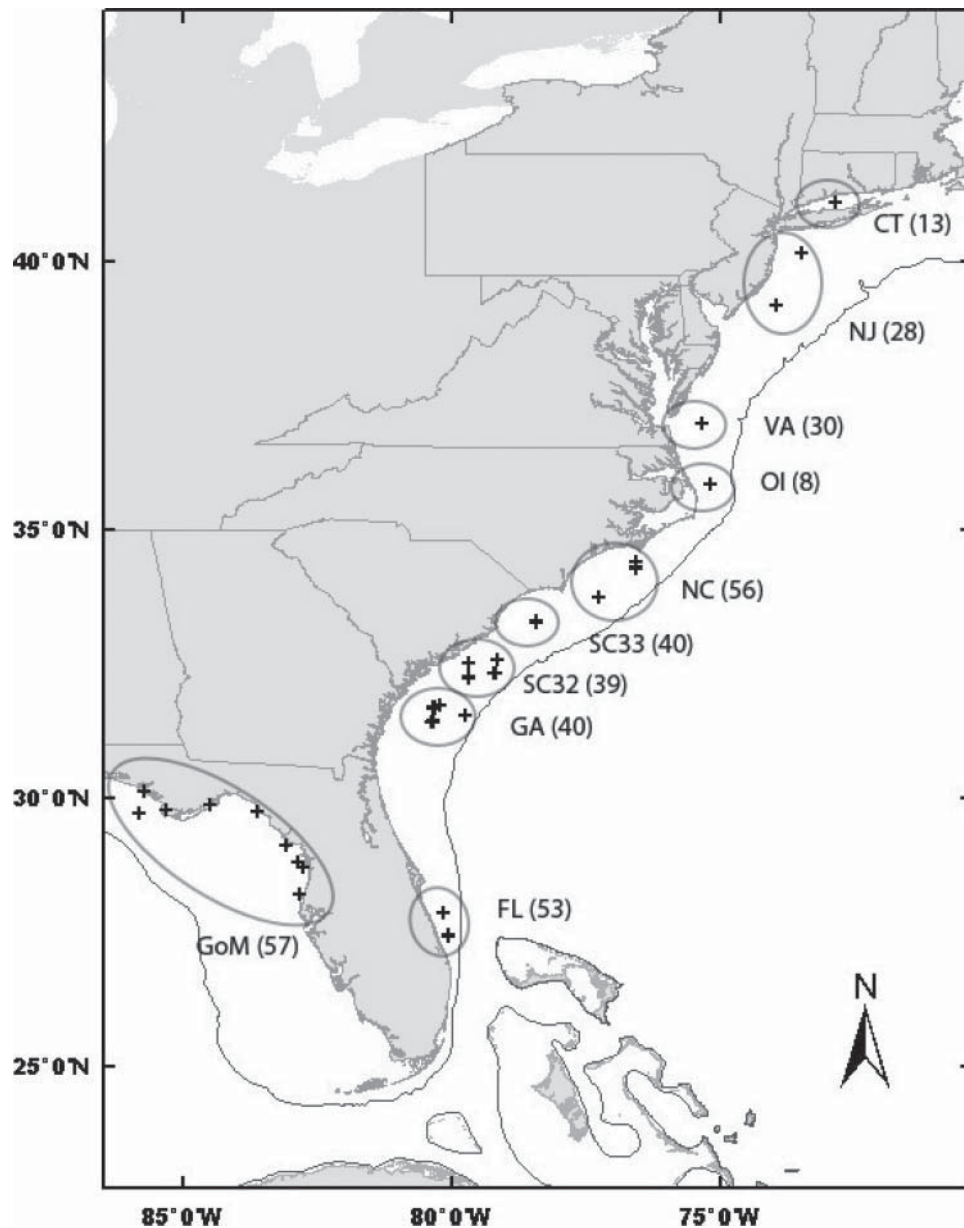


FIGURE 1. Map depicting the approximate sampling locations (“+” symbols) and population groupings (ovals) of black sea bass along the U.S. Atlantic coast and Gulf of Mexico coast relative to the 200-m isocline. Location codes are defined in Table 1. Sample size (in parentheses) is the total number of samples that were successfully sequenced and analyzed from each location.

(Applied Biosystems, Foster City, California) under the following conditions: 94°C for 5 min; 36 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min; and finishing with 72°C for 7 min.

The PCR amplification products were purified using the exonuclease I–shrimp alkaline phosphatase (ExoSAP) clean up protocol. Four microliters of PCR product were digested in 1 µL of a 3:1 shrimp alkaline phosphatase : exonuclease mix. One microliter of the PCR–ExoSAP product was used in cycle sequence reaction per the manufacturer’s instructions for BigDye Terminator version 3.1 Cycle Sequencing (Applied Biosystems) with

modifications. All samples were sequenced in both the forward and reverse directions in 10-µL reaction volumes using 1.6 pmol of the same primers for amplification and a 1:4 dilution of the dye terminators (1 µL). Cycle sequencing profile was as follows: 96°C for 30 s; and 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Sequencing reaction products were purified either by using Centrisept columns (Princeton Separations, Freehold, New Jersey) per the manufacturer’s instructions or by following a standard ethanol–EDTA–sodium acetate precipitation protocol.



**Sequence and population analyses.**—Sequences were initially edited in Sequencher version 4.7 (Gene Codes Corp., Ann Arbor, Michigan), and the nucleotide–nucleotide Basic Local Alignment Search Tool (blastn) was applied to the sequences to verify the region of DNA. Edited sequences were aligned by eye and imported into MEGA version 3.1 (Kumar et al. 2000). Neighbor-joining analyses using  $p$ -distances (proportion of nucleotide site differences between sequences) were conducted in MEGA to examine phylogenetic signal and to identify related groups of haplotypes. Genetic diversity indices, including haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ), were calculated using Arlequin version 3.1 (Excoffier et al. 2005).

Population differentiation was examined through a hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) using both conventional  $F$ -statistics (Wright 1965) and  $\Phi$  statistics (distance based analogs of  $F$ -statistics). Prior to population-level assessment, a subset of sample locations was examined for temporal variability to ensure that any detectable genetic differences were due to differences between populations rather than to temporal variation. These included samples collected along the Atlantic coast at 27.7°, 31.6°, 32.3°, 39.5°N and from the Gulf of Mexico coast at 29.5°N during 1996 and 2006. To examine temporal stability, a hierarchical AMOVA was performed in Arlequin version 3.1, nesting collection years within sample location. The  $\Phi_{sc}$  (sampling location temporal differentiation within population) and the corresponding significance value ( $P$ -value) were calculated to determine the difference between collection years within a location. Results were considered significant at a  $P$ -value of less than 0.05.

After assessing temporal stability, a hierarchical AMOVA was performed in Arlequin version 3.1 to analyze partitioning of genetic variability using two different analysis regimes. In the first analysis, samples were divided into region of sampling: MAB, SAB, or Gulf of Mexico (i.e., both subspecies, *C. striata striata* and *C. striata melana*). The samples were further subdivided into populations by sampling location to examine within-region population structuring. In the second analysis, samples collected in the Gulf of Mexico were removed and only those collected from the MAB and SAB were examined (i.e., only the Atlantic subspecies *C. striata striata*).

**Haplotype distribution and gene flow.**—Groups of related haplotypes (see Results) collected along the U.S. Atlantic coast were examined from each sampling location and plotted against latitude to determine whether a cline in haplotype groups existed between the MAB and SAB regions. Mantel and partial Mantel tests were performed using Isolation by Distance Web Service version 3.21 with 10,000 permutations to test for statistical correlations between the genetic differentiation index  $F_{ST}$  and geographic distance (km; Smouse et al. 1986). Partial Mantel tests were conducted because Mantel tests often show a false correlation due to another variable, such as population isolation (Reynolds and Houle 2002). These correlations included the addition of an indicator matrix that was reflective of regional sample origin (coded as 0 = same region or 1 = different region). This allowed us to examine whether an isolation-by-distance or

regional differentiation model provided the best fit to the data. The degree of gene flow ( $M$ ) occurring between the MAB and SAB regions was estimated using maximum likelihood as implemented in the program MIGRATE within LAMARC version 2.0 (Beerli and Felsenstein 1999, 2001; Kuhner 2006). Default settings were used in a total of five replicate runs using a migration model consisting of joint maximum-likelihood estimates of all  $n \times n$  parameters.

## RESULTS

Amplification with the black sea bass control region primers yielded a 342-base-pair product from 363 individuals. There were 88 variable sites and 3 insertion/deletion events that defined a total of 144 haplotypes (GenBank accession number JQ249073–JQ249216). Phylogenetic analysis revealed two groups of haplotypes resolving the split between the two proposed black sea bass subspecies (not shown). Within the *C. striata striata* group (C<sub>SS</sub>CR designation,  $n = 306$  fish), two haplotypes (C<sub>SS</sub>CR1 and C<sub>SS</sub>CR17) dominated sampling and accounted for 55% (170 of 306) of the total C<sub>SS</sub>CR haplotypes tallied (Table 1). Examination of variable sites revealed two differences (at positions 177 and 203) that separated these two most common haplotypes (Table 1). All other haplotypes were observed at a much lower frequency; the majority occurred only once (singletons) and could be classified as variants from the two most commonly occurring haplotypes (C<sub>SS</sub>CR1 and C<sub>SS</sub>CR17; the exception was haplotype C<sub>SS</sub>CR18, which was intermediate to both). Within the *C. striata melana* group (C<sub>SM</sub>CR designation,  $n = 57$  fish), no single haplotype accounted for greater than 6% of the observed totals and the majority of haplotypes occurred as singletons. Likewise,  $h$  and  $\pi$  were highest in the Gulf of Mexico (*C. striata melana*;  $h = 0.997$ ,  $\pi = 0.015$ ; Table 2) and lowest in the MAB (*C. striata striata*;  $h = 0.424$ ,  $\pi = 0.002$ ; Table 2).

## Population Differentiation

The AMOVA results revealed no significant difference in temporal samples collected during 1996 and 2006 at five independent locations (four along the U.S. Atlantic coast and one in the Gulf of Mexico;  $\Phi_{sc} = -0.19$ ,  $P = 0.655$ ). Therefore, within-region samples collected across years were pooled and analyzed for genetic differences between the three regions (Gulf of Mexico, SAB, and MAB). Traditional  $F$ -statistics revealed a significant difference between regions, with 20.4% of the variability attributed to the differences between samples from the Gulf of Mexico, SAB, and MAB ( $F_{ST} = 0.204$ ,  $P = 0.002$ ). Atlantic-only comparisons found that 26.0% of the variability was explained by the differences between the SAB and MAB samples ( $F_{ST} = 0.260$ ,  $P = 0.001$ ). When examining genetic differentiation using  $\Phi$  statistics, between-region variability ( $\Phi_{ct}$ ) increased and explained 64.2% of the variability between the Gulf of Mexico, SAB, and MAB ( $\Phi_{ct} = 0.642$ ,  $P = 0.0000$ ). When the Gulf of Mexico samples were removed, 40.9% of the variability was attributable to differences between SAB and MAB samples ( $\Phi_{ct} = 0.409$ ,  $P = 0.006$ ).

TABLE 1. Mitochondrial control region variable sites and sample composition for black sea bass (CsmCR = *Centropomus striata striata* control region; CsmCR = *C. striata melana* control region). Other abbreviations are as follows: CT = Connecticut (41.1°N); NJ = New Jersey (39.5°N); VA = Virginia (36.9°N); NC = North Carolina (34.3°N); SC33 = South Carolina (33.3°N); SC32 = South Carolina (32.3°N); GA = Georgia (31.6°N); FL = Florida (27.7°N); GoM = Gulf of Mexico (29.5°N). Variable site position is in relation to reference haplotype CsmCR1; dots represent identical bases and hyphens represent insertion/deletion events.

Haplotype	Sequence	Middle Atlantic Bight										South Atlantic Bight								FL	GoM	Total
		CT	NJ	VA	NC	SC33	SC32	GA	FL	GoM	Total											
ATA-CACA-TTGAGCACTGAATTCACCTAGTAACTGATCAACATCTTAACCTAATGCCTCT-AATTAAATGAACATACTTCTAAGTCTC	111																					



TABLE 1. Continued.

[illegible]

TABLE 1. Continued.

CsmCR92	.....-C.....	.....A.....	.....G.....	.....	1	1
CsmCR93	.....-A.....	.....A.....	.....G.....	.....G.....	1	1
CsmCR1	.....-A.....	.....A.....	.....GG.....	.....G.....	3	3
CsmCR2	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR3	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR4	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR5	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR6	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR7	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR8	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR9	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR10	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR11	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR12	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR13	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR14	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR15	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR16	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR17	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR18	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR19	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR20	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR21	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR22	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR23	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR24	.....-A.....	.....A.....	.....GG.....	.....G.....	2	2
CsmCR25	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR26	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR27	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR28	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR29	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR30	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR31	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR32	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR33	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR34	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR35	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR36	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR37	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR38	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR39	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR40	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR41	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR42	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR43	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR44	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR45	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR46	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR47	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR48	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR49	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
CsmCR50	.....-A.....	.....A.....	.....GG.....	.....G.....	2	2
CsmCR51	.....-A.....	.....A.....	.....GG.....	.....G.....	1	1
Total					13	57



TABLE 2. Black sea bass haplotypic diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) and the associated SD (in parentheses) for each location and for all samples combined within a region. Cape Hatteras (35.1°N) separates the Middle Atlantic Bight (MAB) and South Atlantic Bight (SAB) management units. Abbreviations are defined in Table 1.

Location	$h$	$\pi$
<b>SAB sites</b>		
27.7°N (FL)	0.861 (0.040)	0.007 (0.005)
31.6°N (GA)	0.887 (0.043)	0.008 (0.005)
32.3°N (SC32)	0.815 (0.061)	0.006 (0.004)
33.3°N (SC33)	0.846 (0.051)	0.005 (0.003)
34.3°N (NC)	0.870 (0.038)	0.006 (0.004)
<b>MAB sites</b>		
35.8°N (OI)	0.250 (0.180)	0.001 (0.001)
36.9°N (VA)	0.361 (0.109)	0.002 (0.002)
39.5°N (NJ)	0.442 (0.116)	0.003 (0.002)
41.1°N (CT)	0.641 (0.150)	0.002 (0.002)
<b>GoM site</b>		
29.5°N (GoM)	0.999 (0.004)	0.015 (0.008)
<b>Regions</b>		
GoM	0.999 (0.004)	0.015 (0.008)
SAB	0.853 (0.021)	0.006 (0.004)
MAB	0.424 (0.071)	0.002 (0.002)

### Distribution of Haplotypes and Gene Flow

The proportion of haplotype frequencies varied across the U.S. Atlantic and Gulf of Mexico coasts. The C<sub>ss</sub>CR1 haplotype and closely related variants occurred in 89–100% of the fish sampled from the MAB but only 8–21% of the fish sampled from the SAB (Figure 2). The reverse was seen for C<sub>ss</sub>CR17 variants, which occurred in 79–93% of the fish sampled from the SAB but only 0–11% of the fish sampled from the MAB (Figure 2). The C<sub>sm</sub>CR haplotypes were only found in the Gulf of Mexico samples, occurring in 96% of the fish sampled from this region. Neither the C<sub>ss</sub>CR1 haplotype nor its variants were observed in the Gulf of Mexico samples, whereas C<sub>ss</sub>CR17 variants (2 observations) accounted for 4% of the fish sampled in the Gulf of Mexico (see Table 1).

All Mantel tests for Atlantic samples revealed a positive correlation for comparisons of genetic distance and geographic distance, whether using collection location ( $r = 0.57$ ,  $P = 0.009$ ) or regional assignment ( $r = 0.67$ ,  $P = 0.009$ ). Although these correlations remained significant for all partial Mantel tests ( $P < 0.01$ ), it was observed that regional assignment ( $r = 0.47$  when controlling for geographic distance) was a better fit than geographic distance ( $r = 0.17$  when controlling for regional assignment). This relationship was also observed from plotting the two primary groups of C<sub>ss</sub>CR haplotypes, as an abrupt shift in proportions was clearly present (Figure 2).

The number of immigrants per generation between the two regions was calculated by multiplying the estimated  $\theta$

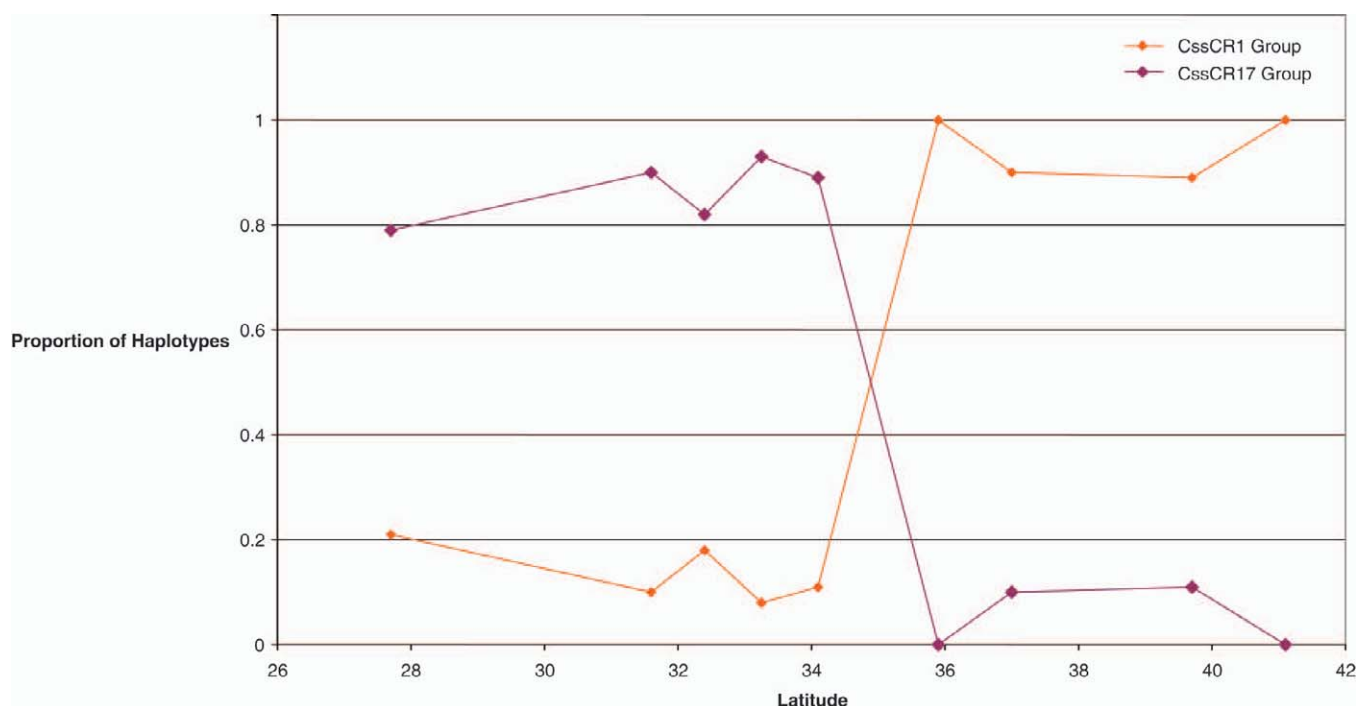


FIGURE 2. Proportions of the black sea bass haplotypes C<sub>ss</sub>CR1 and C<sub>ss</sub>CR17 (and their variants; C<sub>ss</sub>CR = *Centropristis striata striata* control region) plotted against latitude along the U.S. Atlantic coast.

TABLE 3. Gene flow ( $M$ ) and theta ( $\theta$ ) estimates for black sea bass residing in the two Atlantic coast regions (SAB = South Atlantic Bight; MAB = Middle Atlantic Bight). Estimated number of immigrants into each region is also shown.

Analysis or region	$\theta$	$M$ : SAB to MAB	$M$ : MAB to SAB	Immigrants per generation
1				
SAB	0.15825		484.3054	76.64
MAB	0.00521	0.0000		0.00
2				
SAB	0.23771		502.4583	119.44
MAB	0.00402	218.8317		0.88
3				
SAB	0.21910		481.7304	105.55
MAB	0.00307	468.8115		1.44
4				
SAB	0.29331		556.3892	163.19
MAB	0.00302	71.3616		0.22
5				
SAB	0.13319		478.7537	63.77
MAB	0.00466	236.6724		1.10

(coancestry coefficient) value for one region by its respective  $M$ -value. The average number of immigrants from the MAB to the SAB was 105.72 (range = 63.77–163.19), whereas the average number of immigrants from the SAB to the MAB was 0.73 (range = 0.00–1.44; Table 3). All five analyses revealed the general pattern of larger  $\theta$  estimates for the SAB versus the MAB. In addition, calculated  $M$  from the MAB to the SAB was greater than  $M$  from the SAB to the MAB.

## DISCUSSION

A significant difference was detected among black sea bass sampled in the Gulf of Mexico, MAB, and SAB, identifying significant genetic structuring among fish from these regions. Phylogenetic analyses uncovered two distinct groups of haplotypes, resolving subspecies and deep phylogeographic differentiation between Gulf of Mexico samples and Atlantic samples. Focusing on the Atlantic coast samples, our results rejected the null hypothesis that black sea bass along the U.S. Atlantic coast comprise a single panmictic population. The difference between Atlantic coast regions was not only detected in the AMOVA results but was further supported by the distribution of group haplotype frequencies. In combination, these results support the subspecies designation of black sea bass from the Gulf of Mexico and along the U.S. Atlantic coast and indicate that at least two well-differentiated populations exist along the Atlantic coast, with Cape Hatteras likely serving as a geographic barrier that disrupts gene flow between black sea bass from the two regions.

The results presented in this study support the previous findings of Bowen and Avise (1990) in that the largest levels of sequence divergence are observed between black sea bass col-

lected from the Gulf of Mexico and the Atlantic Ocean. However, the current study uncovers a much greater level of nucleotide divergence and haplotypic diversity in both regions. It is likely that differences in molecular techniques (restriction fragment analysis versus DNA sequencing), increased sampling, and the highly polymorphic nature of the locus employed in the present study account for the observed discrepancies. It is interesting to note that while limited in sampling, the study by Bowen and Avise (1990) did appear to capture, albeit subtly, the heterogeneity of samples collected north and south of Cape Hatteras as reported herein. In their study, of the 19 fish sampled along the Atlantic coast, the majority (17 fish) had the same haplotype (C). The two observed variants (haplotypes D and E) were sampled from fish that were collected south of Cape Hatteras. These data follow the pattern observed in the present study—that is, decreased haplotypic diversity in samples collected north of Cape Hatteras relative to samples collected south of Cape Hatteras.

The AMOVA results revealed significant genetic differentiation among the three regions examined. This differentiation was evident not only through traditional  $F$ -statistic estimates but also through estimates of  $\Phi$  statistics. When considering all three regions, the differences among regions explained 64.15% of the variability using  $\Phi$  statistics. However, once the Gulf of Mexico (presumably *C. striata melana*) samples were removed, the estimate was reduced, with between-region differences explaining 40.9% of the variability. Although the subspecies-level differentiation explained a large portion of the variability between regions, the differentiation between the SAB and MAB samples remained significant ( $\Phi_{ct} = 0.409$ ,  $P = 0.006$ ). Furthermore, the results from this analysis are comparable to or larger than estimates typically observed for marine fishes. For example, in a study examining a reef species with similar life history characteristics (i.e., the blue rockfish *Sebastes mystinus*), a statistically significant genetic break occurring around Cape Mendocino in the Pacific Ocean was detected, with a  $\Phi_{ct}$  value of 0.116 ( $P = 0.035$ ; Cope 2004). To our knowledge, the data presented here for black sea bass illustrate one of the largest levels of population differentiation observed in a broadly distributed marine fish species.

Similarly interesting results were found when examining the distribution of primary haplotypes and their variants along the U.S. Atlantic coast. There was a distinctive shift in the proportion of the C<sub>SS</sub>CR1 and C<sub>SS</sub>CR17 haplotypes and their variants in the SAB and MAB regions, with a proposed break occurring near Cape Hatteras. The C<sub>SS</sub>CR1 haplotype was more common in samples collected north of Cape Hatteras, occurring in 75% of the fish sampled from the MAB region. Although this haplotype also occurred in fish from the SAB, it was observed at a much lower proportion (11.5% of the fish sampled). Inversely, the C<sub>SS</sub>CR17 haplotype was the most commonly observed haplotype south of Cape Hatteras, occurring in 35.7% of the fish sampled, whereas only 3.8% of the fish sampled north of Cape Hatteras were observed to possess this haplotype. Partial Mantel tests showed that this distribution was best explained

by regional assignment rather than by an isolation-by-distance model. As such, this shift in haplotype frequencies suggests that black sea bass along the U.S. Atlantic coast comprise two separate management units that are likely separated in the vicinity of Cape Hatteras; thus, the two-stock designation currently in place along the Atlantic coast is supported (Moritz 1994).

The role of biogeographic barriers in the establishment of genetic structuring within the marine realm has been well documented (Bowen and Avise 1990; Avise 2000; Bernardi et al. 2003; Cope 2004). Along the southeastern coast of the United States, there are two notable biogeographic barriers that are largely affected by the currents of the Gulf Stream: Cape Canaveral, Florida, and Cape Hatteras. The Gulf Stream, which transports warm tropical waters into the Atlantic, travels close to the coastline along southeastern Florida until the point of Cape Canaveral, where it is deflected further offshore. This allows fish larvae originating from the Gulf of Mexico to enter the south Atlantic but prevents their further dispersal within the Atlantic (Avise 1992). Among the marine species that demonstrate a genetic break between the Gulf of Mexico and Atlantic Ocean are the Atlantic horseshoe crab *Limulus polyphemus* (Saunders et al. 1986), eastern oyster *Crassostrea virginica* (Reeb and Avise 1990), and red drum *Sciaenops ocellatus* (Seyoum et al. 2000). Black sea bass from the Gulf of Mexico and the Atlantic are probably under similar behavioral and oceanographic constraints as their pattern in genetic partitioning is concordant with those of the aforementioned species. However, for black sea bass, it appears that their limited southern distribution in the eastern Gulf of Mexico has lessened the impact of Gulf Stream transport of larvae around the Florida peninsula, as samples collected south of Cape Canaveral consisted solely of *C. striata* haplotypes. In fact, the presence of Atlantic haplotypes in the Gulf of Mexico sample suggests that exchange of individuals from the Atlantic into the Gulf of Mexico may not be that uncommon or has occurred in the recent past.

From Cape Canaveral, the Gulf Stream continues northward along the continental shelf of the southeastern United States. At Cape Hatteras, the current nears the coastline and is then deflected toward the northeastern Atlantic. The warm waters are then replaced by much cooler water from the Labrador Sea, which is transported southward to Cape Hatteras by the Labrador Current (Briggs 1974). As a result, sharp temperature and salinity gradients occur at this confluence, leading to a major break between coolwater and warmwater faunal assemblages (described as the Virginian and Carolinian provinces by Briggs 1974). While zoogeographic differences north and south of Cape Hatteras have been well described, the ability to serve as a barrier to gene flow for species whose ranges extend beyond Cape Hatteras has revealed mixed results. For example, genetic studies on marine species within the Atlantic, such as weakfish *Cynoscion regalis* (Graves et al. 1992) and summer flounder *Paralichthys dentatus* (Jones and Quattro 1999), have failed to demonstrate a genetic break across Cape Hatteras. In contrast, studies investigating population structure in the north-

ern quahog *Merceneria merceneria* (Baker et al. 2008;  $\Phi_{ct} = 0.076$ ,  $P = 0.013$ ), Atlantic croaker *Micropogonias undulatus* (Baker et al. 2007; using parasites), and Atlantic spotted dolphin *Stenella frontalis* (Adams and Rosel 2006;  $\Phi_{st} = 0.198$ ,  $P < 0.001$ ) have implicated Cape Hatteras as the cause of genetic differentiation. It is important to note that of these studies examining population subdivision, none reported a degree of genetic differentiation exceeding that observed herein ( $\Phi_{ct} = 0.409$ ).

The results obtained from gene flow estimates showed a consistent pattern of more immigrants per generation from the MAB to the SAB. There are two factors that may contribute to this observed pattern of migration. First, as previously mentioned, black sea bass in the MAB and SAB differ in their degree of site fidelity. Adult and juvenile black sea bass in the MAB migrate offshore and southward during the onset of fall, whereas those in the SAB undertake very little migration and their movement is mainly confined to offshore regions. Therefore, it is possible that during the winter, black sea bass in the MAB could migrate further south of Cape Hatteras, where there are warmer waters facilitating southward transport. Conversely, black sea bass in the SAB are more likely to move offshore and further south to warmer waters instead of moving northward, where the waters are much cooler. Thus, given this difference in behavior, adult migration from the MAB to the SAB has a greater likelihood than the opposite scenario. The second important factor is the transport of larvae between the two regions. A study examining the flux of larvae across the area of convergence at Cape Hatteras revealed that MAB-spawned larvae could drift into the SAB via net water flow from the MAB to the SAB along the coastline (Grothues et al. 2002). However, SAB-spawned larvae could not move past the area of convergence between the Labrador Current and Gulf Stream in the manner of MAB-spawned larvae. While it is not uncommon for SAB-spawned fish larvae to be transported into the MAB, their fate is largely determined by physiological tolerances and environmental conditions favorable for across-shelf transport (Hare and Cowen 1991, 1996; McBride and Able 1998; Hare et al. 2002). Since black sea bass spawn on the inner continental shelf, the likelihood of larval entrainment within the Gulf Stream is low, thus reducing the occurrence of larval transportation into the MAB. Regardless, the data presented here would suggest that this rate of exchange must be low relative to effective population sizes as it is currently insufficient to genetically homogenize the stocks or to buffer adjacent stocks from overfishing.

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