2014

Genetic Stock Identification and Migration in Black Sea Bass (Centropristis striata) along the Western Atlantic Coast and Gulf of Mexico

Justin Francis Lewandowski
University of South Carolina - Columbia

GENETIC STOCK IDENTIFICATION AND MIGRATION IN BLACK SEA BASS (*Centropristis striata*) ALONG THE WESTERN ATLANTIC COAST AND GULF OF MEXICO

by

Justin Lewandowski

Bachelor of Science
University of South Carolina, 2009

Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in

Biological Sciences

College of Arts and Sciences

University of South Carolina

2014

Accepted by:

Joseph Quattro, Director of Thesis

Jerry Hilbish, Reader

Dennis Allen, Reader

Lacy Ford, Vice Provost and Dean of Graduate Studies

Acknowledgements

I would like to acknowledge the many people who helped bring this project to this point. My advisor, Dr. Joseph Quattro, in addition to the equipment, funding, and lab space needed provided me with the guidance and support to help me get through my research. My committee members, Dr. Jerry Hilbish and Dr. Dennis Allen, likewise gave me guidance and support in making it this far. My lab mates, including Mark Roberts, Katie Allen, Kate Levasseur, Mohammad Alqahtani, Max Bangs, Kyra Marsigliano, Sarah Grasty, and Shane Benson further helped me through my work. I would also like to thank Thomas Greig and NOAA, and SCDNR, for providing the samples that made this work possible. Finally, I would like to thank my parents who continually supported and motivated me throughout my time at the University of South Carolina.

Abstract

The black sea bass, *Centropristis striata*, is a member of the family Serranidae that is commercially important throughout its range, which extends throughout the western Atlantic (from Cape Cod, Massachusetts to Cape Canaveral, Florida) and Gulf of Mexico (from Mobile Bay, Alabama to Tampa Bay, Florida). There are two known subspecies, *C. striata striata* in the Atlantic and *C. striata melana* in the Gulf, and through behavioral and morphological evidence two separate stocks are managed in the Atlantic, north and south of Cape Hatteras, NC. Recent genetic studies on mitochondrial DNA (mtDNA) have supported this. To further investigate the relationships of *C. striata* in the Atlantic (as well as the Gulf), more individuals were analyzed looking at mtDNA as well as nuclear DNA (nDNA) to determine differentiation between them, as well as if any migration was occurring. DNA from specimens was extracted, amplified, and sequenced in order to compare results, which were run through Arlequin v3.5, SPADE, and Beerli's Migrate. The results for mtDNA confirmed a noticeable separation between *C. striata* in the Atlantic and Gulf, and a smaller but still significant difference between *C. striata* north and south of Cape Hatteras in the Atlantic. nDNA showed smaller differences between regions, which supports male-mediated gene flow occurring for this species. Migration was shown to be low but still occurring between different regions indicating that there is still some connection occurring but likely not nearly enough to warrant a change in stock management.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AMOVA ..... Analysis of Molecular Variance

GOM .....Gulf of Mexico

MAB .....Mid-Atlantic Bight

MLE ..... Maximum Likelihood Estimate

SAB..... South-Atlantic Bight

SPADE.....Species Prediction and Diversity Estimation

Chapter 1: Introduction

The ability of populations to rebound from depleted conditions depends upon management strategies that incorporate crucial information such as the reproductive capacity within (self-recruitment) and among (migration, larval dispersal) distinct population segments (DPS). It is of extreme importance that population structure and demographics of managed species are adequately understood to assess and refine current management plans.

There is great potential in the field of genetics to determine conditions of stocks of various fish. Genetic research allows us to look at the relationships of individuals and to determine the health of a population (the higher the genetic diversity, the better off a species is likely to be in the future). Genetic data can also be used to describe basic genetic stock (genetically distinct population segments among which migration/gene flow is demonstrably limited) structure and allow for estimates of the magnitude and direction of gene flow (dispersal) between populations.

The black sea bass (*Centropristis striata*) is a demersal fish found throughout the western Atlantic and Gulf of Mexico (Musick and Mercer 1977). It is a member of the family Serranidae (sea basses and groupers, as well as numerous smaller relatives), and shares most of the features typical of the family, including being a protogynous hermaphrodite (beginning life as a female and changing sex to male later in life) (Wenner et al. 1986). Its prefers structured benthic habitats, including rocky reefs and artificial

reefs, shellfish beds, and wrecks in warm temperate waters (Steimle et al. 1999). It is an important predator in these habitats, feeding on a variety of prey including fish, crustaceans, and mollusks (Steimle et al. 1999).

The fisheries of Serranids are extremely popular, and many are overfished to the point that they are critically endangered. An example is the Goliath Grouper (*Epinephelus itajara*), a species that had been fished to the point of extinction on many reefs and had a very patchy distribution across its entire range (Koenig et al. 2007). Although measures were put into place to protect the goliath grouper, recovery is extremely slow and it is unlikely to ever reach its former levels. Being protogynous hermaphrodites, Serranids may be particularly vulnerable to overfishing due to skewed sex ratios and fishing size selection (Armsworth 2001; Alonzo and Mangel 2004).

C. striata is not endangered; however, it has been overfished in many areas in the past including the South Atlantic Bight, as determined by a reduction in individual mean size through time (Cupka et al. 1973). Continued overfishing can result in a dramatic decrease of genetic diversity within the species, which could lead to undesirable stocks (Conover and Munch 2002). It was estimated by the Southeast Data, Assessment, and Review (SEDAR) and the South Atlantic Fishery Management Council (SAFMC) that a 62% reduction in catch was needed to address this problem (SEDAR Update 1 2005, SAFMC 2006). Fortunately, stocks were determined to have recovered recently, with catch limits set to more than double. Specifically, the annual catch limit of 847,000 pounds (whole weight) was proposed to increase to 1,814,000 pounds (SAFMC 2013).

Because of this drop and recovery, *C. striata* is an appropriate model to use in a population study. Since it has undergone a rebound collecting specimens is not a

problem as it would be for less common and more threatened Serranid species. However, since its fishery did experience a decline, it is still of importance to determine its population structure to help avoid that happening again and to assess and refine management plans. Identifying different populations and determining if mixing is occurring between them is an important part of this need. It could mean the difference between high or low genetic diversity within populations, especially if they suffer a decrease in numbers. Also, over-exploitation and/or fishery practices applied to any single population have potential to impact populations far-removed, as long as migration, dispersal and gene-flow are sufficient. Furthermore, *C. striata* is a typical member of the family Serranidae in terms of its reproductive life cycle, diet, and habitat. Because of this, techniques used in this experiment have the potential to be applied to other Serranid species that are in peril.

Three recognized stocks for black sea bass exist: two in the Atlantic and one in the Gulf of Mexico. Black sea bass in the Gulf are recognized as a different subspecies (*Centropristis striata melana*) from those in the Atlantic (*Centropristis striata striata*). These are distinguished by morphological (Miller 1959) and genetic characters (Bowen and Avise 1990, Chapman et al. 1999). Morphological differences among subspecies are pronounced and include morphometric (upper-jaw, pectoral fin length) and meristic (gill rakers, pectoral-fin rays) characteristics (Miller 1959). Morphological differentiation is supported genetically; phenetic analyses of mtDNA restriction profile frequency data indicate two very distinct genetic clusters that differentiate the Atlantic and Gulf of Mexico groups (Bowen and Avise 1990). The estimated degree of differentiation between clades of Atlantic and Gulf mtDNA haplotypes (~ 0.9% sequence divergence)

suggests complete genetic isolation between groups that was initiated some 350,000 years before present (Bowen and Avise 1990). Subsequent direct nucleotide sequence analysis of the mitochondrial 16S rRNA and ND1 loci and allele frequency differences at two microsatellite DNA loci further support subspecies distinction (Chapman et al.1999).

C. striata in the Atlantic are managed as two distinct stocks, one in the Middle Atlantic Bight (MAB: Cape Cod, Massachusetts to Cape Hatteras, North Carolina), and one in the South Atlantic Bight (SAB: Cape Hatteras to Cape Canaveral, Florida) (Mercer 1978, Wenner et al. 1986). Cape Hatteras is a known geographical boundary in the Atlantic, with the meeting of the Gulf Stream from the south and the Labrador Western Boundary Current from the north (Pickart and Watts 1990). Previous studies have shown that various types of fish (Schwartz 1989) as well as other marine species (Fornshell et al. 1984) have northern and southern boundaries at Cape Hatteras. However, there are also numerous types of fish that display no genetic differences north and south of Cape Hatteras (Avise et al. 1987; Bowen and Avise 1990; Jones and Quattro 1999). In between, there are species with distributions ranging across Hatteras but with it serving as a barrier to gene flow (Avise et al. 1987; Baker et al. 2007). It is with this group that the black sea bass is thought to belong.

A reason for this could be spawning location. Many species that have unbroken gene flow across Hatteras seem to be offshore spawners on the outer continental shelf (Jones and Quattro 1990; Hare and Cowen 1996) which could lead to a greater likelihood of young being caught up in offshore currents and pushed across the boundary point of Hatteras. By comparison, species that spawn nearer to shore (Thorrold et al. 1997), which black sea bass are included among (Bowen and Avise 1990), seem to have a

population break at Hatteras. Thus Cape Hatteras can be looked at as a frequent, but not absolute, boundary for many species in the western Atlantic.

In the case of *C. striata*, strong morphological and behavioral evidence has supported the existence of a barrier between populations situated north and the south of the Cape Hatteras boundary. The two populations can be distinguished by such characteristics as growth rate, size at sexual maturity, and maximum size, with the northern population growing faster and attaining greater sizes in all accounts than the southern population (Mercer 1978, Wenner et al. 1986, Steimle et al. 1999). Furthermore, the northern population appears to undergo migration whereas the southern population is more sedentary, and the southern population appears to spawn earlier in the year.

Management of the northern stock of *C. striata* is overseen by the Mid-Atlantic Fishery Management Council, while the southern stock is overseen by the South Atlantic Fishery Management Council (Roy et al. 2012). Both of these councils conduct independent stock assessments. The most recent stock assessments for each unit suggest the northern stock is not overfished, while the southern stock had previously been overfished (SAFMC 2006; Shepherd 2009) but has made a strong recovery (SAFMC 2013). While genetic studies have been successful in documenting the differences between Gulf of Mexico and western Atlantic specimens, they had previously been less convincing in supporting the differences in *C. striata* north and south of Cape Hatteras (Bowen and Avise 1990; Chapman et al. 1999).

However, further studies of genetic data by Roy et al. (2012) showed a distinct difference between Atlantic and Gulf populations of *C. striata*, as well as differences

between MAB and SAB populations of *C. striata* by analyzing the mitochondrial control region. This was most likely due to increased sampling size, differences in molecular techniques (DNA sequencing as opposed to the previous restriction fragment analysis), and the highly polymorphic nature of the mtDNA control region as the locus employed (Roy et al. 2012). These results were concordant with life history and morphological studies and supported the management of *C. striata* in the Atlantic as two separate stocks. Also of note was that no temporal variation was noted between samples collected in 1996 and 2006. Tests of gene flow showed some mixing occurring between stocks, with more migration from the MAB to SAB, but with a low rate of exchange relative to effective population size and thus insufficient to homogenize the stocks or buffer adjacent stocks from overfishing (Roy et al. 2012).

Although genetic research has now supported previous studies done on black sea bass, it has only been through mitochondrial DNA (mtDNA). The genealogical history of mtDNA is specialized, as it is maternally inherited (Giles et al. 1980) and thus can only trace along female lineages. This is in contrast to nuclear DNA (nDNA) which is biparentally inherited (Melnick and Hoelzer 1991) and thus can trace along both female and male lineages. The significance in the differences between the two can be seen in scenarios such as female philopatry (Carreras et al. 2006), in which females consistently return to their place of birth to reproduce while males travel freely between populations. In addition, instances involving male-mediated gene flow in which females aren't returning to a predefined point but are nonetheless remaining in a population while males are migrating long distances can lead to a lack of nDNA variation while a good deal would be present in mtDNA (Pardini et al. 2001).

The goal of this project was to use both mtDNA (D-Loop locus) and multiple nDNA loci (SREB2, MYH6, and ITS2) to test the assumption that populations of black sea bass residing north and south of Cape Hatteras, NC represent distinct genetic stocks (strongly supported for at least some combination of populations by previous data). In addition, temporal variation in black sea bass was looked at, since the use of temporal analyses in population genetic samples can be used to verify patterns in data (Heath et al. 1990). Finally, the magnitude and direction of dispersal among populations north and south of Cape Hatteras and among populations within the southern Atlantic were estimated. These analyses were carried out using Arlequin v3.5 (Excoffier et al. 1992; Excoffier and Lischer 2010), SPADE (Chao and Shen 2010), and Beerli's Migrate (Beerli 1998; Beerli and Felsenstein 1999, 2001).

Based on previous data and the knowledge of Cape Hatteras as a gene flow barrier, it was expected that populations of *C. striata* north and south of Cape Hatteras would represent distinct genetic stocks but with limited mixing occurring between them, supporting previous genetic and morphological research, and that the addition of nDNA analysis would allow a more in-depth look at migration patterns and relationships between stocks.

Chapter 2: Methods

Field Collections

Samples were obtained from various sites in the western Atlantic north and south of Cape Hatteras, NC, as well as from the Gulf of Mexico in order to get a general sweep of each population. Individuals were collected from the same age group, and collected in the late summer/early autumn so as to avoid coinciding with breeding/spawning periods (typically January-June). This increased the likelihood that similar genes would be expressed from the individuals and thus avoided a bias in the data. Collaborations with environmental agencies, specifically the South Carolina Dept. of Natural Resources and NOAA, were undertaken in order to obtain these samples.

Extractions from specimens that were collected in 2006 were provided by Thomas Greig, with 53 from 27.7°N (Florida), 40 from 31.6°N (Georgia), 39 from 32.3°N (South Carolina), 40 from 33.3°N (South Carolina), 56 from 34.3°N (North Carolina), 8 from 35.8°N (Oregon Inlet), 30 from 36.9°N (Virginia), 38 from 39.5°N (New Jersey), 13 from 41.1°N (Connecticut), and 57 from 29.5°N (Gulf of Mexico) (Appendix A.1) For these, fin clips had been taken and preserved in 100% ethanol. More recently, collections were organized by Dennis Allen in 2008, with 39 from 40°N (New York), 103 from 39°N (New Jersey), 34 from 34°N (North Carolina), 40 from 33°N (South Carolina), and 101 from 29°N (Gulf of Mexico) (Appendix A.2). Arrangements were made to acquire adult sea bass from headboats, commercial trap and trawl fishers, and MARMAP agencies where appropriate.

Fish were caught using shell-filled habitat trays (Lehnert and Allen 2002), small mesh traps, and hook and line. Additional material was acquired through local fishers and fisheries workers. A clip from each fish (small portions of muscle or fin or entire juveniles) was snipped and preserved in ethanol to prevent degradation.

Laboratory Methods

DNA extractions were performed using established protocols (Jones and Quattro 1999). Dneasy Qiagen kits (QIAGEN Corporation, Maryland, USA) were used following the manufacturer's protocol to extract DNA from fin clippings. Agarose gel electrophoresis with ethidium bromide staining (Oswald 2007) was used to confirm presence of DNA.

Once the extractions were completed, the genomic DNA was amplified by polymerase chain reaction (PCR) (Oswald 2007; Bangs 2011). Both mitochondrial (mt) DNA amplifications and nuclear (n) amplifications were done on the samples. The mtDNA control region was amplified using Dloop primers (forward primer CstrCR-F2: 5' – GAA CCA GAT GCC AGG AAT A – 3' and reverse primer Cstr-CR-R1: 5' – ATA TCA GCA TAC ATC TGT GTC – 3'). The nuclear DNA was analyzed by looking at three different loci. The first set of primers targeted the G-protein coupled receptor (SREB2) (in a nested PCR, with forward primers sreb2_F10: 5'- ATG GCG AAC TAY AGC CAT GC -3' for round 1 and sreb2_F27: 5'- TGC AGG GGA CCA CAM CAT -3' for round 2, along with reverse primers sreb2_R1094: 5'- CTG GAT TTT CTG CAG TAS AGG AG -3' for round 1 and sreb2_R1082: 5'- CAG TAS AGG AGC GTG GTG CT -3' for round 2). The second set targeted the myosin heavy chain 6 (MYH6) (also in a nested PCR, with forward primers myh6_F459: 5'- CAT MTT YTC CAT CTC AGA

TAA TGC -3' for round 1 and myh6_F507: 5'- GGA GAA TCA RTC KGT GCT CAT CA -3' for round 2, along with reverse primers myh6_R1325: 5'- ATT CTC ACC ACC ATC CAG TTG AA -3' for round 1 and myh6R_1322: 5'- CTC ACC ACC ATC CAG TTG AAC AT -3' for round 2). Finally, the third set targeted an internal transcribed spacer (ITS2) (forward primer BSB-ITS-F2: 5'- GGG GCA GTC GCA GGC GCA TCG CGT -3' and reverse primer ITS R: 5'- ATA TGC TTA AAT TCA GCG GG -3'). PCRs were run at 94°C for 5 min; 35 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min; and finishing with 72°C for 6 min. As with extractions, successful PCR amplifications were confirmed using gel electrophoresis with ethidium bromide staining.

Successfully amplified samples were cleaned using the exonuclease I–shrimp alkaline phosphatase (ExoSAP) protocol to purify the products, with five microliters of the PCR product being mixed with one microliter of the exosap mix. Then the samples were prepped for sequencing using BigDye Terminator version 3.1 (Applied Biosystems, Inc), with 1.5 microliters of the exosap product being mixed with 10.5 microliters of the BigDyev3.1 stock solution. BigDye cycle sequencing was carried out at 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Sequencing reaction products were purified using standard ethanol–EDTA–sodium acetate precipitation protocol.

Samples were sent to Functional Biosciences, Madison, WI for sequencing, and the obtained DNA sequences were assembled into contigs and edited using Sequencher (version 4.1; Genecodes Corporation, Michigan, USA). Once manually edited, sequences were exported to BioEdit version 7.0 (Hall 1999) and aligned using ClustalW (Thompson et al. 1994). They were then collapsed using Collapse GUI v2.1 in order to identify haplotypes and alleles.

Analytical Methods

All individuals were assigned designated numbered haplotypes based on their Collapse run for both mtDNA and nDNA. Before being analyzed they were run though Haplotype Inference by Maximum Parsimony (HAPAR) (Wang and Xu 2003) in order to confirm that haplotype calls were correct.

They were then arranged into input files to be interpreted using an Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) run in Arlequin v3.5 (Excoffier and Lischer 2010) which partitioned the total genetic variance into components attributable to within and among populations. mtDNA and nDNA were run separately (with all three nuclear loci both done in the same run and individually). Spatial comparisons were made between the MAB, SAB, and Gulf of Mexico, as well as temporal comparisons made between time 1 and time 2 samples. For the spatial comparisons, FCT values were determined for groups predefined (in this case, North Atlantic, South Atlantic, and Gulf of Mexico), FSC for populations within those groups, and FST for variation within those populations. For temporal comparisons, time 1 and time 2 were the predefined groups (FCT). Variance components were also determined in these runs.

In addition to AMOVA tables, Arlequin was used to construct Tajima's D tables for mtDNA and heterozygosity tables for nDNA for each sampling site as well as each overall region (MAB, SAB, and GOM). Further info was summarized as figures showing gene diversity, allele count tables showing number of alleles of each locus per site, and condensed Fst tables.

Data were also run through SPADE (Chao and Shen 2010), specifically Estimating Allelic Differentiation/Similarity Among Subpopulations, in order to determine D values (Jost 2008), which are more accurate than Fsts in determining divergence when gene diversity is high.

Furthermore, results were run through a Migrate analysis (Beerli 1998; Beerli and Felsenstein 1999, 2001) in order to estimate migrations rates and lengths of divergence time. A maximum likelihood (MLE) method was used to determine migration rates.

Chapter 3: Results

AMOVA Tests

The FCT value (0.664) and variance component (2.285) for mtDNA showed distinct differences between the North Atlantic, South Atlantic, and Gulf of Mexico (Table 1). The FST value (0.663) and variance component (1.157) were also high indicating high levels of variation within populations. A very low FSC value (-0.002) and variance component (-0.002) showed almost no variation between different populations within each group (with Connecticut, New York, New Jersey, Virginia, and North Carolina north of Cape Hatteras comprising the different populations of group 1, the MAB; North Carolina south of Cape Hatteras, South Carolina, Georgia, and Florida comprising group 2, the SAB; and eastern and western Gulf of Mexico comprising group 3, the Gulf itself) (Figure 1). The same was true when the Atlantic stocks were pooled together and compared with the GOM (FCT=0.714, variance component=3.592) (FSC=0.19498, variance component=0.279) (FST=0.770, variance component=1.157)) (Table 1).

For comparisons between just the MAB and SAB (Table 1), differences between the two groups were also high (FCT=0.426, variance component=0.616), though not as high as when the Gulf of Mexico was included as a third group. Similar trends were seen in the FST and FSC values (FSC=0.002, variance component=0.002) (FST=0.428, variance component=0.828), with variation within each population being high but variation between different populations in the same groups being very low (Figure 2).

When comparing just the MAB with the GOM (FCT=0.783, variance component=4.377) (FSC=-0.012, variance component=-0.014) (FST=0.781, variance component=1.225) (Table 4) and just the SAB with the GOM (FCT=0.701, variance component=3.476) (FSC=0.002, variance component= 0.003) (FST=0.701, variance component=1.483) (Table 1), the results were again similar to the Atlantic as a whole compared with the GOM.

For temporal variation (Table 1), FCT value denoted differences in time periods, in this case showing that there was ultimately no difference between time 1, in the late 1990s, and time 2, in the late 2000s (FCT=-0.083, variance component=-0.217). Variation between populations was noted to be high (FSC=0.591, variance component=1.673) (in this case including all populations from the MAB to the Gulf of Mexico since they were all included in their group which was either time period 1 or 2), and variation within each population was noted to be high just as it was with the spatial AMOVA tests (FST=0.557, variance component=1.157) (Figure 3).

nDNA for combined loci was much more uniform across all three regions (Table 2), with FCT (-0.004), FSC (0.00590), and FST (0.01000) values being extremely low, along with their corresponding variance components for the most part (-0.001, 0.003, and 0.334 respectively). Of the variation that did exist, the majority of it was found within populations (Figure 4). Likewise, comparisons between pooled Atlantic samples and the GOM showed low variation (FCT=-0.00361, variance component=-0.001) (FSC=0.00692, variance component=0.002) (FST=0.00333, variance component=0.338) (Table 2). Variation within just the Atlantic showed a similar trend (FCT=-0.003, variance component=-0.001) (FSC=0.00824, variance component=0.004) (FST=0.011,

variance component=0.334) (Table 2), with low variation among groups, among populations within groups, and within groups. Again, of the variation that did exist, the largest amount was within groups (Figure 5). Comparisons of the MAB with the GOM (FCT=-0.003, variance component=-0.001) (FSC=0.003, variance component=0.001) (FST=-0.001, variance component=0.333) (Table 2) and the SAB with the GOM (FCT=-0.004, variance component=-0.001) (FSC=0.009, variance component=0.003) (FST=0.006, variance component=0.341) (Table 2) showed similar trends.

Temporal variation was also low for all FCT, FSC, and FST values (FCT=-0.004, variance component=-0.002) (FSC=0.006, variance component=0.003) (FST=0.010, variance component=0.334) (Table 2), indicating low variance across both time sets, as well as between and within populations (Figure 6).

The trends for combined nDNA were also seen when looking just at the SREB2 locus in Table 3. Comparing MAB, SAB, and GOM, (FCT=-0.003, variance component=-0.001), (FSC=0.008, variance component=0.003), and (FST=0.005, variance component=0.338). Comparing pooled Atlantic and GOM, (FCT=-0.004, variance component=-0.001), (FSC=0.007, variance component=0.002), and (FST=0.003, variance component=0.338). Comparing MAB and SAB, (FCT=-0.001, variance component=-0.001), (FSC=0.008, variance component=0.003), and (FST=0.007, variance component=0.340). Comparing MAB and GOM, (FCT=-0.003, variance component=-0.001), (FSC=0.003, variance component=0.001), and (FST=-0.001, variance component=0.333). Comparing SAB and GOM, (FCT=-0.004, variance component=-0.001), (FSC=0.009, variance component=0.003), and (FST=0.006, variance component=0.341). Comparing Time 1 and Time 2, (FCT=0.001, variance

component=0.000), (FSC=0.005, variance component=0.002), and (FST=0.006, variance component=0.338).

For MYH6 the trends were overall similar, but with a slightly higher FST value as seen in Table 4. Comparing MAB, SAB, and GOM, (FCT=0.020, variance component=0.008), (FSC=0.013, variance component=0.005), and (FST=0.033, variance component=0.384). Comparing pooled Atlantic and GOM, (FCT=0.039, variance component=0.016), (FSC=0.014, variance component=0.005), and (FST=0.053, variance component=0.384). Comparing MAB and SAB, (FCT=0.001, variance component=0.001), (FSC=0.015, variance component=0.006), and (FST=0.016, variance component=0.377). Comparing MAB and GOM, (FCT=0.042, variance component=0.018), (FSC=0.016, variance component=0.006), and (FST=0.057, variance component=0.390). Comparing, SAB and GOM, FCT=0.038, variance component=0.015), (FSC=0.010, variance component=0.004), and (FST=0.047, variance component=0.389). Comparing Time 1 and Time 2, (FCT=0.008, variance component=0.003), (FSC=0.022, variance component=0.009), and FST=0.030, variance component=0.384).

Finally, for ITS2 there were overall higher FST and FCT values but overall the trends were still the same when comparing populations with variation relatively low as seen in Table 5. Comparing MAB, SAB, and GOM, (FCT=0.145, variance component=0.058), (FSC=0.018, variance component=0.006), and (FST=0.161, variance component=0.334). Comparing pooled Atlantic and GOM, (FCT=0.257, variance component=0.118), (FSC=0.024, variance component=0.008), and (FST=0.274, variance component=0.334). Comparing MAB and SAB, (FCT=0.011, variance

component=0.004), (FSC=0.019, variance component=0.007), and (FST=0.029, variance component=0.361). Comparing MAB and GOM, (FCT=0.312, variance component=0.143), (FSC=0.034, variance component=0.011), and (FST=0.335, variance component=0.306). Comparing SAB and GOM, (FCT=0.241, variance component=0.103), (FSC=0.006, variance component=0.002), and (FST=0.246, variance component=0.323). Comparing Time 1 and Time 2, (FCT=-0.002, variance component=-0.001), (FSC=0.120, variance component=0.046), and (FST=0.118, variance component=0.334).

Counts of the each allele present in each population for all loci can be seen in Tables 6-9, showing that gene variability is relatively constant across sampling sites. This is also seen when averaging gene diversity across all four loci (Figure 7). Summarized FST values for population comparisons for all loci can be found in Appendix B.

Tajima's D values were analyzed for the D-loop locus at each sampling site (Table 10). All sites showed negative values, with most being significant. In addiction to each sampling site, Tajima's D values were analyzed in each overall region (Table 11), again showing negative values of significance.

Also analyzed were the heterozygosities for all of the nuclear loci. As with Tajima's D with the D-loop locus, heterozygosities were first looked at each sampling site (Tables 12-14). Observed and expected heterozygosities were not significantly different for almost all loci per site, with the exceptions of MYH6 at NJ Year 1 (p-value=0.039), SC33 Year 1 (p-value=0.001), and SC32 Year 1 (p-value=0.016) (Table 13) (all of which involved a deficit of observed heterozygotes). Heterozygosities were

then analyzed for all nuclear loci at each overall region (MAB, SAB, and GOM) (Tables 15-17), and there were no significant differences found for any of the regions.

Spade

SPADE yielded results showing high diversity between regions in the Atlantic compared to the GOM for MYH6 and ITS2, while still revealing some diversity between the MAB and SAB in the Atlantic as well (Table 18; Figure 7).

Beerli Tests

Beerli's Migrate using Maximum Likelihood tests yielded results shown in Table 19 and Figure 8. Mutation rates (Θ) were very low for the MAB and SAB .Migration rates were roughly three times higher from the SAB to the MAB than the other way around.

Table 3.1: Fixation indices and variance components for the D-loop locus (FCT, Va=variation between groups; FSC, Vb = variation between populations within groups; FST, Vc=variation within populations).

	FCT	FSC	FST	Va	Vb	Vc
MAB: SAB: GOM	0.664	-0.002	0.663	2.285	-0.002	1.157
Pooled Atl: GOM	0.715	0.194	0.770	3.592	0.279	1.157
MAB: SAB	0.426	0.002	0.428	0.616	0.002	0.828
MAB: GOM	0.783	-0.012	0.781	4.377	-0.014	1.225
SAB: GOM	0.701	0.002	0.701	3.476	0.003	1.483
Time 1: Time 2	-0.083	0.591	0.557	-0.217	1.673	1.157

Table 3.2: Fixation indices and variance components for combined nuclear loci (FCT, Va=variation between groups; FSC, Vb = variation between populations within groups; FST, Vc=variation within populations).

	FCT	FSC	FST	Va	Vb	Vc

MAB: SAB: GOM	-0.004	0.006	0.010	-0.001	0.003	0.334
Pooled Atl: GOM	-0.004	0.007	0.003	-0.001	0.002	0.338
MAB: SAB	-0.003	0.008	0.011	-0.001	0.004	0.334
MAB: GOM	-0.003	0.003	-0.001	-0.001	0.001	0.333
SAB: GOM	-0.004	0.009	0.006	-0.001	0.003	0.340
Time 1: Time 2	-0.004	0.006	0.010	-0.001	0.003	0.334

Table 3.3: Fixation indices and variance components for the SREB2 locus (FCT, Va=variation between groups; FSC, Vb = variation between populations within groups; FST, Vc=variation within populations).

	FCT	FSC	FST	Va	Vb	Vc
MAB: SAB: GOM	-0.003	0.008	0.005	-0.001	0.003	0.338
Pooled Atl: GOM	-0.004	0.007	0.003	-0.001	0.002	0.338
MAB: SAB	-0.001	0.008	0.007	-0.001	0.003	0.340
MAB: GOM	-0.003	0.003	-0.001	-0.001	0.001	0.333
SAB: GOM	-0.004	0.009	0.006	-0.001	0.003	0.341
Time 1: Time 2	0.001	0.005	0.006	0.000	0.002	0.338

Table 3.4: Fixation indices and variance components for the MYH6 locus (FCT, Va=variation between groups; FSC, Vb = variation between populations within groups; FST, Vc=variation within populations).

	FCT	FSC	FST	Va	Vb	Vc
MAB: SAB: GOM	0.020	0.013	0.033	0.008	0.005	0.384
Pooled Atl: GOM	0.039	0.014	0.053	0.016	0.005	0.384
MAB: SAB	0.001	0.015	0.016	0.001	0.006	0.377
MAB: GOM	0.042	0.016	0.057	0.018	0.006	0.390
SAB: GOM	0.038	0.010	0.047	0.015	0.004	0.389
Time 1: Time 2	0.008	0.022	0.030	0.003	0.009	0.384

Table 3.5: Fixation indices and variance components for the ITS2 locus (FCT, Va=variation between groups; FSC, Vb = variation between populations within groups; FST, Vc=variation within populations).

	FCT	FSC	FST	Va	Vb	Vc
MAB: SAB: GOM	0.145	0.018	0.161	0.058	0.006	0.334
Pooled Atl: GOM	0.257	0.024	0.274	0.118	0.008	0.334
MAB: SAB	0.011	0.019	0.029	0.004	0.007	0.361

MAB: GOM	0.312	0.034	0.335	0.143	0.011	0.306
SAB: GOM	0.241	0.006	0.246	0.103	0.002	0.323
Time 1: Time 2	-0.002	0.120	0.118	-0.001	0.046	0.334

Table 3.6: Counts of each allele present in each population of the D-Loop locus.

D-Loop

Allele	CT Yr1	NY Yr2	NJ Yr1	NJ Yr2	VA Yr1	OI Yr1	NC Yr1	NC Yr2	SC33 Yr1	SC32 Yr1	SC Yr2	GA Yr1	FL Yr1	GM Yr1	GM Yr2	**Allele**
1	8	26	21	46	22	7	6	5	3	4	2	4	9			1
2													1			2
3			2													3
4			1	1												4
5	1	1		2		1		1								5
6	1															6
7	1	1														7
8													1			8
9	1			2												9
10	1							1								10
11				2						1						11
12										1						12
13										1						13
14			1													14
15					2											15
16				1	1											16
17		1	2	2	2		19	8	15	16	13	13	18			17
18							1									18
19													1			19
20							1				1					20
21													1			21
22													1			22
23													1			23
24							1									24
25							1									25
26							5		5	3		2	3			26
27													1			27
28							2	1	1		2					28
29							3	1	2			1	1			29
30			1													30
31														1		31
32				1									1	1		32
33													1			33
34													1			34
35											1		1			35
36													1			36
37													1			37
38							1						1			38

39							1			1	1		1			39
40													1			40
41									1				1			41
42													1			42
43													1			43
44												1	1			44
45													1			45
46													1			46
47												1				47
48												1				48
49									1							49
50												1				50
51									1							51
52									1		1	2				52
53												1				53
54												1				54
55									1			1				55
56							1			1						56
57								1		1						57
58												2				58
59												1				59
60							1			1						60
61										1						61
62										1						62
63									1							63
64												1				64
65								3		1						65
66									1							66
67												1				67
68									1							68
69								1			1	1				69
70										1						70
71									2							71
72							2		1	2						72
73									1							73
74								1			3	1				74
75										1						75
76												1				76
77												1				77
78										1						78
79												1				79
80									1							80
81							1									81
82							1									82
83							1									83
84							1									84
85							1									85

86							1									86
87							1									87
88							1									88
89					1		1	1								89
90							1									90
91							1									91
92			1													92
93									1		1					93
94														3	3	94
95														1		95
96														1		96
97														1		97
98														1		98
99														1		99
100														1		100
101														1		101
102														1		102
103														1		103
104														1	3	104
105														1		105
106														1		106
107														1		107
108														1		108
109														1		109
110														1		110
111														1		111
112														1		112
113														1		113
114														1		114
115														1		115
116														1		116
117														2	1	117
118														1		118
119														1		119
120														1		120
121														1		121
122														1		122
123														1		123
124														1		124
125														1		125
126														1		126
127														1		127
128														1		128
129														1		129
130														1		130
131														1		131
132														1		132

133														1		133
134														1		134
135														1		135
136														1	7	136
137														1		137
138														1		138
139														1		139
140														1		140
141														1		141
142														1		142
143														2	6	143
144														1		144
145		1		1												145
146				1												146
147				4												147
148				1												148
149				1												149
150				1												150
151				1												151
152				1												152
153				1												153
154																154
155																155
156																156
157																157
158								1								158
159								1								159
160								1								160
161								1								161
162											1					162
163											1					163
164											1					164
165											1					165
166											1					166
167											1					167
168											1				2	168
169															1	169
170															1	170
171															1	171
172															1	172
173															1	173
174															1	174
175															1	175
176															1	176
177															1	177
178															1	178
179															1	179

180															1	180
181															1	181
182															1	182
183															1	183
184															1	184
185															1	185
186															1	186
187															3	187
188															1	188
189															1	189
190															1	190
191															2	191
192															1	192
193															1	193
194															1	194
195															1	195
196															1	196
197															1	197
198															1	198
199															1	199
200															1	200
201															1	201
202															1	202
203															1	203
204															1	204
205															1	205
Sum:	13	30	29	69	28	8	56	28	40	38	33	39	53	57	62	

Table 3.7: Counts of each allele present in each population of the SREB2 locus.

SREB2

Allele	CT Yr1	NY Yr2	NJ Yr1	NJ Yr2	VA Yr1	OI Yr1	NC Yr1	NC Yr2	SC33 Yr1	SC32 Yr1	SC Yr2	GA Yr1	FL Yr1	GM Yr1	GM Yr2	**Allele**
1	4	18	14	52	28	2	28	20	24	21	12	20	26	24	39	1
2							1			1				1	1	2
3								1	1				1			3
4							1									4
5	9	38	15	65	45	11	36	26	39	30	47	40	31	33	59	5
6	1									1						6
7						2										7
8	1			2			1	3	6	3	3		4	2	4	8
9	1			1			1								1	9
10		1		3	2		3	3	2			3	2	2	1	10
11									1				2	3		11
12						1										12

13										1		2				13
14		4	2	7	2			1	6	4	3	4	3	5	5	14
15													1			15
16	1	2		3			2		1				3	1		16
17							1			1						17
18			1	3												18
19		1	3	7	2	1	1		1			1	1	3	3	19
20					1											20
21						1	1			2			1			21
22													1			22
23									1					1	1	23
24										1	2					24
25										2						25
26					2		1		1	1	1	1	1			26
27														1		27
28							1									28
29														1		29
30									1			1	1			30
31										1			1			31
32										1						32
33												1				33
34	1				1											34
35													1			35
36					1							1	1			36
37								2					1			37
38				1						1		1				38
39												1				39
40												1				40
41									1			1				41
42						1				1						42
43										1						43
44	1															44
45										1		1				45
46												1				46
47					1											47
48	1				1		1						1			48
49						1										49
50								2								50
51			1	2	1		1		1							51
52													1			52
53					1											53
54														1		54
55				1												55
56				1												56
57		2														57
58		2														58
Sum:	20	68	36	148	88	20	80	58	86	74	68	80	84	78	114	

Table 3.8: Counts of each allele present in each population of the MYH6 locus.

MYH6

Allele	CT Yr1	NY Yr2	NJ Yr1	NJ Yr2	VA Yr1	OI Yr1	NC Yr1	NC Yr2	SC33 Yr1	SC32 Yr1	SC Yr2	GA Yr1	FL Yr1	GM Yr1	GM Yr2	Allele
1	3	31	11	60	22	8	26	25	30	24	29	29	40	30	33	1
2		1				1			1			1	3	1		2
3		2		2												3
4		3	1	4				3			2		2	2	1	4
5	1				1		2			1			1		2	5
6	2				1		2		3			5	1		2	6
7	1	5	3	8	5	1	3				5			7	17	7
8	6	16	13	53	25	4	22	20	22	13	20	18	18	4	9	8
9										1	1					9
10									2	1			2	3	1	10
11	3	2	5	11	22	2	12	7	13	23	2	18	10	6	7	11
12					1			1							1	12
13	1	2	2	2	2		4		7	7	5	2	2	10	16	13
14		1					2									14
15					3											15
16	2	2		3			1	2			2			3	13	16
17		2	1	5	1				1			1				17
18	1					4	1		1		1				2	18
19							3		3			3		4	3	19
20		1					1								2	20
21									1		1		1	5	4	21
22					3											22
23					2							1	3			23
24							1		1			2				24
25									1	1						25
26										1						26
27										1						27
28										1				2		28
29													1			29
30															1	30
31														1		31
Sum:	20	68	36	148	88	20	80	58	86	74	68	80	84	78	114	

Table 3.9: Counts of each allele present in each population of the ITS2 locus.

ITS2

Allele	CT Yr1	NY Yr2	NJ Yr1	NJ Yr2	VA Yr1	OI Yr1	NC Yr1	NC Yr2	SC33 Yr1	SC32 Yr1	SC Yr2	GA Yr1	FL Yr1	GM Yr1	GM Yr2	Allele
1	1	11	3	22	10	2	23	16	25	25	11	13	22	59	86	1

2		7		25		1	1		4		5	4	2	9	15	2
3		7		16				3			5				3	3
4	6	19	21	40	42	12	32	17	27	22	23	28	39	4	8	4
5	2	2		1					1	1	1	3	4	2		5
6										1						6
7				2							1	1				7
8	11	22	12	40	34	5	22	21	24	21	19	23	14			8
9				2	1		2		4	3	2	6	1			9
10														2		10
11														2	1	11
12					1											12
13									1							13
14								1							1	14
15											1					15
16										1		1	2			16
17												1				17
Sum:	20	68	36	148	88	20	80	58	86	74	68	80	84	78	114	

Table 3.10: Tajima's D and corresponding p-values for all sampling locations for the D-loop locus.

	Tajima's D	p-value
CT Yr1	-1.863	0.008
NY Yr2	-2.008	0.005
NJ Yr1	-1.802	0.011
NJ Yr2	-2.210	0.001
VA Yr1	-1.314	0.082
OI Yr1	-1.055	0.203
NC Yr1	-2.023	0.005
NC Yr2	-1.639	0.033
SC33 Yr1	-2.140	0.001
SC32 Yr1	-1.751	0.022
SC Yr2	-2.291	0.005
GA Yr1	-2.059	0.005
FL Yr1	-2.409	0.000
GM Yr1	-1.737	0.012
GM Yr2	-1.987	0.007
Mean	-1.886	0.027
s.d.	0.359	0.053

Table 3.11: Tajima's D and corresponding p-values for all regions for the D-loop locus.

	Tajima's	p-value

	D	
MAB	-2.285	0.000
SAB	-2.444	0.000
GOM	-2.005	0.000
mean	2.245	0.001
s.d.	0.222	0.002

Table 3.12: Observed vs expected heterozygosity and corresponding p-values for all sampling locations for the SREB2 locus.

	Obs. Het.	Exp. Het.	P-value
CT Yr1	0.800	0.621	1.000
NY Yr2	0.500	0.614	0.065
NJ Yr1	0.833	0.683	0.789
NJ Yr2	0.690	0.676	0.392
VA Yr1	0.591	0.663	0.514
OI Yr1	0.500	0.647	0.088
NC Yr1	0.675	0.654	0.936
NC Yr2	0.655	0.675	0.896
SC33 Yr1	0.674	0.713	0.698
SC32 Yr1	0.730	0.757	0.291
SC Yr2	0.559	0.508	0.264
GA Yr1	0.650	0.687	0.196
FL Yr1	0.690	0.714	0.979
GM Yr1	0.711	0.727	0.725
GM Yr2	0.625	0.631	0.279

Table 3.13: Observed vs expected heterozygosity and corresponding p-values for all sampling locations for the MYH6 locus.

	Obs. Het.	Exp. Het.	P-value
CT Yr1	1.000	0.889	0.714
NY Yr2	0.794	0.648	0.911
NJ Yr1	0.722	0.767	0.039
NJ Yr2	0.577	0.627	0.814
VA Yr1	0.750	0.765	0.745
OI Yr1	0.900	0.847	0.899
NC Yr1	0.700	0.723	0.870
NC Yr2	0.655	0.621	0.056
SC33 Yr1	0.698	0.791	0.001
SC32 Yr1	0.649	0.767	0.016
SC Yr2	0.618	0.661	0.332
GA Yr1	0.750	0.761	0.114

FL Yr1	0.619	0.663	0.573
GM Yr1	0.711	0.764	0.082
GM Yr2	0.804	0.781	0.309

Table 3.14: Observed vs expected heterozygosity and corresponding p-values for all sampling locations for the ITS2 locus.

	Obs. Het.	Exp. Het.	P-value
CT Yr1	0.700	0.626	0.644
NY Yr2	0.647	0.639	0.050
NJ Yr1	0.722	0.557	0.277
NJ Yr2	0.676	0.646	0.050
VA Yr1	0.727	0.614	0.639
OI Yr1	0.600	0.642	0.543
NC Yr1	0.550	0.690	0.311
NC Yr2	0.655	0.666	0.640
SC33 Yr1	0.605	0.743	0.129
SC32 Yr1	0.568	0.725	0.170
SC Yr2	0.735	0.706	0.745
GA Yr1	0.725	0.755	0.298
FL Yr1	0.690	0.689	0.763
GM Yr1	0.500	0.450	0.817
GM Yr2	0.304	0.369	0.095

Table 3.15: Observed vs expected heterozygosity and corresponding p-values for all regions for the SREB2 locus.

	Obs. Het.	Exp. Het.	P-value
MAB	0.621	0.662	0.073
SAB	0.664	0.681	0.898
GOM	0.660	0.669	0.412

Table 3.16: Observed vs expected heterozygosity and corresponding p-values for all regions for the MYH6 locus.

	Obs. Het.	Exp. Het.	P-value
MAB	0.710	0.716	0.070
SAB	0.660	0.720	0.127
GOM	0.766	0.774	0.056

Table 3.17: Observed vs expected heterozygosity and corresponding p-values for all regions for the ITS2 locus.

	Obs. Het.	Exp. Het.	P-value
MAB	0.680	0.637	0.055
SAB	0.645	0.715	0.070
GOM	0.383	0.403	0.618

Table 3.18: Jost's D values run in SPADE for nuclear loci for all region comparisons.

	SREB2	MYH6	ITS2
Total (MAB, SAB, and GOM)	-0.002	0.189	0.447
Pooled Altantic vs GOM	-0.003	0.179	0.562
MAB vs GOM	-0.005	0.203	0.685
SAB vs GOM	-0.002	0.168	0.478
MAB vs SAB	0.000	0.014	0.041

Table 3.19: Migrate summary of profile likelihood percentiles for all parameters (Θ=mutation rates, M=migration, 1=MAB, 2=SAB).

Parameter	Percentiles								
	0.005	0.025	0.05	0.25	MLE	0.75	0.95	0.975	0.995
Θ_1	0.0002	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003
Θ_2	0.0007	0.0007	0.0007	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008
M_21	0.2928	0.2969	0.2989	0.3054	0.3100	0.3146	0.3213	0.3235	0.3278
M_12	0.1174	0.1190	0.1198	0.1223	0.1241	0.1259	0.1286	0.1294	0.1311

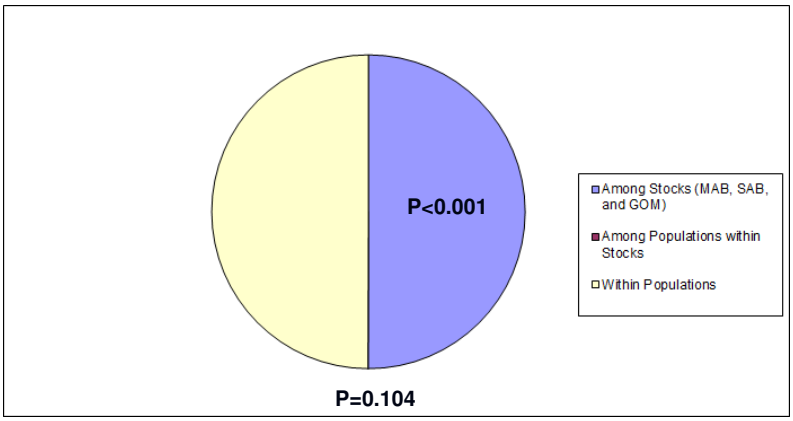

Figure 3.1: Proportion of spatial variation based on Fixation Indices between the MAB, SAB, and GOM stocks of *C. striata* for the D-loop locus.

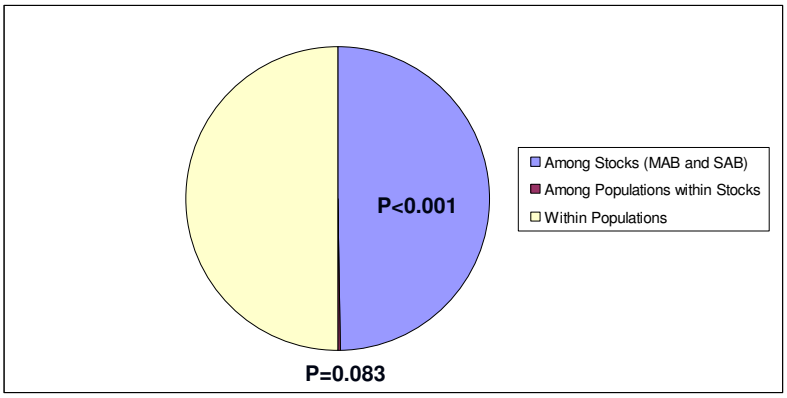

Figure 3.2: Proportion of spatial variation based on Fixation Indices between the MAB and SAB stocks of *C. striata* for the D-loop locus.

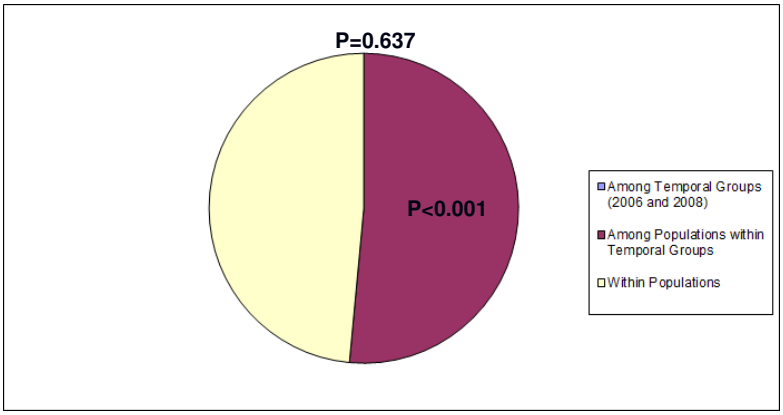

Figure 3.3: Proportion of temporal variation based on

Fixation Indices between 2006 and 2008 samples of *C. striata* for the D-loop locus.

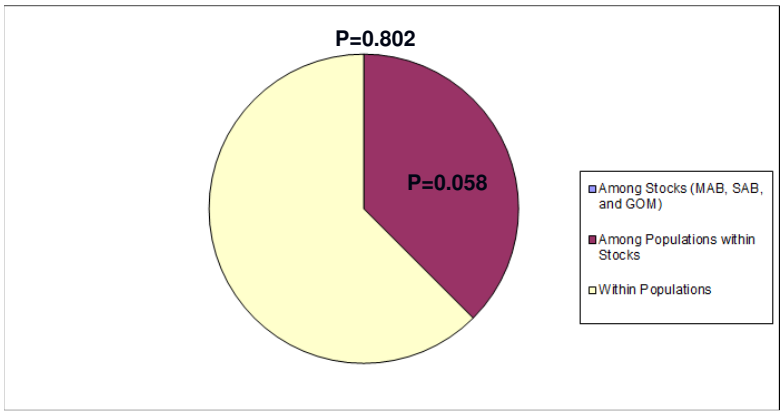

Figure 3.4: Proportion of spatial variation based on Fixation Indices between the MAB, SAB, and GOM stocks of *C. striata* for the combined nuclear loci (SREB2, MYH6, and ITS2).

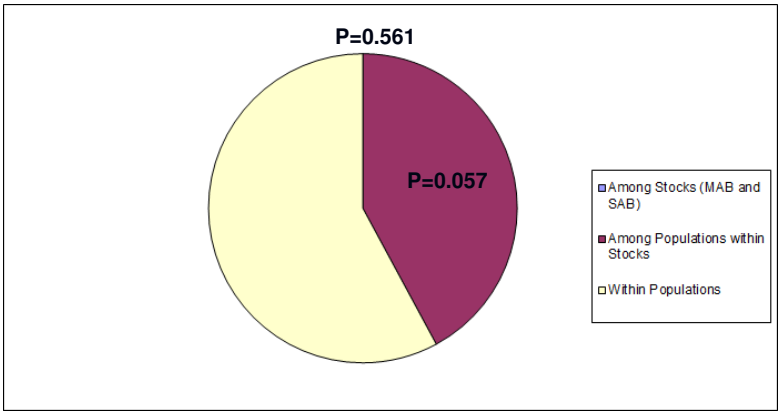

Figure 3.5: Proportion of spatial variation based on Fixation Indices between the MAB and SAB stocks of *C. striata* for the combined nuclear loci (SREB2, MYH6, and ITS2).

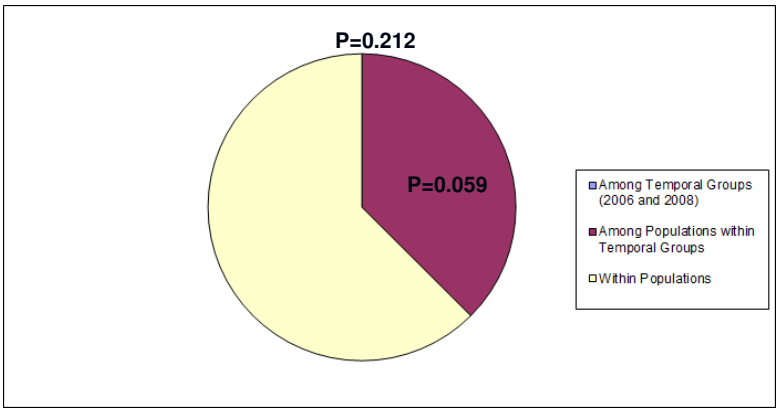

Figure 3.6: Proportion of temporal variation based on Fixation Indices between 2006 and 2008 samples of *C. striata* for combined nuclear loci (SREB2, MYH6, and ITS2).

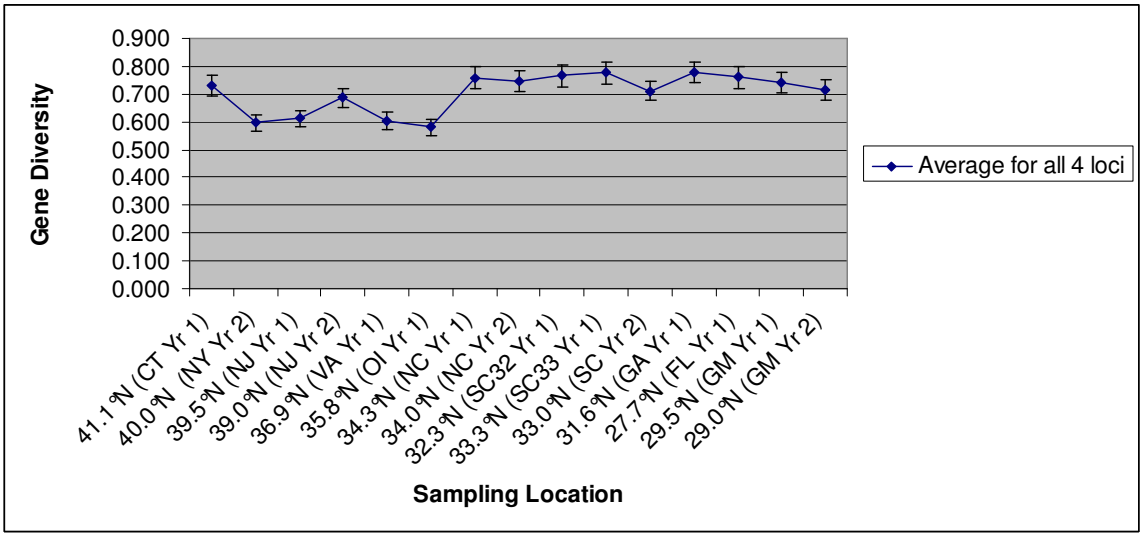

Figure 3.7: Average gene diversity across all four loci for each sampling site.

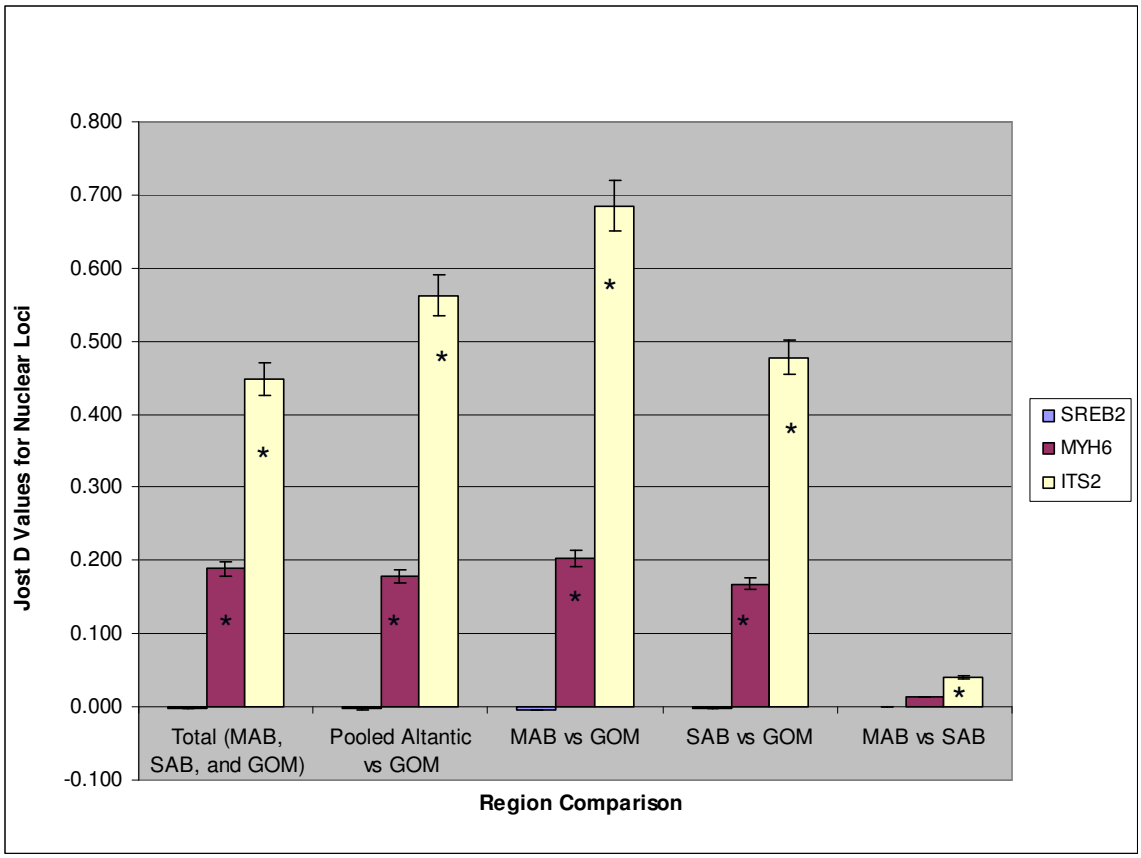

Figure 3.8: Jost D values for all nuclear loci (* indicate significant values).

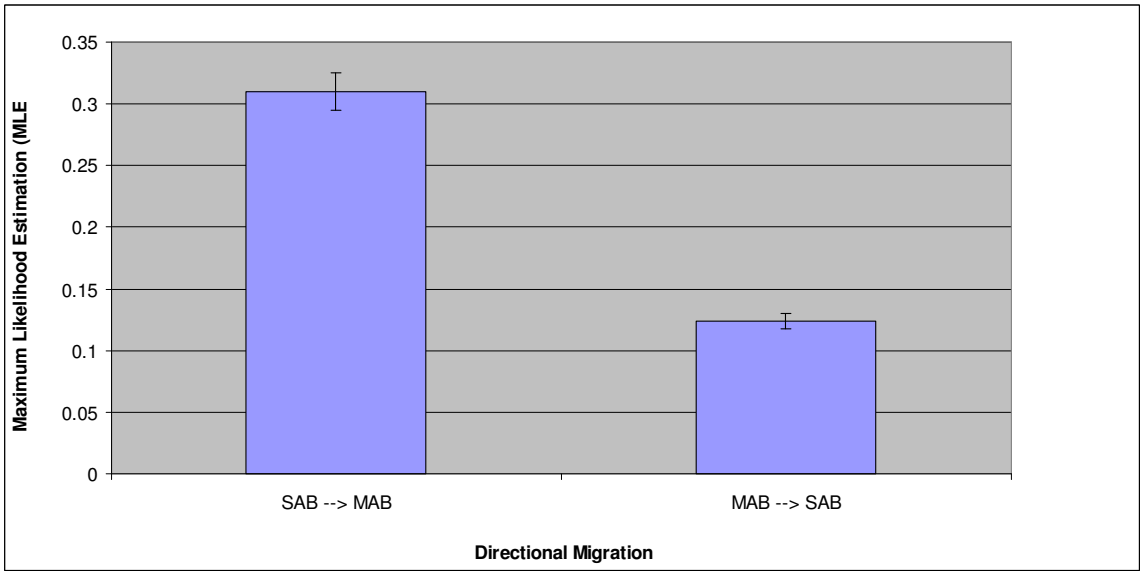

Figure 3.9: Directional migration based on maximum likelihood estimates (MLE).

Chapter 4: Discussion

This study confirmed that there is distinct variation between the two subspecies, which was to be expected. Of interesting note in the case of *C. striata* however, is that individuals found in the Atlantic south of Cape Canaveral are of the Atlantic (*C. striata striata*) subspecies. This is in contrast to other species known to have a split between the Atlantic and Gulf of Mexico, such as the horseshoe crab *Limulus polyphemus* (Saunders et al. 1986) and the American oyster *Crassostrea virginica* (Reeb and Avise 1990), in which individuals south of Cape Canaveral correspond more to Gulf specimens, likely due to the Gulf Stream moving offshore to the continental shelf at that point. It is likely that a limited southern distribution in the eastern Gulf of Mexico has lessened the movement of larvae around the Florida peninsula (Roy et al. 2012). Furthermore, the presence of Atlantic haplotypes in the Gulf of Mexico suggests that exchange of individuals from the Atlantic into the Gulf of Mexico may have occurred recently or may not be entirely absent even today (Roy et al. 2012). However, the large split between the Atlantic and Gulf indicates this is likely still an uncommon occurrence.

Focusing only on western Atlantic samples, with the Gulf samples omitted, also yielded high diversity between groups. Though not as noticeable as when Gulf specimens were included, the diversity was high enough to indicate a very clear distinction between the MAB and SAB based around Cape Hatteras. This finding agrees with the determination made by Roy et al (2012).

Variation within populations was also very high, which is often seen in various species, including humans (Bowcock et al. 1994). However, variation between populations within regions was extremely low. All of these values indicate a great deal of gene flow between specimens in the MAB (off Connecticut, New York, New Jersey, Virginia, and North Carolina north of Cape Hatteras) and also specimens in the SAB (North Carolina south of Cape Hatteras, South Carolina, Georgia, and Florida).

All of this indicates that *C. striata* is a species that undergoes a good deal of gene flow under normal circumstances, but is largely blocked by the barrier of Cape Hatteras. While it is a clear zoogeographic barrier, the effectiveness of Cape Hatteras as a gene flow barrier has varied between species. For instance, studies on mtDNA restriction fragment length polymorphisms in oyster toadfish *Opsanus tau* have shown that it has a break at Hatteras (Avise et al. 1987). However, oyster toadfish have non-planktonic eggs and larvae and are thus more likely to be affected by barriers since their dispersal ability is limited.

For fish that do have planktonic egg and larval stages, Cape Hatteras has often been shown to not be a barrier to gene flow. Studies on mtDNA RFLPs on summer flounder *Paralichthys dentatus* (Jones and Quattro 1999), weakfish *Cynoscion regalis* (Graves et al. 1992a), and bluefish *Pomatomus saltatrix* (Graves et al. 1992b), have shown no genetic break across Cape Hatteras. One potential reason that differentiates black sea bass from these other fish in this regard is spawning location. Black sea bass tend to spawn more inshore (Bowen and Avise 1990), even in the MAB where migration offshore takes place during winter, not during the primary spawning time of summer. Summer flounder spawn offshore on the continental shelf thoughout their range (Jones

and Quattro 1990). Bluefish are known to spawn on the outer Carolina shelf during summer in the SAB, and the young of these have shown up in estuaries in the MAB (Hare and Cowen 1996).

The varying types of migrations taken by different species may be another factor in black sea bass having a split at Cape Hatteras. Bluefish in the MAB migrate south in winter, leading to them crossing into the SAB (Graves et al. 1992b). While black sea bass migrating offshore in the MAB may move south somewhat, it is more commonly to areas still within the MAB, such as the Chesapeake Bight (Musick and Mercer 1977). This is not to say that black sea bass in the most southern parts of the MAB never make it across Cape Hatteras, but based on the genetic split seen north and south it must happen rarely.

While there have been multiple cases of fish having a planktonic larval stage not being split at Cape Hatteras, studies on other species with this type of larvae, such as Atlantic croaker *Micropogonias undulates* have given an indication of Cape Hatteras being the cause of genetic differentiation (Baker et al. 2007). Though they were originally thought to spawn at mid- and outer-shelf locations throughout their range, there is an indication that a significant amount of spawning may occur nearshore at least in the MAB due to postovular follicles in adults collected in the Chesapeake Bay (Thorrold et al. 1997). This further supports nearshore spawning as one potential factor for limiting gene flow across Cape Hatteras.

Nuclear DNA yielded noticeably different results from mitochondrial. Variation was extremely low both comparing the Atlantic and GOM as well as the MAB and SAB. One way this can be accounted for is by looking at the differences between mitochondrial

and nuclear DNA. mtDNA is maternally inherited while nDNA is inherited from both parents (Giles et al 1980). Maternal inheritance of mtDNA is particularly useful in tracking what females of a species are doing (Roberts et al 2005). In the case of Serranids, however, the fact that they are protogynous hermaphrodites means that all individuals are female at some point which complicates the use of mtDNA to study them. Another point of interest for the maternal inheritance of mtDNA is its effect on mutation rate. mtDNA is more likely to undergo mutations than nuclear DNA, in large part to the fact that it has only one-fourth the effective population size of nDNA and thus genetic drift is going to have a much greater effect on it (Reeb and Avise 1990). In addition, mtDNA has a higher turnover rate than nDNA, which provides more rounds of replication that lead to a greater chance of errors occurring (Brown et al. 1979). This higher mutation rate thus leads to faster evolution for mtDNA.

By comparison, nuclear DNA is much more conservative in its rate of mutation. mtDNA typically has only ¼ the effective population size of nDNA since it is inherited maternally as opposed to both parents (Crease et al. 1990). This can lead to genetic drift having a much greater effect on it and thus a faster divergence once populations have split. In the case of *C. striata*, this effect may not be as great since it is a protogynous hermaphrodite, starting maturity as a female and then changing sex to male later on. This leads to a likely greater number of females since all fish must pass through this stage before becoming males, thus leading to a larger than normal effective population size for mtDNA. Nonetheless, it is still likely significantly smaller than for nDNA.

Looking at the specific nuclear genes themselves, SREB2 is one of the G-protein coupled receptors, a family known for a high degree of sequence conservation throughout

vertebrate evolution (Matsumoto et al. 2005). MYH6 is a highly conserved region of the alpha myosin motor domain (Posch et al. 2011). ITS2 is an internal transcribed spacer found between two structural ribosomal RNAs (Chen et al. 2001).

Although nDNA is more conserved than mtDNA, there were still numerous alleles present when all three nuclear loci were taken into account. Fst values don't necessarily accurately reflect differentiation when gene diversity is high (Jost 2008). To account for this in the nDNA, a SPADE analysis (Chao and Shen 2010) was used to generate Jost D values for nDNA. These results showed higher divergence between the Atlantic and Gulf for ITS2, a lower but demonstrable divergence between the two regions for MYH6, and a low but present divergence between the MAB and SAB for both loci. SREB2 results were extremely low for all comparisons made. Although SREB2 results were low, the MYH6 Jost D values show a significant difference between the Atlantic and Gulf, and ITS2 values show significant differences between the Altantic and Gulf as well as north and south of Cape Hatteras. The significant differences in ITS2 can likely be explained by lower effective population size compared to other nDNA due to clustered evolution (Navajas and Boursot 2003).

The differentiation seen in nDNA further supports a split at Cape Hatteras. However, the noticeably lower differentiation than seen in mtDNA provides strong support for male-mediated gene flow. Since nDNA is inherited from both parents and mtDNA is inherited only maternally, it could be that males are the individuals traveling between populations when such migration does occur. Thus mtDNA would be isolated in each population, while nDNA would still have some degree of mixing. This type of gene flow has been observed in other marine organisms including loggerhead sea turtles

(*Caretta caretta*) (Carreras et al. 2006) and great white sharks (*Carcharodon carcharias*) (Pardini et al. 2001). In the case of *C. striata*, the fact that they are protogynous hermaphrodites means the older and larger individuals are males, which may further add to the likelihood of them being the ones to travel long distances.

Using both mtDNA and nDNA allows for insight into divergence among multiple populations. In this case, mtDNA shows strong evidence that there is a split in the Atlantic, whereas nDNA shows that such a split is not easily recognizable though still evident. Because nDNA shows less divergence between the Atlantic and Gulf, the lack of strong observed differences between the Atlantic regions indicated by this test might not be as reliable as tests with mtDNA. Alternatively, noticeable differences in nDNA could be used to justify a split into multiple species. However, the lack of variation between all regions refutes those possibilities. Because mtDNA shows a differentiation between the north and south Atlantic that is smaller than when including the Gulf, a noticeable split between the populations in the Atlantic that may not be large enough to justify subspecies distinction. Nevertheless, the difference is large enough to indicate that a separation occurred many thousands of years ago.

Temporal variation was almost nonexistent in *C. striata* for all loci indicating that variation seen was neither year-to-year variation nor chance differentiation in the spawning stocks. Although the samples were only a few years apart, this finding agrees with Roy et al. (2012), in which samples were collected ten years apart, which addresses the absence of a temporal split. Further supports of this lies in the similarity of gene diversity across the entire range. Clinal variation in gene diversity can indicate secondary intergradation of different populations as seen in mummichogs *Fundulus heteroclitus*

(Gonzalez-Villasenor and Powers 1990) and lake cisco *Coregonus artedi* (Turgeon and Bernatchez 2001), as well as be evidence of recent range expansion, as seen in blue crabs *Callinectes sapidus* (Mcmillen-Jackson and Bert 2004). Lack of this in black sea bass counters this and thus supports populations that have been split for some time.

The heterozygosity tables showed almost no significant differences between observed and expected heterozygosity, indicating that all of the regions and almost all of the sites were in Hardy-Weinberg equilibrium. The only exception was the MYH6 locus at NJ Year 1, SC33 Year 1, and SC32 Year 1. All of these sampling sites had a deficit of observed heterozygotes. This can be caused by selection against heterozygotes, assortative mating and migration between divergent populations, as well as by inbreeding (Jiggins and Mallet 2000). However, because it only occurred in one of the loci for the few populations, it may be sampling chance.

In terms of how much these stocks are still mixing and in what ways, greater migration occurs between populations in the MAB and SAB than between either Atlantic stock and the Gulf of Mexico, as would be expected. Migration was greater south to north than north to south. This is the opposite of what was seen in Roy et al. 2012, which showed greater migration north to south, likely due to the northern stocks undergoing migration and thus being more likely to travel to a new region, as well as the ability to drift off the area of convergence at Cape Hatteras along the coastline (Roy et al. 2012). However, there are also mechanisms with which *C. striata* could drift north as well. It has been seen in bluefish that larvae spawned south of Cape Hatteras can drift from the SAB to the MAB by warm-core ring streamers (Hare and Cowen 1996). Black sea bass spawn further inshore in the SAB than bluefish and are thus less likely to be carried north

this way, however it is possible that larvae occasionally drift offshore and thus might be brought to the MAB by this mechanism. Further research on the likelihood of black sea bass larvae in the SAB drifting offshore would help to support or refute this.

Conversely, bluefish have also been shown to migrate north across Cape Hatteras by direct swimming (Hare and Cowen 1996). If black sea bass are doing this as well, it could likely be the larger males making the journey and thus further support male-mediated gene flow and also explain why migration rates were opposite to what was seen in Roy et al. 2012. Males migrating from the SAB to MAB would only be seen via analysis of nDNA, and thus with only mtDNA the migration may seem greater from the MAB to SAB. Whether it is planktonic larvae drifting across Cape Hatteras or fish actively swimming across or both, it is likely not a common occurrence as mixing between the MAB and SAB remains low.

Conclusions

Based on the findings of this study, the genetic and basic biological information on *C. striata* are concordant. It appears to be under an appropriate management strategy, which recognizes two different stocks in the Atlantic. However, the genetic information adds a new dimension beyond identification of stocks, that which there is migration occurring and it appears to be biased (with a greater amount occurring from the SAB to MAB than the other way around). Although some migration is occurring between them, it is minimal and not enough to warrant a change in management. Fortunately the stocks have rebounded, but continued monitoring of populations will help avoid another drop in the future. The techniques used in this experiment can be put to use with other species of Serranids, especially those that are endangered, to better understand the structure of their

populations. On an even broader scale, continued genetic research on marine species using both mitochondrial and nuclear DNA and the tests conducted here can help provide meaningful suggestions for fisheries management.

References

Alonzo, S. H., and Mangel, M. 2004. The effects of size-selective fisheries on the stock dynamics of and sperm limitation in sex-changing fish. *U.S. National Marine Fisheries Service Fishery Bulletin* 102: 1–13.

Armsworth, P. R. 2001. Effects of fishing on a protogynous hermaphrodite. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 568–578.

Avise, J.C., Reeb, C.A. and Saunders, N.C. 1987. Geographic population structure and species differences in mitochondrial DNA of mouthbrooding marine catfishes (Ariidae) and demersal spawning toadfishes (Batrachoididae). *Evolution*, 41: 991-1002.

Avise, J. C. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos*, 63: 62–76. [CrossRef], [Web of Science ®], [CSA]

Baker, T. G., Morand, S., Wenner, C. A., Roumillat, W. A. and de Buron, I. 2007. Stock identification of the sciaenid fish *Micropogonias undulatus* in the western North Atlantic Ocean using parasites as biological tags. *Journal of Helminthology*, 81: 155–167. [CrossRef], [Web of Science ®]

Bangs, M.R. 2011. Decline of the Savannah River Redeye Bass (*Micropterus coosae*) due to Introgressive Hybridization with Invasive Alabama Spotted Bass (*Micropterus*

punctulatus henshalli). Master's Thesis, University of South Carolina.

Beerli, P. (1998) Estimation of migration rates and population sizes in geographically structured populations. In: Advances in molecular ecology (Ed. G. Carvalho). NATO-ASI workshop series. IOS Press, Amsterdam. Pp. 39-53.

Beerli, P. and J. Felsenstein (1999) Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics*, 152(2): 763-73,1999.

Beerli, P. and J. Felsenstein (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences of the USA*, 98(8): 4563-4568.

Bowcock, A.M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J.R. and Cavalli-Sforza, L.L. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, 368: 455-457.

Bowen, B. W. and Avise, J. C. 1990. Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: influence of zoogeographic factors and life-history patterns. *Marine Biology*, 107: 371-381.

Brown, W.M., George Jr., M. and Wilson, A.C. 1979. Rapid evolution of animal mitochondrial DNA. *Proc. Natl. Acad. Sci. USA*, 76(4): 1967-1971.

Carreras, C., Pascual, M., Cardona, L., Aquilar, A., Margaritoulis, D., Rees, A., Turkozan, O., Levy, Y., Gasith, A., Aureggi, M., and Khalil, M. 2006. The genetic structure of the loggerhead sea turtle (*Caretta caretta*) in the Meditteranean as revealed by nuclear and mitochondrial DNA and its conservation implications. *Conserv Genet* 8: 761-775.

Chao, A. and Shen, T.-J. (2010) Program SPADE (Species Prediction And Diversity Estimation). Program and User's Guide published at http://chao.stat.nthu.edu.tw.

Chapman R.W., Sedberry, G.R., Koenig, C.C. and Eleby B.M. 1999. Stock identification of gag, *Mycteroperca microlepsis*, along the southeast coast of the United States. *Mar Biotechnol*, 1: 137-146.

Chen, Y., Eisner, J.D., Kattar, M.M., Rassoulian-Barrett, S.L., Lafe K, Bui, U., Limaye, A.P. and Cookson, B.T. 2001. Polymorphic internal transcribed spacer region 1 DNA sequences identify medically important yeasts. *Journal of Clinical Microbiology*, 39:4042–4051.

Conover, D.O. and Munch, S.B. 2002. Sustaining fisheries yields over evolutionary time scales. *Science*, 5:94-96.

Crease, T.J., Lynch, M., and Spitze, K. 1990. Hierarchial analysis of population genetic variation in mitochondrial and nuclear genes of *Daphnia pulex*. *Mol. Biol. Evol.* 7(5): 444-448.

Cupka, D. M., Diaz, R. K. and Tucker, J. 1973. Aspects of the fishery for and biology of *Centropristis striata* in South Carolina waters. *South Carolina Dep. Wild. Resour. Annu. Rep. Proj.,* No. 2-138-R01. 64p.

Excoffier, L. E., Smouse, P.E. and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, 131:479-491.

Excoffier, L. and Lischer, H.E. L. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10: 564-567.

Fornshell, J.A., Frydenlund, D.D. and Christensen, P.D. 1984. Winter distribution of four species of the genus *Ceratium* from Cape Canaveral, Florida to Cape Henlopen, Delaware. *Journal of Plankton Research*, 6(1): 15-27.

Giles, R.E., Blanc, H., Cann, H.M. and Wallace, D.C. 1980. Maternal inheritance of human mitochondrial DNA. *Proc. Natl. Acad. Sci. USA*, 77(11): 6715-6719.

Gonzalez-Villasenor, L.I. and Powers, D.A. 1990. Mitochondrial-DNA restriction-site polymorphisms in the teleost *Fundulus heteroclitis* support secondary intergradation. *Evolution*, 44(1): 27-37.

Graves, J. E., McDowell, J.R., and Jones, M.L. 1992a. A genetic analysis of weakfish (*Cynoscion regalis*) stock structure along the mid-Atlantic coast. *U.S. National Marine Fisheries Service Fishery Bulletin,* 90: 469–475.

Graves, J.E., McDowell, J.R., and Jones, M.L. 1992b. Stock structure of the bluefish *Pomatomus saltatrix* along the mid-Atlantic coast. *U.S. National Marine Fisheries Service Fishery Bulletin*, 90: 703-710.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program from Windows 95/98/NT. *Nucleic Acids Symposium Series*, No 41. 95-98.

Hare, J.A. and Cowen, R.K. 1996. Transport mechanisms of larval and pelagic juvenile bluefish (*Pomatomus saltatrix*) from South Atlantic Bight spawning grounds to Middle Atlantic Bight nursery habitats. *Limnol. Oceanogr,* 41(6): 1264-1280.

Heath, D.D., Busch, C., Kelly, J., and Atagi, D.Y. 2002. Temporal change in genetic structure and effective population size in steelhead trout (*Oncorhynchus mykiss*). *Mol Ecol* 11(2): 197-214.

Innan, H. and Stephan, W. 2000. The coalescent in an exponentially growing metapopulation and its application to *Arabidopsis thaliana. Genetics*, 155(4): 2015-2019.

Jones, W. J. and Quattro, J. M. 1999. Genetic Structure of Summer Flounder (*Paralichthys dentatus*) Populations North and South of Cape Hatteras. *Marine Biology*, 133:129-135.

Jost L. 2008. G_{ST} and its relatives do not measure differentiation. *Molecular Ecology* 17(18), 4015-4026.

Jiggins, CD and Mallet, J. 2000. Bimodal hybrid zones and speciation. *Trend in Ecology and Evolution*, 15(6): 250-255.

Koenig, C.C., Coleman, F.C., Eklund, A.M., Schull, J. and Ueland, J. 2007. Mangroves as essential nursery habitat for goliath grouper (*Epinephelus itajara*). Bulletin of Marine *Science*, 80(3): 567-586.

Lehnert, R.L. 2000. Subtidal oyster rubble as overlooked essential fish habitat: with emphasis on age and growth of young of the year black sea bass (*Centropristis striata*) in that habitat. MS Thesis. Marine Science Program. University of South Carolina. 165 pp.

Lehnert, R.L. and Allen, D.M. 2002. Nekton use of subtidal oyster shell habitat in a southeastern U.S. estuary. *Estuaries*, 25:1015-1024.

Matsumoto, M., Beltaifa, S., Weickert, C.S., Herman, M.M., Hyde, T.M., Saunders, R.C., Lipska, B.K., Weinberger, D.R. and Kleinman, J.E. 2005. A conserved mRNA expression profile of SREB2 (GPR85) in adult human, monkey, and rat forebrain. *Brain Res Mol Brain Res*, 138(1)**:**58-69.

McGovern, J. C., Collins, M. R., Pashuk, O., and Meister, H. S. 2002. Temporal and spatial differences in life history parameters of black sea bass in the southeastern United States. *North American Journal of Fisheries Management*, 22: 1151-1163.

McMillen-Jackson, A.L. and Bert, T.M. 2004. Mitochondrial DNA variation and population genetic structure of the blue crab *Callinectes sapidus* in the eastern United States. *Marine Biology*, 145: 769-777.

Melnick, D.J. and Hoelzer, G.A. 1991. Differences in male and female macaque dispersal lead to contrasting distributions of nuclear and mitochondrial DNA variation. *International Journal of Primatology*, 13(4): 379-393.

Mercer, L. P. 1978. The reproductive biology and population dynamics of the black sea bass, *Centropristis striata*. Doctoral dissertation, College of William and Mary, Williamsburg, VA. 195p.

Miller, R.J. 1959. A review of the sea basses of the genus *Centropristes* (Serranidae) *Tul. Stud. Zool*, 7:35-68.

Musick, J.A. and Mercer, L.P. 1977. Seasonal distribution of black sea bass, *Centropristis striata*, in the Mid-Atlantic Bight with comments on the ecology and fisheries of the species. *Transactions of the American Fisheries Society*, 106(1): 12-25.

Navajas, M. and Boursot, P. 2003. Nuclear ribosomal DNA monophyly versus mitochondrial DNA polyphyly in two closely related mite species: the influence of life history and molecular drive. Proceedings of the Royal Society Biological Sciences.

Oswald, K. J. 2007. Phylogeography and Contemporary History of Redeye Bass

(*Micropterus coosae*). Ph.D. Dissertation, University of South Carolina.

Pardini, A.T., Jones, C.S., Noble, L.R., Kreiser, B., Malcolm, H., Bruce, B.D., Stevens, J.D., Cliff, G., Scholl, M.C., Francis, M., Duffy, C.A.D., and Martin, A.P. 2001. Sex-based dispersal of great white sharks. *Nature* 412: 139-140.

Pickart, R.S. and Watts, D.R. 1990. Deep western boundary current variability at Cape Hatteras. *Journal of Marine Research*, 48(4): 765-791.

Posch, M.G., Waldmuller, S., Muller, M., Scheffold, T., Fournier, D., Andrade-Navarro, M.A., De Geeter, B., Guillaumont, S., Dauphin, C., Youseff, D., Schmitt, K.R., Perrot, A., Berger, F., Hetzer, R., Bouvagnet, R. and Ozcelik, C. 2011. Cardiac Alpha-Myosin (*MYH6*) Is the Predominant Sarcomeric Disease Gene for Familial Atrial Septal Defects. *PLOS One*, 6:e28872.

Reeb, C. A. and Avise, J.C. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster *Crassostrea virginica*. *Genetics*, 124: 397–406.

Roberts, M.A., Anderson, C.J., Stender, B., Sergars, A., Whittaker, J.D., Grady, J.M. and Quattro, J.M. 2005. Estimated contribution of Atlantic Coastal loggerhead turtle nesting populations to offshore feeding aggregations. *Conservation Genetics*, 6:133-139.

Roy, E.M., Quattro, J.M. and Greig, T.W. 2012. Genetic Management of Black Sea Bass: Influence of Biogeographic Barriers on Population Structure. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science*, 4(1): 391-402.

SAFMC (South Atlantic Fishery Management Council). 2006. Final Amendment 13C to the Fishery Management Plan for the Snapper Grouper Fishery of the South Atlantic

Region Including a Final Environmental Impact Statement, Biological Assessment, Initial Regulatory Flexibility Analysis, Regulatory Impact Review and Social Impact Assessment/Fishery Impact Statement. South Atlantic Fishery Management Council, 1 Southpark Circle, Suite 306, Charleston, South Carolina, 29407-4699.

SAFMC (South Atlantic Fishery Management Council). 2013. Snapper Grouper Regulatory Amendment 19: Adjustment to Black Sea Bass catch limits. Council Staff. South Atlantic Fishery Management Council, 4055 Faber Place, Suite 201, North Charleston, SC 29405.

Saunders, N. C., L. G. Kessler, and J. C. Avise. 1986. Genetic variation and geographic differentiation in the mitochondrial DNA of the horseshoe crab, *Limulus polyphemus*. *Genetics*, 112: 613–627.

Schwartz, F.J. 1989. Zoogeography and ecology of fishes inhabiting North Carolina's marine waters to depths of 600 meters. In: George RY, Hulbert AW (eds) North Carolina Coastal Oceanography Symposium. U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, Oceanic and Atmospheric Research, Office of Undersea Research, Rockville, Maryland, pp 335±374

SEDAR (SouthEast Data, Assessment, and Review) Assessment Update #1. 2005. Report of Stock Assessment: Black Sea Bass. SEDAR Update Process #1. Assessment Workshop of March 15–17, 2005. Beaufort.

Seyoum, S., Tringali, M. D., Bert, T. M., McElroy, D. and Stokes, R. 2000. An analysis of genetic population structure in red drum, *Sciaenops ocellatus*, based on mtDNA control region sequences. *U.S. National Marine Fisheries Service Fishery Bulletin*, 98: 127–138. [Web of Science ®]

Shepherd, G. R. 2009. Black sea bass 2009 stock assessment update. *National Marine Fisheries Service, Northeast Fisheries Science Center, Reference Document 09-16, Woods Hole, Massachusetts*.

Steimle, F. W., Zetlin, C. A., Berrien, P. L. and Chang, S. 1999. Essential fish habitat source document: black sea bass, *Centropristis striata*, life history and habitat characteristics. *NOAA Tech. Rep. NMFS*, 143. 42p.

Tajima, F. 1989. Statistical method for testing neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123 (3): 585-595.

Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research.* 22:4673-4680.

Thorrold, S.R., Jones, C.M., and Campana, S.E. Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic Croaker (*Micropogonias undulates*). *Limnol. Oceanogr.*, 42(1): 102-111.

Turgeon, J. and Bernatchez, L. 2001. Clinal variation at microsatellite loci reveals historical secondary intergradation between glacial races of *Coregonus artedi* (Teleosti: Coregoninae). *Evolution*, 55(11): 2274-2286.

Wang, L. and Xu, Y. 2003. Haplotype inference by maximum parsimony. *Bioinformatics*, 19:1773–1780.

Wenner, C. A., Roumillat, W. A. and Waltz, C. W. 1986. Contributions to the life history of black sea bass, *Centropristis striata*, off the southeastern United States. *U.S. National Marine Fisheries Service Fishery Bulletin*, 84: 723-741.

Appendix A: Sampling Locations

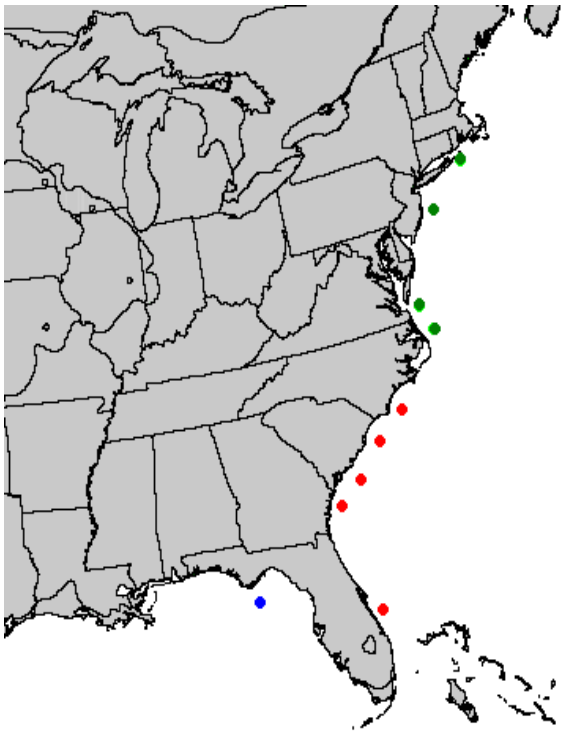

Figure A.1: Sampling Locations for "Year 1" (2006).

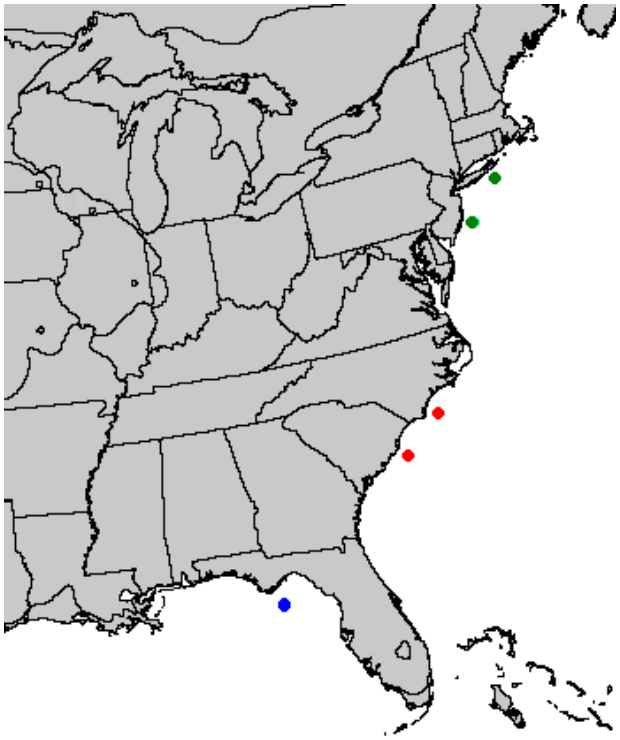

Figure A.2: Sampling Locations for "Year 2" (2008).

Appendix B: Summary of Fst and p-values

Table B.1: Fst (lower left) and accompanying p-values (upper right) between the different regions for the D-Loop locus.

	MAB	SAB	GOM
MAB	X	0.00	0.00
SAB	0.428	X	0.00
GOM	0.781	0.701	X

p-value:=.00

	GOM
Combined Atlantic	0.770

Table B.2: Fst (lower left) and accompanying p-values (upper right) between the different regions for combined nuclear loci.

	MAB	SAB	GOM
MAB	X	0.04	0.39
SAB	0.011	X	0.05
GOM	0.000	0.005	X

p-value:=.05

	GOM
Combined Atlantic	0.003

Table B.3: Fst (lower left) and accompanying p-values (upper right) between the different regions for the SREB2 locus.

	MAB	SAB	GOM
MAB	X	0.03	0.40
SAB	0.007	X	0.04
GOM	0.000	0.005	X

p-value:=.06

	GOM
Combined Atlantic	0.003

Table B.4: Fst (lower left) and accompanying p-values (upper right) between the different regions for the MYH6 locus.

	MAB	SAB	GOM
MAB	X	0.01	0.00
SAB	0.016	X	0.00
GOM	0.057	0.047	X

p-value:=.00 **GOM**

Combined Atlantic 0.053

Table B.5: Fst (lower left) and accompanying p-values (upper right) between the different regions for the ITS2 locus.

ITS2

	MAB	SAB	GOM
MAB	X	0.00	0.00
SAB	0.029	X	0.00
GOM	0.335	0.246	X

p-value=.00 **GOM**

Combined Atlantic 0.274