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The Role of Artificial Structures in Facilitating Range Expansion of the Introduced Barnacle Megabalanus Coccopoma in the Southeastern U.S.A.

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THE ROLE OF ARTIFICIAL STRUCTURES IN FACILITATING RANGE EXPANSION OF THE INTRODUCED BARNACLE *MEGABALANUS COCCOPOMA* IN THE SOUTHEASTERN U.S.A.

by

Alicia M. Reigel

(Under the direction of Daniel F. Gleason and J. Scott Harrison)

ABSTRACT

The barnacle *Megabalanus coccopoma* is a recent invader of the southeastern U.S.A. from the tropical eastern Pacific. In Georgia, *M. coccopoma* populations along the immediate coastline often suffer extensive mortality during the winter, but population rebuilding is common after these events suggesting that there may be nearby larval sources. I investigated the hypothesis that artificial structures (i.e., buoys, towers), occurring far enough offshore of Georgia for water temperatures to be moderated by the Gulf Stream, provide refuges for breeding adults of *M. coccopoma* and can serve as the larval source. I investigated this hypothesis by first developing thirteen microsatellite primer pairs specific to *M. coccopoma*. I also developed the polymerase chain reaction (PCR) and sequencing protocols for use with the primers. These 13 primer pairs were tested on 42 individuals from two populations of *M. coccopoma*. The results indicated high allelic diversity in all of the loci making these primers useful in evaluating population genetics questions related to *M. coccopoma*. To further evaluate the role of artificial structures in the range expansion of *M. coccopoma*, I collected demographic information on existing populations, monitored temperature and salinity both on and offshore, and assessed genetic diversity and structure at 8 research sites ranging from the shoreline to ~50km offshore in the southeast.

Demographic information and abiotic parameter monitoring indicated that offshore artificial structures are suitable habitats for *M. coccopoma* adults and these structures also house *M. coccopoma* populations that are composed of stable, mature individuals that can serve as an abundant source of larvae. The genetic assessment revealed high allelic diversity and significant deviations from Hardy-Weinberg Equilibrium (HWE) in all subpopulations. The analysis of genetic structure indicated that the *M. coccopoma* population in the Georgia Bight is panmictic and suggested that a Wahlund Effect is acting to increase allelic diversity and causing HWE deviations. The combined results support my hypothesis that offshore structures in the Georgia Bight can act as refuges for breeding adults, however there are likely additional larval sources from beyond the region examined that are facilitating the range expansion of *M. coccopoma* in the southeastern U.S.A.

INDEX WORDS: *Megabalanus coccopoma*, Introduced species, Barnacle, Microsatellite analysis, Genetics, Self-recruitment, Georgia Bight

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LITERATURE REVIEW

Invasive species

Biological invasions are defined as the arrival, establishment and diffusion of any non-native species to an area where they did not previously exist through one of two methods: natural range expansion or transport by human activity (Carlton 1987). There are several characteristics common among successful invaders including *r*-selected life histories (high fecundity, fast growth rates, and short generation times), tolerance to broad ranges of environmental conditions, competitiveness, and the ability to occupy empty niches in an ecosystem (Williamson and Brown 1986, Rejmanek and Richardson 1996, Ruiz et al. 2000, Sax and Brown 2000, Sakai et al. 2001). Additionally the ability to efficiently colonize disturbed habitats may increase the invasive potential of a species (Cohen and Carlton 1998, Stachowicz et al. 1999, Sax and Brown 2000, Levin et al. 2002). Current estimates suggest that 1 in 10 introduced species will become established, and of those that establish 1 in 10 will become pests (Williamson and Brown 1986, Williamson and Fitter 1996). Invasive species have the potential to trigger ecosystem change within the invaded region and become a threat to native biodiversity through competition and/or predation (Wilcove 1998, Sax et al. 2005). This threat to native species makes understanding the pathways that facilitate the range expansion of invaders an important area of focus.

Most biological invasions are the result of human activities, either deliberate or accidental (OTA 1993, Pimental 2005). The increase in shipping and transportation in recent decades has correlated with an increase in successful aquatic invertebrate invasions (Elton 1958, Carlton 1987, OTA 1993, Ruiz 2000, Pimental et al. 2005). Power et al. (2006) estimate that at least 7,000 different marine species, from many phyla, are commonly carried in ballast water and on ship hulls. A reported 298 species of introduced marine algae and invertebrates are found in

coastal environments of North America with shipping being the sole vector of introduction for 51% of those species (Ruiz et al. 2000). Mytilid bivalves such as *Perna viridis*, *Mytilus galloprovincialis* and *Perna perna* have successfully established invasive populations within North America due in large part to their ability to survive in ballast water and on ship hulls (Carlton 1996, Hicks and Tunnell 1993, Hicks et al. 2001, Baker et al. 2007). Shipping is also responsible for the successful establishment of invasive populations of cnidarians (Darling et al. 2004), echinoderms, ctenophores (Carlton 1996), tunicates (Lambert and Lambert 1998) and arthropods (Yamaguchi et al. 2009).

Invasive invertebrates are more successful than native invertebrates at recruiting to and establishing populations in disturbed habitats, including artificial structures (Cohen and Carlton 1998, Glasby et al. 2007, Dafforn et al. 2012). Non-native ascidians have also been highly successful invaders by establishing populations on artificial structures such as docks and pilings (Simkanin et al. 2012). Astudillo et al. (2009) surveyed the biota on aquaculture buoys in the SE Pacific and found that suspension-feeders were the most common species in both number and biomass. Foster and Willan (1979) assessed the marine community on an oil rig traveling from Osaka, Japan to New Zealand. Upon arrival in New Zealand the rig was inhabited by twelve species of barnacles, including six species that were previously unrecorded in New Zealand.

Barnacles, in particular, have considerable invasive potential due to their life-history characteristics. They often have high fecundities coupled with larvae that are able to disperse long distances, and the ability to adapt to novel environments (Stanley and Newman 1980, Becker et al. 2007, Gilg et al. 2010). Barnacles have been documented both as post-larvae attached to ships hulls and as larvae within ballast water (Zardus and Hadfield 2005). Most balanomorph barnacle species live above 100 m water depths (Doyle et al. 1997). The sessile

adult phase is generally typified by settlement directly to hard substrates such as rocks, concrete, wood, and metal (Doyle et al. 1997). These life cycle characteristics make artificial structures within the intertidal and subtidal zones highly viable habitats for invasive barnacle species. Several successful barnacle invasions have been documented. Zabin (2009) examined four invasive barnacle species in Hawaii and found that not only are their populations likely to persist, but they are compressing the niches of native barnacles. Additionally, Lawson et al. (2004) established that the invasive *Elminius modestus* was the most dominant barnacle within the Lough Hyne Marine Nature Reserve in Ireland. The success barnacles have shown as invaders coupled with life history characteristics that favor their invasive potential, makes them a group of biological concern within many intertidal locations.

History of Megabalanus coccopoma *in the Southeastern U.S.A.*

The Titan Acorn Barnacle, *Megabalanus coccopoma*, native to the tropical eastern Pacific (Laguna 1990), was recently introduced to the southeastern U.S. In 2006, the barnacle was first sited in both Brunswick, GA (Gilg et al. 2010) and St. Augustine, FL (Spinuzzi et al. 2013). Perreault (2004) found dead specimens of *M. coccopoma* in Louisiana in July 2001 and suggested that shipping was the likely vector for their arrival to the Gulf area due to the well-traveled commercial shipping routes that connected in the region. Very little is known about *M. coccopoma* in either its native or introduced ranges, but in northeast Florida an annual settlement pattern beginning in March, with a peak during the summer months and ending in August or early September, occurred in two consecutive years (Gilg et al. 2010). Consecutive yearly settlement within northeast Florida suggests a local or regional population of breeding adults (Gilg et al. 2010). In contrast, within their warmer invasive range of Brazil, settlement peaks in

September-November but may occur in all months, suggesting that the cooler winter months in the temperate U.S. may inhibit reproduction and/or settlement (Gilg et al. 2010). Temperate winter waters are also thought to cause the death of first-generation *M. coccopoma* along coastlines. Perreault (2004) found that *M. coccopoma* on the coast of Louisiana suffer "winterkills." Low salinity may also inhibit settlement of *M. coccopoma* spat along the coastline. Gilg et al. (2010) found no *M. coccopoma* spat settlement in lower salinity feeder creeks of the Intercoastal Water Way (Florida) whereas spat was present at higher salinity main channel sites. However, to date, no salinity tolerance testing has been completed on *M. coccopoma* larvae.

In the southeastern U.S.A., *M. coccopoma* currently has a northern range limit of Cape Hatteras, NC and has extended as far south as Fort Pierce, FL (Crickenberger and Moran 2013). Its range has expanded further northward annually since 2010; however, population surveys completed along the southeastern coastline between 2010 and 2012 indicated that there were subsequent die-offs at coastal locations during winter months (Crickenberger and Moran 2013). Recent laboratory testing suggested a lower temperature range limit of about 21° C for juveniles (Sam Crickenberger, pers. comm..). In spring 2013, I observed many dead *M. coccopoma* at several upper intertidal sites along the southeastern U.S. coastline (Edisto Beach, SC to St. Simons Island, GA). This observation was consistent with a study done on Tybee Island, GA, where *M. coccopoma* appeared to be restricted to the lower intertidal (Lacey Haley, unpublished). Combined these observations suggest that low temperatures and salinities along immediate coastlines may limit *M. coccopoma* settlement and subsequent survival (Foster 1971, Newman and McConnaughey 1987, Gilg et al. 2010). Though coastal populations of *M. coccopoma* do suffer die-offs in colder months, the population surveys completed in 2010-2012 also indicated settlement of a new cohort of juveniles during the warmer summer and autumn

months (Crickenberger and Moran 2013). This finding corroborated the previously mentioned study completed in Florida by Gilg et al. (2010), in which a settlement peak was observed in the summer months.

The lack of second-generation specimens (mating adults) inhabiting shoreline locations in the southeastern U.S.A. may point to offshore sources of larvae for yearly population rebuilding and range expansion (Perreault 2004). Furthermore, the large size of acorn barnacles and their ability to adapt to novel environments suggests that *M. coccopoma* has the potential to outcompete native barnacles for both space and resources (Stanley and Newman 1980, Roy et al. 2002, Zabin 2009, Spinuzzi et al. 2013). Determining the methods of range expansion employed by *M. coccopoma* may help to create management plans aimed at slowing or preventing the spread of the barnacle in its non-native range. For example, if artificial structures are housing breeding adult populations, their removal may directly decrease the invasion potential of *M. coccopoma* in the southeast. Knowledge related to the spread of *M. coccopoma* may also be employed to decrease the potential for introduction and range expansion of other sessile invertebrates that may be employing similar tactics.

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CHAPTER 1
TETRANUCLEOTIDE MICROSATELLITES FOR THE BARNACLE *MEGABALANUS COCCOPOMA* (DARWIN 1854)

INTRODUCTION

The barnacle *Megabalanus coccopoma* (Darwin 1854) is indigenous to the tropical eastern Pacific Ocean where it ranges from Ecuador to northern Mexico and Baja California (Laguna 1990, Newman and McConnaughey 1987). The introduction of this species to the southeastern U.S.A. was documented simultaneously in St. Augustine, FL and Brunswick, GA in 2006 (Gilg 2010, Spinuzzi et al. 2013). The current range of *M. coccopoma* in the southeastern U.S. extends from Fort Pierce Inlet, FL to Cape Hattaras, NC, where it primarily inhabits artificial structures including rock jetties, piers, buoys and offshore towers (Crickenberger and Moran 2013, Cohen et. al 2014).

Megabalanus coccopoma appears to grow rapidly, have high fecundity (Crickenberger pers. comm.), and can reach sizes far exceeding native southeastern barnacle species. Specimens of *M. coccopoma* I have collected in Georgia waters have a maximum shell height of 8.8 cm and a maximum basal diameter of 6.8 cm. Like many species of barnacles, *M. coccopoma* has the potential for long-range dispersal through planktonic larvae (Severino and Resgalla 2005). Range expansions as large as 794 km have been documented along the southeastern U.S. coast in a single mating and settlement season (Crickenberger and Moran 2013). The combined life-history characteristics of *M. coccopoma* have heightened concerns that this species will outcompete native barnacles along the eastern seaboard (Spinnuzzi et al. 2013). In addition, *M. coccopoma* is an ideal organism for studying the effects of rapid range expansion and contraction on population genetic variation during biological invasions.

Little is known about the population structure of *M. coccopoma* in both its native and introduced range. Recent studies using the mitochondrial genes cytochrome c oxidase subunit I (COI) and 16S rRNA, to assess genetic variation in the introduced populations of *M. coccopoma*, indicated a single, large population in the introduced range with high gene flow (Williamson 2010, Cohen et al. 2014, Crickenberger, unpublished). Mitochondrial markers are valuable tools for investigating population structure and the influence of historical processes on the distribution of genetic variation. However, these markers can have limited utility when evaluating recent and more rapid evolutionary processes, including those acting on nuclear markers (Wang 2010). Microsatellite markers, because of their higher variability, can be more effective tools for studying contemporary evolutionary processes and associated demographics, including cases of recent and ongoing species introductions into non-native areas (Wang 2010, 2011). In this study I describe 13 highly variable microsatellite markers developed to study contemporary evolutionary processes and their consequences on the population structure and demographics of the introduced barnacle *M. coccopoma*.

METHODS

Using morphological characters, I collected a single individual of *M. coccopoma* from the fishing pier at Tybee Island, GA (31º59’31”N, 80º50’42”W) in July 2013. Total genomic DNA was extracted from this individual using the DNeasy tissue kit (Qiagen) following manufacturer protocols, including RNAse treatment to remove RNA from the sample. The purified sample was sent to the Savannah River Ecology Lab to prepare an Illumina paired-end shotgun library. The resulting sequences were analyzed using the program *PAL_FINDER_v0.02.03* (Castoe et al. 2012) to identify genome sequences that contained di-, tri-, tetra-, penta- and hexanucleotide

microsatellite loci. I successfully identified >7,000 loci that met the designated criteria, including appearing no more than twice in the genome to avoid multiple priming sites and have approximately 50-75 repeats of the cloned allele. Of these loci, I chose to test primer pairs for 30 loci with tetranucleotide repeats.

Initial primer screening of the 30 loci for amplification was completed on 8 individuals collected in September 2013, from two locations within the introduced range of the barnacle: four specimens from Navy Tower R2, located approximately 50 km off the coast of Georgia, USA (31º22'30"N, 80º34'01"W), and four specimens from the fishing pier on Saint Simons Island, GA (31º08'02"N, 81º23'48"W)**.** PCR was performed in 10µl reactions including: 0.625 units of *Taq* DNA Polymerase (Apex), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.5 µmol each of forward and reverse primers, and 3.5 µl diH_20. PCR reactions were performed using the following conditions: initial denaturing step at 95°C for 5 minutes, followed by 30 cycles of 95°C denaturing for 10 s, 60°C annealing for 10 s, 72°C extension for 10 s, and a 72°C final annealing step for 5 min. The PCR products were separated using an agarose gel.

Thirteen of the initial 30 primer pairs tested showed consistently clean, strong amplification product for all 8 specimens and were chosen for further characterization using 36 to 42 individuals. Samples used for loci characterization were collected from Navy Tower R2 (N=18), and St. Simons Island Fishing Pier, GA (N=24). PCR was performed using the same conditions and protocol described above. All forward primers were fluorescently labeled and all reverse primers included a GTTT 'pigtail' to the 5' end of the primer in order to standardize the addition or deletion of adenosine by *Taq* polymerase (Brownstein et al. 1996). Primers compatible in multiplex reactions are indicated in Table 1. Amplified PCR products were sized using an ABI 3500 Genetic Analyzer with an internal size standard. Alleles were scored

manually using GENEMAPPER software (PE Applied Biosystems). I calculated expected (H_E), observed heterozygosity (H_O), and polymorphic information content (PIC) using the Microsatellite Toolkit Add-in for Microsoft Excel (Park 2001a; Park 2001b). PIC is the expected proportion of informative offspring that cosegregates by phenotype for the locus being examined. Tests for deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted using GENEPOP v4.0.10 (Rousset 2008).

RESULTS AND DISCUSSION

The number of alleles per locus ranged from 9-45 (mean = 24.46±8.57, Table 1.1) and observed heterozygosity ranged from 0.42-1.0 (mean = 0.675±0.021, Table 1.1). All loci pairs were tested for linkage disequilibrium, but no significance was detected after Bonferonni corrections. All but two loci (MC-3 and MC-29) showed significant deviations from Hardy-Weinberg expectations (Table 1.1). Population admixture, founder events, and null alleles are all possible factors that can result in deviations from Hardy-Weinberg equilibrium. The samples used in this study are from recently introduced populations most likely established from multiple sources. This is a reasonable explanation for the large number of loci not conforming to Hardy-Weinberg expectations, although null alleles cannot be ruled out. Comparison with an established native population will add valuable insight to this conclusion. The high variation observed with these microsatellite primer pairs indicates that they are a useful tool for measuring genetic variation within introduced populations of *M. coccopoma*.

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TABLES AND FIGURES

Table 1.1: Characterization of 13 primer pairs amplified from microsatellite loci for the barnacle, *Megabalanus coccopoma*, collected along the coast of Georgia, USA. *N*, indicates the number of individuals that were successfully genotyped at each locus; *A*, number of alleles for each locus; H_O, is the observed heterozygosity; H_E, the expected heterozygosity; P_{HW}, the probability that the genotype proportions meet the expectation of Hardy-Weinberg equilibrium; and PIC, the polymorphic information content.

Locus	Primer Sequence (5'-3')	Dye	Annealing start temp. (°C)	Repeats in cloned allele	Amplified concurrently in a multiplex	*N*	*A*	Size range (bp)	H_O	H_E	P_{HW}	PIC
MC-1	F: GAGCCGGACTAGATCACATGG R: GACTTCAATCGGCTCGTGG	FAM	60	$(ATAC)_{72}$	MC-15	42	20	195-291	0.60	0.94	<0.0001	0.92
MC-3	F: CCCTGAGAATCCAAACACGG R: AGATACGTTGCAGGAACCAGG	FAM	60	$(AGTG)_{64}$	NA	36	23	185-317	1.0	0.95	0.98	0.94
MC-4	F: CCTTGGTTCCCGAAATAATCC R: AAGGTCACATTGCAAACAATAGC	HEX	60	$(ATAC)_{64}$	NA	38	21	165-270	0.49	0.87	<0.0001	0.84
MC-5	F: GACGTAGACGACCATCAGCC R: GGTTGTTCTCAGTACATACGCCC	FAM	60	$(ATAC)_{64}$	MC-28	36	33	160-324	0.59	0.97	0.01	0.96
MC-9	F: CAATCGTAGGAATCCAGCGG R: CTCAGGTCAGCTGGCAAGG	HEX	60	$(ACTG)_{56}$	NA	30	27	509-729	0.43	0.97	<0.0001	0.95
MC-13	F: GCGTGCAATCCACTATCG R: CTAGATCGCGAGGCATCC	HEX	60	$(ATAC)_{72}$	NA	42	45	144-416	0.76	0.98	0.006	0.97
MC-15	F: GGTTCGCGAGACAATTCTAAATACC R: CGCTCTGAAACACAAACATGG	NED	60	$(ATAC)_{72}$	MC-1	41	26	213-417	0.76	0.95	<0.0001	0.94
MC-22	F: GCGTCATGTATTCAGGTTCAGG R: TAAGAATCGCAACCCGATGG	HEX	60	$(ATAC)_{60}$	MC-24	41	20	167-233	0.66	0.94	<0.0001	0.92
MC-24	F: GAGCACATACAGCAGAGCGG R: GGGAGGACTAATTTCCGTTGC	FAM	60	$(TCTG)_{60}$	MC-22	41	9	172-208	0.59	0.79	0.006	0.75
MC-26	F: CTCCGGAGGGTCCAATCC R: ATGAATGCGCACATAAACGC	NED	60	$(ATAC)_{60}$	NA	41	31	213-359	0.83	0.96	<0.0001	0.95
MC-27	F: CCTCTGACCTCTGACCTATGACG R: ACGCGAAACACACTATTGCC	HEX	60	$(ACTG)_{56}$	NA	41	23	265-397	0.81	0.93	0.03	0.91
MC-28	F: CAGTACAGTACAGTTGAGATAGTTCACCC R: AAATCAGTCCTCCTGACAGTGC	NED	60	$(ATAC)_{56}$	MC-5	39	20	286-450	0.42	0.91	<0.0001	0.88
MC-29	F: AGGAGCATCGACAGTGACTAGC R: TGCTAAAGCATTGCTCCTCC	NED	60	$(ATAC)_{56}$	NA	41	20	164-244	0.85	0.93	0.39	0.91

CHAPTER 2

USING DEMOGRAPHIC INFORMATION AND GENETIC ANALYSIS TO ASSESS THE ROLE OF ARTIFICIAL STRUCTURES IN THE RANGE EXPANSION OF *MEGABALANUS COCCOPOMA* IN THE GEORGIA BIGHT

INTRODUCTION

Historically, populations in marine communities were considered to be open, meaning that currents and tides dispersed larvae readily between distant locations (Thorson 1950, Roughgarden et al. 1985, Pineda et al. 2009). More recent studies have provided substantial support for local retention, whereby larvae remain near or within the natal habitat (Cowen et al. 2000, Levin 2006, Becker et al. 2007). A Working Group at the National Center for Ecological Analysis and Synthesis defined local retention as occurring at spatial scales of <100km and on a time scale less than one generation (Warner and Cowen 2002). While local larval retention is not surprising for species lacking pelagic development, there is also evidence for its existence in species having long planktonic larval durations including crustaceans (Gaines and Bertness 1992, Grosholz and Ruiz 1995, Barber et al. 2000), copepods (Burton and Feldman 1981), and urchins (Palumbi et al. 1997). Open recruitment can be very risky due to the unpredictably of oceanic conditions and has been called a genetic sweepstakes in which chance events determine which adults are successful each reproductive season (Flowers et al. 2002). Local retention may enhance survival of pelagic larvae by reducing the heightened risks incurred during long distance dispersal in the open ocean.

Understanding pelagic larval transport is vital when deciphering the invasive potential and range expansion capabilities of non-native marine organisms. Biological invasions are defined as the arrival, establishment and diffusion of a species to an area where it did not

previously exist through one of two methods: natural range expansion or transport by human activity (Carlton 1987). It has been proposed that some level of local larval retention is required for the successful establishment of invasive marine species because of their low initial population densities and lack of alternate established larval sources (Swearer et al. 2002). Many successful marine invertebrate invaders including algae (Bulleri and Airoldi 2005), ascidians (Simkanin et al. 2012; Dafforn et al. 2012) and crustaceans (Foster and Willan 1979; Astudillo et al. 2009), have been known to establish on permanent artificial structures such as docks, buoys and oil rigs. When deployed, these structures provide unoccupied space for settlement and sometimes a shallow water refuge where one did not exist previously, thus increasing the subtidal and intertidal surface area for sessile invertebrates to colonize (Fauvelot and Bertozzi 2009, Fauvelot et al. 2012, Simkanin et al. 2012). Once established, mature adults have the ability to adhere to these hard substrates and release larvae into conditions favorable for dispersal (Yamaguchi et al. 2009).

In the southeastern U.S., the South Atlantic Bight (SAB) is an oceanic region extending from Cape Hatteras, North Carolina to Cape Canaveral, Florida (Bumpus 1973). The continental shelf in this area is extensive and is bordered by the warm Gulf Stream current originating at tropical latitudes and running north. The study described here takes place within the Georgia Bight region of the SAB, an area extending from approximately Charleston, South Carolina to Jacksonville, Florida (Lee and Brooks 1979). In the Georgia Bight, the continental shelf reaches its widest extent in the SAB, stretching out to 120 km offshore (Edwards et al. 2007). This region of the continental shelf is characterized by a series of circulating frontal eddies that occur as protrusions of the Gulf Stream current (Lee et al. 1981). Recent studies have used computer models and released planktonic drifters in the Georgia Bight and have found that pelagic larvae

can be entrained within this region for up to 60 days (Edwards et al. 2006, Edwards et al. 2007, Hare and Walsh 2007), thus the potential for local recruitment within the Georgia Bight and favorable conditions for the establishment of invasive and introduced species.

There are many on and offshore artificial structures in the Georgia Bight that could host invasive species. Onshore structures include docks, rock jetties and public piers, while offshore structures consist mainly of buoys and abandoned permanent structures such as Navy Towers that were used previously to train military personnel. In 2006, an introduced species of tropical barnacle, *Megabalanus coccopoma*, was discovered on artificial structures along the southeastern U.S.A. coast (Spinuzzi et al. 2013). This barnacle is native to the tropical eastern Pacific and is thought to have been introduced to the U.S. via shipping (Perrault 2004). The current range of *M. coccopoma* in the southeastern U.S. is from Cape Hatteras, NC to Fort Pierce, FL (Crickenberger and Moran 2013). Very little is known about this species in either its native or introduced ranges, however, there are indications that its reproduction, settlement and survival may be limited by seasonally low temperatures and salinities found along the coastline in the SAB (Gilg et al. 2010, Spinuzzi et al. 2013, Crickenberger and Moran 2013). Extensive coastal winter diebacks appear to be common along the immediate shoreline, but they are often followed by range re-expansions. In a single mating and settlement season a range expansion of >790 km has been observed along the southeastern coastline (Crickenberger and Moran 2013). These rapid range expansions may be indicative of successful breeding populations within the SAB that are able to provide sufficient larvae to repopulate the coastline.

Genetic analysis, along with demographic information, may be able to give insight into the recruitment tactics and source-sink dynamics within the local region (Kolbe et al. 2004, Bronnenhuber et al. 2011, Ciosi et al. 2008, Excoffier and Ray 2008, Peacock et al. 2009).

Source populations should exhibit higher genetic diversity because of many generations of stable population growth and recruitment. In contrast, sink populations, synonymous with a series of yearly founder populations (i.e. smaller, younger populations of invasive species) are expected to have lower genetic diversity (Ciosi et al. 2008, Peacock et al. 2009, Ramakrishan et al. 2010). Hedgecock (1994) also suggests that there may be temporal variation in the genetic composition of sink populations. As new individuals are recruited yearly, the genetic diversity will remain small, but may be significantly different each settlement season. As the coastal populations of *M. coccopoma* appear to be dying off each winter and repopulating each spring it is feasible to consider them as a series of yearly founder populations that have a strong genetic signature linking them to their source population.

In this study I investigated the hypothesis that artificial structures offshore of the southeastern U.S. provide refuges for breeding adults of the invasive barnacle, *M. coccopoma*, thus facilitating its ability to repopulate habitats along the immediate shoreline. Specifically, I addressed four predictions. (1) Temperature and salinity reaches lower minimums inshore than offshore. Low salinity and temperature have been suggested previously as limiting factors to *M. coccopoma*'s population expansion and success (Gilg et al. 2010, Crickenberger and Moran, 2013). (2) *M. coccopoma* established on artificial offshore structures are reflective of more mature subpopulations than those inshore. A mature subpopulation will have a higher density and abundance of large individuals signifying an ample mating pool. (3) *M. coccopoma* subpopulations offshore have higher genetic diversity (i.e. number of different alleles and expected heterozygosity (H_E) values) than inshore subpopulations, and genotype frequencies that conform more consistently to Hardy-Weinberg Equilibrium (HWE). If offshore structures are winter refuges for mating adults and provide favorable habitat for settling larvae, they are likely

inhabited by several overlapping generations of *M. coccopoma*, thus will show higher genetic diversity and conformity to HWE than inshore subpopulations. Inshore subpopulations are anticipated to be dominated by first-generation individuals that only display a subset of the possible alleles within the population, and will not be in HWE due to genetic drift and/or founder effects. (4) Onshore subpopulations, thought to be sinks, will display a subset of the genetic structure based on allele frequencies of the offshore subpopulations, thought to be sources, that provide the larvae. This genetic structure will act as a signature that can be traced between the sink and source subpopulations.

METHODS

Samples were collected from eight artificial structures (i.e. research sites) that span a range of temperature and salinity across the Georgia Bight (Figure 2.1). Four sites were chosen along the shoreline, where temperature and salinity are thought to be periodically lower, and included the public fishing piers at Tybee Island, GA (31º59'31"N, 80º50'42"W), St. Simon's Island, GA (31º08'02"N, 81º23'48"W) and Folly Beach, SC (32°39'12"N, 79°56'19"W), and a walking bridge at Jekyll Island, GA (31°07'02"N, 81°24'59"W). One site occurring approximately 20 km offshore, the Gray's Reef National Marine Sanctuary (GRNMS) Buoy (31º24'00"N, 80º52'05"W), was expected to have more moderate temperature and salinity. The GRNMS Buoy is a 3 m diameter disk buoy that is domed on the bottom and has a bridle attached underneath made of 6.35cm diameter pipe; 3 legs (Figure 2.2). Due to a shortage of buoys of similar size and distance from shore, this particular site lacks replication. Lastly, three sites approximately 50 km offshore, where temperature and salinity are expected to remain higher than coastal sites due to proximity to the Gulf Stream Current, consisted of the abandoned Navy

Towers R2 (31º22’30”N, 80º34’01”W), R8 (31º37’59”N, 79º55’29”W), and M2R6 (31º32’01”N, 80º14’09”W; Figure 2.3).

To evaluate the prediction that there are lower minimum temperatures and salinities inshore than offshore I monitored these parameters at six sites. I chose three coastal sites (Jekyll Bridge, Tybee Pier and Folly Pier) and three offshore sites (Navy Towers R2, M2R6 and R8). I deployed submersible Odyssey Temperature/Conductivity (Data Flow Systems, Christchurch, New Zealand) data loggers. Each Odyssey logger was encased in a 3.81 cm diameter PVC tube with a series of 1 cm holes drilled into it to allow water to flow in and out freely, but to minimize the settlement of sessile organisms on the loggers. Odyssey probes were programmed to measure temperature in degrees Celsius and salinity in millisiemens (mS/cm). Additionally, I used an existing temperature (no salinity available) probe at the GRNMS Buoy to obtain temperature data. The GRNMS data probe is a custom probe created by the National Data Buoy Center based on the YSI thermilinear thermistor series 44212. Data recorded by the GRNMS Buoy temperature probe was obtained from historical records available on the National Data Buoy Center website (www.ndbc.noaa.gov). Temperature and salinity were monitored once each hour for approximately 13 months from September 2013 to October 2014. The coastal site data loggers were placed during spring low tide to make sure they were in an area that would remain subtidal. The Navy Tower data loggers were placed ~3.5m below surface level to protect them from storm surge around the metal pilings. The data loggers were first placed in the water during September and October 2013. Temperature/salinity probe placement/retrieval dates for each site varied due to weather conditions and the tidal cycle, but every attempt was made to reach sites as close together in time as possible (Table 2.1). Upon attempting to retrieve the loggers in spring 2014, four of the six loggers including Tybee Pier, Folly Pier, M2R6 and R8 were not located.

Rough winter sea conditions and series of winter storms may have dislodged them from their locations despite the heavy-duty zip ties used to secure them. The two remaining data loggers at the Jekyll Bridge and R2 Tower were collected, the data was uploaded, loggers were cleaned of encrusting organisms, and immediately re-deployed. Loggers were collected for the final time in October 2014.

To test the prediction that offshore subpopulations were more mature and thus could act as larval sources I collected population demographic information including density and shell sizes. Densities of *M. coccopoma* were assessed at each site twice, once in fall 2013 and once in spring 2014 (Table 2.1). In fall 2013 sea conditions were too dangerous for quadrat photographs to be taken at the GRNMS Buoy. Densities were determined by photographing a 20x20 cm PVC quadrat that was placed haphazardly over the surface of the artificial structure in the intertidal and upper portion of the subtidal zone. A minimum of 15 quadrats were photographed at each site; sites that had larger area were documented with additional quadrats to ensure that as much of the structure as possible was assessed (maximum 40 quadrats). Quadrats were analyzed using the Microsoft Paint program to mark each *M. coccopoma* as it was counted. Only live *M. coccopoma* were counted in the density measurement.

To investigate maturity of the subpopulations the shells of *M. coccopoma* that were collected for DNA analysis in both fall 2013 and spring 2014 were measured. Approximately 25-30 individuals were collected at each site in fall 2013 and spring 2014 (Table 2.1) and immediately placed in 95% ethanol for preservation. Barnacles at onshore sites were collected at spring low tide to ensure that as much of the available space on the structure was sampled as possible. *M. coccopoma* on offshore structures were sampled from the surface to approximately three meters deep. Shells of *M. coccopoma* that were collected and positively identified by a

restriction enzyme digest method (Tyson 2015) were measured to the nearest 0.0001 cm using digital calipers. Shell measurements included the diameter of the opercular cavity opening at the widest point, the basal plate diameter at the widest point, the basal plate diameter at a 90° angle from the widest point, and the height at the tallest point (Figure 2.4). The two basal plate measurements were averaged to estimate the plate diameter because this structure is often not circular. A series of one-way ANOVAs or non-parametric Kruskal-Wallis tests were used to assess both the temporal and spatial differences in population density, and barnacle shell size among the subpopulations. A posteriori tests for significance between subpopulations were completed using either Tukey HSD (parametric) or Steel-Dwass (non-parametric) tests.

To further examine the settlement and growth of *M. coccopoma* density and shell size measurements were also completed on the GRNMS Buoy at three time points: spring 2013, fall 2013 and spring 2014. Between the spring 2013 and fall 2013 assessments the buoy was replaced and a new substrata became available for *M. coccopoma* settlement. This allowed me to measure density and shell sizes based on the previously described methods to quantify growth of the population over the course of the study.

To assess the predictions that offshore sites have higher genetic diversity and genotype frequency values that conform to HWE, and that onshore sites display a subset of the genetic structure of the offshore sites, the *M. coccopoma* specimens that were collected underwent genetic testing. Only *M. coccopoma* specimens collected from each research location and positively identified to the species level were used in the genetic analysis. When possible, at least 25 specimens were genotyped to accurately estimate the allele frequency of each subpopulation (Hale et al. 2012). DNA was extracted from each specimen and purified using DNA extraction kits according to the manufacturer's protocol (DNeasy tissue kit QIAGEN or Zymo Genomic

DNA Tissue Miniprep). Thirteen microsatellite primers specific to *M. coccopoma* were used to assess genetic variation and are described along with PCR protocols and sequencing conditions in Chapter 1. PCR products were run on an ABI 3500 Genetic Analyzer with an internal size standard and alleles were scored by eye using GeneMapper (Applied Biosystems).

To specifically address the prediction that offshore sites have higher diversity and conform to HWE values the observed (H_O) and expected heterozygosities (H_E) were calculated for each subpopulation and each individual locus using the Excel Microsat Toolkit add-in (Park 2001a; Park 2001b). FSTAT v 2.9.3 (Goudet 1995) was used to calculate allelic richness for each locus and allelic richness for each subpopulation. Statistical analyses including linkage disequilibrium and Hardy-Weinberg Equilibrium (HWE) were performed using GENEPOP v4 (Rousset 2008). Linkage disequilibrium and deviations from HWE were checked using Markov chain analysis with 10,000 dememorization steps, 500 batches and 10,000 iterations in each batch.

Two different experiments were used to investigate similarities in genetic structure between the subpopulations on and offshore. The first was to follow the natural "extinction" and re-colonization of *M. coccopoma* on a new, suitable substrate and the second was to assess genetic structure among the eight collection sites across the Georgia Bight. I was given a unique opportunity to investigate natural extinction and re-colonization when in spring 2013, the GRNMS Buoy that was harboring a dense population of *M. coccopoma* (Scott Noakes, pers. comm.), was scheduled to be removed and replaced with a similar buoy in the same location. The buoy from which I sampled initially in spring 2013 (before removal) had been in the water since 2009 (Scott Noakes, pers. comm.). Population assessments and sample collections were completed at the time of its removal by the U.S. Coast Guard on January 23, 2013. Similar

assessments were completed on the replacement buoy on October 28, 2013, approximately 8 months after it was deployed, and again on May 27, 2014 approximately 16 months after its deployment. By monitoring this re-colonization I was able to assess the genetic makeup in a brand new offshore population. To further examine genetic structure between the potential larval source and sink populations the *M. coccopoma* collected from all eight subpopulations in both fall 2013 and spring/summer 2014 were evaluated using microsatellite loci.

Genetic structure was assessed for both the re-colonization of the buoy and the genetic differentiation was assessed for samples before and after re-colonization of the buoy and between the eight subpopulations using a series of genetic analyses. Pairwise F_{ST} values were calculated using FSTAT v 2.9.3 (Goudet 1995). Significance of F_{ST} values was assessed not assuming Hardy-Weinberg within samples and 10,000 permutations. Because the subpopulations violated several assumptions of traditional F_{ST} analysis population differentiation was also assessed using a contingency table of allele frequencies and the exact G-test using GENEPOP (Raymond and Rousset 1995) with 10,000 dememorization steps, 500 batches and 10,000 iterations. I checked for the presence of null alleles, allelic dropouts, stuttering and mis-scoring using MICRO-CHECKER v2.2.3 (van Oosterhoust et al. 2003). Analysis of molecular variance (AMOVA) was calculated using Arlequin v3.5 (Excoffier and Lischer 2010). AMOVA is a statistical model that can compare molecular variance within a single species in different groupings. The buoy re-colonization AMOVA was grouped by time point and not by before and after removal of the buoy. Three AMOVAs were completed to determine variance components for all eight subpopulations at the two different time points. The first grouped the subpopulations by site type (coastal, buoy and tower), the second by season collected (fall 2013 and spring 2014)

and in the last ANOVA subpopulations were not partitioned into groups, but were compared against one another.

The Bayesian clustering algorithm in STRUCTURE v2.3.4 (Pritchard et al., 2000) was used to examine population structure of the collected specimens both in the buoy re-colonization and the assessment of genetic structure at all subpopulations. I specified the admixture ancestry model and correlated allele frequencies. STRUCTURE was first used to test a range of potential population clusters (*K)* using a burn-in of 10,000 and 20,000 replicates for 20 repetitions. In this study the clusters are considered to be equivalent to the number of different sources that provided larvae to create the subpopulations. I then used STRUCTURE HARVESTER v0.6.93 (Earl and Vonholdt 2012) to determine the most likely value for *K* by examining plots of L(*K*) and Δ*K* (Evanno et al. 2005). After the determination of the most likely *K* value I re-ran STRUCTURE v2.3.4 for a narrower window of potential *K* values and increased the burn-in to 500,000 with 750,000 replicates for 20 repetitions. I then re-tested the data on STRUCTURE HARVESTER v0.6.93 and determined the most likely value for *K*. Using the most likely K value STRUCTURE ran a final time with an increased burn-in of 1,000,000 and replicates of 2,000,000 for 10 replications. I then used CLUMPPAK (Kopelman et al., in press) to summarize the clustering solutions by determining a mean assignment proportion for each individual. The cluster information was plotted and displayed using Microsoft Excel.

RESULTS

Temperature and Salinity

To test the prediction that temperature and salinity reach lower minimums inshore, data loggers were deployed to record temperature and salinity for 13 months. Though loggers were

placed at seven sites, rough sea conditions detached four of the data loggers from their locations and only two sites, one coastal and one offshore, have continuous data for both temperature and salinity (Jekyll Bridge and R2 Tower). At the GRNMS Buoy only temperature data were collected. Sea temperatures (°C) varied on a daily basis from 4.4 ºC to 31.7 ºC (Figure 2.5). The coldest water temperatures occurred from December 2013 to February 2014 and the warmest from June to September 2014 (Figure 2.5). During the coldest winter months the water temperatures surrounding the R2 Tower remained higher than either site closer to shore (Figure 2.6). Temperatures during this time period averaged 12.50, 13.71, and 16.32 ºC at Jekyll Bridge, GRNMS Buoy and the R2 Tower, respectively (Figure 2.6). The single lowest recorded temperature at each site was 4.4 °C at the Jekyll Bridge, 10.0 ºC at the GRNMS Buoy, and 11.8 ºC at the R2 Tower. For 27 days, from May 24 to June 19, 2014, there was a gap in temperature collection at the R2 Tower due to a technical malfunction of the data logger. Hourly collections were resumed when the equipment was collected and cleaned on 19 June, 2014.

Salinity (mS/cm) was monitored hourly at two of the collection sites from September 2013 to March 2014. In March 2014, the salinity probe at the R2 Tower failed to record accurate data, as the mS/cm dropped suddenly from positive values ranging from 30 to 55 mS/cm to negative values and remained that way for the remainder of the study. Therefore I have shown only salinity data collected when both loggers were functioning correctly. Salinity was lower at the coastal Jekyll Bridge site than at the R2 Tower offshore (Figure 2.7). During the rainy winter months from December to mid-March, both sites reached their lowest minimum salinity. The lowest recorded salinities were 12 mS/cm at Jekyll Bridge and 21.2 mS/cm at R2 Tower. Though the water at R2 Tower showed more variability in daily salinities, it remained higher than the

Jekyll Bridge at all but two data points in March 2013. The temperature and salinity data indicate that both parameters are higher with increasing distance from the coast.

Population Demographics

To investigate the prediction that offshore sites were characterized by more mature *M. coccopoma* populations capable of acting as larval sources, density and shell size were assessed as indicators of population age. Density was assessed at eight collection sites in both fall 2013 and spring 2014 (Figure 2.8). I was unable to assess density at the GRNMS Buoy in fall 2013 as rough sea conditions prevented obtaining usable photographs, and no live *M. coccopoma* were found at the Jekyll Bridge during either collection. Densities at the coastal sites were higher in fall 2013 than spring 2014, while there was no significant difference in densities at offshore sites between the seasons (Figure 2.8). During both the fall 2013 and spring 2014 surveys, the Tower sites had significantly higher overall *M. coccopoma* densities than the coastal sites (Kruskal-Wallis test; DF=14, $p<0.0001$; Figure 2.8). However, in fall 2013, the single site with the highest barnacle density was the Tybee Pier, a coastal site. This contrasts with those obtained in spring 2014, where densities were highest at the GRNMS Buoy, an offshore site. Between fall 2013 and spring 2014, coastal subpopulations of *M. coccopoma* appeared to suffer significant mortality. In fact, only two live *M. coccopoma* were found across all four coastal locations in spring 2014, and both were located at the St. Simons Pier.

I collected and measured a total of 337 *M. coccopoma* during fall 2013 and spring 2014. Due to the near absence of live *M. coccopoma* at the coastal locations in spring 2014, I was only able to make statistical comparisons among locations in fall 2013 (n=104). I found no significant differences in shell size measurements among coastal sites or among the Tower sites so the data

have been collapsed into three site types for statistical comparison: coastal (piers and bridge), midshelf (buoy) and Navy Tower (towers) (Figure 2.9). Opercular cavity opening (Kruskal-Wallis non-parametric test, χ^2=78.21, df=2, p<0.001) and basal diameter (Kruskal-Wallis non-parametric test, χ^2=88.44, df=2, p<0.001) increased with increasing distance from shore (Figure 2.9). A posteriori tests for both measurements show that the Navy Tower shells were the largest (p<0.05). Navy Tower shells were also taller than both other site types (Kruskal-Wallis non-parametric test, χ^2=75.91, df=2, p<0.001; Figure 2.9) but there was no difference in height between the coastal shells and the buoy shells.

Assessment of M. coccopoma *settlement*

To gain further insight into the settlement and growth rates of *M. coccopoma*, density and shell sizes were measured on the barnacles from the GRNMS Buoy before and after its removal. Barnacle densities were assessed on both the original buoy in spring 2013 (OB) and the new one 16 months after deployment (GRNMS Buoy fall 2013). An attempt was also made to quantify densities 8 months after the new buoy (GRNMS Buoy spring 2014) had been deployed, but conditions were too rough to obtain usable quadrat photographs. There was no significant difference in barnacle density on the original buoy and the new buoy 16 months after deployment (Kruskal-Wallis non-parametric test, χ^2:2.69, DF=1, p=0.1009, Figure 2.10).

A total of 77 *M. coccopoma* were collected from the old and new buoy over the course of the study and evaluated for shell metrics (OB=19, GRNMS Buoy fall 2013=32, GRNMS Buoy spring 2014=26). A nonparametric Kruskal-Wallis test revealed the opercular cavities on the GRNMS Buoy in fall 2013 were smaller than both other time points (χ^2: 24.4, DF: 2, p<0.001; Figure 2.11). Interestingly, basal diameters were larger for shells from both the GRNMS Buoy in

fall 2013 and spring 2014 than the old buoy (One-way ANOVA, F=3.767, DF=2, p=0.0277; Figure 2.11). Shell height also differed significantly among time points (One-way ANOVA, F=25.253, DF: 2, p<0.0001) with the tallest barnacles found on the GRNMS Buoy in spring 2014 (Figure 2.11).

Comparing genetic diversity between subpopulations

Thirteen microsatellite loci were use to investigate the predictions that offshore sites have higher genetic diversity than onshore sites. A total of 243 *M. coccopoma* were genotyped using the microsatellite loci described in Chapter 1. For several subpopulations including the Jekyll Bridge in fall 2013, and the Tybee Pier, Folly Pier and Jekyll Bridge in spring 2014, I was unable to find any live *M. coccopoma* for genotyping. The St. Simons Pier sample in spring 2014 was excluded from genetic diversity analyses due to the small sample size of only two. Additionally, I discovered a second morphologically indistinguishable barnacle species at all three Navy Tower locations that I was unaware existed prior to collections in fall 2013. The inability to distinguish between *M. coccopoma* and this second *Megabalanus* species *in situ* meant that several of the Tower sites had lower sample sizes than originally expected.

All thirteen loci were found to be polymorphic across the subpopulations. The number of alleles per locus ranged from 16 to 82 (mean ± SD = 41.08±18.39). The allelic richness for each individual locus ranged from 7.10 to 18.45 (mean ± SD = 13.00±3.10) and the number of private alleles per locus ranged from 4 to 26 (mean ± SD =13.91±6.46; Table 2.2). The expected heterozygosities (H_E) for subpopulations ranged from 0.68 to 0.93 and observed heterozygosities (H_O) from 0.46 to 0.72 (Table 2.3). The highest diversity (H_E) value, 0.93, occurred at the R2 Tower in fall 2013. The allelic richness for all loci at each subpopulations ranged from 13.23 to

19.08 (mean ± SD =15.99±2.23; Table 2.3). Overall, there were no significant differences in the number of alleles per subpopulation or expected heterozygosity values between subpopulations. Private alleles per subpopulation were counted and ranged in value from 7 to 28 (mean ± SD =11.77±7.28; Table 2.3). Tests for linkage disequilibrium among loci did not reveal any nonrandom associations after bonferroni corrections.

I assessed each subpopulation for Hardy-Weinberg Equilibrium (HWE) and found that all but the *M. coccopoma* at the St. Simons Pier in spring 2014 with a very small sample size ($p=0.75$), were significantly out of HWE ($p<0.0001$). For all subpopulations this was due to a heterozygote deficiency at all thirteen loci. In two instances loci MC-29 was not heterozygote deficient. Deviations from HWE are common in introduced or founder populations, however, I did check for the presence of null alleles and mis-scoring. I found no evidence of mis-scoring, but the analysis could only rule out the presence of null alleles at one locus, MC-29.

Comparing genetic structure between source and sink populations

To examine the prediction that the onshore populations would have a subset of the genetic structure of the source populations I first analyzed the re-colonization of the GRNMS Buoy and second I compared the genetic structure at subpopulations across the Georgia Bight. A total of 75 individuals were collected and genotyped from the GRNMS Buoy over the sixteen months of collection. Pairwise F_{ST} values, based on allele frequencies, for the buoy time points ranged from 0.0045 to 0.0195 and all three pairwise comparisons were significantly different from each other (Table 2.4). Exact tests of allele frequency differences between all time points were also significant. The AMOVA results were used to compare variance among time points and the results indicated that the largest source of variation was ‘within individuals’ (70.834%),

while ‘among populations’ (i.e. time points) accounted for the least variation (1.186%; Table 2.5).

L(K) and ΔK, calculated by STRUCTURE HARVESTER, were used to determine the true number of clusters (K) for the specimens collected at all three time points on the buoy. The L(K) results suggested that the ideal number of clusters would be K=3, as this is where the values were level before decreasing (Figure 2.12), however, ΔK results indicated that the ideal number of clusters was K=6, where the ΔK value peaks (Figure 2.13). Here I chose to plot K=3, as there was a noticeable peak at 3 in the ΔK results and this was corroborated by the L(K) results. Additionally, the user manual for STRUCTURE recommends choosing the smallest K value that captures the major structure in your data (Pritchard et al., 2010). At all three time points I consistently saw that the largest majority of individuals assigned to Cluster 1. The original GRNMS Buoy had the highest number of *M. coccopoma* (n=18 of 19 total individuals) that assigned to that particular Cluster (dark grey bars; Figure 2.14). Though neither Cluster 2 or Cluster 3 saw a majority assignment, the buoy specimens collected after 8 months in the water did have the highest number of individuals assign to Cluster 2 (light grey bars; Figure 2.14), while those collected from the buoy after 16 months in the water had the highest number of individuals assign to Cluster 3 (medium grey bars; Figure 2.14).

Genetic structure was also examined at all subpopulations from both fall 2013 and spring 2014. Pairwise F_{ST} values (excluding the two individuals from St. Simons Pier spring 2014) were computed for each subpopulation pair. These values ranged from 0.0000 to 0.0629 and 12 of the 55 comparisons were significantly different ($p<0.05$; Table 2.6). However, exact tests of allele frequency differences between all subpopulations pairs were all significant ($p<0.05$; Table 2.6). A series of AMOVA tests were run with three different groupings to determine the changes in

variance components for each grouping: subpopulations grouped according to site type (coastal, buoy, Navy Tower), subpopulations grouped according to collection time (fall 2013, spring 2014) and a global population were subpopulations were not partitioned. The AMOVA results for the groupings according to site type and collection season revealed that among groups had the least variation (Table 2.7 a,b). Grouping the subpopulations by site type decreased the among group variance to -0.120% (Table 2.7a). For all three groupings the largest percentage of variance was found 'within individuals' (~67.5%), followed by 'among individuals within populations' (~32.1%) (Table 2.7a,b,c). For this study changing the groupings by site and collection season did not alter the variance components as the majority of variance was found 'within individuals'. In addition, a series of three AMOVAs with the same groupings were completed with three loci (MC-5, MC-9 and MC-13) removed from the analyses. These three loci were highly variable (see Table 2.2) and there was potential that this high variability was skewing the AMOVA tests. When these three loci were removed there was an increase in the 'within individual' percent variation from ~67.5% to ~72.80%, however the 'among individuals within populations' decreased from ~32.1% to ~27.0% (Table 2.7 a,b,c; Table 2.8 a,b.c). There was among group variation for both the groupings by season and by site type, but for all three groupings the highest variation was accounted for 'among individuals within populations' (Table 2.8 a,b.c). When the subpopulations were grouped by site type, with the three loci removed, the 'among populations within groups' percentage of variation decreased from ~0.41% to ~0.23% (Table 2.7a; Table 2.8a). This was the same when subpopulations were grouped by collection season with the 'among populations within groups' decreasing the percentage variation from ~0.26% to ~0.20% (Table 2.7 b; Table 2.8 b).

To assess the genetic structure found in the *M. coccopoma* subpopulations I first used L(*K*) and Δ*K* results from STRUCTURE HARVESTER to determine the true number of clusters (*K*) to which my specimens could be assigned. The L(*K*) results indicated a leveling at *K*=5 and *K*=8 (Figure 2.15), while Δ*K* corroborates a *K*=5 result with a large peak at that value (Figure 2.16). For this study I chose to plot *K*=5 based on the suggestions from the user manual for STRUCTURE which recommends choosing the smallest *K* value that represents the structure of your population (Pritchard et al. 2010). The greatest number of individuals for most subpopulations assigned to Cluster 1 (dark gray near the top of Figure 2.17) and Cluster 5 (medium gray at the bottom of Figure 2.17). Clusters 2-4 saw smaller assignment values in all subpopulations (Figure 2.17).

DISCUSSION

The primary objective of this study was to determine if artificial structures existing offshore in the Georgia Bight are providing refuges for breeding adults of the introduced barnacle *M. coccopoma*, thus facilitating its ability to repopulate habitats along the immediate shoreline. Demographic and abiotic monitoring data indicate that not only are permanent structures offshore of Georgia suitable habitats for adult *M. coccopoma*, but the populations found there are comprised of mature barnacles that have the potential to act as larval sources. Genetic analysis revealed that while the *M. coccopoma* introduction in the southeast is recent, there is no evidence for founder effects and any genetic differentiation amongst the subpopulations is subtle and largely driven by numerous low frequency alleles (i.e. private alleles). Despite high allelic diversity across the entire Georgia Bight, all subpopulations examined in the study showed significant deviations from Hardy-Weinberg Equilibrium. The

combined results of this study indicate that the Georgia Bight *M. coccopoma* are composed of a panmictic population that is employing an open recruitment model with larval sources from within and outside of the Georgia Bight.

As a measure of the environmental suitability of the permanent offshore structures as refuges for breeding adults of *M. coccopoma* I monitored temperature and salinity at on and offshore sites. These parameters have previously been suggested as limiting factors to survival and settlement of *M. coccopoma* in the southeastern U.S.A. (Gilg et al. 2010, Spinuzzi et al. 2013, Crickenberger and Moran 2013). The onshore monitoring site, Jekyll Bridge, had the lowest minimum temperature and salinity. R2 Tower, located approximately 50 km offshore, exhibited higher temperatures and salinities over the course of the study. Overall these data showed that distance from shore correlated positively with water temperature and salinity. Distance from shore also increases proximity to the warm tropical waters of the Gulf Stream Current, which may be moderating the water temperature and salinity at the Navy Towers. Thus, the water conditions occurring farther offshore in the Georgia Bight may be more favorable for the establishment and persistence of populations of *M. coccopoma* are already living at the edge of their thermal and salinity tolerances in the southeastern U.S.A.

Winter minimum temperatures have been indicated as the reason for die backs of several tropical invaders in the southeastern U.S.A. including the green mussel, *Perna viridis*, in Florida and Georgia (Firth et al. 2011, Spinuzzi et al. 2013) the porcelain crab, *Petrolisthes armatus*, in Georgia (Canning-Clode et al. 2011) and the barnacle, *M. coccopoma* (Perrault 2004, Crickenberger and Moran 2013). Newman and McConnaughey (1987) reported that *M. coccopoma* cannot survive without tropical water temperatures from 15-35 ºC (Newman and McConnaughey 1987). During the cooler winter months of this study, water temperatures at the

R2 Navy Tower frequently reached temperatures <15 ºC with a low of 11.8 ºC. Temperatures <15 ºC were sustained for several weeks during the winter months, but there were no significant die offs of the population at this site, indicating that the adult *M. coccopoma* barnacles may be better able to tolerate cold temperatures than previously thought. However, this was not the case for the coastal locations, which suffered severe die offs that corresponded with the cooler months. Jekyll Bridge, reached a minimum temperature >5 ºC below that recorded for the R2 Navy Tower and maintained lower water temperatures year round than both the GRNMS Buoy and R2 Tower. When these temperatures are assessed along with the population density measurements collected in this study it was apparent the winter water temperatures were associated with fewer live specimens at the collection site.

Previous work by Gilg et al. (2010) suggested that salinity may be the limiting factor to *M. coccopoma* spat settlement in the Florida Intercoastal Water Way. When examining settlement plates, the study found that spat did not settle on plates placed in feeder creeks that had lower salinities than the main channels where spat settlement was more abundant. Similarly, salinity results from this study were lowest at the coastal monitoring site, Jekyll Bridge, where several *M. coccopoma* individuals were found alive during preliminary studies in spring 2013, but no live specimens were found subsequently. This result suggests that the lower salinities occurring at the coastal locations may be inhibiting the settlement of new *M. coccopoma* despite suitable water temperatures during recruitment months. *Balanus trigonus* experiences a similar effect where high temperatures and low salinities in the summer months hinder their recruitment (Thiyagarajan et al. 2003). Though low salinity may play a role in reducing settlement of *M. coccopoma* larvae, it is less likely that it is the driver of winter diebacks for adult populations. Spinuzzi et al. (2013) found *M. coccopoma* established in areas with salinities ranging from 2 to

42 ppt (~3.8 to 62.3 mS/cm) and surmised that the barnacles can tolerate large salinity fluctuations for short periods of time without adverse effects. During the rainy winter months of this study, waters adjacent to the R2 Tower did often reach salinities <30 mS/cm, which is similar to salinities present in brackish, estuarine water, that can cause physiological stress to barnacles (Foster 1970). Often these low salinities were sustained for several hours to as long as one full day, but they did not appear to coincide with death of *M. coccopoma* individuals. Thus, I propose that lower water temperatures are more likely the driver behind the coastal winter die offs of *M. coccopoma* and that range expansion of this species along the immediate coast may be held in check by this environmental variable.

Subpopulations of *M. coccopoma* in the Georgia Bight were assessed for population stability and maturity to determine their ability to act as larval providers. The barnacle density data from fall 2013 and spring 2014 showed that the offshore subpopulations were not subject to winter die offs and were thus more stable than those onshore. In addition, shell sizes from fall 2013 increased with increasing distance from shore. The more consistent densities and larger shells of barnacles offshore support my prediction that offshore sites are composed of more mature *M. coccopoma* populations and thus, good sources of larvae.

In this study shell size was used as a proxy for *M. coccopoma* maturity. Crickenberger and Moran (2013) found that *M. coccopoma* along the southeastern U.S. coastline with a basal diameter >3.28 cm settled during the previous year. Barnacles settled in the previous year are likely to be sexually mature. In fall 2013, four of the eight subpopulations in this study, those at the GRNMS Buoy and all three Navy Towers, had average basal diameters exceeding 3.28 cm. The *M. coccopoma* population on the GRNMS Buoy had an average basal diameter of 3.61cm in fall 2013 even though this buoy was not in place prior to January 2013. This result suggests that

barnacles on the buoy reach reproductive size quicker than those settling on the immediate coastline. Perhaps the warmer and more saline water offshore near the buoy is more favorable to *M. coccopoma* growth and allows them to reach maturity more quickly than on the coastline. Another species of balanoid barnacle, *Semibalanus balanoides*, is known to have reproductive size plasticity in different environments (Bertness et al. 1991). It is also possible that differences in aerial exposure can impact growth rates. While the offshore sites in this study are mostly subtidal, coastal sites are intertidal and the barnacles can be exposed to the air for hours at a time. Aerial exposure has been indicated as a factor limiting body size in the barnacle species, *Semibalanus balanoides*, where there is a negative correlation between exposure time and body size (Bell 2010). Though no one has investigated this correlation in *M. coccopoma*, it cannot be ruled out as a reason that offshore barnacles are significantly larger. Overall, the consistently high densities, rapid growth, and larger shells of *M. coccopoma* on offshore structures indicate they are more stable populations than those at onshore structures and are composed of sexually mature individuals that are able to act as larval sources within the Georgia Bight.

This study also assessed the subpopulations for genetic diversity, Hardy-Weinberg Equilibrium and genetic structure to provide a better understanding of the recruitment dynamics within the Georgia Bight. I found that the species has high allelic diversity at all sites and there was no significant difference in allelic diversity between the site types (coastal, buoy and tower). This diversity is due in large part to high numbers of private alleles at many of the loci. The results also showed that genetic variability was randomly distributed among sites suggesting that there is little to no barrier for dispersal of larvae within the region. Furthermore, all subpopulations evaluated were significantly out of Hardy-Weinberg Equilibrium (HWE) and genetic structure was not similar between the subpopulations.

The entire Georgia Bight thus appears to house a panmictic population of *M. coccopoma* with larvae arriving from many outside sources. High allelic diversity is unusual in introduced and invasive populations due to founder effects, genetic drift and bottlenecks, however, the introduced *M. coccopoma* in this region do not appear be suffering from these. Though this is the first study to employ microsatellite markers on *M. coccopoma*, other studies have assessed diversity of the species using mitochondrial COI and the 16S genes. One such study on *M. coccopoma* from both the southeastern U.S. and the barnacle's native area, found very high diversity values with no significant difference between the introduced and native ranges (Cohen et al. 2014). Furthermore, COI analysis done on an introduced *M. coccopoma* population in Japan also indicated high levels of haplotype diversity with 78% of the haplotypes being represented in only a single individual (Yamaguchi et al. 2009). High allelic richness values are a trait exhibited in other barnacle species including *Megabalanus azoricus* ranging from 4.69-10.38 (Girolamo et al. 2013), *Pollicipes elegans* ranging from 2.0-17.41 (Plough and Marko 2014), and *Semibalanus balanoides* ranging from 6.0-22.3 (Flight et al. 2011). The high allelic richness values I found were largely driven by rare or private alleles both in the loci and in the subpopulations. This is not an uncommon trait in barnacles, many species, including other *Megabalanus* species are known to have a bias toward an excess of rare alleles (Wares 2010, Wares 2011, Ewers and Wares 2012). My results, and those from previous studies, indicate that allelic richness is normally high among barnacle species, including *M. coccopoma*, and even if decreased due to an introduction may still not be low enough to affect the overall diversity of the founder population.

While genetic diversity of *M. coccopoma* subpopulations is high in the southeastern introduced range, the subpopulations are not in HWE due to a homozygote excess. This may be

the result of a Wahlund Effect: deviations from HWE due to the mixing of larvae from source populations with different allele frequencies. The Wahlund Effect has been observed in sessile invertebrates such as limpets (Johnson and Black 1984) and mussels (Tracey et al. 1975) where breeding populations are isolated. The subpopulations of *M. coccopoma* in the Georgia Bight are reproductively isolated from one another, and the high diversity coupled with the HWE deviations point to the presence of a Wahlund Effect. If several distinct source populations contribute to an introduction, Wahlund Effect may be seen in the early stages before the introduced population becomes self-sustaining (Holland 2000, Kolbe et al 2008). The results produced by the STRUCTURE analysis are consistent with this hypothesis suggesting that each population may have arisen from multiple HWE populations. Cohen et al. (2014) found little genetic structuring between two native *M. coccopoma* populations using mitochondrial markers. Highly variable markers such as microsatellites are often more powerful tools than mitochondrial marker to detect population structuring (Balloux and Lugon-Moulin 2002). Further analysis of potential source populations will help clarify the significance of the Wahlund Effect in *M. coccopoma* introductions.

A second, but not necessarily mutually exclusive explanation for the homozygote excess observed in *M. coccopoma* populations in the Georgia Bight is self-fertilization. Though the details of *M. coccopoma* reproductive biology remain unpublished, past studies have found that the some members of the *Megabalanus* genus, like many other Thoracica species, are simultaneously hermaphroditic and can self-fertilize (Newman and Abbott 1980). In the majority of balanoid barnacle species studied, outcrossing is preferred and self-fertilization is thought to be rare (Barnes and Crisp 1956, Kelly et al. 2012). Facultative selfing may be a response to low densities during range expansion or introductions and could contribute to the higher levels of

homozygosity than expected from random mating (Robson et al. 2009, Girolamo et al. 2013). *Megabalanus azoricus* populations in their native range demonstrated similar patterns of homozygote excess at microsatellite loci, suggesting the possibility that some fraction of self-fertilization may be more common in *Megabalanus* than other barnacles (Girolamo et al. 2013). The highly variable microsatellite loci developed for this study provide a means to investigate rates of selfing and outcrossing in *M. coccopoma*.

To investigate the degree of localized larval recruitment being employed in the Georgia Bight, this study assessed the genetic structure of a new *M. coccopoma* population on the free subtidal habitat created by the replacement of the GRNMS Buoy and among subpopulations across the Georgia Bight region. The F_{ST} values and the STRUCTURE results for the GRNMS Buoy indicated low levels of genetic differentiation between the original buoy and the new buoy populations. Interestingly, the two sampling periods completed on the new buoy subpopulation (fall 2013 and spring 2014), also showed detectable differences although much smaller than those detected between pre- and post-recolonization. This result was unexpected as it was assumed that the specimens from these two sampling periods were from the same source population sampled twice over a small spatial scale. Furthermore, when both the on and offshore subpopulations of *M. coccopoma* from 2013 and 2014 were assessed there was no evidence of genetic structure. All of the subpopulations were found to be significantly different from one another based on F_{ST} values and STRUCTURE analysis.

Hare and Walsh (2007) and Edwards et al. (2007) found strong evidence to suggest that the Georgia Bight is a relatively closed system where larvae can be retained locally over time scales of 1-2 months. Further, historical oceanographic data from this region shows strong frontal eddies on the continental shelf which would be ideal for retaining larvae and promoting

local recruitment (Bumpus 1971, Atkinson et al. 1978, Lee et al. 1981). Genetic differences I observed across the *M. coccopoma* subpopulations do not provide support for local retention as the main recruitment tactic by this species in the Georgia Bight, but suggest more complex processes. The high genetic diversity and lack of similar genetic structure among subpopulations suggest that *M. coccopoma* in the Georgia Bight are recruiting openly. Due to the unpredictability of ocean conditions it is common for many marine species to experience high larval death rates. Therefore, populations that are employing open recruitment can still show temporal variation in the genetic composition of recruits that can lead to microgeographic genetic heterogeneity (Hedgecock 1994). Larval settlement is essentially a sweepstakes in which only larvae from a small fraction of the breeding population is able to survive and settle, making individual sites highly diverse, while the population over a large scale appears to have low spatial genetic variance. There is strong evidence that this does occur in marine species with planktonic larvae including urchins (Palumbi et al. 1997, Flowers et al. 2002), barnacles (Hedgecock 1982, 1986), limpets (Johnson and Black 1984), and anchovies (Hedgecock et al. 1991, Hedgecock et al. 1994). This theory appears to match much of the evidence from this study in which the Georgia Bight subpopulation had small but significant differences in allele frequencies throughout suggesting the regional population is panmictic, but individual subpopulations in the area show genetic divergence from one another. Though the offshore structures have had many seasons of recruitment, the shell size metrics show low standard errors suggesting that all open intertidal space is quickly colonized by a random assortment of larvae, the sweepstakes winners, and there is no room for new recruits in future seasons. This may explain why the genetic structure remains distinct over multiple recruitment seasons at offshore sites. The unique oceanographic conditions in the Georgia Bight coupled with the recent

introduction and instability of the *M. coccopoma* populations at many sites makes the genetic sweepstakes a highly probable scenario for the region. In order to truly assess this the subpopulations would need to be sampled at least one more time. Hedgecock (1994) suggests that analysis of temporal genetic change is robust over two to ten generations. Sampling again would increase the recruitment seasons (i.e. generations) covered from one to at least two, thus allowing a greater picture of the temporal variation in the region.

This study establishes a baseline assessment of both demographic information, and genetic structure of *M. coccopoma* at both on and offshore artificial structures in the Georgia Bight. The analysis of the abiotic parameters and the demographic data indicates that the Navy Towers offshore are highly suitable refuges for breeding adults of *M. coccopoma*, while the onshore sites are less suitable and experience extreme winter die offs likely due to lower water temperatures. The genetic analysis revealed an introduced population that has high allelic diversity, indicative of long-term success. The Georgia Bight *M. coccopoma* are composed of a large, panmictic population that is likely employing an open recruitment model with sources from inside and outside of the Bight region. The partitioning patterns of this diversity among each individual site, at each time point are complex. STRUCTURE clustering patterns and heterozygote deficiency patterns suggest different source populations with high diversity established the Georgia Bight population with multiple introductions of *M. coccopoma* from different locations. Additionally, there is evidence of microgeographic genetic heterogeneity among the subpopulations suggesting a genetic sweepstakes effect in the region. It is also important to note that the excess of homozygotes in the subpopulations suggests the possibility of considerable self-fertilization in *M. coccopoma*. Though this study found that there are sources of larvae from outside of the Georgia Bight that are key to the continuous coastal

repopulations, it is also clear that the offshore structures in the region are able to support sexually mature *M. coccopoma* subpopulations with high diversity that can, and likely are, providing larvae to the region as well. The combined results of this study lead me to suggest that permanent offshore structures, like the Navy Towers, both in the Georgia Bight region, but also throughout the southeastern U.S.A. need to be removed fully or the structures need to be partially removed from the upper subtidal (~5 or more meters below sea level). This may help to prevent introduced *M. coccopoma* and other non-native marine invertebrates from establishing breeding populations that contribute larvae to expand their ranges along the southeastern coastline.

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TABLES AND FIGURES

Table 2.1: Dates of specimen collection, density photographs and data probe placement and retrieval in fall 2013 and spring/summer 2014. The dates listed for Jekyll Bridge and R2 Tower in fall 2014 were the final retrieval of the temperature/salinity probes. ND = no data collected. Asterisks (*) indicate the deployment and retrieval of a data logger. After initial placement in Fall 2013 only 2 data loggers were retrieved.

	Date of specimen collection, density photographs and data logger placement or retrieval			
Site	**Spring 2013**	**Fall 2013**	**Spring/Summer 2014**	**Fall 2014**
Tybee Pier	ND	October 11*	May 23	ND
St. Simons Pier	ND	September 19*	April 29	ND
Jekyll Bridge	ND	September 19*	April 29*	October 23*
Folly Pier	ND	October 14*	April 27	ND
GRNMS Buoy	January 23	October 28*	May 27	ND
R2 Tower	ND	September 16*	June 19*	October 6*
M2R6 Tower	ND	September 16*	June 19	ND
R8 Tower	ND	September 16*	June 19	ND

Table 2.2: Global summary statistics for each loci averaged across all sites and time points. *A* is the mean allelic richness, A_{TOTAL} is the total number of alleles found for that locus in all populations, and private alleles are alleles that appear only once in all of the sampled subpopulations.

Locus	*A*	A_{TOTAL}	*Private Alleles*
MC-1	12.44	29	6
MC-3	12.42	32	6
MC-4	10.21	33	11
MC-5	16.35	58	19
MC-9	17.27	67	26
MC-13	18.45	82	24
MC-15	13.90	42	14
MC-22	11.16	26	7
MC-24	7.10	16	4
MC-26	14.36	46	10
MC-27	12.59	42	14
MC-28	10.43	34	7
MC-29	12.34	27	5

Table 2.3: The descriptive statistics for all thirteen microsatellite loci across subpopulations in both fall 2013 and spring 2014. *n* is the number of individuals sampled, *A* is the allelic richness, H_E is the expected heterozygosity, H_O is the observed heterozygosity and private alleles are alleles across all thirteen loci that appear only once in that particular subpopulation. ND (no data) indicates that no live specimens of *M. coccopoma* were found at that location and therefore there are no genetics results and NA (not applicable) indicates a site with a small sample size that was not included in the analysis.

Site	*N*	*A*	H_E	H_O	Private Alleles
		FALL 2013			
Tybee Pier	31	19.69	0.92	0.65	22
St. Simons Pier	24	17.38	0.92	0.66	14
Jekyll Bridge	ND	ND	ND	ND	ND
Folly Pier	22	15.54	0.90	0.58	7
GRNMS Buoy	32	19.08	0.92	0.72	17
R2 Tower	18	15.69	0.93	0.68	28
M2R6 Tower	19	14.46	0.91	0.59	8
R8 Tower	15	13.54	0.92	0.62	12
		SPRING 2014			
Tybee Pier	ND	ND	ND	ND	ND
St. Simons Pier	NA	NA	NA	NA	NA
Jekyll Bridge	ND	ND	ND	ND	ND
Folly Pier	ND	ND	ND	ND	ND
GRNMS Buoy	27	17.69	0.91	0.60	11
R2 Tower	15	13.23	0.91	0.55	7
M2R6 Tower	21	15.85	0.91	0.57	13
R8 Tower	17	13.69	0.90	0.56	14

Table 2.4: Pairwise values of F_{ST} for the three time points on the Gray's Reef National Marine Sanctuary Buoy based on allele frequencies of all thirteen loci. All time points were significantly different from one another ($p<0.0001$) as denoted by asterisks (*). OB indicates the original GRNMS Buoy population.

	OB	**GRNMS Buoy fall 2013**
GRNMS Buoy fall 2013	0.0151*	-------
GRNMS Buoy spring 2015	0.0195*	0.0045*

Table 2.5: Results from the analysis of molecular variance (AMOVA) for all *M. coccopoma* collected at the three time points on the Gray's Reef National Marine Sanctuary Buoy and averaged over all 13 loci.

Source of Variation	Sum of Squares	Variance Components	% Variation
Among Populations	22.293	0.072	1.186
Among Individuals within Populations	539.965	1.70	27.980
Within Individuals	319.500	4.299	70.834
Total	881.757	6.0696	

Table 2.6: Pairwise F_{ST} values (below diagonal) and indication of significance levels or exact test of allele frequency differences (above diagonal) for all sites in fall 2013 and spring 2014 for all thirteen loci ($p<0.05$). Bolded F_{ST} values indicates significance at $p<0.05$ and +++ indicates significant allele frequency differences at $p<0.001$. Jekyll Bridge is not included in this analysis as no live individuals were found during fall 2013 or spring 2014. St. Simons Pier spring 2014 was not included due to insufficient sample size (n=2).

	Tybee Pier F13	**Folly Pier F13**	**St. Simons Pier F13**	**GRNMS Buoy F13**	**R2 F13**	**M2R6 F13**	**R8 F13**	**GRNMS Buoy SP14**	**R2 SP14**	**M2R6 SP14**
Tybee Pier F13	---	+++	+++	+++	+++	+++	+++	+++	+++	+++
Folly Pier F13	0.0030	---	+++	+++	+++	+++	+++	+++	+++	+++
St. Simons Pier F13	0.0000	0.0013	---	+++	+++	+++	+++	+++	+++	+++
GRNMS Buoy F13	0.0007	0.0033	0.0021	---	+++	+++	+++	+++	+++	+++
R2 F13	0.0037	**0.0057**	**0.0065**	**0.0058**	---	+++	+++	+++	+++	+++
M2R6 F13	0.0000	0.0000	0.0000	0.0000	**0.0030**	---	+++	+++	+++	+++
R8 F13	0.0003	0.0025	**0.0550**	0.0041	0.0081	0.0000	---	+++	+++	+++
GRNMS Buoy SP14	0.0000	0.0001	0.0005	**0.0045**	**0.0049**	0.0000	**0.0059**	---	+++	+++
R2 SP14	0.0029	0.0013	0.0024	0.0030	0.0000	0.0000	0.0056	0.0000	---	+++
M2R6 SP14	0.0051	0.0021	0.0036	0.0010	0.0067	0.0000	0.0016	0.0035	0.0000	---
R8 SP14	0.0059	0.0077	0.0045	**0.0108**	**0.0135**	0.0000	**0.0144**	0.0042	0.0031	**0.0108**

Table 2.7: The results from the analysis of molecular variance (AMOVA) using all *M. coccopoma* collected from the Georgia Bight area and averaged over all 13 loci. (A) Subpopulations were grouped according to site type: coastal, buoy, Navy Tower. (B) Subpopulations were grouped together by season of collection: fall 2013 and spring 2014 (C) All subpopulations were grouped into one global population.

A.

Source of Variation	Sum of Squares	Variance Components	%Variation
Among Groups	16.023	-0.007	-0.120
Among populations within groups	78.692	0.0243	0.405
Among individuals within populations	1706.135	1.928	32.117
Within Individuals	943.00	4.058	67.598

B.

Source of Variation	Sum of Squares	Variance Components	%Variation
Among Groups	9.813	0.007	0.109
Among Populations Within Groups	84.902	0.016	0.264
Among Individuals Within Populations	1706.135	1.928	32.117
Within Individuals	943.000	4.058	67.598

C.

Source of Variation	Sum of Squares	Variance Components	%Variation
Among Populations	94.714	0.191	0.319
Among Individuals Within Populations	1706.135	1.928	32.088
Within Individuals	943.000	4.058	67.539

Table 2.8: The results from the analysis of molecular variance (AMOVA) using all of the specimens of *M. coccopoma* collected from the Georgia Bight area and averaged over all 10 loci. Loci MC-5, MC-9 and MC-13 were removed from these analyses due to their very high allele diversity. (A) Subpopulations were grouped according to site type: coastal, buoy, Navy Tower. (B) Subpopulations were grouped together by season of collection: fall 2013 and spring 2014 (C) All subpopulations were grouped into one global population.

A.

Source of Variation	Sum of Squares	Variance Components	%Variation
Among Groups	11.931	-0.002	-0.042
Among populations within groups	55.199	0.010	0.228
Among individuals within populations	1282.123	1.226	27.006
Within Individuals	779.000	3.305	72.808

B.

Source of Variation	Sum of Squares	Variance Components	%Variation
Among Groups	6.078	-0.0003	-0.006
Among Populations Within Groups	61.053	0.009	0.201
Among Individuals Within Populations	1282.123	1.226	27.003
Within Individuals	779.000	3.305	72.801

C.

Source of Variation	Sum of Squares	Variance Components	%Variation
Among Populations	67.131	0.009	0.198
Among Individuals Within Populations	1282.123	1.226	27.003
Within Individuals	779.000	3.305	72.800

Figure 2.1: Map of the research sites within the Georgia Bight. Left inset map indicates the location of the Georgia Bight on the United States east coast indicated by the black box. Collection sites are indicated by red dots. There are four coastal collection sites (Folly Pier, Tybee Pier, Jekyll Bridge, St. Simons Pier), one buoy site (GRNMS Buoy) and three offshore Navy Tower sites (R2, M2R6, R8).

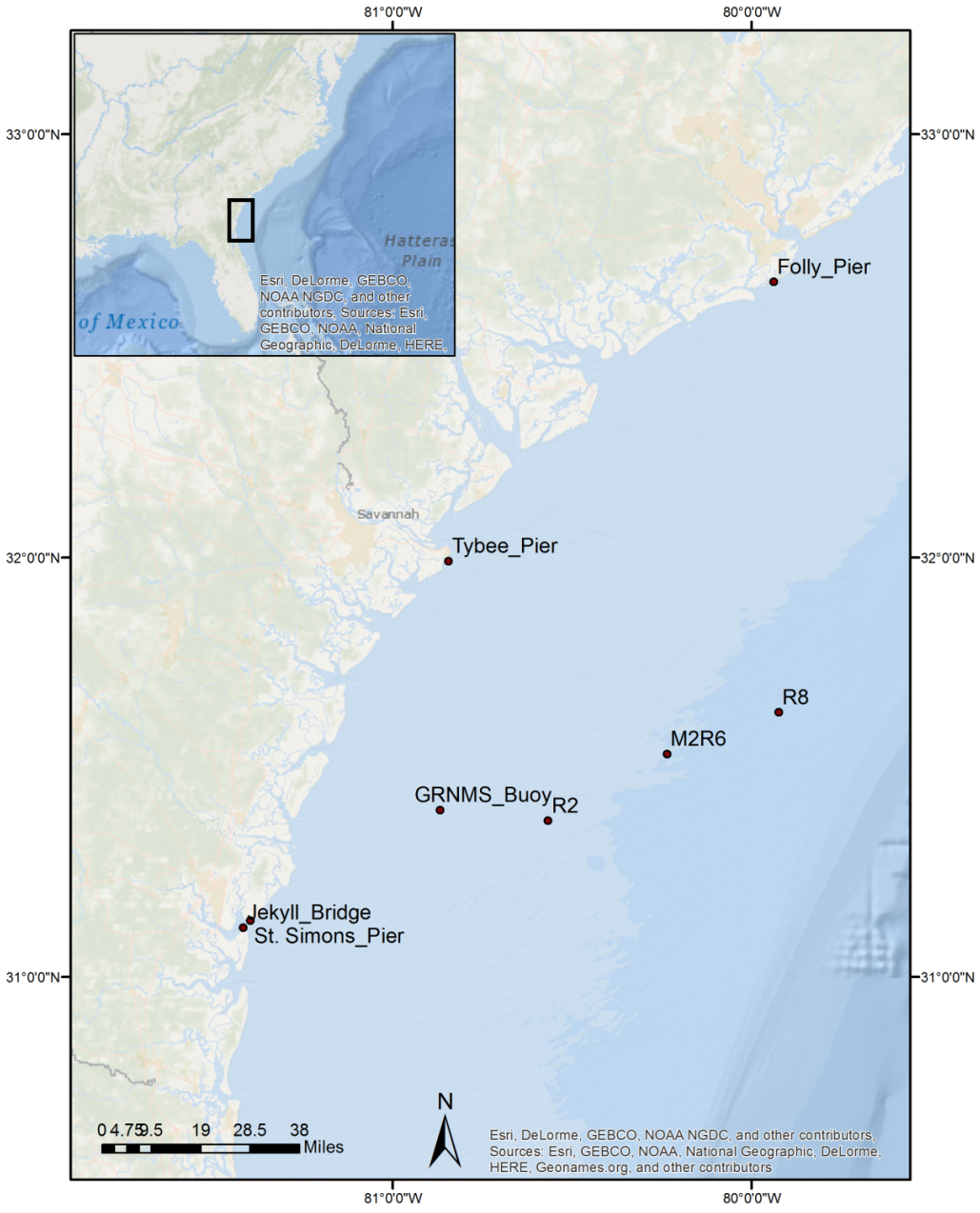

Figure 2.2: Gray's Reef National Marine Sanctuary (GRNMS) Buoy (pictured before it was placed in the water in January 2013; Photo credit to Scott Noakes).

Figure 2.3: Abandoned Navy Tower R2 (31º22'30"N, 80º34'01"W) off the coast of Georgia. All three Navy Towers used in this study (R2, M2R6, and R8) are similar in design and size (Photo credit to Chris Briand).

Figure 2.4: Photographs of an *M. coccopoma* specimen with arrows indicating the location of the three shell size measurements that were taken for this study. From left to right: diameter of opercular cavity opening at the widest point, basal diameter at the widest point and at a 90º angle to the widest point, and height at the tallest point.

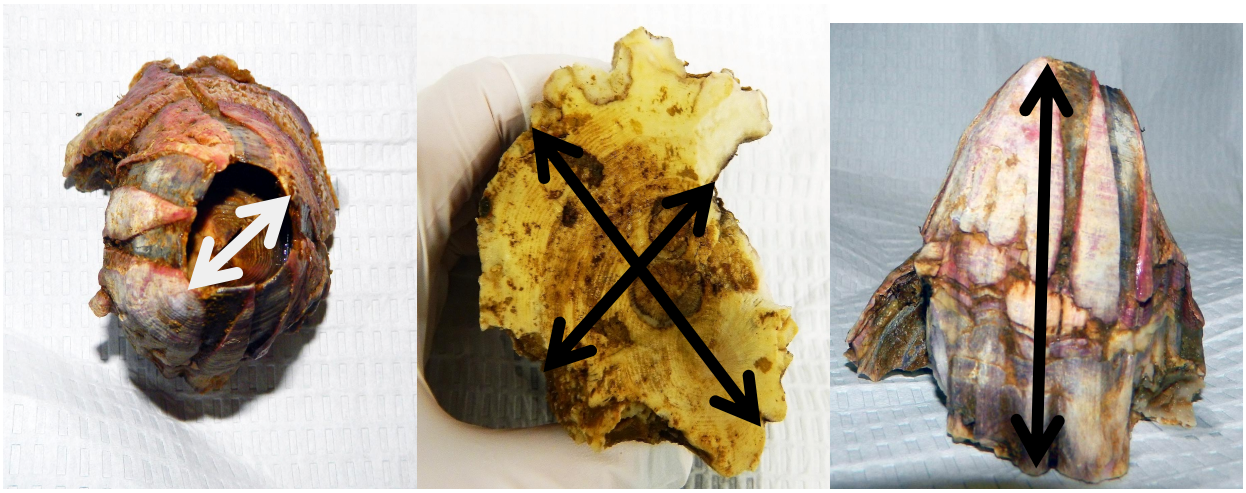

Figure 2.5: Sea water temperatures (ºC) for three sites: R2 Tower (Blue Line), Jekyll Bridge (grey line), GRNMS Buoy (Black Line). Temperatures were recorded hourly at Jekyll Bridge and R2 Tower by a submersible Odyssey Temperature/Conductivity logger from September 9, 2013 to October 16, 2014. There was a gap in collection at R2 Tower during April 2014 when the equipment experienced a technical malfunction. The GRNMS Buoy temperature data was retrieved from the National Data Buoy Center website. The minimum recorded temperature, 4.4 ºC, occurred in January 2014 at Jekyll Island.

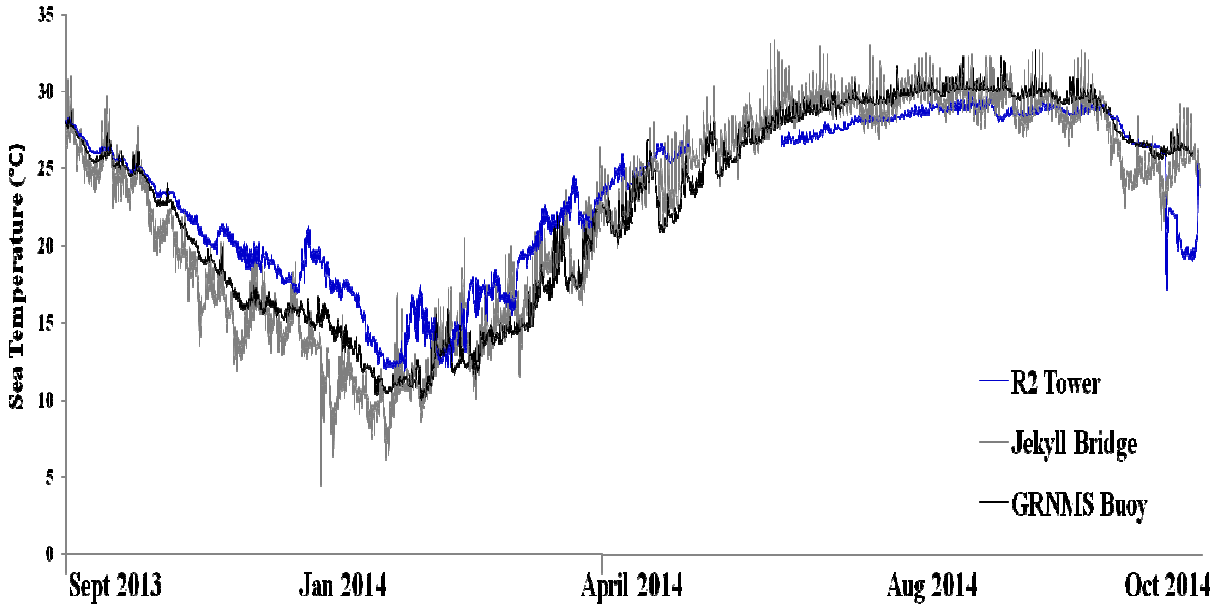

Figure 2.6: Sea temperatures (°C) from December 1, 2013 to February 28, 2014 for three sites: Jekyll Bridge (gray line), R2 Tower (blue Line), and GRNMS Buoy (black line). The lowest temperature (4.4°C) was recorded on January 3, 3014 at Jekyll Island. Throughout the winter months Jekyll Island maintained the overall lowest minimum temperatures.

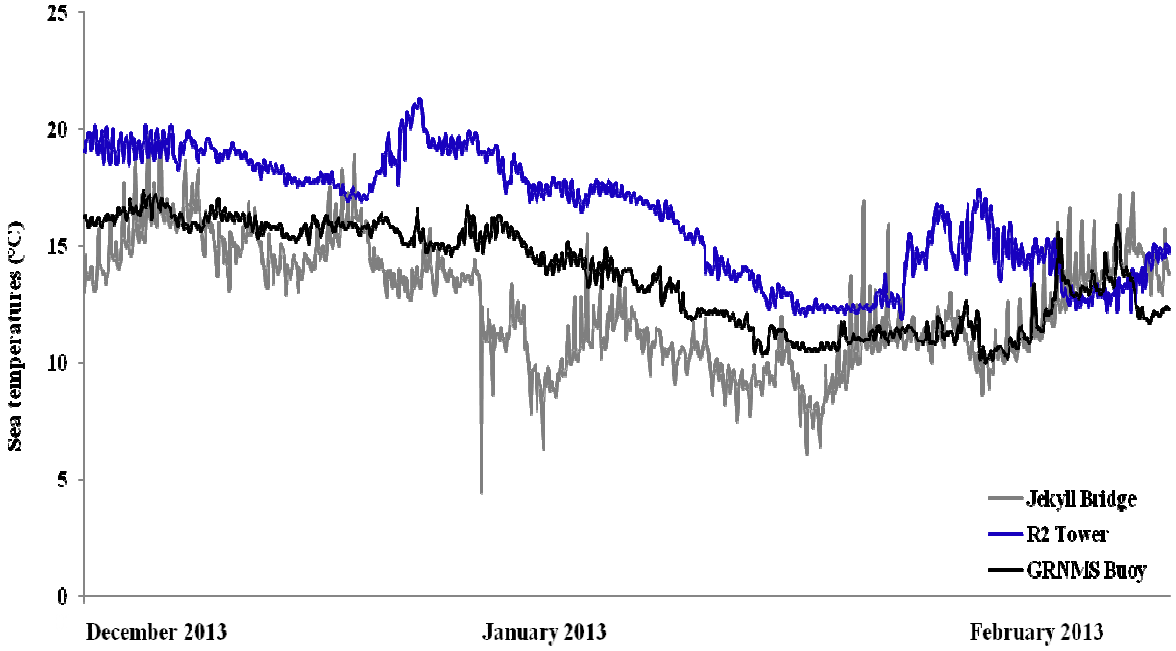

Figure 2.7: Salinity (mS/cm) from September 19, 2013 to March 25, 2014 at two collection sites: Jekyll Bridge and R2 Tower. Salinity measurements were collected hourly at each site with a submersible Odyssey temperature/conductivity data logger.

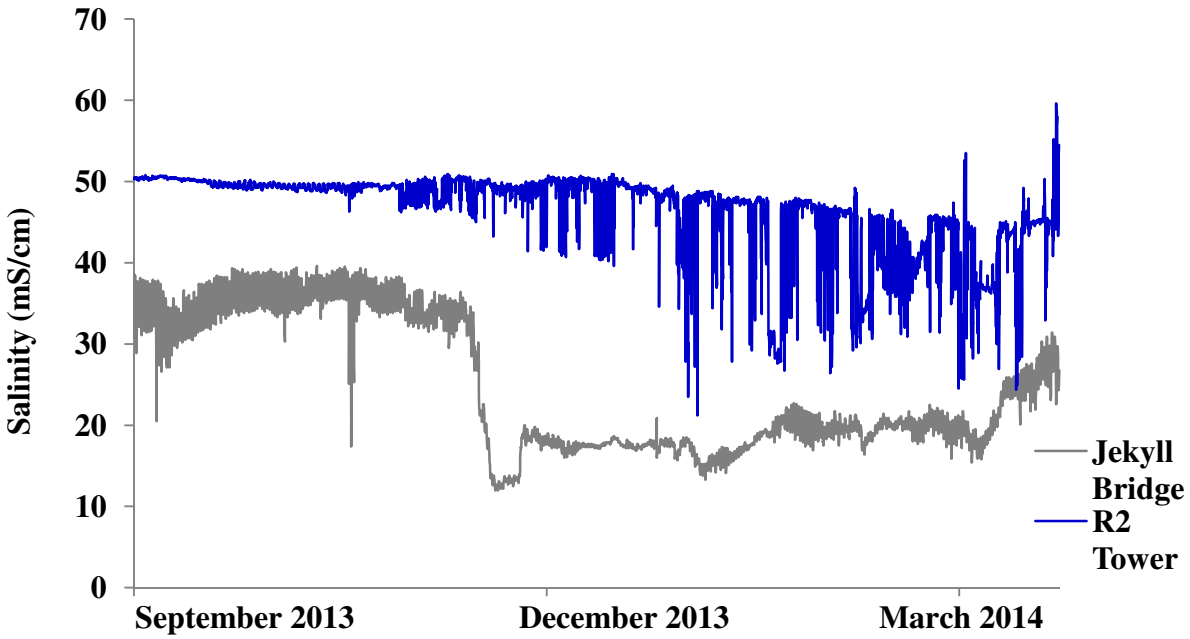

Figure 2.8: Density of *M. coccopoma* (individuals per 20x20cm quadrat) ±SE collected at eight sites in fall 2013 and spring 2014. Significant differences in density were found at Tybee Island and Folly Beach between the two seasons ($p<0.05$). No density photographs were taken at GRNMS Buoy in fall 2013 indicated by NP. No live specimens were found at Jekyll Bridge in fall 2013 or spring 2014.

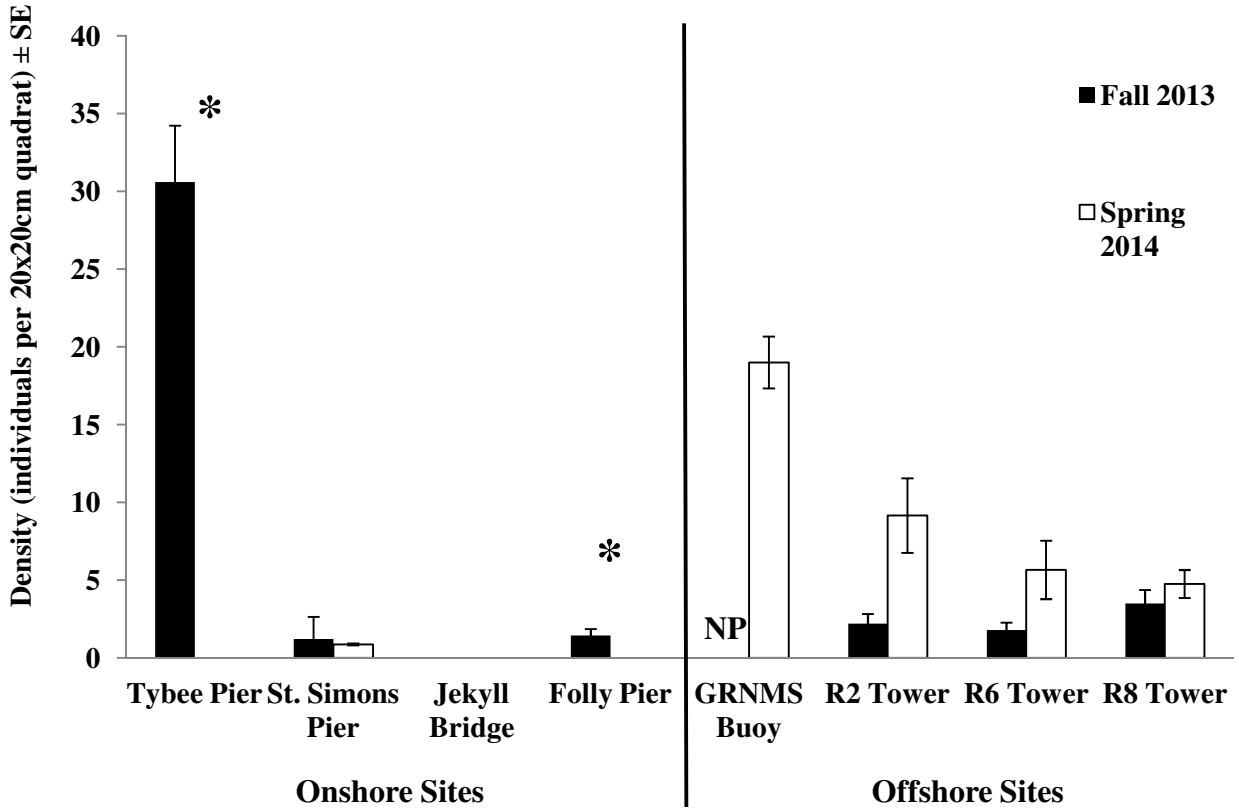

Figure 2.9: *M. coccopoma* shell size measurements (centimeters ± SE) collected from eight research sites in fall 2013. Individual coastal and Navy Towers sites were grouped together for clarity. Opercular cavity opening and basal diameter were significantly different for all three site types ($p<0.05$). The measurement for height showed that the Navy Tower shells were significantly taller than both other site types, but we saw no significant difference between coastal and midshelf heights. Asterisks denote significance and different letters on bars denote significant differences between site types based on a Tukey HSD a posteriori test ($p<0.05$).

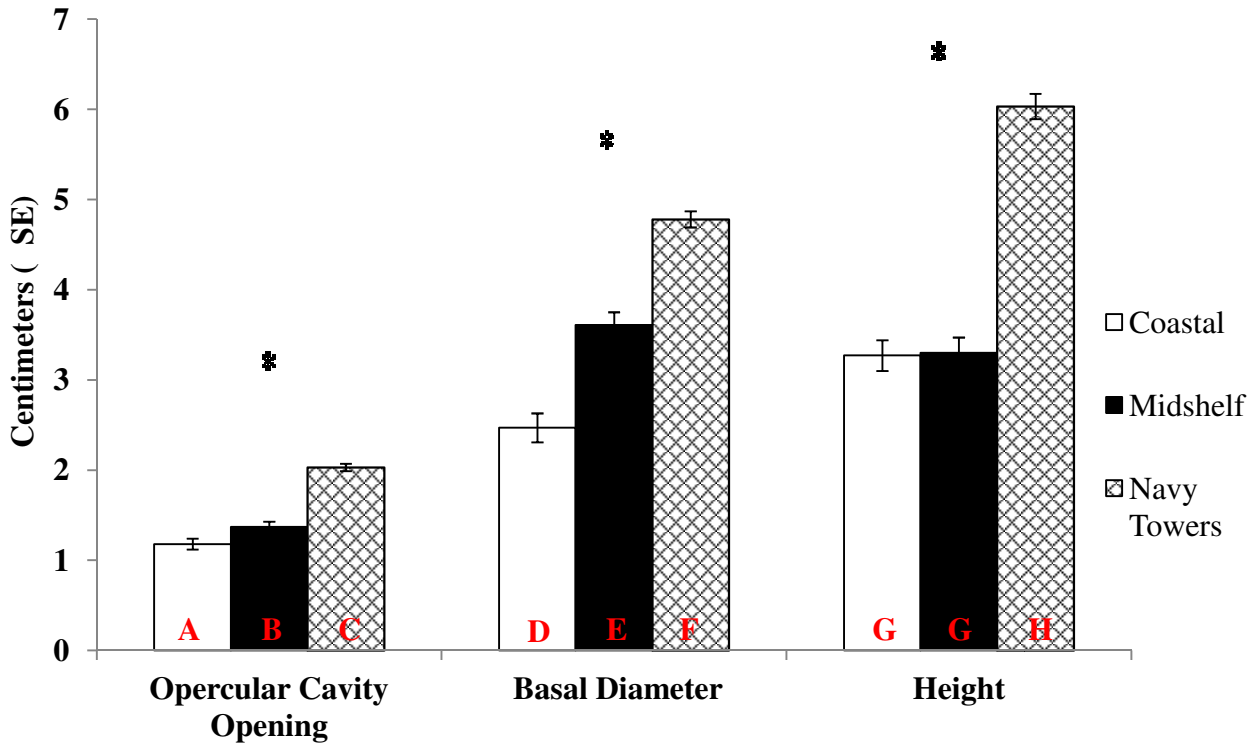

Figure 2.10: Density of *M. coccopoma* (#individuals per 20x20cm quadrat) ± SE collected at the GRNMS Buoy at two time points: January 2013 (OB) and May 2014 (GRNMS Buoy spring 2014). A nonparametric Kruskal-Wallis test indicated no significant difference between the density per quadrat at the two sites (χ^2:2.69, DF=1, p=0.10).

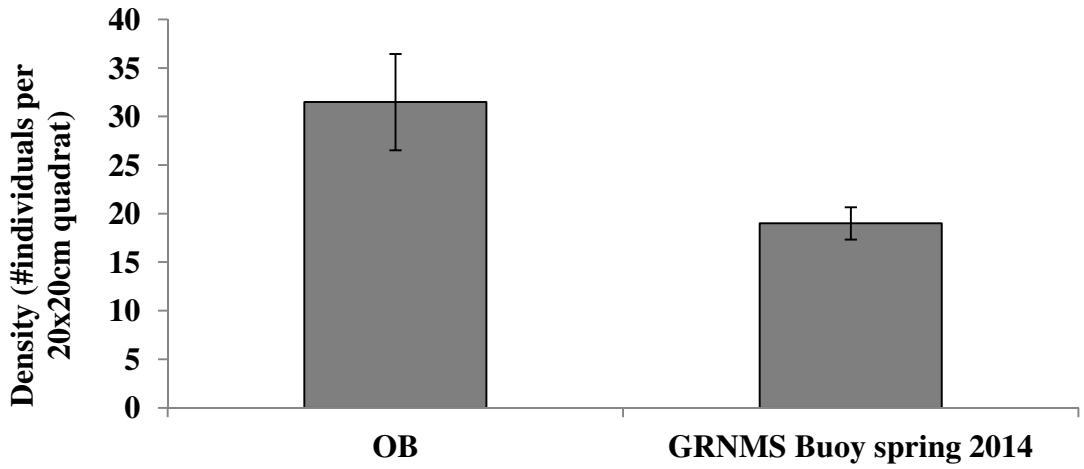

Figure 2.11: *M. coccopoma* shell sizes in centimeters ± SE for specimens collected from the GRNMS Buoy at three time points: Jan 2013 (OB), September 2013 (GRNMS Buoy fall 2013), and May 2014 (GRNMS Buoy spring 2014). Letters on bars denote significant differences based on a posteriori tests ($p<0.05$).

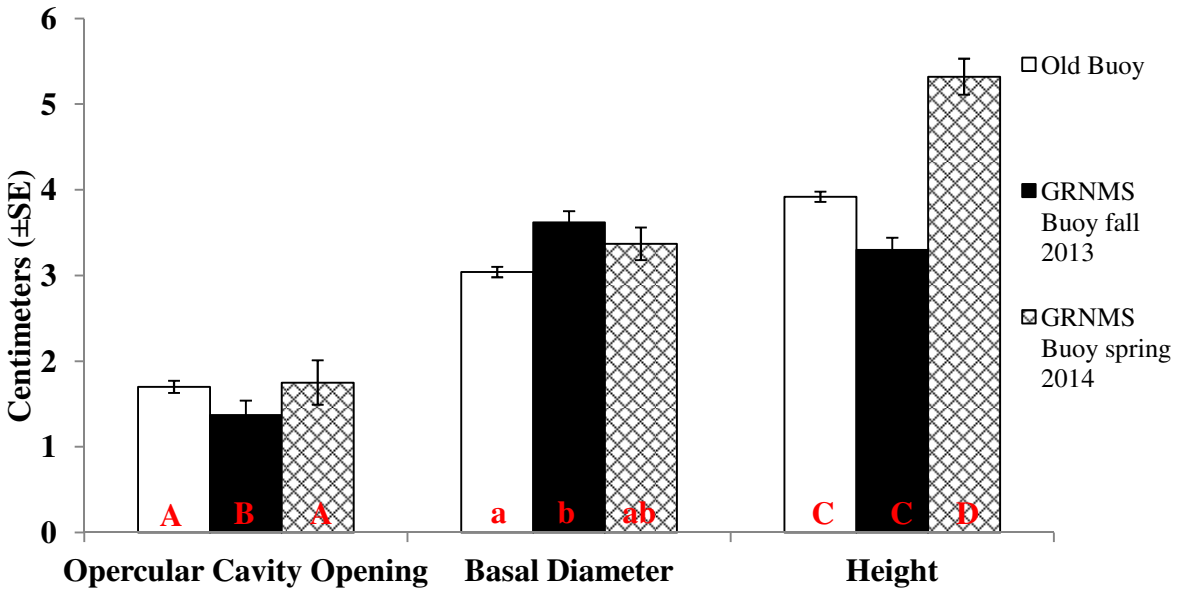

Figure 2.12: The mean log likelihood (L(*K*) ± SD) for potential clusters (*K*) of 1-10 based on the microsatellite analysis from specimens collected from the GRNMS Buoy at three time points. These data were averaged for 20 replicates of each *K* value. The program STRUCTURE HARVESTER (Earl and Vonholdt 2012) was used to average L(*K*) values over replicates.

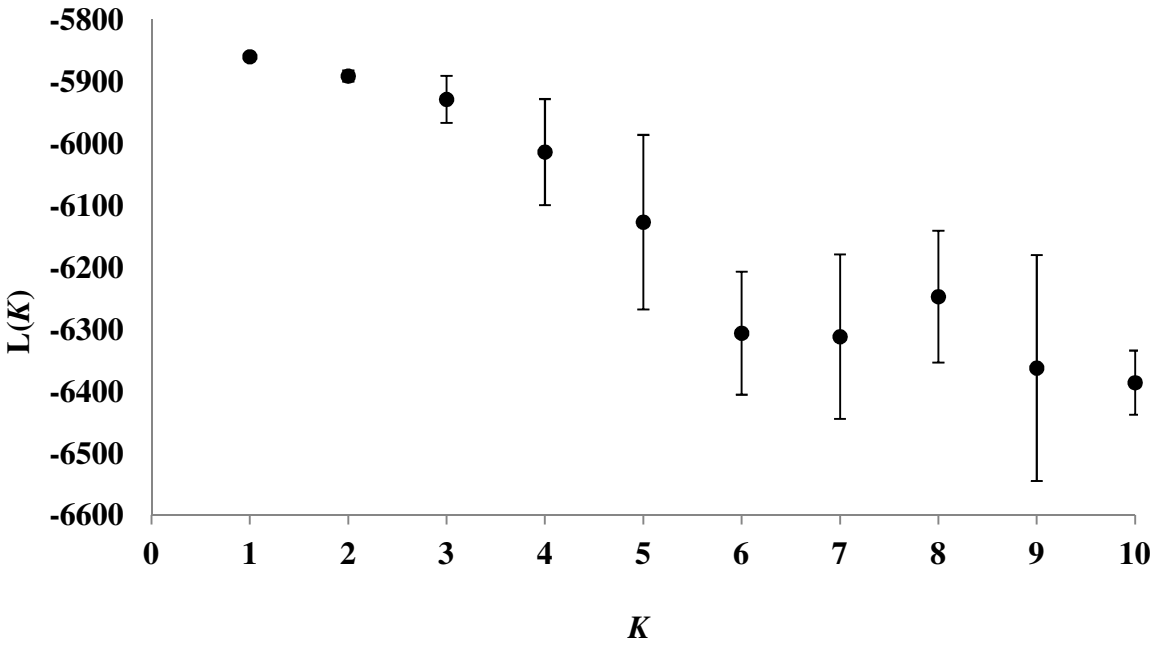

Figure 2.13: Values of Δ*K* for the specimens collected from the GRNMS Buoy at three time points. Δ*K* is an ad hoc value based on the second rate order of change of the likelihood function with respect to *K*, the number of clusters in the population (Evanno et al. 2005). Δ*K* was calculated by the program STRUCTURE HARVESTER (Earl and Vonholdt 2013) for *K* = 1-10.

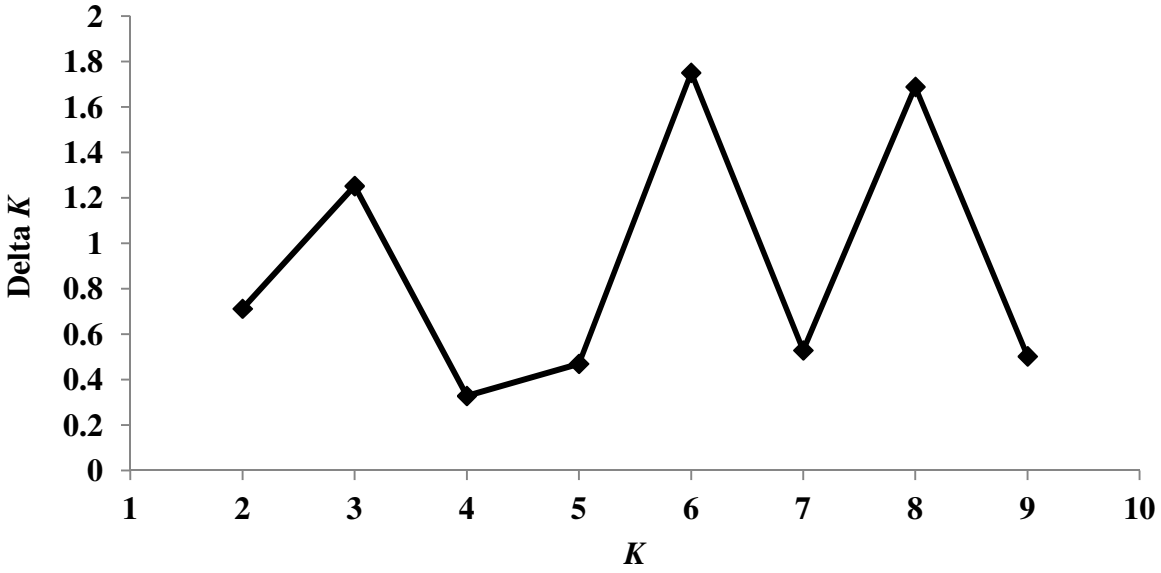

Figure 2.14: STRUCTURE analysis for *M. coccopoma* from three time points (OB = Jan 2013, GRNMS Buoy fall 2013 = September 2013 and GRNMS Buoy spring 2014 = May 2014) at GRNMS Buoy assuming admixture and correlated allele frequencies for clusters *K*=3. Each individual is represented by a vertical line with shading to indicating the posterior probability that an individual is from a given cluster.

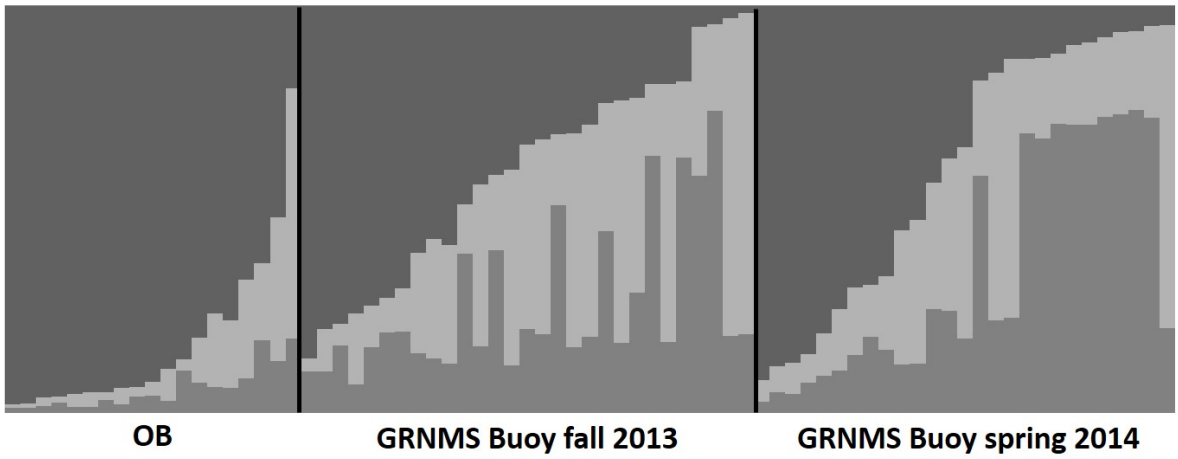

Figure 2.15: The mean log likelihood (L(*K*) ± SD) averaged for 20 replicates of clusters (*K)* 4-10 for all eight subpopulations in both fall 2013 and spring 2014. This data was obtained from the output of the program STRUCTURE HARVESTER (Earl and Vonholdt 2012) which averages L(*K*) values over replicates.

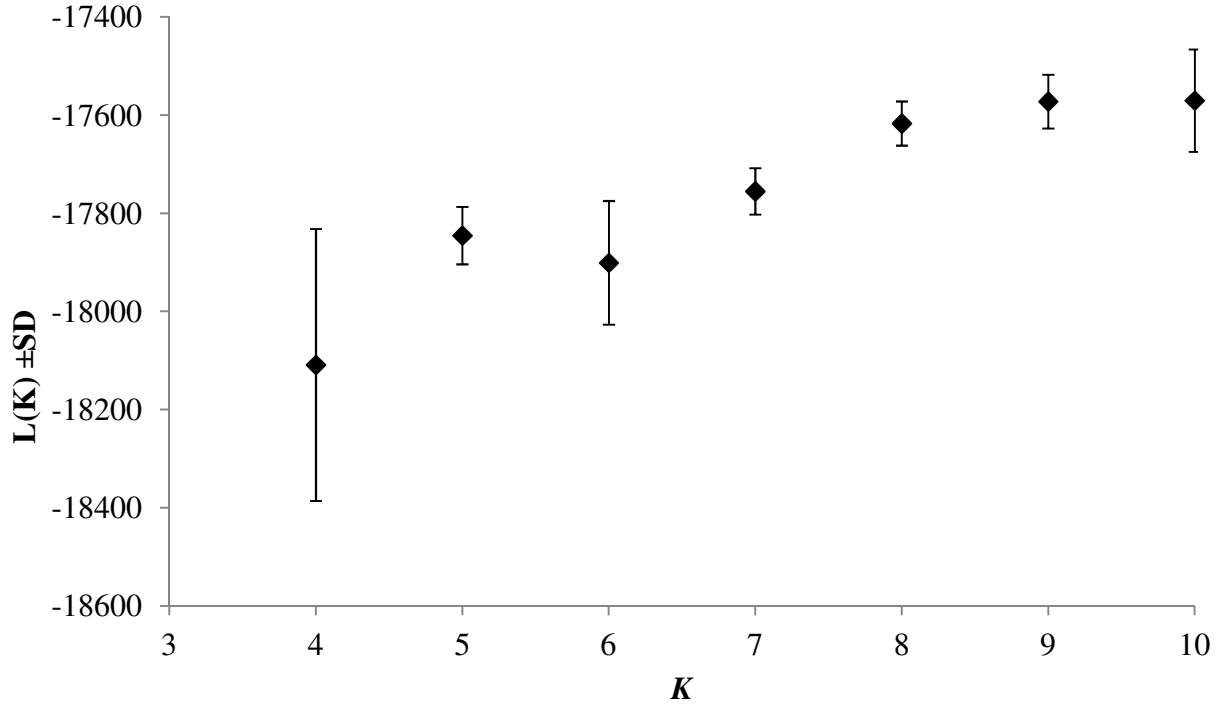

Figure 2.16: Values of Δ*K*, an ad hoc value based on the second rate order of change of the likelihood function with respect to the number of clusters (*K*) in the population. (Evanno et al. 2005). This figure shows results for *K* = 4-10 for all *M. coccopoma* collected at all eight subpopulations in fall 2013 and spring 2014. This data was obtained from the output in the program STRUCTURE HARVESTER (Earl and Vonholdt 2012).

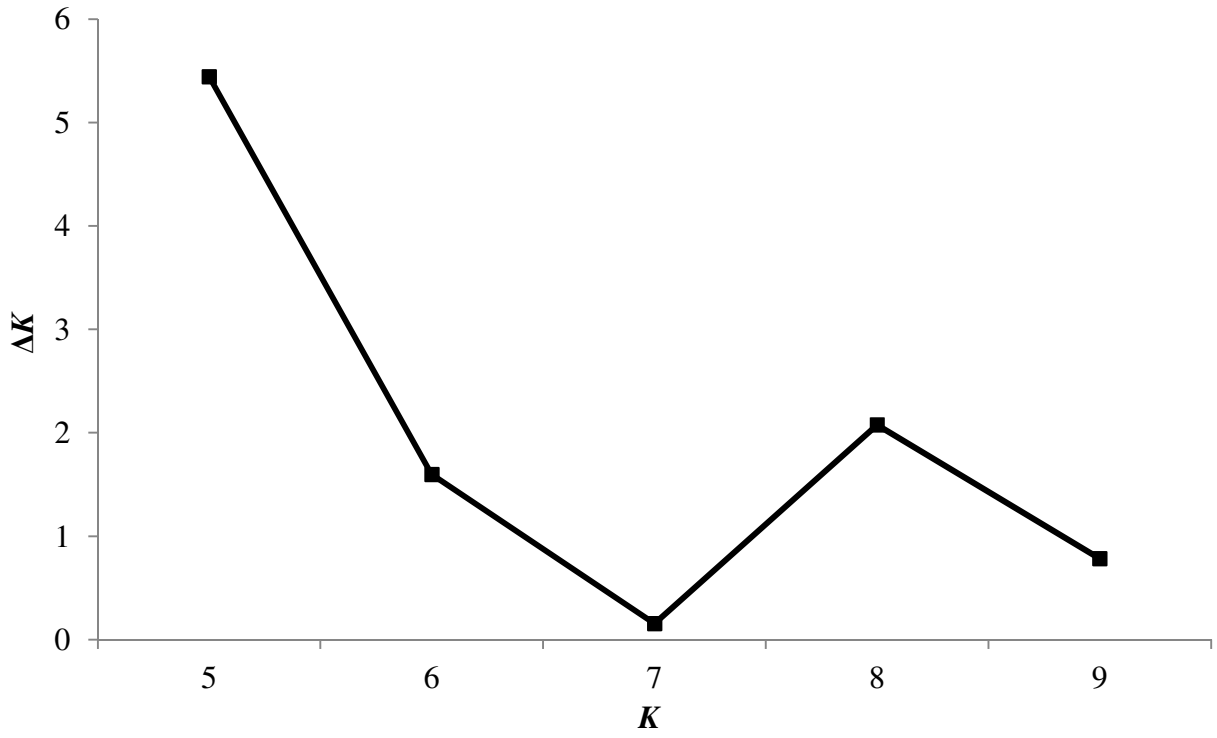

Figure 2.17: STRUCTURE analysis for *M. coccopoma* from the all subpopulations for fall 2013 and spring 2014 assuming admixture and correlated allele frequencies for clusters *K*=5. Each individual is represented by a vertical line with shading indicating the posterior probability that an individual is from a given cluster.