EARLY LIFE HISTORY, HABITAT USE, AND MICROSATELLITE ALLELE FREQUENCY OF TWO COMMON REEF FISHES (*STEGASTES PARTITUS* AND *THALASSOMA BIFASCIATUM*) IN MARINE PROTECTED AREAS OF THE NORTHWESTERN GULF OF MEXICO

By

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DEDICATION

This is dedicated to Daisy and Tika who saw me through the best and the worst of my efforts.

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LIST OF ABBREVIATIONS/SYMBOLS

ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
C_P	Mallow's C_P Statistic
EFGB	East Flower Garden Banks
FGB	Flower Garden Banks
F_{ST}	Fixation Index
GOM	Gulf of Mexico
HAPC	Habitat Area of Particular Concern
IBD	Isolation-by-Distance
IQR	Intra-quartile Range
M	Slatkin's Similarity Measure
MPA	Marine Protected Area
mtDNA	Mitochondrial DNA
NIH	National Institutes of Health
NOAA	National Oceanic and Atmospheric Administration
NW	Northwest
PCR	Polymerase Chain Reaction
PLD	Pelagic Larval Duration
SL	Standard Length
T_A	Annealing Temperature
TL	Total Length
WFGB	West Flower Garden Banks

ABSTRACT

EARLY LIFE HISTORY, HABITAT USE, AND MICROSATELLITE ALLELE FREQUENCY OF TWO COMMON REEF FISHES (*STEGASTES PARTITUS* AND *THALASSOMA BIFASCIATUM*) IN MARINE PROTECTED AREAS OF THE NORTHWESTERN GULF OF MEXICO

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Two common reef fishes, the bluehead wrasse (*Thalassoma bifasciatum*) and the bicolor damselfish (*Stegastes partitus*), are used as model species for understanding the function of marine protected areas (MPAs) in the northwestern Gulf of Mexico (GOM). These species have contrasting life histories, which represent a spectrum of common life histories of reef fish. Here, the early life history, habitat associations, and population genetics of these two common reef fishes, in the northwestern GOM are examined to help acquire a better understanding of the nursery, or larval settlement, value of the region. Because the banks examined here are near the northern limits of reef coral growth, and are isolated from other nursery areas such as seagrasses or mangroves they

could serve as stepping stones for replenishment and dispersal of larvae throughout this region.

East Flower Garden Banks (EFGB) and West Flower Garden Banks (WFGB) are both dominated by scleractinian corals, with higher rugosity, or more varied substrate topography, while Stetson Bank and Sonnier Bank are both dominated by rock, sand, and algae cover, and have lower average rugosities. There does not appear to be a clear substrate preference for either species post-settlement in this region. There was a significant difference in density of bicolor damselfish between banks, but not for bluehead wrasse. There was a significant difference in PLD of bluehead wrasse between banks but not for bicolor damselfish. Bluehead wrasse exhibit a longer less variable PLD than bicolor damselfish. Back-calculations suggested a slightly higher length-at-settlement value for bluehead wrasse compared to surrounding regions, and bicolor damselfish showed an average length-at-settlement value about twice that of surrounding regions. The bicolor damselfish examined here show evidence of local retention while the bluehead wrasse show evidence or broader dispersal, this further supports studies done in surrounding regions such as the Caribbean.

CHAPTER 1

Introduction

In order to properly and effectively manage marine protected areas (MPAs) it is imperative to understand population dynamics of the living resources that occur within their boundaries. The continental shelf in the northwestern Gulf of Mexico is characterized by several reefs and banks, some of which are marine protected areas. The most significant areas are represented by East and West Flower Garden Banks, and Stetson Bank, which collectively comprise a National Marine Sanctuary. Other banks receive less stringent protection, such as Sonnier Bank – a Habitat Area of Particular Concern (HAPC) protected from anchoring and use of bottom impacting fishing gear, and related fishing activities (Chandler and Gillelan 2005). In the 2012 Sanctuary Expansion Action Plan, the National Oceanic and Atmospheric Administration (NOAA) recommended Sonnier Bank to be included in the boundary expansion of the Flower Garden Banks National Marine Sanctuary. This would upgrade the level of protection for this bank within the next few years (Schmahl 2012).

The majority of the northwestern Gulf of Mexico is characterized by low-relief bathymetry covered in soft sediments. Scattered throughout the area are naturally occurring high-relief structures, called banks (Rezak et al. 1985). These banks have resulted from uplift during geological processes, revealing hard substrate composed of siltstone, claystone, or igneous bedrock. This hard substrate provides a habitat for hermatypic corals, gorgonians, sponges, and other sessile invertebrates. The Flower Garden Banks (FGB) are situated at the edge of the continental shelf, and due to stable tropical temperatures and clear water this area harbors a diverse coral reef ecosystem. Reefs here occur at depths of 15 to 52 m (though deeper corals also occur at depths of up to 85 m). About one-third of the western Atlantic reef-building coral species are found at FGB, along with several hundred other invertebrates and fishes (Rezak et al. 1985). The Flower Garden Banks contain the northernmost tropical reefs on the North American continental shelf, and the closest coral reefs are approximately 400 miles away, off the coast of Tampico, Mexico (NOAA 2009b).

Compared to other reefs in adjacent regions, such as the Caribbean, these reefs are poorly understood. The level of connection between these reefs and surrounding areas such as those of the Caribbean is unclear. The fish population in this northwestern Gulf of Mexico may be continuously replenished from larvae that travel from the other areas, such as the Caribbean, or they may sustain

themselves through self-recruitment. Here, the early life history, population structure, and benthic community associations of two common and widespread coral reef fishes in MPAs of the northwestern Gulf of Mexico (GOM) are examined to help achieve a better understanding of the nursery value of the region. Because the FGB are near the northern limits of reef coral growth in the Gulf of Mexico, they are approximately 600 km from the closest coral reefs in the southwestern Gulf, and the banks in this region lack nearby shallow, vegetated habitat such as seagrasses or mangroves that could act as "nursery areas" or larval settlement areas, the banks examined here may act as nurseries for site attached species, such as those examined here (Pattengill-Semmens et al. 2000).

Banks with a high level of self-recruitment and little connectivity to surrounding regions may require a different type of protection than those that are sustained by more distant supplies of recruits. Despite being true tropical reefs with Caribbean fauna, the banks examined in this study are less speciose than other reefs in the Caribbean. The fact that many of the species present in the Gulf of Mexico are also found elsewhere in the Caribbean indicates there is likely a certain level of connection between the regions, however the differences in fish species diversity and density as well as some endemic species imply a certain level of isolation in the Gulf Banks.

Despite being relatively close to the EFGB and WFGB and similar in minimum depths, Sonnier and Stetson Banks are mid-shelf banks with slightly cooler winter temperatures and higher turbidity that prevents significant growth of reef-building corals. Benthic species composition at these mid-shelf banks is mainly comprised of sponges and patchy colonies of *Millepora* and *Pavona* (NOAA 2009b).

Figure 1. Map of study area

Locations of naturally occurring hard bottom habitats in the northwestern Gulf of Mexico mapped on a mosaic of satellite imagery and ocean bathymetry. Sampling sites are indicated by white markers and other surrounding banks are indicated by grey markers. The Flower Gardens Marine Sanctuary is composed of two outer-shelf reefs (East and West Flower Garden Banks) and one mid-shelf reef (Stetson Bank). Sonnier Bank is a habitat area of particular concern (HAPC), and is also a mid-shelf reef. The outer-shelf banks are composed of reef-building corals, while the mid-shelf regions are dominated by sponges and encrusting corals.

At both the outer-shelf and mid-shelf banks, there are dense fish communities composed of predatory fishes of significant economic value and diverse assemblage of small forage (prey) fishes (Rooker et al. 1997, Kraus et al. 2007). Nearly all of these species have a pelagic larval stage that provides a mechanism for long-distance dispersal relative to adults that exhibit site fidelity and more limited movements. After settlement, some of these species may

remain on a single coral head for their entire lives. The pelagic larvae are somewhat passive in the water column; larvae can effectively control dispersal distance through vertical migration, but cannot readily “swim” horizontally to a new location. Therefore, currents of the area play an important role in the recruitment of new individuals to the region.

The long-term net direction of currents on the continental shelf in the Northwestern Gulf of Mexico results in a slow clockwise mixing of waters around the shores of Texas and Louisiana. In addition, the Loop Current travels northward from the Straits of Yucatan into the Gulf of Mexico then loops westward, eventually exiting at the Straits of Florida where it meanders northward and is called the Gulf Stream (Sheinbaum 2002). The Loop Current is present in the Gulf of Mexico about 95% of the time, and it sporadically sheds a clockwise rotating ring of warm water that separates from the main current (Figure 2). This ring, or eddy, slowly and erratically drifts to the southwest towards Texas and Mexico. No preferred paths have been shown for these eddies (Hamilton et al. 1999). Studies have also shown that there is no real periodicity to the formation of these eddies, but they tend to develop 6 or 11 months apart (Sturges and Leben 2000), Eddies in the Gulf usually last from a few months to a year, generally until they re-enter the Gulf Stream. These eddies are large and deep, extending over as much as 100-200km (diameter) (Brown et

al. 1989). As these eddies travel through the Gulf, they exchange heat, water, energy, nutrients and organisms with the surrounding areas (Brown et al. 1989). They can carry with them animal larvae, plant spores, and other floating matter from the Caribbean. These eddies are potentially very important in linking the Gulf of Mexico to the Caribbean. Particles from the Caribbean can be transported and deposited in the northwestern end of the Gulf of Mexico, and potentially represent a significant source of recruits to banks of the northwestern Gulf of Mexico. This creates an important dispersal linkage between the northwestern Gulf of Mexico and the Caribbean, and physical transport models support this long-distance invasion hypothesis (Lugo-Fernández et al. 2001). While this mechanism may explain how the Caribbean corals and fish species originally became established, the shorter-term mechanisms of self-recruitment and local population dynamics in this area are poorly understood. Further, larvae that begin metamorphosis in the water column, must find suitable habitat for settlement (Sale 1991), which is only available in scattered locations represented by petroleum platforms and naturally occurring banks. This situation represents another barrier to long-distance recruitment to the northwestern Gulf of Mexico, especially for species with short pelagic larval durations.

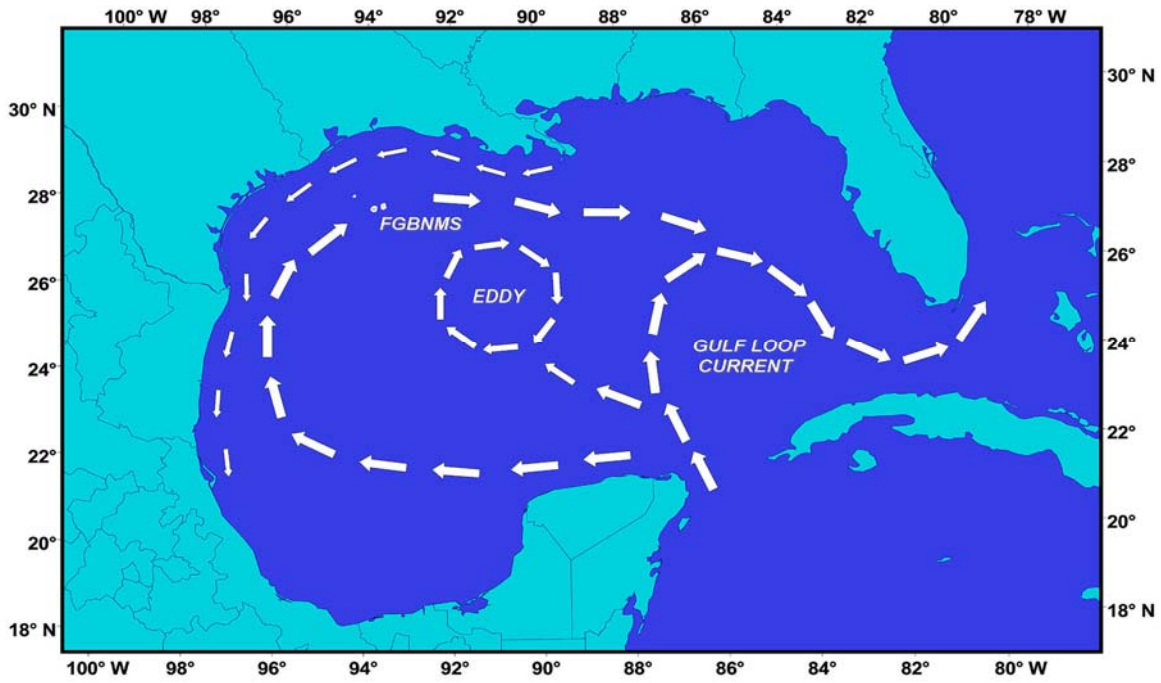

Figure 2. Overarching currents in the Gulf of Mexico.
Courtesy of NOAA. The main currents in the GOM slowly mix in a clockwise direction from Texas to Louisiana. The Gulf Loop enters from the Yucatan, loops around and exits below Florida and becomes the Gulf Stream as it travels northward. Periodically a clockwise rotating ring of warm water, an eddy, separates from the Loop Current and travels erratically to the southwest.

Reef fish reproduction is generally characterized by high risk and almost total mortality of propagules. Spawning adults, who produce large numbers of zygotes, investing little in each, generally compensate for these losses and often species will have repeated spawning episodes. This combination of high fecundity and varying larval success can result in an ever-changing variety of larvae in the replenishment of new generations. This, therefore impacts the population dynamics and genetic structure of reef communities (Doherty and

Fowler 1994). Many adult reef fish inhabit a more or less fixed area on a reef, they are “site-attached”. To arrive at thier locations, the pelagic larvae released from eggs rely heavily on currents and other environmental factors in the region as well as ontogenetic vertical migration and mortality (Paris et al. 2006). Once the larvae reach a suitable area on a reef, they must undergo a metamorphosis from larvae into juveniles. The individuals that survive the highly vulnerable larval stage and metamorphosis into juveniles are termed “recruits” that have settled on the reef, a process is termed “recruitment” (Sale 1991). Previous studies have shown that suitable living space is the limiting resource in most reef communities, and this suitable space is spatially and temporally unpredictable (Sale 1977). Recruitment limitation has also been identified as a model for structuring reef communities. Essentially, the number of recruits determines the local species composition and relative abundances in a particular location (Sale 1991).

It is important to note that overfishing and other human impacts can have profound effects on community structure. Loss of keystone species and overfishing of predators may change the rank order of fish species abundances, and may result in impairment of the potentially important ecosystem functions provided by these predators (Sale 1991, Roberts 1995). Because all of the areas in this study are protected to some degree, human impacts may be relatively minimal, except on species that travel beyond MPA boundaries. However

petroleum production activities happen around and within MPA boundaries, also longline fishing gear is used in the vicinity of the banks and along the entire continental shelf since the late 1800s. Commercial snapper and grouper fishing occurs along the continental shelf edge. All of these could potentially impact the FGB region (Schmahl 2002).

A better understanding of the recruitment structure and metapopulation dynamics can help to evaluate the nursery value of these banks and the other similar banks in this region where there is not similar protection. Shallow reef areas in other regions serve as nursery habitats, they contain high densities of juvenile fish, this is likely due to their structural complexity which provides a hiding place against predators, and because they are often located at a distance from rest of the deeper coral reef and are therefore less frequented by predators. Corals may provide an ideal hiding space and can house relatively high densities of juvenile fish. The shallow reefs in the FGB are also away from the deeper coral reefs and their predators, which may be another reason these are potential nursery areas for juvenile reef fish (Nagelkerken et al. 2000, Hickerson and Schmahl 2005).

Here the early life history, habitat associations, and population genetics of two common reef fishes, bluehead wrasse (*Thalassoma bifasciatum*) and bicolor damselfish (*Stegastes partitus*), in the northwestern Gulf of Mexico are examined. These model species have contrasting life histories and are ideal for

understanding the function of MPAs in the region and the measures needed to protect these marginal northern coral reef habitats. A better understanding of this region may be useful in other nursery areas that may function to replenish fish to surrounding banks.

Both the bluehead wrasse and bicolor damselfish are abundant coral reef fish species. In the Gulf of Mexico both species are among the most common reef fish (Humann and Deloach 2002, Lang 2003, NOAA 2009a). In addition, recent visual surveys of the region show Pomacentridae (damselfishes) and Labridae (wrasses) accounted for 89 – 94% of the total fish composition at each bank (Rooker et al. 1997). Bluehead wrasse were the most abundant in the FGB, and bicolor damselfish were the sixth most abundant species (Rooker et al. 1997). Both of these species exhibit a pelagic larval stage, and, after settlement, are highly attached to particular sites on a reef. Bluehead wrasse have pelagic eggs and are aggregate spawners that spawn year-round. They often migrate midday to down-current reef edges for spawning activities. They are also sequential hermaphrodites (protogynous). Females generally spawn once daily, whereas dominant males can spawn up to forty times a day, and smaller males about twice daily (Wilson and Wilson 1992). Bluehead wrasse from the Pacific have a mean pelagic larval duration (PLD) of approximately 49.3 days, s.d. 5.5d (Victor

1986), which is considered a long PLD, with high variance. These PLD characteristics allow for greater dispersal potential.

In contrast with the pelagic spawning of bluehead wrasse, female bicolor damselfish deposit a cluster of adhesive eggs on a hard surface, and the male fertilizes the eggs and guards the nest until the eggs hatch. After typical 4 to 7 day incubation, larvae hatch and leave the nest for a short planktonic life stage (Thresher 1980, Wilson and Wilson 1992). Bicolor damselfish in the Pacific have a mean PLD of approximately 28.8 days, (s.d. 1.1d; (Wellington and Victor 1989), which is considered a short PLD, with small variance. These PLD characteristics are much more limiting for dispersal potential than the bluehead, and therefore may lead to greater local retention of larvae.

Bluehead release eggs directly into the water column during spawning while bicolor retain eggs nearby until the hatch. Because of these differences in life history traits, and differences seen in other populations, a longer PLD is expected for bluehead compared to bicolor. In addition, because of the isolation of the northwestern Gulf of Mexico, reef fish populations may exhibit a longer PLD to recruit from areas farther away. Geographic variation in PLD has been seen in damselfishes and wrasses, with Pacific congers showing distinctly longer larval durations when compared to Caribbean species. However several more recent studies have not show a correlation between range and PLD. There appears to

be a correlation for ocean-wide dispersal and PLD, but at finer scales the relationship is equivocal. Because these larvae are capable of vertical migration, it is still possible for species with a relatively long PLD to be endemic to a small area (Victor and Wellington 2000).

Fish populations that exhibit longer PLDs correlated with less genetic similarity, or isolation-by-distance (IBD), the tendency of populations that are a geographically closer to be more similar than populations that are further apart (Wright 1943), support the idea that those habitats are dependent upon recruits from distant populations. On the other hand, the relative isolation of this area may require a significantly shorter PLD correlated with more genetic differentiation, or higher IBD, for self-recruitment to these habitats. In this case, MPA's in the region could function as self-sustaining independent populations. It is also possible that this region consists of a mixture of both types of populations where the proximity of banks is as much if not more of an influence as resources and other conditions. Because some populations are potentially responsible for the replenishment of larvae to surrounding regions, it is important to establish which, if any, populations are functioning this way, as they would be critical to protect in order to maintain those species in the region (Pulliam 1988, Crowder et al. 2000).

A better understanding of different life histories related to recruitment dynamics and degree of external replenishment of individuals can lead to better resource management and protection of marine fish populations. Having an understanding of the pelagic stage and potential dispersal of larvae in addition to knowledge of settlement/nursery regions can help to establish how fish populate an area. This is important for maintaining existing populations, as well as re-populating areas that have previously been negatively impacted. To evaluate habitat requirements and understand how recruitment influences population dynamics, here the habitat associations, early life history traits for bluehead and bicolor damselfish, as well as genetic markers for bluehead are examined. Genetic markers will provide an understanding of the relative population size in the GOM. Comparisons between bluehead wrasse and bicolor damselfish are expected to reveal differences consistent with a higher level of isolation-by-distance in species with shorter pelagic larval durations such as bicolor damselfish. Finally, the distribution of suitable habitats in the northwestern GOM is heterogeneous and unequally distributed between mid- and outer-shelf banks; therefore, habitat associations and abundance data generated by this study will provide another dimension of insight for understanding recruitment variability. The behavior of pre- and post-settlement fish as well as post-settlement population densities can also be affected by habitat characteristics. The quality and/or number of refuges from predation will vary with types of substrates as well as rugosity of the habitat,

therefore, settling individuals may suffer lower mortality in habitats with more or higher quality shelter (Levin 1991).

To determine potential nursery areas and habitat associations for individuals on the reef, here the densities and habitat characteristics for bluehead wrasse and bicolor damselfish in MPAs of the northwestern Gulf of Mexico are described. In addition, information on the early life history characteristics (i.e., hatch date distribution, pelagic larval duration, larval and post-settlement growth) of these two reef fishes are described to determine probably dynamics of recruitment. Finally, to determine the relative sizes of bluehead wrasse populations in the Gulf of Mexico allelic variance is quantified.

CHAPTER 2

Methods

Study Area

Visual and photo transects were conducted and fish samples were collected at East and West Flower Garden Banks, Stetson Bank, and Sonnier Bank in the Northwest Gulf of Mexico (Figure 1). These areas are likely critical habitats for corals and reef fishes requiring hard-bottom substrate. Unfortunately, the resident fish assemblages associated with many of these banks have not been thoroughly studied. A better understanding of the recruitment structure and metapopulation dynamics can help to evaluate the nursery value of this region (Rooker et al. 1997). Sites were selected based on access via boat and generally the same sites were visited monthly based on dive conditions and other safety factors. Sites were visited in May to September, spawning season for many fish, 2009 with 75 dives at EFGB, 72 at WFGB, 43 at Sonnier Bank, and 61 dives at Stetson Bank. Surveys were conducted approximately monthly, 5/26-5/28, 6/21-6/23, 7/22-7/23, 8/16-8/18, and 9/14-9/16, dependent on weather conditions and

vessel availability. Local conditions and safe diving considerations also limited the number of transect surveys accomplished. At each site visit a target of three transects in two depth zones (~20m and ~30m) were attempted at each of 2 sites per bank, per month between May 2009 and September 2009.

SCUBA surveys

Visual surveys are a common non-invasive method for measuring fish communities and habitat characteristics in clear water (Sale and Sharp 1983). Conventional survey gear, such as trawls, cannot be used because it would modify and damage to the habitat. Although there are many variations, this study utilized a line transect approach. Because site-attached species, which have very small home ranges, 80-275 m^2 for bluehead wrasse (Tecumseh et al. 1990) and 44-57 m^2 for bicolor damselfish (Knapp and Warner 1991), were the focus of transect counts, line transects were preferred over point counts. Previous studies have shown line transects to give higher estimates of sedentary species, with lower variation than point counts. This, therefore, makes line transects a more suitable method for assessing abundance of site-attached species such as reef fish (Buxton and Smale 1989, Pyle 2007).

To quantify fish population density, substrate composition, and regional rugosity, transect surveys were performed by a two or three person SCUBA team, by placing a 5m tape measure on top of the reef in a straight line. Dives were

conducted using nitrox (oxygen enriched air) to maximize depth and bottom time. The first diver took a visual census by identifying and counting all site-attached species, within one meter on either side of the measuring tape. Since small site-attached fishes were the focus of this study, it is important to note that the tape measure had little effect on the distribution of fishes along the transect. Fish would hide when first approached with the measuring tape, but quickly resumed normal activity (within 3 to 5 minutes) of foraging and defending territories during the count. After this adjustment period, transects were initiated and took about 7 to 10 minutes to complete. These A total of 216 transect counts were conducted.

The second diver followed behind the first to photographically record benthic habitat. This diver placed a 0.5 m x 0.5 m square frame (made from PVC pipe) on the substrate, which was used to scale photographs during digital processing. Two pictures were taken at each meter, for a total of ten pictures per transect. A subset of 5 randomly selected photos (n=1075 total) from each transect were analyzed. In some cases photos were blurry or unusable and these were excluded from the analysis.

Finally, to index complexity of the habitat, a measure of rugosity was recorded by the first (or third) diver placing a 3 meter long weighted chain along the substrate for three 1m intervals along the tape measure. Rugosity was measured as the contour distance of the substrate divided by the straight-line distance between

the ends of the chain. This type of rugosity index has been widely applied, and provides an efficient way to compare this aspect of habitat between sites (Chandler et al. 1985, McClanahan and Shafir 1990, Rooker et al. 1997).

Image analysis software (ImageJ) was used to estimate the percent composition of major sessile benthic species (corals, sponges, algae, etc.) within each transect from the photographs. The distance scale was set according to the 0.5m quadrat used in each photo, and then a measure of the total area in the photograph was taken. Next, polygons were drawn around each coral species and area was recorded along with the corresponding species name. Once all identifiable areas were measured, then the sum of the areas of the species identified in the photo was subtracted from the total area to get the amount of the photo that was “unidentifiable”. Unidentifiable was defined as areas where photos were too blurry, not enough light was available to clearly see the region, or the area was too deep to identify the species (relative to the majority of the photo).

Population density (number of individuals per transect) of bluehead wrasse and bicolor damselfish from the surveys were examined with analysis of variance (ANOVA) for monthly and regional spatial differences (between banks). In addition, multiple regression analysis was used to examine relationships between bluehead wrasse or bicolor damselfish population density and the percent

coverage of benthic organisms. This was accomplished using regression model selection of main effects with Mallow's C_p (Mallows 1973).

Early Life History

Collections of both species, bicolor damselfish and bluehead wrasse, were made during two dive trips, one in May 2009 and the other in September 2009. Fish were collected using dip nets, micro-spears, and slurp guns. Samples were stored on dry ice in the field and kept frozen until they were processed in the lab. Juvenile fish (< 1-year old) were identified by color pattern and size and targeted for collection. A total of 67 bluehead wrasse and 44 bicolor damselfish were collected (n=111) for otolith analysis. A fin clip from each fish was placed in lysis buffer for subsequent genetic analysis. Otoliths were extracted using a stereomicroscope. Extracted otoliths were cleaned to remove any attached tissue, and saved in sample tubes.

Important early life history traits for reef fishes include hatch date, pelagic larval duration, and growth rate. All of these variables can be quantified using otoliths. Otoliths are "ear stones" in fish. They are calcium carbonate structures found in the inner ears of fish that aid in balance and orientation and have incremental growth. Fish have three pairs of otoliths; the sagittae are the largest and are most often used for analysis. A cross section of an otolith reveals growth rings, similar to a tree trunk. Previous papers have validated that these growth rings form with

daily periodicity in the otoliths of bluehead wrasse and bicolor damselfish (Victor 1982, Robertson et al. 1988), and so these growth rings can be counted to estimate age, hatch date, and growth rate for individuals.

Otoliths were mounted to petrographic slides using thermoplastic glue. Otolith sections were polished to a smooth appearance at 450x magnification by hand using 400 and 600 grit abrasive paper and alumnia paste on a felt pad. Polishing was conducted gradually in the saggital plane to reveal the core of the otolith and a visible sequence of rings extending to the edge. Pictures were taken with a microscope camera on a compound microscope. The image scale was calibrated with an optical micrometer. Increments were counted from photographs and increment width is measured using ImageJ, image processing software from NIH (Schneider et al. 2012).

To determine pelagic larval duration and growth rates specific to larval and post-settlement phases, it is necessary to determine when settlement occurred in the otolith chronology. The settlement mark (or check) is described as a change in increment width, with post-settlement increments being generally thinner than nearby pre-settlement marks. According to Wilson and McCormick, bicolor damselfish exhibit an “abrupt settlement mark” which is easily identifiable, with a sharp decrease in increment width across the settlement check. However, bluehead wrasse exhibit a “zonal settlement mark”. This is not as evident as the

abrupt settlement mark of damselfish, and it also shows a larger transition zone (Wilson and McCormick 1999).

A preliminary analysis on several species of damselfish was performed to establish an objective method to analyze the settlement check. The pre-settlement increments were on average about twice the length of the post-settlement increments (Figure 3). Increment widths (microns) in the otoliths of 4 reef fishes, purple reeffish (*Chromis scotti*), cocoa damselfish (*Stegastes variabilis)*, dusky damselfish (*Stegastes adustus)*, and dusky damselfish (*Stegastes adustus)*, are shown along the Y-axis while data are ordered along the horizontal axis by cumulative increment count from the core. This method was used to identify settlement marks and estimate PLD. Finally, using age and standard length at capure, size-at-age curves were created to estimate size-at-settlement.

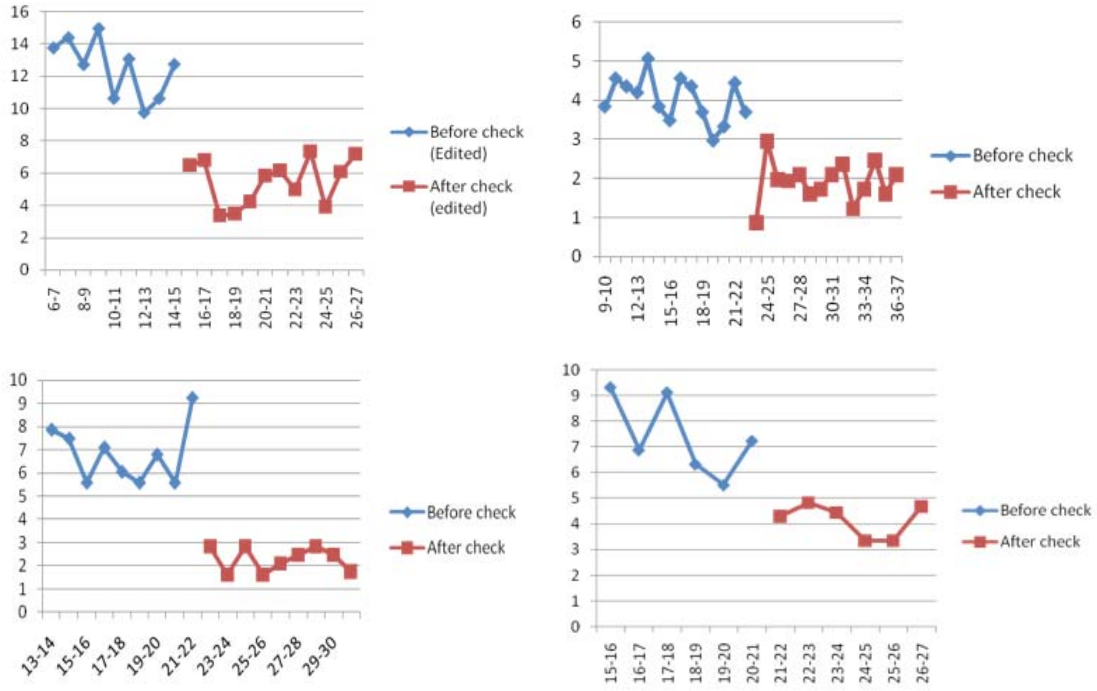

Figure 3. Preliminary otolith increment width data
For all figures, blue (diamonds) is before check and red (squares) is after check increment length. The y-axis is increment width (microns) and the x-axis is daily increment count from the core.

Increment width was graphed to determine the location of the settlement check (Figure 3). The PLD (pelagic larval duration) and total age of each fish was determined by counting increments. Hatch date was calculated by subtracting daily age from the date of capture. Standard length at capture date was plotted against age at capture. With this information hatch date and length-at-age was

estimated using back calculations. Hatch date was determined by subtracting age at capture from capture date and length-at-settlement was determined using the equation for the best fit line of the length at capture date against age at capture data.

Genetic Analysis

Genomic DNA was extracted from fin clips preserved in lysis buffer (pH 8.02) (Longmire et al. 1997) and purified using the Qiagen DNeasy Tissue DNA extraction kit. First, 200μL of digested tissue and lysis buffer were added to buffer solution and proteinase K was added. Because samples were already lysed in buffer solution, samples were kept in the proteinase K solution for one hour, incubated at 55°C. DNA samples were cleaned using 90-100% ethanol and re-suspended in buffer solution. The tissue extraction procedure recommends two elution steps, however, because the amount of tissue relative to the amount of initial lysis buffer solution was small, the samples were only eluted once.

DNA extractions were confirmed through gel electrophoresis and polymerase chain reactions (PCR) using universal 12s and rat 18s primers to ensure the presence of DNA. Three microsatellite primers for *Thalassoma bifasciatum* (Wooninck et al. 1998, Williams et al. 2004) were used for preliminary analysis of heterozygosity. The primers selected for use are listed in Table 1.

Table 1. Primers used for Microsatellite Analysis
with primer specific annealing temperatures listed.

Primer Name	Sequence	T_A
FAM-T3235mod+	AAGCCATGTAGACCAAATATGA	50
T3235mod+-R	AAAGCTCCAACATTAGAACAGA	
FAM-T3333	AGCTGTGGCAGATGGTCATGC	55
T3333-R	GGTGTTTGATTGAGAGATGGTCA	
FAM-TbAAT4	AAGGCATGTCTGTGATTAGTATTA	50
TbAAT4-R	GCAGGATAAAGCAAATAGCA	

DNA was amplified using PCR with 0.5 U *Taq*-gold, the conditions were as follows: initial denature at 95°C for 11 minutes, 40 cycles denaturing at 95°C for 30 seconds, locus-specific annealing temperature (T_A above) for 30 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 30 minutes (Williams et al. 2004, Salas et al. 2010). Fragments were separated on and sizes determined using an ABI 3130xl genetic analyzer and scored using GENEMAPPER software followed by analysis with Structure 2.3 (Pritchard et al. 2000).

According to Longmire et al. (1997) samples can be stored at ambient temperatures, but should be protected from extreme heat. In the case of tissues, no more than 0.3-0.5 grams are added to 5 ml of lysis buffer. Despite not exceeding this amount of tissue, PCR reactions yielded weak results making analysis difficult, for this reason only bluehead wrasse, not bicolor damselfish,

were analyzed here. Samples were stored at ambient temperatures prior to DNA extractions. For a single population, as few as 25-30 individuals can yield meaningful microsatellite data (Hale et al. 2012) and a minimum of 25 individuals were attempted in this analysis, however due to weak PCR products, only n = 69 individuals produced useable results (EFGB = 23, Sonnier = 11, WFGB = 34, Positive = 1, Stetson excluded because no fish were obtained).

Microsatellites are a reliable method for detecting weaker genetic changes than can be seen with mtDNA or alloenzymes, which have previously been used to examine population structure. Microsatellites are 2-6 base repeats in the variable non-coding region of DNA. Primers for bluehead and bicolor damselfish have been established in previous studies (Wooninck et al. 1998, Parker et al. 1998, Williams et al. 2003, 2004, Thiessen and Heath 2007, Purcell et al. 2009). It has been suggested that to produce meaningful results, a minimum of five microsatellite loci should be used (O'Connell and Wright 1997). Here, three loci for bluehead wrasse are examined for preliminary analysis and compared the abundances to previously established abundances of these markers from the Caribbean. These three microsatellite loci are highly abundant and dispersed throughout the genome.

Because microsatellite loci are among the fastest evolving genetic markers available, are highly polymorphic, and are generally believed to be selectively

neutral, they should be ideal markers for analysis of marine populations (Bagley et al. 1999). Microsatellite analysis was employed here to estimate the effective population size of bluehead wrasse in the northwest Gulf of Mexico. First, bank-scale population size of bluehead wrasse was examined to determine whether there is significant gene flow between banks. Then, overall microsatellite data was compared against similar data from the Caribbean to determine the level of connection between the two regions. Puebla et al. (2012) collected samples along the Mesoamerican Barrier Reef in Belize in June-July 2010. Puebla et al. (2012) analyzed 81 individuals and 11 primer sets: TbAAT18, TbAAT4, TbAAT41, TbAAT49, T0101, T3333, TbAAC34, TbAAT42, T0328, T3231, and TbAAC50.

The variance in microsatellites arises from slippage during DNA replication because of DNA polymerase. Similarly, because DNA polymerase (*Taq* polymerase) is used in PCR to amplify DNA, some slippage may occur and result in “stutter” bands, which may be seen when analyzing results. Because of this, data were corrected, using the method created by LeDuc et al. (1995), to account for this stutter (LeDuc et al. 1995). Allelic variance was compared to previously published values for bluehead and bicolor in the Caribbean. This was used to estimate the current effective population size, which represents a level of connection between the GOM and Caribbean. Similar allelic variance would

imply a larger population, and therefore a greater connection between the GOM and Caribbean. A significant difference in allelic variance would imply a smaller population, and therefore greater isolation of the GOM from the Caribbean.

Allelic variance was evaluated using an F_{ST} and isolation-by-distance (IBD) test (Selkoe and Toonen 2011). Analysis was performed using GeneMapper to identify alleles fragment lengths, Structure 2.3.4 (Pritchard et al. 2000) to investigate effective population sizes, as well as IBD software (Bohonak 2002) which has been suggested to more effectively measure population size. Initially, only samples from the Flower Garden Banks were analyzed, allele data from 3 primer sets (T3235mod+, T3333, TbAAT4) and 69 individuals was utilized in the preliminary analysis.

Using Structure software it was possible to analyze the populations regardless of low numbers. Structure software is a model-based clustering method for using multilocus genotype data to infer population structure and assign individuals to populations. In the model there are *K* clusters (where *K* may be unknown), each of which is characterized by a set of allele frequencies at each locus. Individuals in the sample are assigned (probabilistically) to populations, or jointly to two or more populations if their genotypes indicate that they are admixed. The method can produce accurate assignments using few loci (Pritchard et al. 2000).

As outlined by (Evanno et al. 2005), four steps for the graphical method allowing detection of the true number of groups K were used. When K is approaching a true value, L(K) plateaus (or continues increasing slightly) and has high variance between runs. First, mean L(K) over the total number of runs for each K value was determined. Then, the rate of change of the likelihood distribution (mean) calculated as L'(K) = L(K) – L(K – 1) was determined. Next, absolute values of the second order rate of change of the likelihood distribution (mean) calculated according to the formula: |L"(K)| = |L'(K + 1) – L'(K)|. Finally, ΔK was calculated as $\Delta K = m|L''(K)|/s[L(K)]$. The modal value of this distribution is the true K or the uppermost level of structure, in this current case, two clusters.

Recently F_{ST} has been challenged as an effective way to measure marine connectivity (Selkoe and Toonen 2011). Instead, isolation by distance analysis (IBD) may be a better measure of connectivity in marine populations. Another program, IBD (Isolation By Distance), was utilized for FGB data to perform this analysis (Bohonak 2002). This program tests significance by asking whether the pairwise genetic distance matrix is correlated with the pairwise geographic distance matrix using a Mantel test.

IBD software compares genetic distance, with geographic distance and provides Slatkin's (1993) similarity measure $M\hat{} = (1/ F_{ST} - 1)/4$ for each population. Studies of isolation by distance typically seek to ascertain (1) whether there is a

statistically significant relationship between genetic distance (or similarity) and geographic distance; and (2) the strength of this relationship. Significance is usually assessed by asking whether the pairwise genetic distance matrix is correlated with the pairwise geographic distance matrix using a Mantel test. The Mantel test tests the correlation between two distance matrices. It is non-parametric and computes the significance of the correlation through permutations of the rows and columns of one of the input distance matrices. The test statistic is the Pearson product-moment correlation coefficient *r*. *r* falls in the range of -1 to +1, where being close to -1 indicates strong negative correlation and +1 indicates strong positive correlation. An *r* value of 0 indicates no correlation. In his 1993 paper, Slatkin suggests that there is no way to measure significance of M, therefore IBD software calculates a p-value in an attempt to measure significance for the null hypothesis $r \geq 0$ and $r \leq 0$ which can be compared to the correlation results.

CHAPTER 3

Results

SCUBA surveys

Bluehead wrasse were abundant, 52% of all fish species, with 25% adults and 26% juveniles overall (Figure 4). The total number of bluehead was n = 278 at West Flower Garden Banks (26.8% of the bank total), n = 284 (34.9%) at East Flower Garden Banks, n = 88 (24.1%) at Sonnier, and n = 102 (9.7%) at Stetson Bank (Figure 5). Bicolor damselfish were less abundant, but still somewhat common at all banks, 7% of the total fish count overall, with 4% overall each of juveniles and adults (Figure 4). The number of bicolor damselfish was n = 16 at West Flower Garden Banks (1.5%), n = 26 (3.2%) at East Flower Garden Banks, n = 10 (2.7%) at Sonnier, and n = 24 (2.3%) at Stetson (Figure 5).

Observed density remained stable across months for both species (ANOVA: bicolor damselfish: $F_{4, 181} = 1.49$, $p = 0.2084$; bicolor damselfish: $F_{4, 275} = 1.81$, $p = 0.1263$) (Figure 6 & Figure 7). Average number of bicolor damselfish per

transect was relatively constant per month, about 2 individuals per transect (Figure 6). Average number of bluehead wrasse fluctuated slightly month-to-month, with the largest average number of individuals per transect seen in the month of August, this month also had the largest standard deviation (Figure 7).

ANOVA analysis showed there was a significant difference in bicolor damselfish population density between banks in the Gulf of Mexico ($F_{3, 182} = 5.06$, $p = 0.0022$) (Figure 8). There was not, however, a significant difference of density of bluehead wrasse between banks ($F_{3, 276} = 2.56$, $p = 0.0551$). Overall very few bicolor damselfish were seen per transect, and Sonnier Banks had the largest average number of individuals per transect (Figure 8). Most banks had an average of 10 bluehead wrasse per transect, however Stetson Banks showed almost 14 individuals per transect and a large standard deviation (Figure 9).

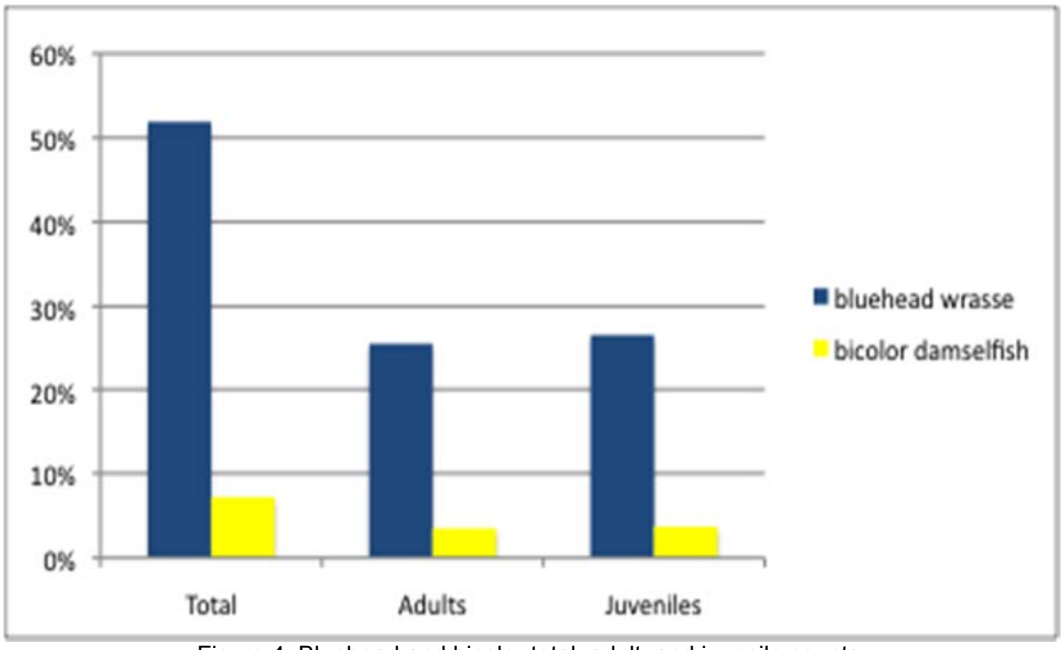

Figure 4. Bluehead and bicolor total, adult, and juvenile counts.

Percent abundance of all bluehead wrasse and bicolor damselfish, adult percent abundance, and juvenile juvenile percent abundance in the northwestern Gulf of Mexico.

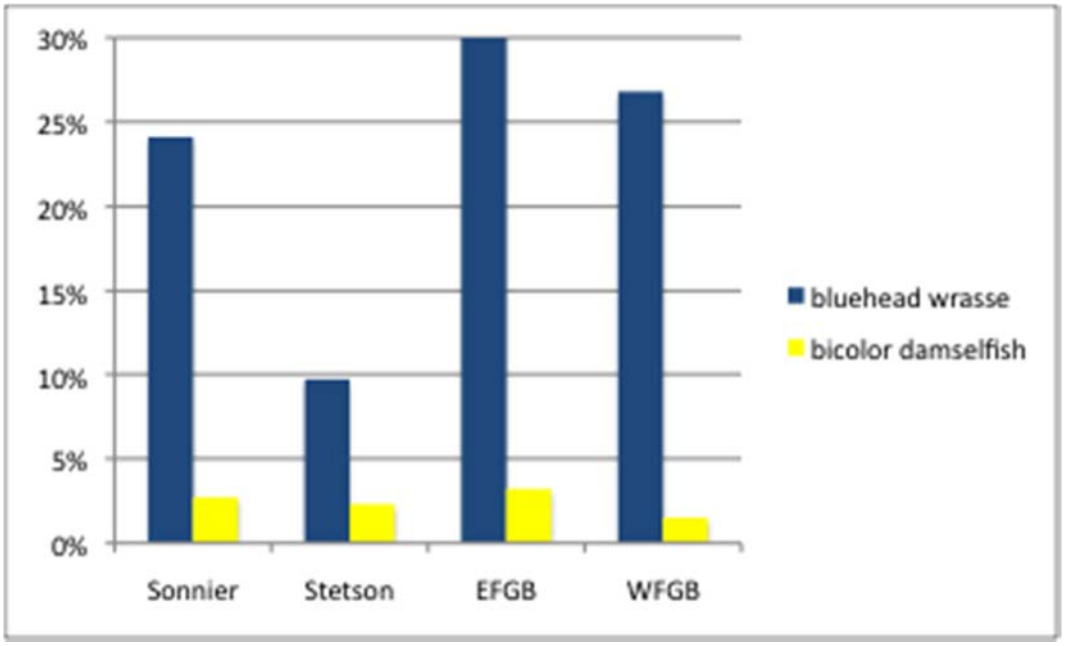

Figure 5. Counts of bluhead wrasse and bicolor damselfish at each bank in the northwestern Gulf of Mexico.

Percent abundance for bluhead wrasse and bicolor damselfish at each bank in the northwestern Gulf of Mexico.

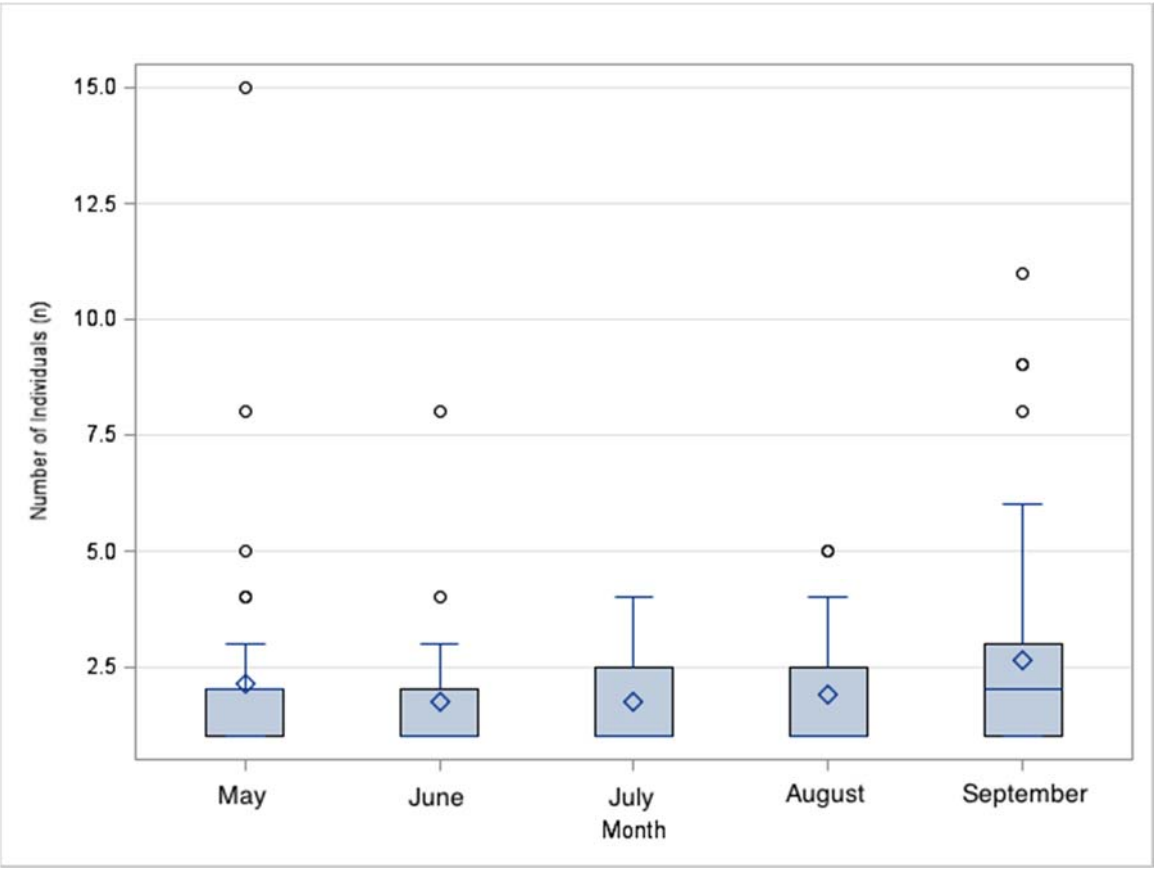

Figure 6. Bicolor damselfish density by month.

The box represents the 25th to 75th percentiles of population density and the median is shown as a line through the box. The diamond represents the mean, and the top of the whiskers represents the max number of individuals in the intra-quartile range (IQR). Circles represent outliers.

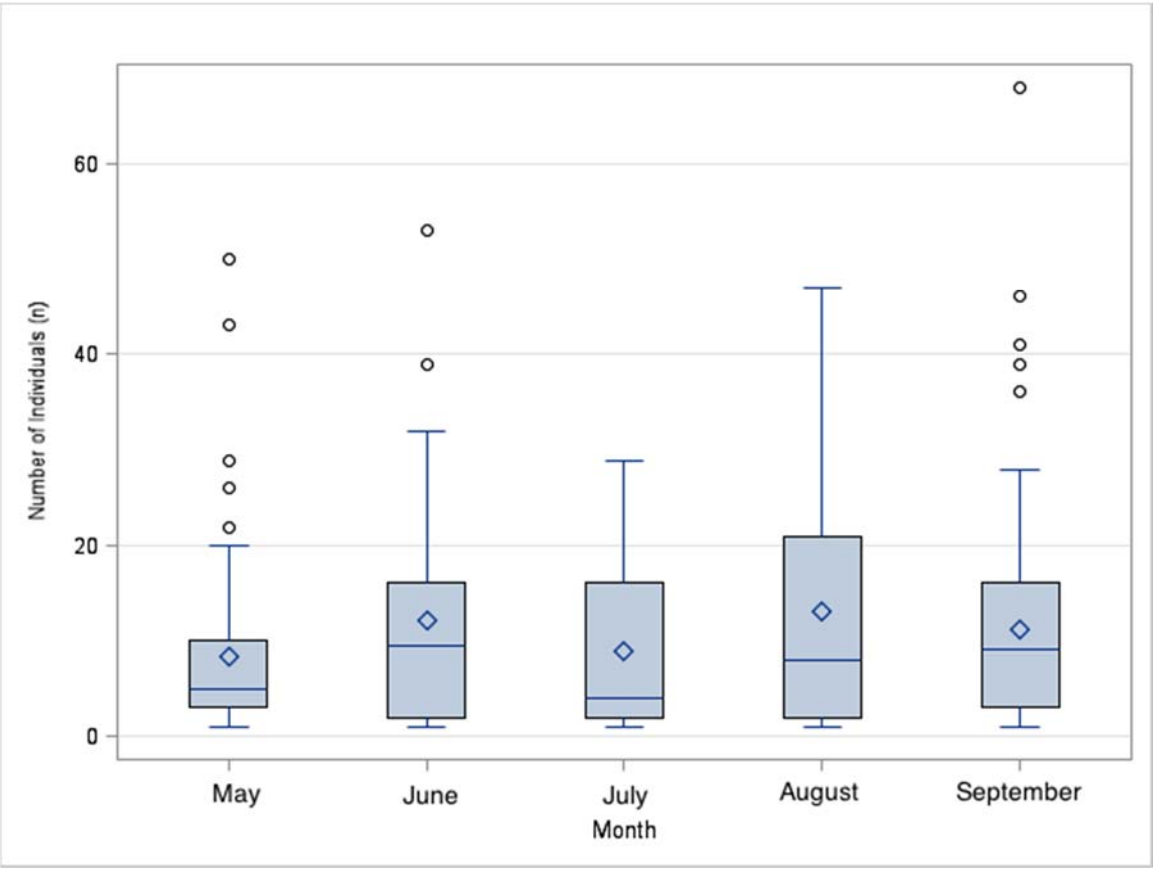

Figure 7. Bluehead wrasse density by month.

The box represents the 25th to 75th percentiles of population density and the median is shown as a line through the box. The diamond represents the mean, and the bottom and top of the whiskers represent the minimum and maximum number of individuals in the IQR, respectively. Circles represent outliers.

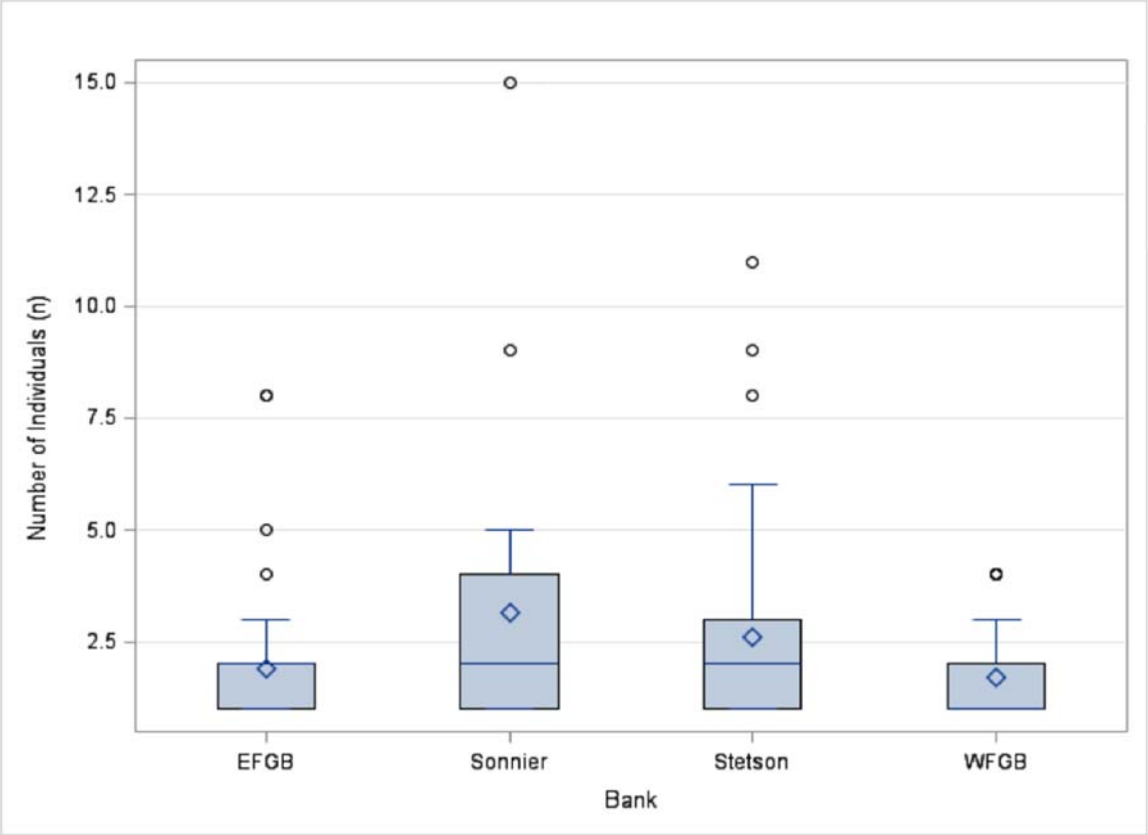

Figure 8. Bicolor damselfish density by bank.

The box represents the 25th to 75th percentiles of population density and the median is shown as a line through the box. The diamond represents the mean, and the top of the whiskers represents the max number of individuals in the IQR. Circles represent outliers.

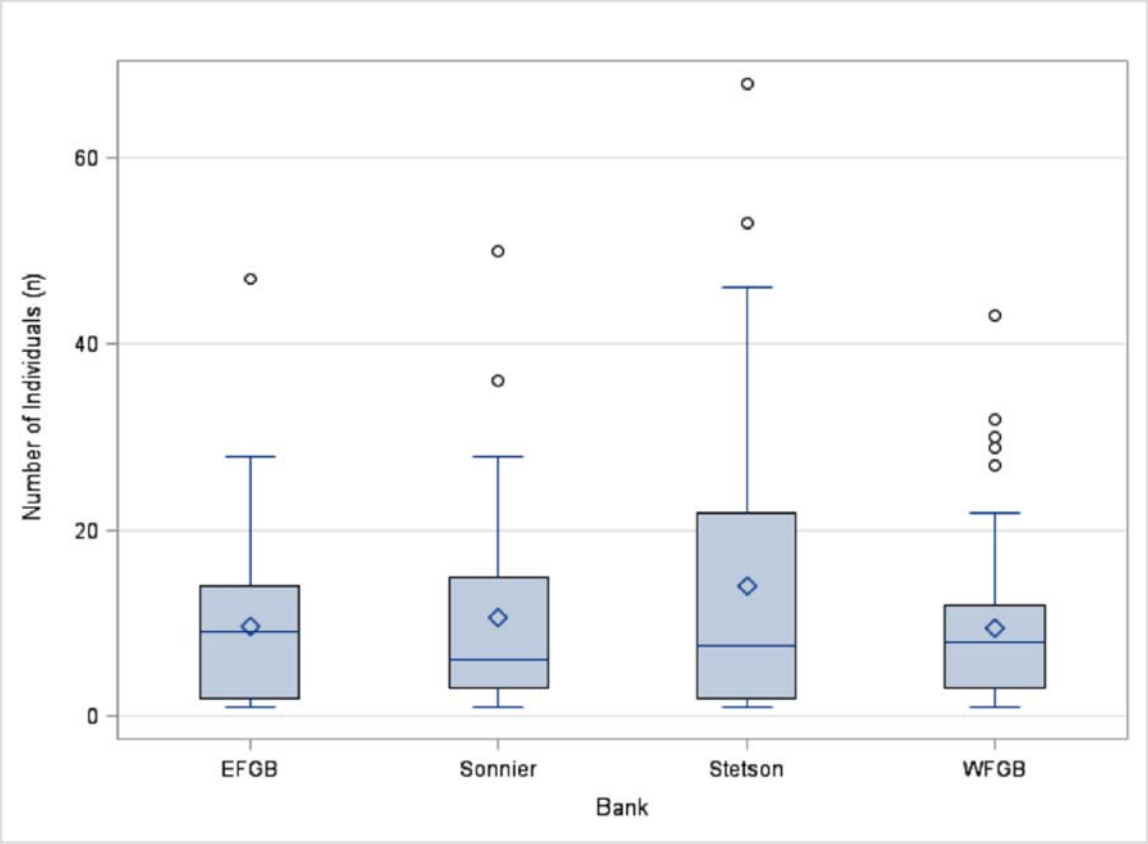

Figure 9. Bluehead wrasse density by bank.

The box represents the 25th to 75th percentiles of population density and the median is shown as a line through the box. The diamond represents the mean, and the bottom and top of the whiskers represent the minimum and maximum number of individuals in the IQR, respectively. Circles represent outliers.

Habitat Preference

Because the number of dives at each location was limited by logistics, the number of benthic photos used to characterize habitat varied at each location: 260 photos were analyzed from Stetson Bank, from 52 transects; 200 photos from Sonnier Bank were analyzed from 40 transects; 315 photos from 63 transects were analyzed from East Flower Garden Banks; and, 300 photos from 61 transects were analyzed from West Flower Garden Banks.

East and West Flower Garden Banks were similar in benthic community composition, mostly composed of hermatypic boulder corals (mainly *Montastraea franksi,* 18.4%, *Montastraea faveolata,* 12.2%, *Colpophyllia natans*, 9.0%, *Montastraea cavernosa,* 7.1%) and some less abundant brain and branching corals (*Diplora strigosa*, 2.1%, *Stephanocoenia interseptsa,* 2.1%, and *Montastraea annularis* and *Madracis decatis* 1.2% combined). Other branching and finger corals were present in small fraction of the area (various species 3.1%, see Appendix A) Algae also accounted for a substantial portion of coverage (*Lobophora*, 15.1%, and Y-branching, 10.5%) sponges accounted for very little of the substrate at East and West Flower Garden Banks, about 3%.

By comparison, Stetson and Sonnier Bank were similar to each other and dominated by algaes (Y-branching & *Halimeda*, combined, 23.5%, filamentous, 23.2%, *Lobophoroa*, 3.9%, and crustose coralline, 3.0%). Some branching and

finger corals (mostly *Millepora alcicornis*, 7.2%) as well as bare substrate (primarily limestone (17.4%) and sand (7.8%) also dominated these banks. Sponges were also more prevalent at Stetson and Sonnier as compared to EFGB and WFGB, they accounted for almost 7% of the substrate composition.

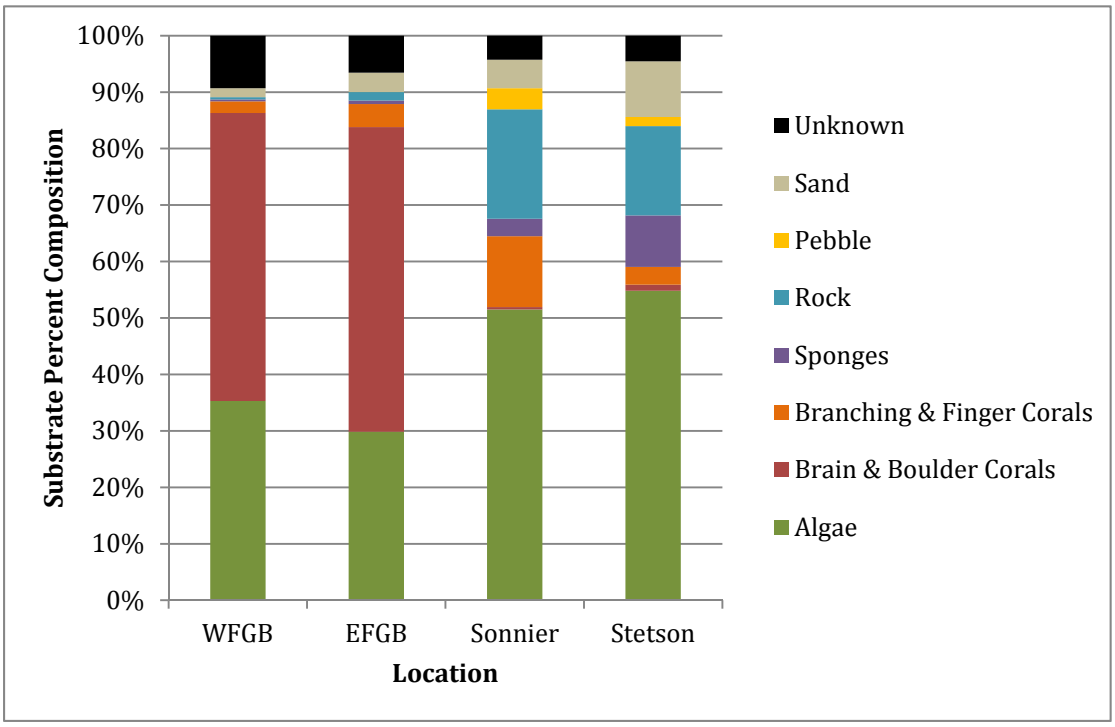

Figure 10. Substrate percent composition
Substrate percent composition for West Flower Garden Banks, East Flower Garden Banks, Sonnier, and Stetson in the Northwest Gulf of Mexico. WFGB and EFGB are mainly composed of brain and boulder corals in addition to algal cover. Stetson and Sonnier and mainly algae covered substrate as well as bare substrates such as sand and rock.

The regression analysis between substrate composition and density of bluehead wrasse and bicolor damselfish showed an overall weak association between fish and substrate. The best-fit models most often selected for bluehead wrasse contained branching corals and rock, though they were rarely significant and generally negative relationships. The best-fit model for bluehead wrasse at EFGB was shown to be branching corals and rock (C_p = -0.1870, R^2 = 0.2957), which showed lower R^2 values than the next closest models (branching corals, rock, & rugosity and branching corals, rock, & sand). At WFGB, branching corals and rock was the best-fit model with bluehead wrasse (C_p = -0.0306, R^2 = 0.1023). Rock and branching corals individually were somewhat similar in results to branching corals & rock combined, but not quite as close (C_p = -0.0012, R^2 = 0.0589; and C_p = 1.0994, R^2 = 0.0353, respectively). Sonnier's best-fit model for bluehead wrasse was rock & sand (C_p = 1.2012, R^2 = 0.2508). However it was very close to the next two best-fit models (rock, sand, & rugosity; C_p = 1.9951, R^2 = 0.2780; and non-branching corals, rock, & sand; C_p = 2.5157, R^2 = 0.2663). Branching corals was the best-fit model at Stetson for bluehead wrasse (C_p = -1.5900, R^2 = 0.0861). Non-branching & branching corals was similar (C_p = -1.5776, R^2 = 0.1322). However the next two models were not nearly as close as the first two (non-branching corals; C_p = -0.2201, R^2 = 0.0544; and branching corals & rock; C_p = 0.0009, R^2 = 0.0956).

After selecting the branching corals & rock model as the best-fit for bluehead wrasse at EFGB, an ANOVA was run to see if the relationship was significant. There was an inverse relationship between branching corals and rock presence and density of bluehead wrasse at EFGB ($F_{2, 60} = 6.95$, $p = 0.0019$). There was not a significant relationship between the presence of branching corals and rock on density of bluehead wrasse at WFGB ($F_{2, 57} = 0.06$, $p = 0.9419$). The ANOVA for Sonnier showed that there was a significant negative correlation of rock and sand on density of bluehead wrasse ($F_{2, 37} = 6.52$, $p = 0.0037$). There was also a slightly significant relationship between the presence of branching corals on density of bluehead wrasse ($F_{1, 50} = 4.13$, $p = 0.0475$). The R^2 is marginal, however, and likely does not represent a good correlation between branching corals and density of bluehead wrasse at Stetson bank (Figure 11).

Bicolor damselfish most often showed sand and algae in the selected best-fit models, these were also rarely significant as well as negative. The best-fit model for bicolor damselfish at EFGB was shown to be algae ($C_p = 1.1316$, $R^2 = 0.0804$), which showed lower R^2 values than the next closest models (algae & branching corals and non-branching corals & rock). However, the C_p values for the next closest models (1.2016 and 1.4598, respectively) were not as close to the number of parameters as the model for algae. At WFGB, sand and sponge was the best-fit model with bicolor damselfish ($C_p = 0.0842$, $R^2 = 0.0405$). Sand, sponge & rugosity, and sand were somewhat similar in terms of results to sand &

sponge, but not quite as close (C_p = 0.0515, R^2 = 0.0405; C_p = 0.0.7490, R^2 = 0.0.0689; and C_p = 0.9985, R^2 = 0.0199, respectively). Sonnier's best-fit model for bicolor damselfish was algae, rock, sand, and rugosity at Sonnier (C_p = 3.0666, R^2 = 0.5300). However it was very close to the next two best-fit models (branching coral, rock, sponge, & rugosity; C_p = 3.2173, R^2 = 0.5278; and algae, branching coral, sponge, & rugosity; C_p = 3.3568, R^2 = 0.5257). Finally, rock was the best-fit model at Stetson for bicolor damselfish (C_p = -1.9908, R^2 = 0.1638). Sand was similar (C_p -1.0315, R^2 = 0.1432). However the next two models were not nearly as close as the first two (rock & sand; C_p = -0.7545, R^2 = 0.1801; and rock & rugosity; C_p = -0.0604, R^2 = 0.1652).

There was not a significant relationship between algae presence and density of bicolor damselfish ($F_{1, 61}$ = 3.56, p = 0.0639) (Figure 12). There was also not a significant correlation between the presence of sand and sponge, as well as rugosity on density of bicolor damselfish at WFGB ($F_{2, 57}$ = 0.85, p = 0.4347). The ANOVA for Sonnier showed that there is a significant relationship between presence of algae, rock, and sand, as well as rugosity on density of bicolor damselfish ($F_{4, 33}$ = 9.31, p < 0.0001). There was a positive correlation between rugosity and density, but a negative relationship between algae, rock, and sand density with bicolor density at Sonnier. There was a significant correlation between rock presence and density of bicolor damselfish at Stetson ($F_{1, 50}$ = 12.46, p = 0.0009). There was a positive correlation between rock presence and

density, this contrasts with the negative relationship seen at Sonnier bank (Figure 13).

Both EFGB and WFGB had a higher average than overall average rugosity for the region (EFGB = 161, WFGB = 162, Overall = 151) (Figure 14). Considering these are coral dominated banks, a higher rugosity was expected (Sleeman et al. 2005). The scleractinian corals that comprise most of this region contribute significantly to the reef structural framework, rugosity, and habitat complexity (Garcia-Sais et al. 2011). Stetson and Sonnier both had lower average rugosities than for the region overall (Stetson = 140, Sonnier = 132, Overall = 151) (Figure 14). Considering that algae, rocks, and sand as opposed to corals, which grow vertically, dominate these banks, a lower rugosity would be expected. *Millepora* was also the most common coral in these regions. Fine branching *Millepora* coral colonies often form continuous and relatively flat surfaces (Knudby and LeDrew 2007), which correlates with the low rugosity seen in these regions.

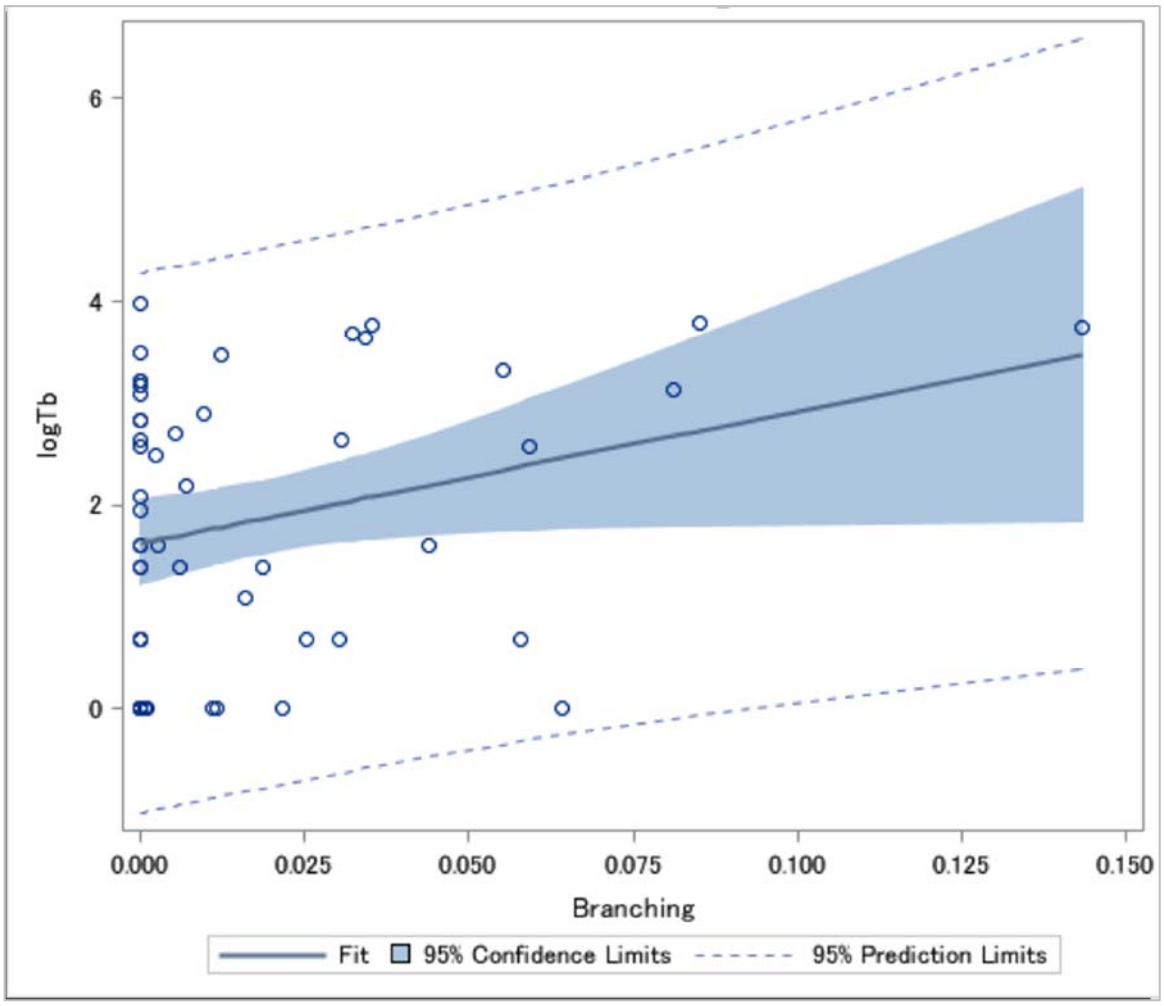

Figure 11. Branching corals and presence of bluehead wrasse regression.
This shows a slight, positive regression between bluehead wrasse and density of branching corals at Sonnier.

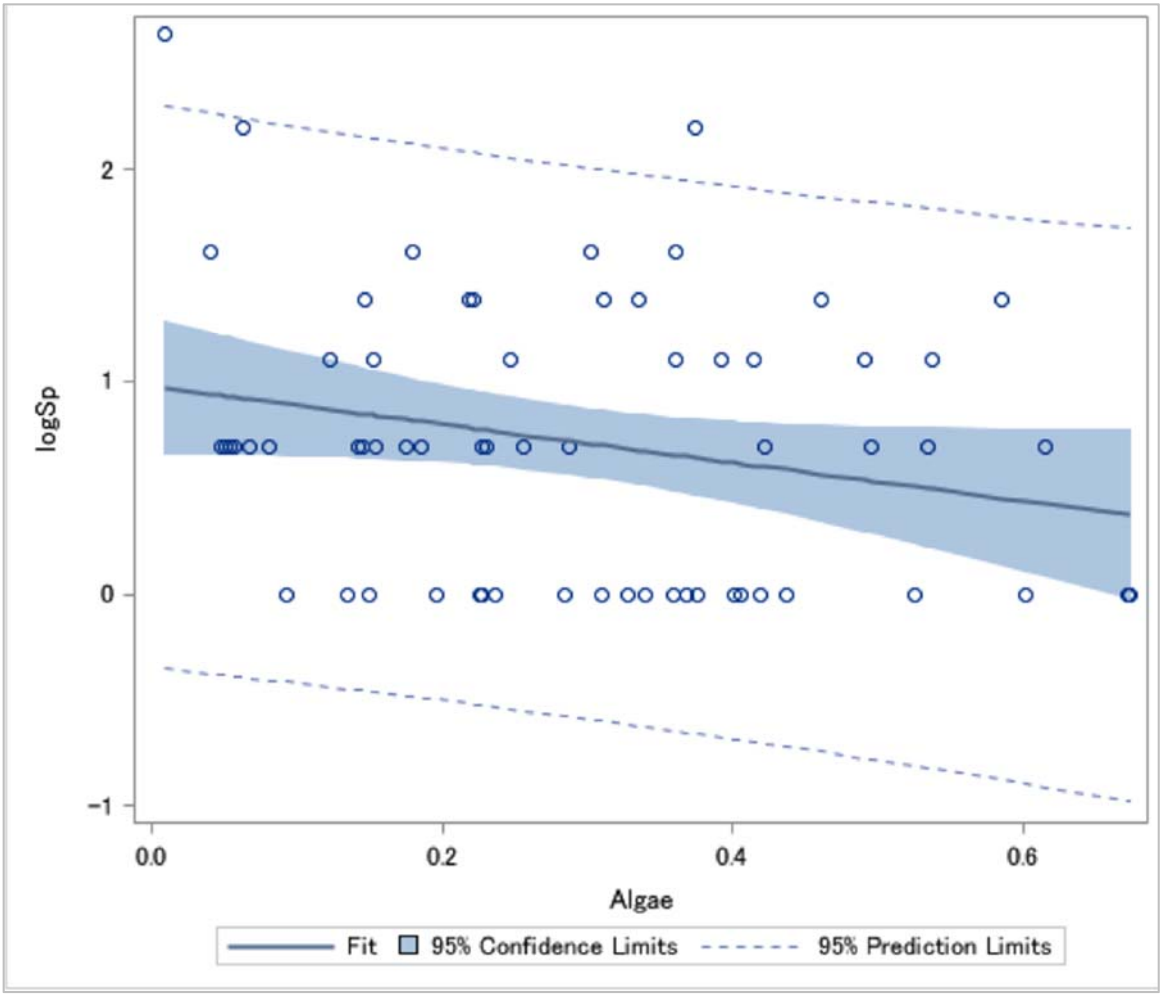

Figure 12. Algae presence and bicolor damselfish density regression.
A slight negative regression between bicolor damselfish density and algae density at EFGB.

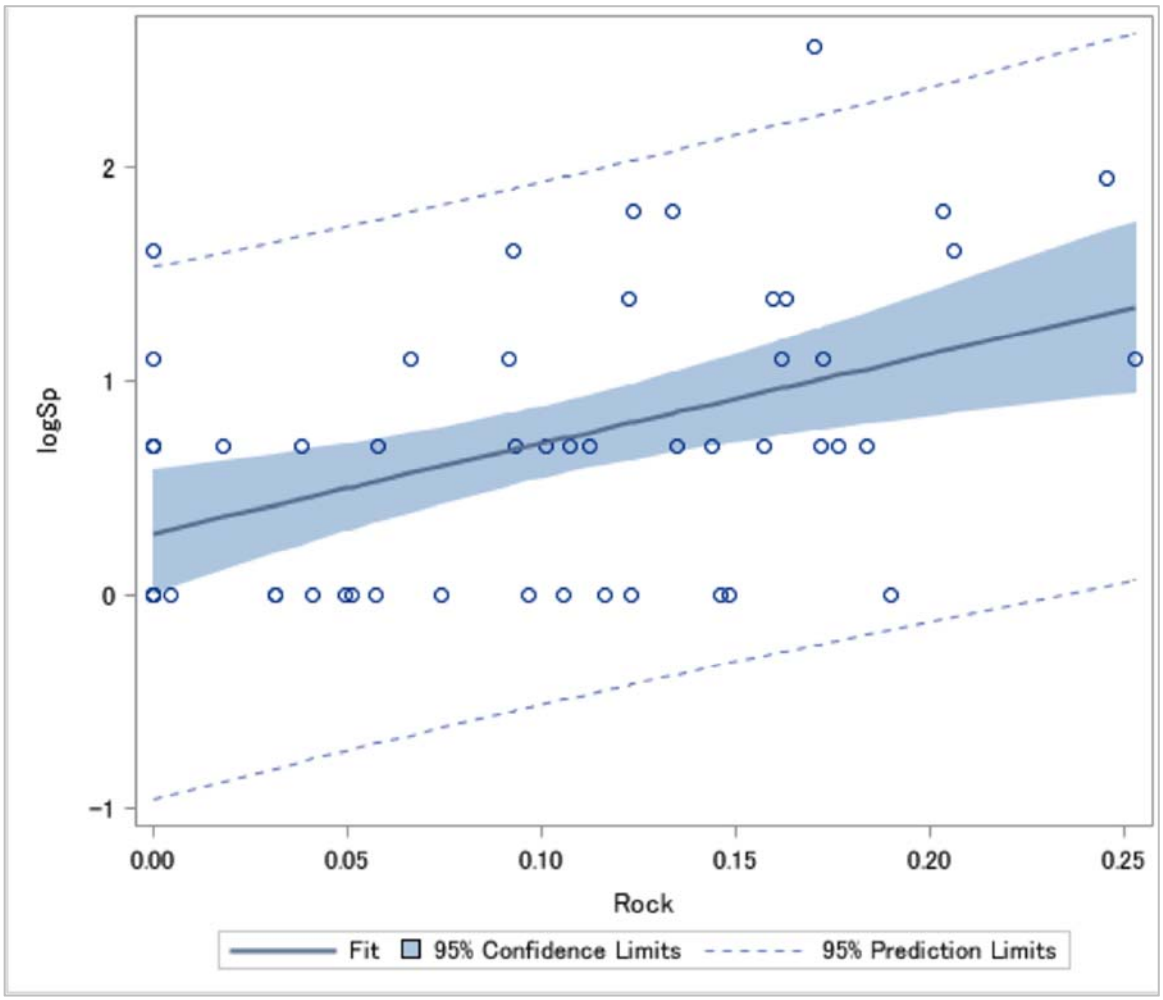

Figure 13. Rock presence and bicolor damselfish density regression.
A positive regression between bicolor damselfish density and rock presence at Stetson.

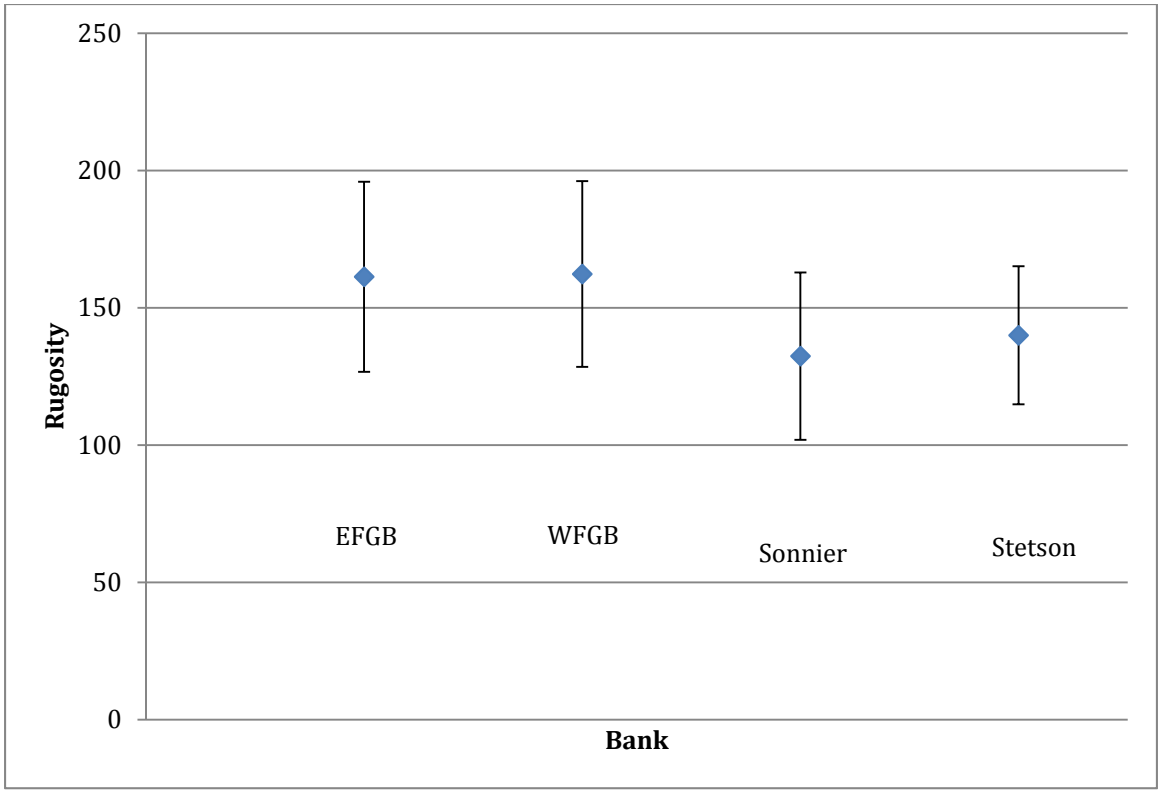

Figure 14. Average rugosity
with standard deviations for banks in the NW Gulf of Mexico. EFGB and WFG have higher mean rugosity while Stetson and Sonnier have lower mean rugosity than the pooled mean rugosity.

Early Life History

Using the standard length at age equations derived from graphing SL at capture date age for bluehead wrasse (Figure 15) and bicolor damselfish (Figure 16), the average standard length at settlement (mm) for each species at each bank was back-calculated (Table 2).

Table 2. Back-calculated Average Length at Settlement FGB

Bank	Bluehead Wrasse SL (mm)	Bicolor Damselfish SL (mm)
EFGB	14.48	19.33
WFGB	12.83	20.34
Stetson	NA	21.13
Sonnier	14.32	21.51
Overall	*13.72*	*20.62*

An ANCOVA analysis showed no significant difference of length at age between banks for bluehead wrasse ($F_{3, 34}$ = 53.62, p = 0.2171) (Figure 15). This implies a similar growth-rate for bluehead wrasse at all banks in the study region. Another ANCOVA showed a significant effect of bank on length at age of bicolor damselfish ($F_{4, 18}$ = 9.42, p = 0.0274) (Figure 16). This implies a different growth-rate for bicolor damselfish at the banks in the study region.

There was a significant difference between bicolor and bluehead PLD ($F_{1,63}$ = 87.74, p < 0.0001) (Figure 17). There was a significant difference between PLD of bluehead wrasse between banks ($F_{2, 35}$ = 4.13, p = 0.0245). The overall mean PLD for bluehead wrasse was 47.08±8.95 days; 50.60±8.38 d at EFGB,

43.00±7.81 d at WFGB, and 49.86±9.28 d at Sonnier (Figure 18). By comparison, there was not a significant difference between PLD of bicolor damselfish between banks ($F_{3, 23}$ = 0.70, p = 0.0.5608). Bicolor had an overall mean PLD of 28.44±6.10 days; 24.00±1.41 d at EFGB, 31.50±3.54 d at Sonnier, 30.22±4.58 d at Stetson, and 27.50±7.27 d at WFGB (Figure 19).

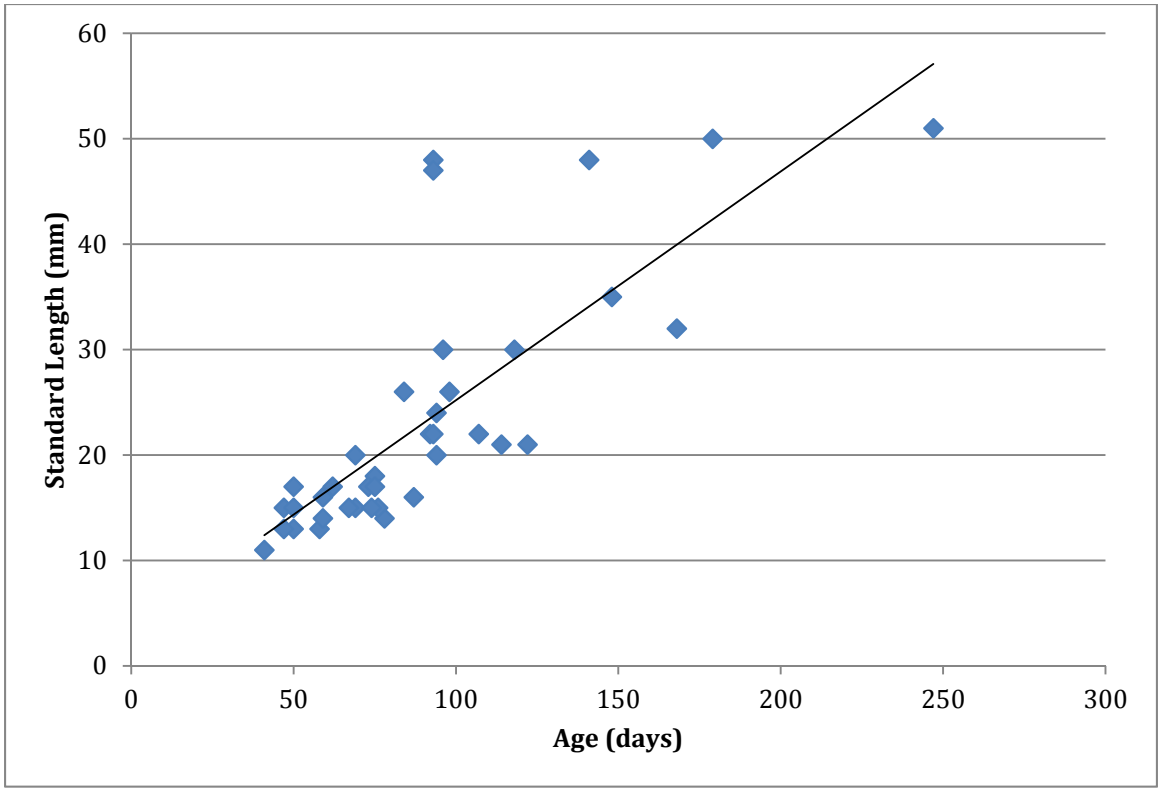

Figure 15. Standard length at age bluehead wrasse
The equation derived from this correlation, SL = (0.217 x Age) + 3.501 (R^2 = 0.61496), was used to back-calculate SL at settlement from all individuals.

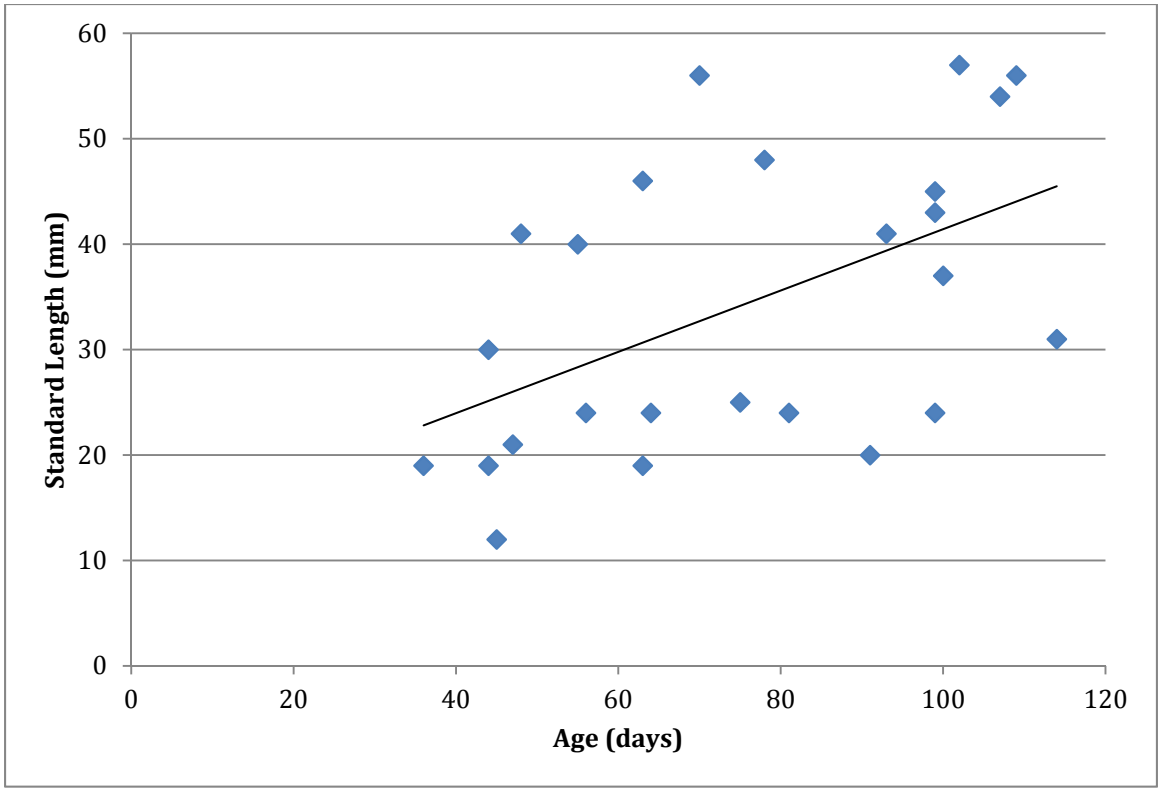

Figure 16. Standard length at age bicolor damselfish
The equation derived from this correlation, SL = (0.2908 x Age) + 12.347 (R^2 = 0.26519), was used to back-calculate SL at settlement from all individuals.

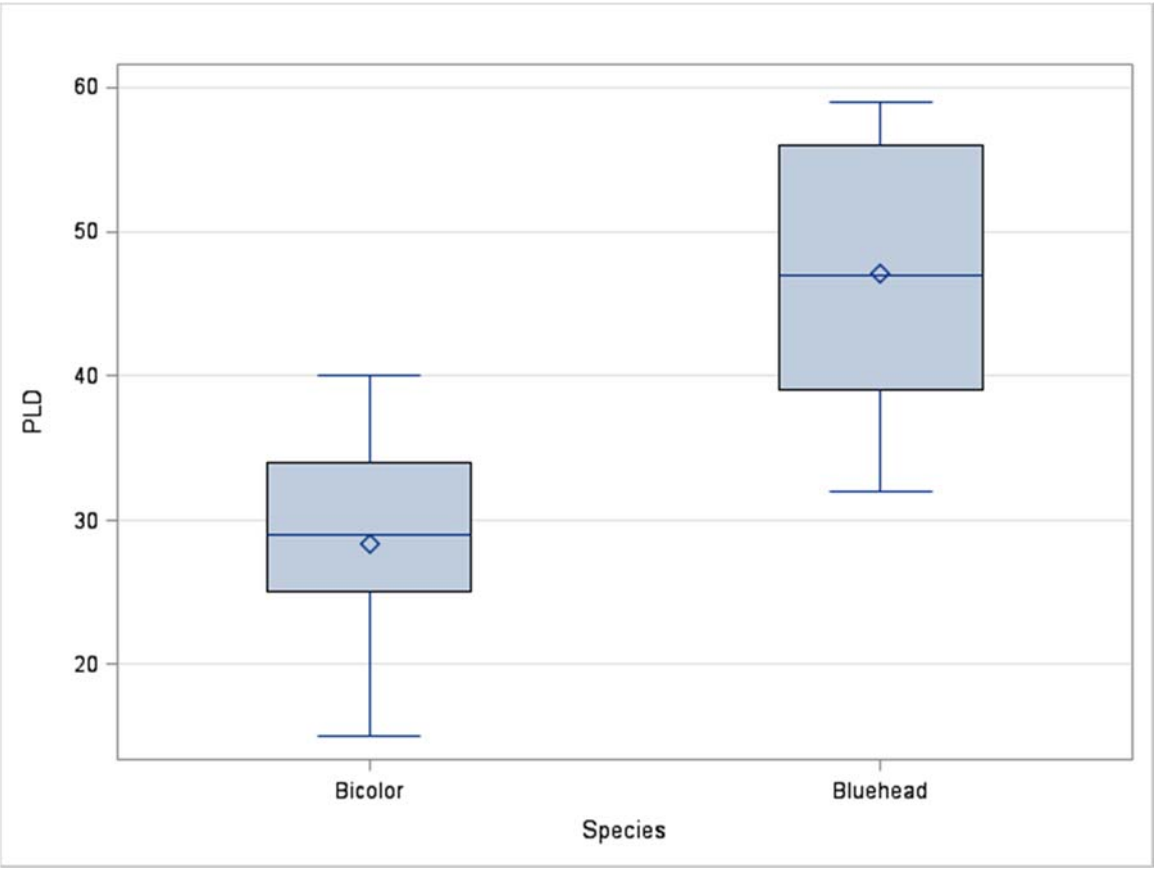

Figure 17. Bicolor vs. bluehead PLD (days).

The box represents the 25th to 75th percentiles of population density and the median is shown as a line through the box. The diamond represents the mean, and the bottom and top of the whiskers represent the minimum and maximum number of individuals in the IQR, respectively. Circles represent outliers.

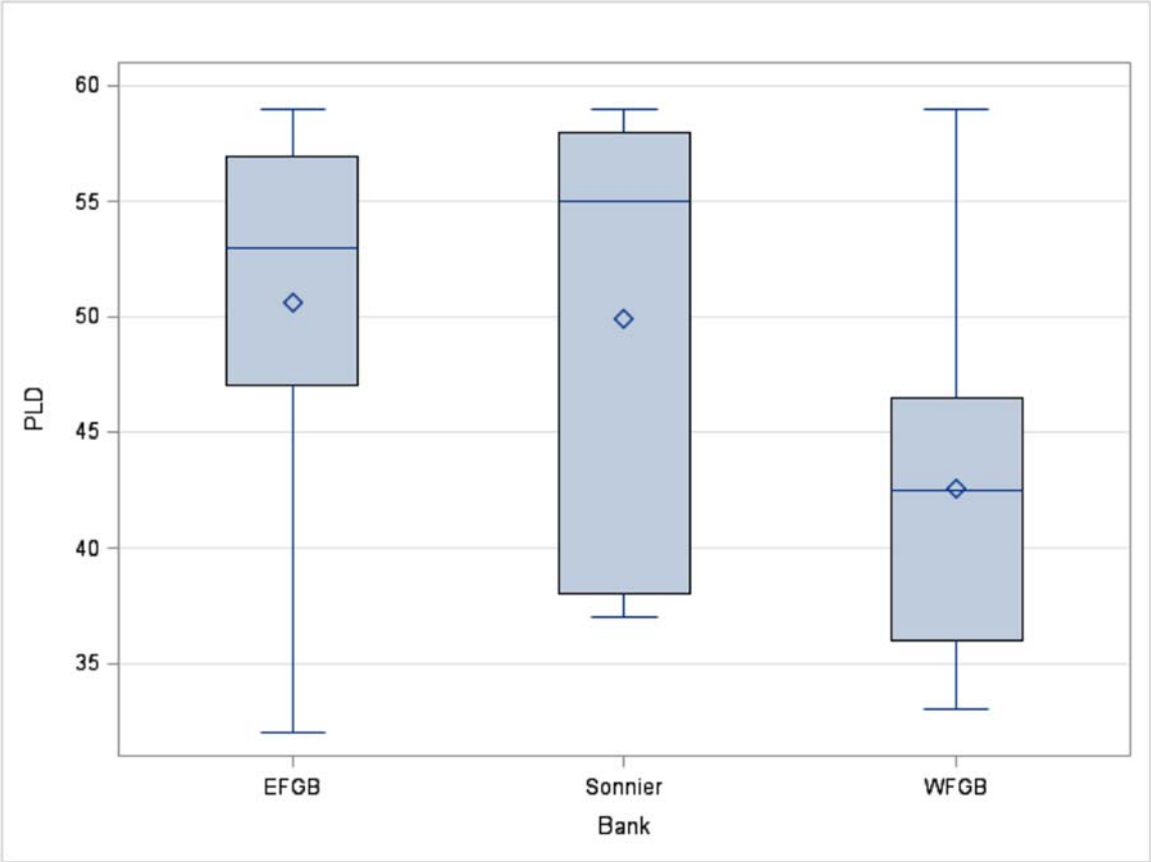

Figure 18. Distribution of PLD (days) across banks for bluehead wrasse.

The box represents the 25th to 75th percentiles of population density and the median is shown as a line through the box. The diamond represents the mean, and the bottom and top of the whiskers represent the minimum and maximum number of individuals in the IQR, respectively. Circles represent outliers.

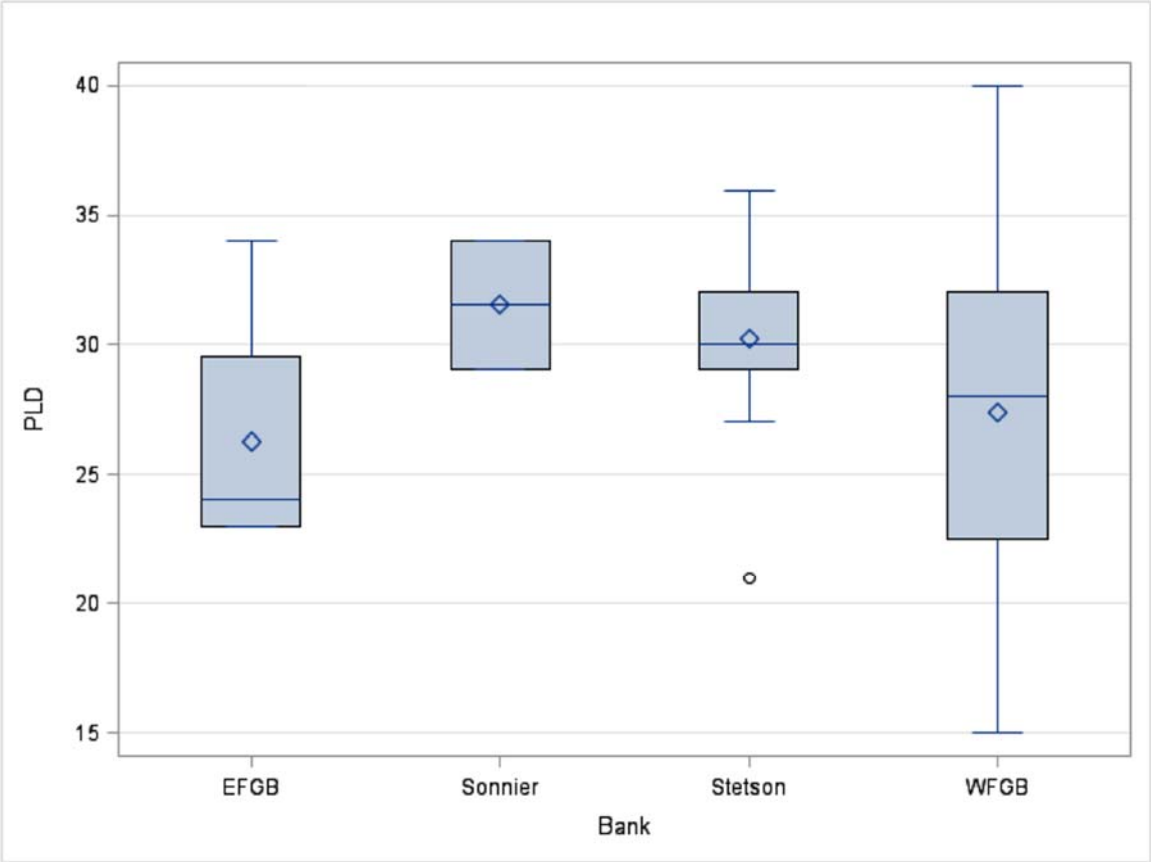

Figure 19. Distribution of PLD (days) across banks for bicolor damselfish.

The box represents the 25th to 75th percentiles of population density and the median is shown as a line through the box. The diamond represents the mean, and the bottom and top of the whiskers represent the minimum and maximum number of individuals in the IQR, respectively. Circles represent outliers.

Genetic Analysis

Due to weak PCR products for all sample, only bluehead wrasse were analyzed here, bicolor could not be analyzed. First, GOM samples alone were examined at the bank-scale population level. Initially, the true value of K, ΔK, is determined using methods described by (Evanno et al. 2005) (Figure 20); here true K was determined to be 1. Structure analysis software can be evaluated at the K = 1 level, the F_{ST} value for the population was 0.000575 (Figure 21). This very low F_{ST} value likely means little genetic isolation of populations within the GOM.

After the preliminary analysis, samples from the Gulf of Mexico were compared to those from the Caribbean (Puebla et al. 2012) to determine if the effective population extended into more distant regions. For the combined GOM data compared to Puebla et al. (2012), the true K value was again determined to be 1 (Figure 22). The structure output for each possible K is was then more closely examined with a multiple line output (Figure 23). At K = 1, the F_{ST} value for the population was 0.0004. This very low F_{ST} value likely reflects little genetic isolation of individuals in the GOM from the Caribbean, however, these numbers are higher than the F_{ST} for GOM alone, and only 2 loci were examined, therefore there may be slightly less gene flow between the Caribbean and the GOM than within the GOM alone especially when more loci are analyzed.

Next, using IBD software, first GOM only samples were examined at the bank-scale population level. For GOM only, both genetic distance versus geographic distance as well as genetic distance versus log(geographic distance), exhibit a negative relationship, however the p-values suggest that the relationship may not be significant ($r = -0.71$ $p > 0.05$ and $r = -0.64$ $p > 0.05$, respectively), (Figure 24).

Analysis for combined GOM data and data from Puebla et al. 2012 were also analyzed. Genetic distance vs. geographic distance and log(geographic distance) as well as log(genetic distance) vs. geographic distance and log(geographic distance) were all compared. Results were for each analysis are as follows: 1) genetic distance vs. geographic distance ($r = -0.52$, $p > 0.05$); 2) genetic distance vs. log(geographic distance) ($r = -0.55$, $p > 0.05$); 3) log(genetic distance) vs. geographic distance ($r = 0.19$, $p > 0.05$); and 4) log(genetic distance) vs. log(geographic distance) ($r = 0.01$, $p > 0.05$). This shows a negative relationship between genetic distance and geographic and log(geographic) distance. It shows no relationship, or a slightly positive relationship between log(genetic distance) and geographic and log(geographic) distance. (Slatkin 1993) suggests that a log-log graph of M against geographic distance would be approximately linear in a population at equilibrium under restricted dispersal. The log-log analysis for the GOM and Puebla et al. 2012 shows no correlation between genetic distance and geographic distance (Figure 25). This supports the Structure software analysis results.

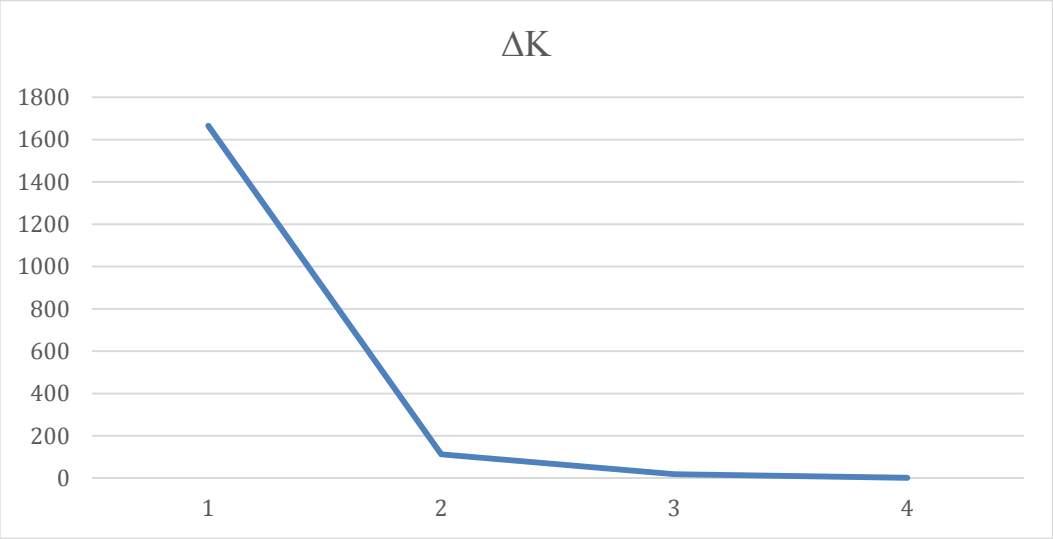

Figure 20. Selection of true K (number of clusters), FGB only.
ΔK calculated as ΔK = m|L″(K)|/s[L(K)]. When K is approaching a true value, L(K) plateaus (or continues increasing slightly) and has high variance between runs. The modal value of this distribution is the true K, or the number of populations, the uppermost level of population structure, here one cluster (Evanno et al. 2005).

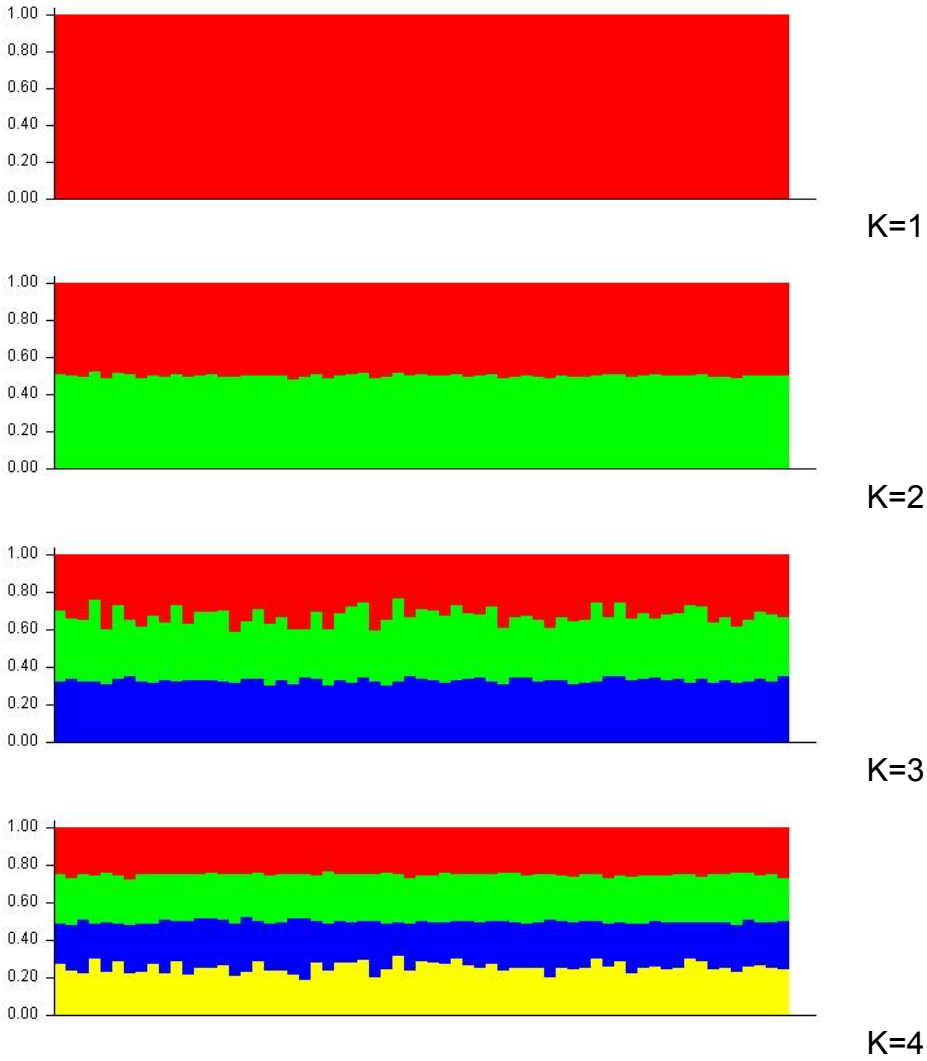

Figure 21. Structure software estimated population structure, FGB only.
Each individual is represented by a thin vertical line, which is partitioned into K colored segments that represent the individual's estimated membership fractions in K clusters. True K is selected as described by (Evanno et al. 2005) using ΔK, here 1 cluster. Bar length is proportional to the inferred ancestry values into each group for each individual.

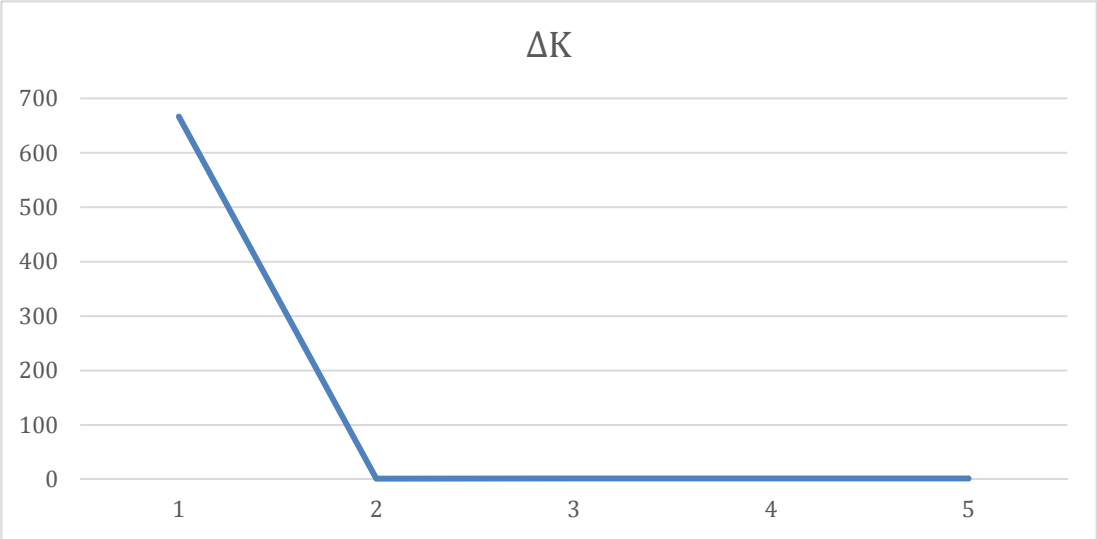

Figure 22. Selection of true K (number of clusters), combined GOM & (Puebla et al. 2012). ΔK calculated as ΔK = m|L″(K)|/s[L(K)].)]. When K is approaching a true value, L(K) plateaus (or continues increasing slightly) and has high variance between runs. The modal value of this distribution is the true K(*), the uppermost level of population structure, here two clusters (Evanno et al. 2005).

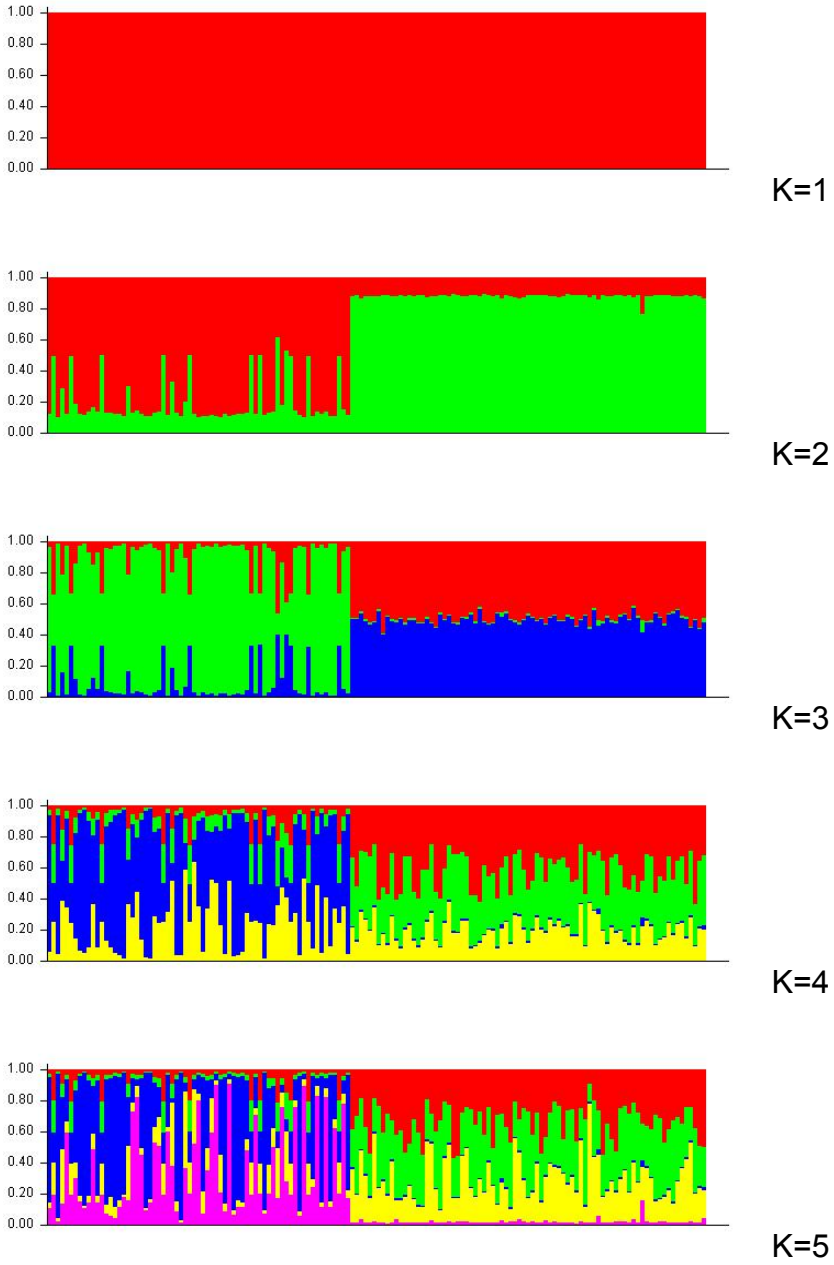

Figure 23. Structure software estimated population structure, combined GOM & (Puebla et al. 2012). Each individual is represented by a thin vertical line, which is partitioned into K colored segments that represent the individual's estimated membership fractions in K clusters. True K is selected as described by (Evanno et al. 2005) using ΔK, here 1 cluster. Bar length is proportional to the inferred ancestry values into each group for each individual.

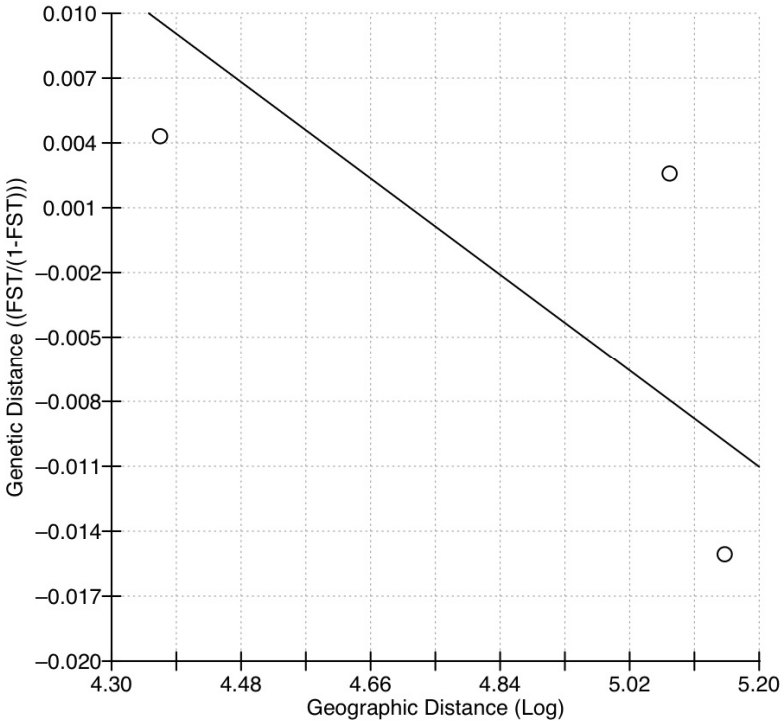

Figure 24. IBD Software Analysis FGB Only
Genetic Distance (M) vs. log(Geographic Distance) (meters). Circles represent outliers.

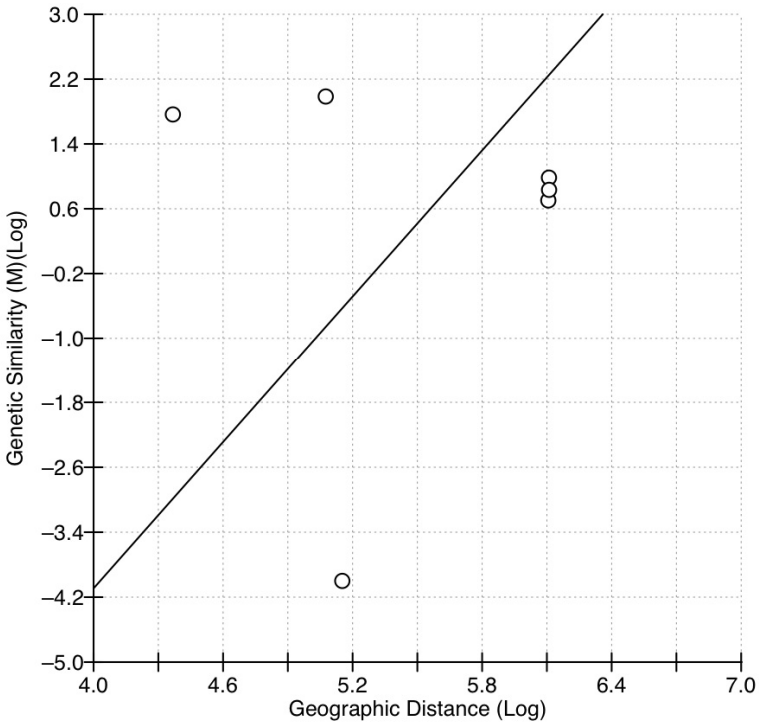

Figure 25. IBD Software Analysis FGB and Puebla et al. Data
log(Genetic Distance) vs. log(Geographic Distance). Circles represent outliers.

CHAPTER 4

Discussion

SCUBA Surveys

Density of bluehead wrasse and bicolor damselfish did not significantly vary month-to-month. This relatively consistent population size likely means that recruitment of fish is not significantly variable across months. Similar results were seen in St. Croix, where the processes resulting in the large-scale distribution of recruits were consistent over at least 4 yr. Seasonality in recruitment of coral reef fishes is common on widely separated geographic areas, but it was not evident at the smaller scale examined here, or in St. Croix (Caselle and Warner 1996).

Density of bluehead wrasse did not vary between banks, however density of bicolor damselfish did vary significantly between banks. This supports the hypothesis of wide-range dispersal of bluehead wrasse compared to local retention of bicolor damselfish. It has been suggested that only in situations in which mortality is constant from site to site (and/or generation to generation) that a strong relationship between recruitment levels and the size of the resulting

adult population will be observed (Caley et al. 1996). Therefore, assuming similar mortality rates for both bicolor damselfish and bluehead wrasse at all banks in the NW GOM, then the difference in density counts of bicolor damselfish at each bank may be a result of local retention and little external recruitment in the region. If each bank is functioning to replenish itself and each population is an independent source population, then density would be variable across banks.

Habitat preference

East Flower Garden Banks (EFGB) and West Flower Garden Banks (WFGB) are both dominated by scleractinian corals, which contribute to higher rugosity in these regions. Stetson Bank and Sonnier Bank are both dominated by rock sand and algae cover and have lower average rugosities than EFGB and WFGB. There does not appear to be a clear substrate preference for either species post-settlement in this region for general substrate types (boulder corals, branching corals, algae, rock, etc.). However, it is possible that competition for reef space has a greater influence on distribution and density of fish on the reef, or size-at-settlement influences.

Bicolor damselfish at Sonnier appear to prefer varied substrate, but avoid areas where algae, rock, or sand are present. At Stetson bicolor damselfish appear to have some preference for rock in the region, which is opposite the preference of bicolor damselfish at Sonnier. Bicolor damselfish are smaller planktivores that can fit into the interstitial spaces in lower rugosity environments, such as at

Stetson Bank, however may not be a preference for rock that results in this correlation, but competitive exclusion from other areas (Neely 2008). This may explain the positive correlation at Stetson versus the negative correlation at Sonnier for rocky substrate. It is also possible that size-at-settlement influences distribution and density of bicolor damselfish at these banks. In another study larger bicolor damselfish larvae showed a preference for *Montastraea* coral over *Porites* rubble. The advantage of larger larvae selecting coral substrate over rubble may be related to size-dependent preferences in food and shelter (Nemeth 2005). Avoidance of sandy or algae covered substrate is likely due to a lack of shelter and therefore protection from predators.

There is an inverse relationship between branching corals and rock presence and density of bluehead wrasse at EFGB. There appears to be a rejection of rock and sand by bluehead wrasse at Sonnier. The other banks showed no significant preference or rejection of substrate by bluehead wrasse. In other regions bluehead wrasse actually displayed a preference for rocky substrate. In the Virgin Islands bluehead wrasse preferred rocky areas within lagoons. This was related to position on the reef. On the forereef, where rock cover and structure was abundant, bluehead were more often found on rocky substrates. However, on the seaward reef, large aggregations of juveniles were seen around tall coral heads (Gratwicke et al. 2006). Bluehead were also found around rocky substrates in Curacao. Their fusiform body shape likely allowed them access to

the interstitial spaces of rubble piles, making it a refuge that would not be selected by the more species. (Neely 2008). This may mean that similar to bicolor, habitat preference may be related to selective pressure or size-at-settlement pressures. With more pressures in surrounding regions, bluehead utilize smaller alternate regions on the reef that are not utilized by competing larger fish. If these fish encounter less pressure in the GOM, they may tend to avoid these areas, dominated by rocks and branching corals, more than in surrounding regions.

Early Life History

Reef fish species which exhibit high parental investment, including nest builders such as bicolor damselfish, should enhance self-recruitment by retaining young in the vicinity of the parent for an extended period of time (Sponaugle et al. 2002). On the other hand, pelagic-spawners, like bluehead wrasse, who invest less in offspring, are presumed to have eggs that are smaller, less active, and less capable of remaining near the source population (Sponaugle et al. 2002). Therefore, it is likely that bicolor damselfish will show more local retention while bluehead wrasse may exhibit broader dispersal of larvae.

Longer PLDs are necessary for greater dispersal, and therefore more external recruitment, while shorter PLDs would imply more local retention. In other papers average PLD's for bluehead wrasse were as follows:

Table 3. PLD's for Bluhead Wrasse

Location	PLD (days)	Source
GOM	47.08	This study
Panama	46	(Victor 1982)
Caribbean	49.3	(Victor 1986)
Belize	45	(Philibotte 2002)
Barbados	42-56	(Searcy and Sponaugle 2001)

The overall mean PLD of 47.8 days seen in this study fits in well with PLD's seen in surrounding regions. EFGB and Sonnier both exhibited mean PLD's on the higher end of those seen in other regions, while EFGB showed a mean PLD on the very low end of those seen in surrounding regions. This may imply EFGB acts as a source population with more local retention while the other banks receive more external replenishment and act as sink populations. The location of EFGB relative to WFGB and the overarching currents in the region may support the idea that WFGB receives more external replenishment while EFGB relies more on local retention to support the population of bluehead wrasse.

Similar PLDs at all banks may suggest local retention for bicolor damselfish in the northwestern Gulf of Mexico. The mean PLD for all banks in this study fits well within the ranges seen in surrounding areas:

Table 4. PLDs for Bicolor Damselfish

Location	PLD (days)	Source
GOM	28.44	This study
Barbados	26-35	(Sponaugle and Cowen 1996)
Panama	33.91	(Wilson and Meekan 2002)
St. Croix	28.8	(Wellington and Victor 1989)

PLD of bicolor damselfish from this region appeared to be more variable between banks than those of bluehead wrasse (r2 = 0.1886 Figure 16 and r2 = 0.5988 Figure 15, respectively), but the standard deviation of bluehead wrasse (overall ±8.95d) PLDs were much higher than bicolor damselfish (overall ±6.1d) implying more variable PLDs across in general. More variable PLDs supports other research that has shown pelagic spawners exhibit a longer and more variable PLD (Sponaugle and Pinkard 2004).

In addition to the length of PLD, size-at-settlement can be an indicator of local retention or external recruitment. Rapidly growing fish are able to escape the pelagic environment earlier and are more likely to settle closer to their natal regions. These fish are known to be better to exhibit some form of active swimming behavior, which likely enhances local retention (Sponaugle et al. 2002). Bicolor damselfish exhibited a much higher growth rate (Figure 16) than bluehead wrasse (Figure 15), which supports the idea of more local retention in this nest-building species.

The average back-calculated length-at-settlement for bluehead wrasse in the GOM (13.72mm) is slightly higher than the average's seen for surrounding regions (Table 5). Previous studies have suggested that rapidly grown larvae show better condition and higher lipid reserve at recruitment than slowly grown ones (Searcy and Sponaugle 2001, Sponaugle et al. 2006). This suggests that larger larvae found in the GOM may be better suited for survival on the reef at settlement.

Table 5. Bluehead Wrasse SL at Settlement Other Regions

Location	Mean SL at Settlement (mm)	Reference
GOM	13.72	This study
Barbados	10.9	(Searcy and Sponaugle 2001)
Barbados	11.7	(Searcy and Sponaugle 2001)
Barbados	11.8	(Searcy and Sponaugle 2001)
Panama	11.0	(Victor 1986)
Barbados	10.8	(Sponaugle and Cowen 1997)
Virgin Islands	8.48*	Masterson *et al.* 1997
Virgin Islands	8.65*	(Masterson et al. 1997)
Virgin Islands	8.91*	(Masterson et al. 1997)

* SL calculated from TL using the equation TL = (1.15*SL)+1.15 established from measured SL and TL of FGB samples.

Similarly, bicolor damselfish were much larger at settlement in the GOM than in surrounding regions (Table 6). From back-calculations, it appears that bicolor damselfish in the GOM are almost twice the size of the same species in surrounding regions.

Table 6. Bicolor Damselfish SL at Settlement Other Regions

Location	Mean SL at Settlement (mm)	Reference
GOM	20.62	This study
Barbados	11.6	(Sponaugle and Cowen 1996)
Florida Keys	10.1	(Sponaugle et al. 2005)
Mexican Caribbean, eastern Yucatan Peninsula	10.5	(Villegas-Hernández et al. 2008)
Mexican Caribbean, eastern Yucatan Peninsula	11.1	(Villegas-Hernández et al. 2008)
Mexican Caribbean, eastern Yucatan Peninsula	12.1	(Villegas-Hernández et al. 2008)

Despite a fast growth rate, demersal spawners' larvae tend to settle quickly at relatively small sizes. They should be at a disadvantage post-emergence, as mortality, especially through predation, is widely seen to be size-based, therefore fish settling rapidly at smaller sizes, such as bicolor damselfish, should suffer higher mortality on the reef (Sponaugle and Pinkard 2004). However, it seems bicolor damselfish in the GOM are much larger at settlement, therefore this may not be as much of an issue in this region as it is in surrounding regions.

Genetic Analysis

The preliminary genetic analysis on bluehead wrasse implies that the populations from the three banks sampled in the northwest Gulf of Mexico, East Flower Garden Banks, West Flower Garden Banks, and Sonnier Banks are interbreeding freely and represent one effective population. Initial Structure software F_{ST} analysis was further validated using a more robust IBD analysis, in both analyses no significant relationship between banks and genetic similarity

was evident. Similar results were seen when GOM data was compared against data from the Caribbean, Structure analysis again showed that the populations from the northwest Gulf of Mexico are interbreeding freely with individuals from Belize. However, the F_{ST} value for the GOM vs. Caribbean is slightly higher than that seen for the GOM alone. Because the data presented here are very preliminary, it is possible that additional loci will result in a stronger F_{ST} value, which could reflect a higher degree of isolation in the GOM from Belize. Even with some degree of isolation these regions may not be interbreeding freely and may represent two effective populations. The IBD analysis, however, showed no correlation between genetic distance and geographic distance. This further supports the hypothesis of broader dispersal distances for pelagic spawners than demersal spawners. Further microsatellite or other genetic research on bicolor damselfish, both species, or other similar species could further support this hypothesis.

Summary

In summary, it appears that external recruitment and local retention in the northwest Gulf of Mexico supports evidence seen in other regions, with pelagic spawners (bluehead wrasse) likely exhibiting more external recruitment than demersal spawners (bicolor damselfish) who likely exhibit greater local retention. Further microsatellite or other genetic research could further support this hypothesis. There does not appear to be a clear post-settlement substrate

preference for either species in this region, which may imply that other factors affect density and distribution of these species. It is possible that pre-settlement suitable substrate and/or competition for available territory have greater influence on the density and distribution of individuals on the reef.

Microsatellite analysis was very preliminary and indicated there is likely open gene flow both within the GOM and between the GOM and Belize. However, a slightly higher F_{ST} value seen when samples were compared to Belize, may indicate some degree of isolation, this could potentially be confirmed with analysis of additional loci. If there were some degree of isolation in the GOM, then replenishment of bluehead wrasse would mainly come from within the region where source populations with broad dispersal can effectively replenish sink populations in the region. In the GOM, a shorter PLD for bluehead at WFGB and longer PLDs at EFGB and Sonnier, coupled with overarching clockwise currents in the GOM, may mean that WFGB is functioning as a source population replenishing EFGB and Sonnier with larvae. On the other hand, bicolor damselfish likely have less external recruitment and more local retention of larvae; this could further be confirmed with subsequent microsatellite research on this species in the GOM. Local retention of these fish coupled with variable densities across banks likely means that each of these populations functions as a self-sustaining independent population.

Understanding the recruitment strategies for these representative species can affect MPA functionality in that populations of pelagic spawners can function as source or sink populations, whereas demersal spawners are likely more independent and self-sustaining populations. Therefore, source regions of pelagic spawners that likely function as stepping stones to replenish surrounding populations would be essential to protection of these species. Regions utilized by demersal spawners are likely individual source populations that may not require as stringent protection, as each population could function to replenish itself, however banks with endemic species that are also demersal spawners would also require more stringent protection

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APPENDIX A Substrate Composition Data

	WFGB	EFGB	EFGB & WFGB Total	Sonnier	Stetson	Stetson & Sonnier Total
Unknown/Unidentifiable:	9.34%	6.55%	6.55%	4.29%	4.55%	4.44%
Algae:						
Crustose Coraline:	5.76%	1.10%	3.41%	7.00%	0.00%	3.01%
Filamentous:	4.02%	2.77%	3.39%	18.43%	26.87%	23.24%
Lobophora:	14.41%	15.71%	15.07%	8.93%	0.04%	3.86%
Y-branching & Halimeda:	11.23%	10.24%	10.73%	17.66%	27.90%	23.50%
Ventricaria ventricosa:	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Branching/Finger Corals:						
Madracis Mirabilis:	1.58%	1.82%	1.70%	0.19%	0.00%	0.08%
Millepora alcicornis:	0.52%	0.78%	0.65%	12.48%	3.14%	7.15%
Madracis decactis:	0.00%	0.04%	0.02%	0.00%	0.00%	0.00%
Madracis formosa:	0.00%	0.04%	0.02%	0.00%	0.00%	0.00%
Madracis pharensis:	0.00%	0.94%	0.48%	0.00%	0.00%	0.00%
Porites divaricata:	0.00%	0.45%	0.23%	0.00%	0.00%	0.00%
Brain & Boulder Corals:						
Colpophyllia natans:	7.21%	10.73%	8.98%	0.00%	0.00%	0.00%
Diplora strigosa:	0.82%	3.30%	2.07%	0.00%	0.00%	0.00%
Montastraea annularis & *Madracis decatis:*	1.97%	0.41%	1.18%	0.00%	0.59%	0.34%
Montastraea cavernosa:	6.72%	7.39%	7.06%	0.00%	0.00%	0.00%
Montastraea faveolata:	7.81%	16.49%	12.19%	0.03%	0.00%	0.01%
Montastraea franksi:	24.72%	12.27%	18.44%	0.27%	0.00%	0.12%
Siderastrea siderea:	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Stephanocoenia intersepta:	0.01%	0.22%	0.12%	0.15%	0.51%	0.35%
Porites astreoides:	0.00%	0.45%	0.23%	0.00%	0.00%	0.00%
Solenastrea bournoni:	0.00%	0.38%	0.19%	0.00%	0.00%	0.00%

	WFGB	EFGB	EFGB & WFGB Total	Sonnier	Stetson	Stetson & Sonnier Total
Sponges:						
Mussa angulosa:	0.22%	0.58%	0.40%	0.00%	0.00%	0.00%
Pseudoceratina crassa:	0.02%	0.03%	0.02%	0.20%	1.51%	0.94%
Other/Unknown sponges:	0.05%	0.00%	0.03%	0.02%	0.00%	0.01%
Haliscara sp.:	0.01%	0.00%	0.01%	0.15%	0.01%	0.07%
Verongula rigida:	0.02%	0.00%	0.01%	0.00%	0.00%	0.00%
Scolymia wellsi:	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Agelas clathrodes:	0.00%	0.00%	0.00%	0.84%	0.68%	0.75%
Red Sponge:	0.00%	0.00%	0.00%	0.60%	0.30%	0.43%
Spheciospongia vesparium:	0.00%	0.00%	0.00%	0.28%	0.05%	0.15%
Verongula rigida:	0.00%	0.00%	0.00%	0.25%	0.21%	0.22%
Anthosigmella varians:	0.00%	0.00%	0.00%	0.05%	0.00%	0.02%
Diplastrella megastellata:	0.00%	0.00%	0.00%	0.47%	3.96%	2.46%
Geodia neptuni:	0.00%	0.00%	0.00%	0.05%	0.08%	0.07%
Holopsamma helwigi:	0.00%	0.00%	0.00%	0.23%	0.26%	0.24%
Agelas conifer:	0.00%	0.00%	0.00%	0.00%	0.06%	0.03%
Aplysina archeri:	0.00%	0.00%	0.00%	0.00%	0.12%	0.07%
Black Sponge:	0.00%	0.00%	0.00%	0.00%	1.85%	1.05%
Callyspongia vaginalis:	0.00%	0.00%	0.00%	0.00%	0.02%	0.01%
Ircinia strobilina:	0.00%	0.00%	0.00%	0.00%	0.02%	0.01%
Rock:	0.40%	1.51%	0.96%	19.58%	15.82%	17.44%
Pebble:	0.00%	0.01%	0.00%	3.76%	1.61%	2.54%
Sand:	1.60%	3.41%	2.52%	5.12%	9.86%	7.82%

APPENDIX B Preliminary Genetics Data

Thalassoma bifasciatum, 3 loci and 69 individuals

Flower Garden Banks						
npops = 4						
nloci = 3						
	T3235		T3333		TbAAT4	
pop = EFGB						
TB021	200	210	?	?	150	150
TB022	140	194	?	?	?	?
TB023	182	182	205	227	153	168
TB024	236	196	193	211	?	?
TB025	162	192	195	207	?	?
TB026	214	250	?	?	?	?
TB027	140	140	215	215	?	?
TB028	162	182	?	?	150	165
TB029	138	174	207	211	153	165
TB030	?	?	?	?	144	144
TB031	188	228	225	225	?	?
TB032	?	?	?	?	144	144
TB033	?	?	?	?	?	?
TB034	164	182	205	205	?	?
TB035	?	?	205	213	147	147
TB036	182	190	213	235	144	153
TB037	162	182	197	207	?	?
TB038	?	?	201	217	162	162
TB039	160	170	191	211	?	?
TB043	138	192	203	217	?	?
TB044	160	182	203	219	?	?
TB045	162	182	217	217	?	?
TB046	182	182	201	201	144	153
pop = Positive						
+	186	214	197	217	162	165
pop = Sonnier						
TB001	162	192	203	211	?	?
TB002	?	?	199	209	?	?
TB040	?	?	?	?	?	?
TB041	166	196	195	225	165	165
TB042	184	184	219	257	?	?

TB063	182	182	197	197	?	?
TB064	192	210	205	227	153	153
TB065	166	228	219	239	138	165
TB066	140	194	?	?	?	?
TB067	160	182	221	229	159	186
TB068	194	202	199	217	159	183
pop = WFGB						
TB003	160	192	197	247	150	156
TB004	170	198	211	211	177	183
TB005	160	182	195	207	172	186
TB006	160	182	199	219	159	171
TB007	182	202	205	217	177	180
TB008	162	192	207	213	?	?
TB009	162	182	195	231	159	171
TB010	140	214	?	?	150	153
TB011	138	194	197	207	?	?
TB012	182	182	?	?	153	165
TB013	170	182	207	225	?	?
TB014	?	?	?	?	?	?
TB015	170	192	199	217	?	?
TB016	?	?	?	?	?	?
TB017	166	182	207	213	150	150
TB018	186	220	205	221	?	?
TB019	180	180	199	215	?	?
TB020	154	194	187	187	?	?
TB047	140	182	?	?	192	192
TB048	?	?	?	?	174	174
TB049	?	?	?	?	?	?
TB050	160	180	199	199	?	?
TB051	188	194	?	?	153	153
TB052	182	194	195	231	171	192
TB053	?	?	?	?	?	?
TB054	190	204	209	217	150	165
TB055	184	202	195	219	?	?
TB056	?	?	?	?	153	165
TB057	?	?	195	203	?	?
TB058	162	192	211	211	153	156
TB059	160	182	207	221	159	165
TB060	160	200	?	?	?	?
TB061	146	160	203	203	?	?
TB062	?	?	201	215	162	168

CURRICULUM VITAE

Anne M. Hansen graduated from West Springfield High School, Springfield, VA in 1999. She received her Bachelor of Science in Biology from George Mason University in 2005. She was employed as an environmental scientist in Springfield, VA for six years and received her Master of Science in Environmental Science and Policy from George Mason University in 2013.