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Species Responses to Climate Change and Environmental Heterogeneity: a Multi-Focal Approach

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SPECIES RESPONSES TO CLIMATE CHANGE AND ENVIRONMENTAL HETEROGENEITY: A MULTI-FOCAL APPROACH

by

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DEDICATION

This dissertation is dedicated to my parents, John and Susan Riley, for their unequivocal support throughout the years.

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ABSTRACT

Changes in global climate trends have the potential to influence diverse ecosystems at multiple levels of organization, from the species to the community. Changes in other environmental variables, such as those resulting from pollution and habitat modification, also have the potential to impact species fitness and alter species interactions. This dissertation employs a multi-focal approach to investigate species responses to two main topics: climate change and environmental heterogeneity. To investigate the first topic, this work focuses on the climate change-induced range expansion of the mangrove tree crab *Aratus pisonii* into novel salt marsh habitats. It investigates latitudinal patterns of life history traits, as well as alterations of these life history characteristics associated with the species' range expansion. This work compares fitness metrics and selection pressures in historical and novel habitats, and also investigates phylogeographic patterns of genetic variation in *A. pisonii* to provide insight into the relative roles of phenotypic plasticity and local adaptation in the phenotypic changes facilitating its range expansion. To investigate the second topic, this dissertation provides a mechanistic understanding of how diet, which is easily influenced by changing environmental conditions, impacts the physiology and reproduction of *A. pisonii*. Finally, this dissertation investigates the impact of past niche construction (i.e. physical habitat modification) on the intensity and spatial variation of current plant-animal interactions. Collectively, this work assesses the diverse impacts of various environmental changes on multiple levels of organization, from the genetic to the individual to the community level.

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CHAPTER 1

GENERAL INTRODUCTION

In recent decades, changes in global climate trends have led to profound alterations in natural systems. These climate change impacts span a wide array of taxa in diverse ecosystems, from the species to the community level (Walther 2002, Bellard et al. 2012). In particular, climate change has the potential to influence the type and timing of species interactions (Walther 2010). Complex species interactions are essential for maintaining ecosystem functioning, stability, and biodiversity; therefore, global change impacts on these interactions can have influential consequences (Tylianakis et al. 2008, Cahill et al. 2012).

For instance, because the geographic ranges of most plants and animals are limited by climatic factors (Parmesan et al. 2006), a common response to changing climate trends is a poleward shift or expansion in species distribution (Chen et al. 2011, Poloczanska et al. 2013). Such range expansions have been widely documented in an array of taxa. However, species differ in the type and timing of their responses to climate changes. As a result, the range expansions of many animal species outpace that of their habitat, and this can lead to novel ecosystems with unique compositions and plant-animal interactions (Walther 2010, Lurgi et al. 2012).

In addition to global climate changes, variation in other environmental factors has the ability to influence individual behavior, species fitness, and community interactions (Sih et al. 2011). For example, organisms are also impacted by widespread habitat

modification and pollution (Sih et al. 2011). Pollution has the potential to modify trophic interactions and species fitness via changes in behavior, which in turn can influence foraging activities (e.g. Reichmuth et al. 2009). Habitat modification has also been shown to disrupt species interactions, modify community composition, and alter food webs (Tyliankis et al. 2007 and references therein). Thus, like recent changes in global climate trends, spatial and temporal heterogeneity in other environmental factors can have important consequences for natural systems.

This dissertation furthers our knowledge of species responses to climate change and environmental heterogeneity by focusing on the following:

CHAPTER 2 investigates spatial mismatches in species interactions resulting from differences in the timing of climate-induced range expansions. Specifically, this chapter focuses on the range expansion of the mangrove tree crab *Aratus pisonii* relative to that of mangroves, the foundation species with which it has historically been associated. The current distribution of *A. pisonii* is established via field surveys spanning the ecotone (i.e. transition in vegetation) between mangrove and salt marsh habitats, and compared to historical reports of the species' range limit to determine the relative rate of range expansion.

CHAPTER 3 assesses changes in life history traits associated with the climate change-induced range expansion of *A. pisonii* into novel salt marsh habitats. This chapter establishes latitudinal patterns of life history traits in *A. pisonii* populations throughout a large portion of its range, including historical mangrove and novel marsh habitats. Additionally, characteristics associated with these life history traits, including fecundity,

offspring quality, and potential selection pressures are compared between the two habitat types.

CHAPTER 4 examines phylogeographic patterns of genetic variation in *A. pisonii* across a broad geographic gradient, spanning from mangrove habitats near the center of its range to novel marsh habitats near the northernmost margin of its current distribution. This chapter identifies patterns of genetic differentiation, molecular diversity, and historical demographics using two mitochondrial loci (COI and control region). This study contributes to an understanding of the mechanisms underlying phenotypic differences in native and novel habitats (detailed in CHAPTER 3).

CHAPTER 5 provides a mechanistic understanding of the impact of an omnivorous diet on the physiological condition and reproductive investment of *A. pisonii*. Organisms often initially respond to environmental changes by altering their diet. Thus, a mechanistic understanding of how diet influences physiology and reproduction provides important insight into the potential impact of environmental changes on species fitness and population dynamics.

CHAPTER 6 investigates how past niche construction (e.g. physical habitat modification) activities influence spatial variation in plant-herbivore interactions. Specifically, this chapter examines the impact of tree hole refuges, which are the result of past herbivory by wood-boring insects, on the current herbivory patterns of three herbivores in a Belizean mangrove system.

CHAPTER 7 provides a general conclusion to the dissertation.

CHAPTER 2

RANGE EXPANSION OF *ARATUS PISONII* (MANGROVE TREE CRAB) INTO NOVEL VEGETATIVE HABITATS[1]

[1]Riley ME, Johnston CA, Feller IC, Griffen BD (2014) Range expansion of *Aratus pisonii* (mangrove tree crab) into novel vegetative habitats. *Southeastern Naturalist* 13(4):43-38. Reproduced here with permission of the publisher, subject to conditions (Appendix A).

2.1 Abstract

As ecological communities expand poleward with climate change, associated species are expected to accompany habitat-forming, foundation species. However, differences in physiological limitations and/or sensitivity to climatic cues can cause spatial or temporal mismatches in the expansion of foundation species and associated inhabitants. Here, we document novel habitat switching by an inhabitant that has outpaced its traditional habitat. We provide the first report of the typically mangrove-associated crab *Aratus pisonii* in temperate salt marsh habitats along the Florida Atlantic coast. *Aratus pisonii* are present in salt marshes as far north as Little Satilla Creek, GA (31°5'32"N), substantially further north than the northernmost mangrove (~30°N). Based on historical records of the range limit of *A. pisonii* and its current distribution, we calculate that the species has moved poleward at a rate of 62 km per decade over the last century, outpacing the range expansions of the foundation species (13-45 km/decade) with which it has traditionally been associated.

2.2 Introduction, Methods, Results, and Discussion (combined)

The geographic ranges of most species are limited by climactic factors, and changes in global climate trends, particularly warming temperatures, have enabled marine and terrestrial species from a wide array of taxa to shift or expand their distributions poleward (Chen et al. 2011, Poloczanska et al. 2013). The responses of habitat-forming marine species such as coral, sea grass, oysters, mangroves, and salt marsh grasses to climate change are of particular interest, as these foundation species provide habitat for a

plethora of resident species (Hoegh-Guldberg and Bruno 2010). Poleward range expansions have been documented for a number of foundation species (Kim et al. 2012, Saintilan et al. 2014, Yamano et al. 2011), and in some cases these range expansions have facilitated the poleward movement of associated or obligate inhabitants (e.g. Yamano et al. 2012). However, differences in the type and timing of species' responses to climate change enable some species to expand their ranges more quickly than others, allowing certain animal species to expand their ranges faster than the foundation species with which they have traditionally been associated. Here, we provide the first report of the range expansion of *Aratus pisonii* H. Milne Edwards (Mangrove tree crab), a tropical and subtropical mangrove-associated species, whose range expansion has outpaced that of its native habitat and led to its establishment in novel temperate salt marsh habitats.

The Mangrove tree crab *Aratus pisonii* is abundant throughout Neotropical mangrove systems from Brazil to Florida, including the Caribbean, as well as Nicaragua to Peru along the eastern Pacific coast (Chace and Hobbs 1969, Rathbun 1918). These grapsid crabs are highly adapted to the complexity of mangrove habitats. They have a unique arboreal lifestyle and consume large amounts of fresh mangrove leaves, which make up an estimated 84% of their diet (Beever et al. 1979, Erickson et al. 2003). They are the dominant folivore on *Rhizophora mangle* L. (Red mangrove) in Florida, Belize, and Panama (Feller et al. 2013). Although *A. pisonii* preferentially feed on leaves of Red mangroves, they are also found on *Avicennia germinans* Jacq. (Black mangroves) and *Laguncularia racemosa* Gaertn. (White mangroves) in mixed mangrove stands throughout Florida (Beever et al. 1979). Previously published reports of *A. pisonii*'s range indicate that their distribution was once limited to mangroves (Beever et al. 1979,

Chace and Hobbs 1969, Rathbun 1918, Warner et al. 1967) and commercially developed areas adjacent to mangrove habitats (i.e. docks and pilings) (Beever et al. 1979).

Observations of numerous (>50) *A. pisonii* clinging to *Spartina alterniflora* Loisel (Smooth cordgrass) among mixed marsh and mangrove vegetation at Fort Matanzas National Monument (29°43'8"N, 81°14'32"W) in August 2012 prompted the authors to speculate that *A. pisonii*, like many other marine and terrestrial species, is expanding its range poleward in response to a warming climate. Mangroves themselves are expanding their ranges poleward and encroaching on salt marsh vegetation globally (Saintilan et al. 2014), and mangroves in Florida have experienced a substantial poleward expansion along the Atlantic coast in the last 30 years due to a decrease in the frequency of extreme cold events (Cavanaugh et al. 2014). Recent estimates of their range expansions indicate that White mangroves are expanding northward at a rate of 13 km per decade, Red mangroves are expanding northward at a rate of 37 km per decade, and Black mangroves are expanding northward at a rate of 45 km per decade (Williams et al. 2014). However, the presence of *A. pisonii* in mixed marsh and mangrove vegetation near the northernmost limit of mangrove habitat suggested that the range of *A. pisonii* might be expanding poleward more quickly than its mangrove habitat, leading to its establishment in salt marshes north of the northernmost mangrove trees.

In order to determine whether the Mangrove tree crab *A. pisonii* has expanded its range poleward into salt marsh habitats beyond the range limit of mangroves, we conducted a distributional survey in June, July, and August 2013 along the Atlantic coast of Florida and southern Georgia that included the current mangrove-salt marsh ecotone (i.e. transition zone). The ecotone extends from 28°N to 30°N, with the northernmost

mangrove occurring just north of St. Augustine, FL (Cavanaugh et al. 2014, Williams et al. 2014). Our survey spanned 27°33'2" N to 31°5'32" N latitude, and included two pure mangrove sites, three mixed sites with marsh and mangrove vegetation, and five pure salt marsh sites (Table 1, Fig. 1). *Aratus pisonii*, including ovigerous females, were present at nine of the ten survey sites (Table1, Fig. 1, Fig. 2). Our two northernmost survey sites, Little Satilla Creek and Jekyll Island, were both at approximately 31°N. We found a low abundance of *A. pisonii* at Little Satilla Creek (n=5), and no individuals at Jekyll Island (Table 1), suggesting that the current northernmost limit of *A. pisonii* occurs at approximately 31°5' N latitude.

Here, we provide the first report of *A. pisonii* establishment in mixed mangrove and salt marsh habitats, as well as exclusively salt marsh vegetation, as far north as Little Satilla Creek, GA (31°5'32"N). This is 152 km north of the northernmost White mangrove, 128 km north of the northernmost Red mangrove, and 109 km north of the northernmost Black mangrove. A historical record from 1918 documents the northernmost limit of *A. pisonii* on the eastern coast of Florida as Miami, FL (25°48'N) (Rathbun 1918). This suggests that *A. pisonii* has expanded its range at a rate of 62 km per decade over the last century. This rate of range expansion, which is faster than that of all three of the true mangrove species present in Florida, is consistent with reports of other marine species expanding their range at an average rate of 72 km per decade (Poloczanska et al. 2013). Future work is necessary to understand the ecological impact of *A. pisonii*'s establishment in salt marshes, as well as the potential alterations in resource use and life history strategy associated with *A. pisonii*'s range expansion. Species-specific responses to climate change that lead to spatial mismatches between

animals and foundation species have the potential to create novel community assemblages, such as the one described here. Novel communities resulting from decoupled species interactions may present serious challenges to policy makers and resource managers, and thus should be of particular interest as climate change advances (Hoegh-Guldberg and Bruno 2010).

2.3 Acknowledgments

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2.4 Tables

Table 2.1. Location of survey sites, including descriptions of habitat type, vegetation present at site, and distribution of *A. pisonii*. Y=yes, N=no.

				Vegetation				
Survey Site	**Latitude (°N)**	**Longitude (°W)**	**Habitat Type**	***Laguncularia racemosa***	***Rhizophora mangle***	***Avicennia germinans***	***Spartina alterniflora***	***A. pisonii***
Avalon State Park	27.55	80.33	Mangrove	Y	Y	Y	N	Y
Sebastian Inlet State Park	27.85	80.45	Mangrove	Y	Y	Y	N	Y
Fort Matanzas National Monument	29.73	81.24	Mixed Marsh	N	Y	Y	Y	Y
Devil's Elbow	29.75	81.25	Mixed Marsh	N	N	Y	Y	Y
Anastasia State Park	29.87	81.27	Mixed Marsh	N	N	Y	Y	Y
Big Talbot Island State Park	30.51	81.46	Salt Marsh	N	N	N	Y	Y
Fernandina Beach	30.67	81.47	Salt Marsh	N	N	N	Y	Y
Crooked River State Park	30.85	81.56	Salt Marsh	N	N	N	Y	Y
Jekyll Island	31.05	81.42	Salt Marsh	N	N	N	Y	N
Little Satilla Creek	31.09	81.57	Salt Marsh	N	N	N	Y	Y

2.5 Figures

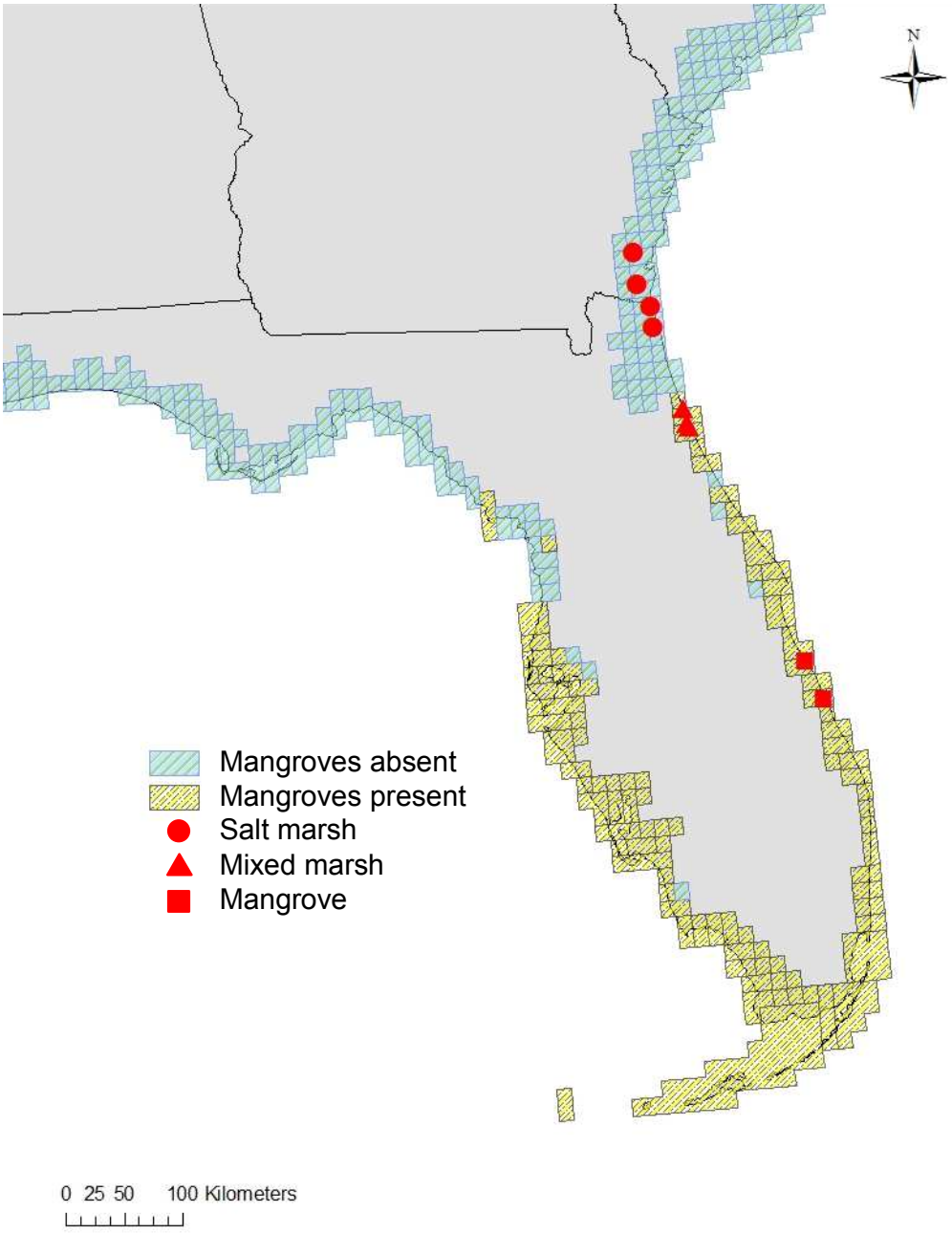

Figure 2.1. Sites along the Atlantic coast of Florida and southern Georgia where *Aratus pisonii* were documented during surveys in June-August 2013. Sites (north to south): Little Satilla Creek, Crooked River State Park, Fernandina Beach, Big Talbot Island State Park, Anastasia State Park, Devil's Elbow, Fort Matanzas National Monument, Sebastian Inlet State Park, Avalon State Park. Mangrove distribution based on Osland et al. 2013.

Figure 2.2. *A. pisonii* clinging to *Spartina alterniflora* at (A) Fort Matanzas National Monument and (B) Big Talbot Island State Park in August 2013.

CHAPTER 3

CLIMATE CHANGE-INDUCED RANGE EXPANSION ALTERS TRADITIONAL ECOGEOGRAPHIC PATTERNS OF LIFE HISTORY[2]

[2]Riley M. and B. Griffen. Climate change-induced range expansion alters traditional ecogeographic patterns of life history. *In review* at *Global Ecology and Biogeography*.

3. 1 Abstract

Range shifts and expansions resulting from global climate change have the potential to create novel communities with unique plant-animal interactions, and organisms expanding their range into foreign biotic and abiotic environments may encounter selection pressures that alter traditional ecogeographic patterns of life history traits. Here, we examined latitudinal patterns of life history traits (body size and size at maturity) in a broadly distributed ectotherm (mangrove tree crab *Aratus pisonii*) that has recently expanded its range into a novel habitat type. Additionally, we compared characteristics associated with these life history traits, including fecundity, offspring quality, and potential selection pressures between historical and novel habitats. Consistent with traditional ecogeographic concepts (i.e. Bergmann's Rule), size at maturity and mean body size of reproductive females increased with latitude within the historical habitat. However, they decreased significantly in novel habitats at the highest latitudes of the species' range, which was consistent with habitat-specific differences in both biotic (predation) and abiotic (temperature) selection pressures. Although initial maternal investment (egg volume and weight) did not differ between habitats, fecundity was lower in novel habitats as a result of differences in size at reproduction. Offspring quality (larval starvation resistance) was likewise degraded in novel habitats relative to historical habitats. These differences in offspring quality may have enduring consequences for species success and persistence in novel habitats. Life history characteristics such as those investigated here are fundamental organismal traits; consequently, understanding the potential impacts of climate change responses on latitudinal patterns of these traits is of the utmost importance in predicting climate change

impacts and properly managing climate-induced novel ecosystems. Therefore, ecologists and resource managers should consider the prospective impact of climate change on both traditional concepts of ecogeographic processes and on life history characteristics as climate change advances.

3.2 Introduction

In recent decades, changes in global climate trends have led to radical alterations in natural ecosystems. A number of systems have experienced losses in biodiversity as well as changes in the composition and dynamics of communities (Walther 2002; Cahill et al. 2012), both of which are predicted to continue in the future (Parmesan et al. 2006; Bellard et al. 2012). One of the most common responses to a warming climate is a shift in the range or distribution of species. Because the geographic ranges of most plants and animals are limited to some extent by climatic factors (i.e. temperature, light, precipitation) (Parmesan et al. 2006), changes in global climate trends have caused many species to shift or expand their distributions to include higher altitudes and more poleward latitudes (Chen et al. 2011; Poloczanska et al. 2013). For example, species whose ranges are constrained by the cold are expanding their distributions poleward in response to warming temperatures, with terrestrial species expanding at an average rate of 16.9 km each decade (Chen et al. 2011) and marine species expanding at an average rate of 72 km each decade (Poloczanska et al. 2013). Such range expansions have been widely documented in an extensive array of taxonomic groups, including insects (e.g. Thomas et al. 2001; Hill et al. 2002), plants (e.g. Piermattei et al. 2012; Cavanaugh et al. 2014), mammals (e.g. Jannett et al. 2007), and marine invertebrates (e.g. Pitt et al. 2010).

Variation in the timing and spatial response of different taxonomic groups to changes in climate can cause species to shift their ranges at different speeds, and thus has the potential to lead to spatial mismatches between previously interacting species (le Roux and McGeoch 2008; Schweiger et al. 2008; Lurgi et al. 2012). This creates novel ecosystems with unique community assemblages and plant-animal interactions (Parmesan et al. 2006; Lurgi et al. 2012). For instance, the range expansion of a herbivorous insect species may outpace that of its historical host, thereby exposing naïve host plants with inferior secondary defenses to severe herbivory by the range-expanding insect (e.g. Raffa et al. 2013). Similarly, the range expansion of a flowering plant species may outpace that of its native pollinators, leading to fitness declines as a consequence of the decreased success of sexual reproduction (e.g. González-Varo et al. 2013).

Novel communities often represent a suite of new selection pressures, and as a result range-expanding organisms may experience rapid changes in plastic life history characteristics (Burton et al. 2010; Phillips et al. 2010). Because many organisms already exist across historically broad ranges in which they display latitudinal clines in life history traits, the selection pressures associated with their range expansion (predation pressure, temperature) may override historical biogeographic life history patterns. For example, body size is among the most fundamental life history characteristics of an organism, influencing key traits such as individual fitness, population dynamics, and species adaptability. Biologists have long documented variations in both body size and size at maturity across latitude, particularly increases in body sizes and size at maturity with latitude. Bergmann (1847) originally addressed this topic by noting that organisms in cooler temperatures (i.e. higher latitudes) tend to have larger body sizes than those in

warmer temperatures (i.e. lower latitudes). Bergmann's Rule, which originally described this pattern interspecifically, is now used to describe intraspecific patterns in body size as well (also referred to as James's Rule, see Blackburn et al. 1999). Although the generality of and mechanisms underlying Bergmann's Rule remain a topic of debate (e.g. Mousseau et al. 1997; Blackburn et al. 1999; Angilletta and Dunham 2003, but see Horne et al. 2015 for some consensus), the existence of latitudinal clines in body size is broadly recognized.

Nevertheless, selection pressures associated with climate change-induced range expansions into novel habitats may also influence ecogeographic phenomena such as Bergmann clines. Yet relatively few studies have provided empirical evidence of altered life history traits associated with range expansions (Gaston 2009, but see Gutowski and Fox 2012; Hassall et al. 2014). Here, we examine the potential for range expansion into a novel habitat to alter life history traits within the context of existing latitudinal life history clines using the model organism *Aratus pisonii* (mangrove tree crab). It is one of numerous species whose range expansion has led to a spatial mismatch with a habitat-forming plant species. Within its native habitat, this neotropical mangrove crab exists in close association with the red mangrove *Rhizophora mangle,* which provides both food and shelter for this arboreal species. Although mangroves in Florida have experienced a substantial recent poleward expansion along the Atlantic coast due to a decrease in the frequency of extreme cold events (Cavanaugh et al. 2014), the poleward range expansion of *A. pisonii* (estimated at 62 km/decade in the last century) has outpaced that of its native habitat and the species has established itself in novel vegetation in temperate salt marshes (Riley et al. 2014a). The transition between mangroves and salt marshes occurs

along a gradient, and thus it is difficult to pinpoint a specific observed time of observed time of arrival for this species in salt marshes. However, estimates of the rate of its range expansion (Riley et al. 2014a) suggest that the species first colonized the southernmost novel habitat type in our study (Fort Matanzas) in approximately 1990.

We identified latitudinal patterns of life history traits (female size at maturity and average size at reproduction) of *A. pisonii* across a broad (13.73°) latitudinal gradient that included its native mangrove habitat and novel salt marsh vegetation. Additionally, for a representative of each habitat type (native mangrove and novel mixed marsh) we compared the proportion of females actively reproducing at multiple time points as well as fecundity and metrics of maternal investment (egg volume and weight) and offspring quality (larval starvation resistance). Finally, because selection pressures such as predation may vary between habitats, and this may influence the life history traits of interest here (e.g. Reznick et al. 1990, Walsh and Reznick 2009), we assessed the ability of individuals to utilize the vegetation structure in novel habitats as a potential predator evasion strategy, and compared size-specific predation risk in both native and novel habitats.

3.3 Methods

3.3.1 Determination of latitudinal life history trends

In order to determine latitudinal patterns of body size and size at maturity, we conducted population surveys at 11 sites spanning a 13.73° latitudinal range in the northern hemisphere (Table 1). These sites included seven pure mangrove habitats, three hybrid mangrove-salt marsh habitats (hereafter referred to as mixed marsh), and one pure

salt marsh habitat. The number of mixed and pure salt marsh sites surveyed was constrained by the extent of hybrid marsh sites and the limited distribution of *A. pisonii* in pure salt marsh habitats. Here, the term "mixed marsh" refers to a site that is dominated by salt marsh cordgrass *Spartina alterniflora* with isolated dwarf mangrove trees occasionally interspersed among the cordgrass. However, because mangroves themselves have only recently begun expanding their range into these salt marsh habitats (Cavanaugh et al. 2014, Williams et al. 2014), these dwarf trees are younger and smaller than those found further south in mature mangrove forests. As a result, the mangrove trees in mixed marshes are highly connected to the surrounding salt marsh, and their small size and isolated nature likely do not lend themselves to the same microhabitat creation that has been documented in mature mangrove forests (e.g. Chapperon and Seuront 2011). These mixed marshes therefore more closely resemble salt marshes than mangrove forests.

At each site, crabs (mean sample size ± SE=112.36 ± 6.93 at each site) were randomly collected during daytime high tides. We measured their carapace width (CW) to the nearest tenth of a millimeter, identified their sex, and determined the reproductive status (gravid or not gravid) of females. In order to expand our latitudinal coverage of size at maturity data, we also included published reports of size at maturity from two additional sites in mangrove habitats (Diaz and Conde 1989; Warner 1967). In order to determine the influence of latitude and habitat type on (1) size at maturity and (2) average size of ovigerous females, we used two separate general linear models with latitude and habitat type (native mangrove or novel mixed marsh/ marsh) as explanatory variables.

3.3.2 Comparison of body size distributions and reproductive effort at representative sites

We conducted additional surveys throughout the summer reproductive peak (June, July, and August 2013) to further investigate body size distributions and reproductive effort. Due to logistical constraints, these additional surveys were restricted to two representative sites: a representative mature mangrove forest (Avalon State Park, Fort Pierce, FL, 27.55°N) and a representative mixed marsh (Anastasia State Park, St. Augustine, FL, 29.87°N), which were chosen after careful consideration of several factors. Both sites (1) exemplify the prominent characteristics of each habitat type, (2) occur at relatively similar latitudes but are neither the northernmost nor southernmost site surveyed for each habitat type, and (3) initial surveys demonstrated that body size patterns at the two sites were consistent with the overall latitudinal patterns described here.

At each of these representative sites, we collected female crabs (mean sample size ± SE=57.17 ± 4.14 at each time point) as described previously, measured their carapace width (CW) to the nearest tenth of a millimeter, and determined their reproductive status (ovigerous or not ovigerous). Because *A. pisonii* carry multiple broods each year, we took advantage of *A. pisonii*'s reproductive lunar synchronization (Warner 1967) and conducted all monthly surveys in the week preceding the full moon to capture periods of maximum reproductive effort for both populations. From these surveys, we compared body size distribution patterns of reproductively mature and gravid females, as well as the proportion of gravid females (hereafter referred to as reproductive effort) at each of the 3 monthly samples.

Size at maturity for each habitat was considered to be the smallest gravid female out of the total surveyed. Body size data was analyzed using a Wilcoxon rank-sum test

and Fisher's F-test in order to compare mean size and size variability between the two populations, respectively. Additionally, Hartigan's dip test for unimodality was calculated to determine the body size distribution (unimodal, bimodal, etc.) of both reproductively mature and gravid females in the population in order to elucidate whether a single cohort or multiple cohorts were reproducing simultaneously in each habitat type. The proportion of gravid females in each habitat type was determined for each monthly survey by dividing the total number of gravid females by the total number of females larger than the site-specific size at maturity. This data was analyzed using a generalized linear model with a binomial distribution and month and habitat as categorical explanatory variables.

3.3.3 Comparison of maternal investment, offspring quality, and fecundity at representative sites

In order to determine offspring quality, we collected ovigerous females from the representative mangrove (n=21) and mixed marsh (n=18) sites described in the previous section and transported them live to the Smithsonian Marine Station in Fort Pierce, FL. Crabs were maintained at ambient temperature with a natural light:dark cycle in individual plastic aquaria (22.8 cm L x 15.2 cm W x 16.5 cm H) containing approximately 200-300 mL of filtered seawater (salinity ~31 ppt) and a piece of plastic mesh (20 cm x 4.5 cm) that provided a substrate on which crabs could exit the water. Crabs were fed plant material (*Spartina alterniflora* for crabs collected from the mixed marsh habitat and *Rhizophora mangle* for crabs collected from the mangrove habitat*) ad libitum*. Both food and water were changed every 48 hours.

A small portion (<1/3) of each female's egg mass was gently removed from the pleopods with forceps within 24 hours of collection from the field, placed into filtered sea water (~31 ppt), and visually examined under a Leica M205C dissecting microscope at 80X to determine egg developmental stage. For females carrying recently extruded, non-eyed eggs (mangroves n= 10, mixed marsh n=15), which are reflective of initial maternal investment in quantity of egg yolk, a subsample of the removed eggs (n=20-30) was photographed using a Jenoptik ProgRes C14+ camera attached to the microscope and ProgRes CapturePro v2.8.0 software. Egg size was then determined by randomly selecting 10 eggs from the photographed egg mass and measuring the circular area of each egg with imageJ software. Capitalizing on the spherical nature of *A. pisonii* eggs (Diaz et al. 1983), these measurements of circular area were used to determine spherical egg volume and averaged to provide the mean egg size for each female. The impact of maternal habitat on mean egg volume was determined using a T-test.

The remaining eggs in each brood were allowed to remain on the pleopods and continue normal development. Crabs were monitored twice daily for larval hatching. Twelve newly hatched larvae from each brood (mangrove n=21 and mixed marsh n=18 as described previously) were randomly selected and pipetted into individual Kimble borosilicate glass culture tubes (15 mm x 85 mm) with ~80 mL of 0.2 μm filtered seawater. Water was partially changed and larval mortality was monitored daily to determine starvation resistance (days) as a proxy for maternal energy provisioning (e.g. Sato and Suzuki 2010). The impact of habitat on larval starvation resistance was determined using a mixed effects Cox model, with unique maternal identity included as a random effect, thus preventing pseudoreplication by accounting for the twelve larvae

collected from each individual female while simultaneously focusing on the importance of maternal habitat on larval starvation resistance.

In order to determine egg weight and fecundity, additional ovigerous females (n=22 per site) were collected from the same representative sites and placed immediately upon ice. Eggs were removed from the pleopods of each female, egg stage (eyed/noneyed) was determined, and crabs with recently extruded, noneyed eggs were dissected to ensure that they were post-vitellogenic (i.e. all eggs were extruded). A subset of eggs was taken from 15 randomly selected crabs from each habitat type and counted under a dissecting microscope. These subsets were dried at 65°C for 48 hours and weighed. We then determined the relationship between egg count and dry egg mass using a linear regression, and used the slope of this relationship (which indicates the average dry mass of a single egg) along with total brood dry weight to calculate total fecundity for individuals from both habitats. The impact of habitat on individual egg weight was analyzed with a general linear model with dry mass of the subset of eggs as the response variable and egg count and habitat as explanatory variables. Fecundity was analyzed using a general linear model with maternal body size (CW) and habitat as explanatory variables.

3.3.4 Determination of predator avoidance capability and size-specific predation pressure

In order to investigate the shift in body size distribution to smaller individuals in the mixed and pure salt marsh habitats revealed by our population surveys (see Results), we first assessed the ability of large (20-25 mm CW) *A. pisonii* collected from mangrove

habitats to climb the habitat structure available in novel salt marsh habitats, as arboreality is one of the most important predator evasion techniques employed by this species in native mangrove systems. We conducted laboratory trials in which individuals (n=12, mean ± SE= 22.6 ± 0.4 mm CW) were placed in 5-gallon buckets containing sediment to a height of 50 mm with a single stalk of *S. alterniflora* (mean ± SE= 1.0 ± 0.4 mm basal stem diameter) placed upright in the center of the bucket to simulate the habitat structure available in marsh habitats. Seawater was then added up to a height of 100 mm to simulate a high tide event, and individuals were placed in the bucket and their behavior (climbing/not climbing habitat structure) and survival were observed hourly for 6 hours (the length of a high tide cycle). If a crab had not demonstrated the ability to climb the habitat structure by the end of the 6 hours, the water was gently disturbed by hand to encourage the crab to climb the stalk of *S. alterniflora* and verify its ability to do so.

Next, we investigated size-specific predation pressure in both mangrove and mixed marsh habitats. Individuals of two size classes (small and large) were collected from the representative mixed marsh and mangrove sites, respectively. We brought both small (8-15 mm CW) and large (18-25 mm CW) *A. pisonii* into the laboratory and affixed monofilament tethers (45 cm of 10 lb test) by tying a loop in the monofilament and attaching this loop to the back of the carapace using cyanoacrylate glue. Crabs were maintained in individual aquaria overnight and observed the following morning to ensure that tethers remained secure. Crabs were then transported to the field at low tide. Tethers were attached to mangrove roots or dead *Spartina* stalks above the low tide line such that individuals could access the water to stay moist at low tide and exit the water at high tide. Individuals were left in the field for 24 hours and then checked for survival as well as any

indications of predation (e.g pieces of carapace remaining on tether) during low tide. This tethering protocol was repeated four times in each habitat for a total of 149 individuals (mangrove n=78, mixed marsh n=71). To avoid any differences in tethering artifacts between habitat types, we restricted our analyses to comparisons of predation on the two size classes within each habitat using separate generalized linear models with a binomial distribution.

3.4 Results

3.4.1 Latitudinal life history trends

Female size at maturity increased significantly with latitude, but this trend was countered by the species' range expansion into novel marsh habitats, as size at maturity was smaller in mixed and pure salt marshes at the highest latitudes of the species range than in any of the mangrove habitats surveyed (GLM, Latitude: mean ± SE=0.373 ± 0.097, P=0.003; Habitat: mean ± SE= 7.986 ± 1.202, P=0<0.001, Fig. 1). Similarly, the average size of ovigerous females increased with latitude, but this trend was reversed at the highest latitudes of the species' range, where gravid individuals were significantly smaller in novel marsh habitats than native mangrove habitats (GLM, Latitude: mean ± SE=0.312 ± 0.046, P<0.001; Habitat: mean ± SE= 8.358 ± 0.313, P=0<0.001).

3.4.2 Body size distributions and reproductive effort at representative sites

Female size at maturity occurred at 8.9 mm CW in the representative mixed marsh habitat and 14.4 mm CW in the representative mangrove habitat (Fig. 2). The mean size of gravid females was smaller in the mixed marsh habitat (mean ± SD= 11.4 ±

1.3 mm CW, n=119) than the mangrove habitat (mean ± SD= 19.2 ± 2.6 mm CW, n=70) (Wilcoxon rank-sum test, $p< 0.001$, Fig. 2A). Additionally, gravid females in the mangrove population displayed 3.53x more variation in body size than those in the mixed marsh population (Fisher's F-test, $p<0.001$, Fig. 2A). In the marsh, the body size distribution of all females larger than the site-specific size at maturity was unimodal (Hartigan's dip test, D=0.031, P=0.227, Fig. 2A), while body size distribution of these females was bimodal in the mangroves (Hartigan's dip test, D=0.046, P=0.016, Fig.2A).

Although the proportion of the females that were gravid increased at both sites over the monthly summer sampling dates, it was consistently higher in the population from the mixed marsh habitat than the population from the mangrove habitat (GLM, month: P=0.001; site: P<0.001, Fig. 3).

3.4.3 Maternal investment, offspring quality, and fecundity at representative sites

Initial maternal investment in eggs, as measured by mean volume of non-eyed eggs, was not impacted by maternal habitat (T-test, P=0.903, Fig. 4). Similarly, individual egg weight (0.00729 mg), as measured by the slope of the regression between egg count and weight, did not differ between habitat types (GLM, P=0.343). Larval starvation resistance was significantly impacted by habitat type, with offspring from mothers in the mangroves demonstrating enhanced starvation resistance compared to those from mothers in the mixed marsh. Larval starvation was not impacted by maternal body size (mixed effect Cox model, maternal habitat: Coefficient ± SE= 0.855 ± 0.300, P=0.004, Fig. 5).

Fecundity increased significantly with body size but did not differ between habitats (GLM, Maternal body size: mean ± SE=1754.7 ± 9.171, P<0.001; Habitat: P=0.274, Fig. 6). Therefore, although there was no difference in standardized fecundity (mean no. eggs/ mm CW) between habitats, crabs in mangroves had a larger mean nonstandardized fecundity (mean no. eggs/ individual ± SE=15215.9 ± 1027.6) than those in the mixed marsh (mean no. eggs/ individual ± SE=6649.7 ± 404.9) due to their larger body size (Fig. 6).

3.4.4 Predator avoidance capability and size-specific predation pressure

All individuals survived the predator avoidance laboratory study and demonstrated the ability to climb *S. alterniflora* for predator evasion. Field tethering results indicate that predation pressure is nearly twice as high on large crabs than small crabs in novel marsh vegetation (GLM, Habitat: mean ± SE= 0.860 ± 0.501, P=0.086, Fig. 7), although this was only marginally significant, potentially due to insufficient replication. However, tethering experiments revealed no differences in size-selective predation in native mangrove habitats (GLM, Habitat: P=0.927, Fig. 7).

3.5 Discussion

We assessed the potential for range expansion into a novel habitat to alter latitudinal patterns of life history characteristics by investigating these patterns in a broadly distributed ectotherm across a wide latitudinal gradient that included both historical and native habitats. Traditional ecogeographic patterns of body size variation (i.e. Bergmann's Rule) describe latitudinal increases in body size that have been widely

documented intraspecifically and interspecifically (Ray 1960; Meiri and Dayan 2003). Although the literature surrounding Bergmann's Rule has generally been contentious and the mechanism(s) underlying this ecogeographic phenomenon remain a topic of debate (Mousseau et al. 1997; Blackburn et al. 1999; Angilletta and Dunham 2003), several factors have received attention as potential drivers of Bergmann clines.

The first proposed mechanism, temperature, is a result of the thermal plasticity of body size. Recent evidence provides a conceptual unification of these latitudinal clines in body size with the temperature-size rule, one of the most prominent mechanisms proposed as an explanation for latitudinal patterns of body size (Horne et al. 2015). The temperature-size rule describes the inverse relationship between body size at different developmental stages, such as reproductive maturity, and rearing temperature (Ray 1960). As a result of this phenotypic response to temperature during ontogeny, individuals reared at low temperatures delay maturity, reaching maturity at a larger body size and growing to be larger overall than conspecifics reared at warmer temperatures, possibly as a result of temperature-dependent reaction norms and growth efficiency (Angilletta and Dunham 2003; Kingsolver and Huey 2008). The temperature-size rule has been supported in more than 80% of the ectothermic species examined (Atkinson 1994; 1995). Additionally, recent evidence also indicates that terrestrial multivoltine arthropods, such as the species described here, generally follow the patterns predicted by both Bergmann's Rule and the temperature-size rule (Horne et al. 2015).

Variation in resource quality and availability also has the potential to interact with latitudinal clines in body size by altering the relationship between temperature and body size. For instance, Diamond and Kingsolver (2010) demonstrated that insect larvae reared

on high quality host plant diets in the lab followed the temperature-size clines predicted by the temperature-size rule. However, when larvae were reared on inferior quality host plant diets, they actually demonstrated a reversal of the temperature-size rule, achieving larger final body size at warmer temperatures and suggesting the importance of diet in the applicability of the temperature-size rule under natural conditions.

Finally, predation pressure has also been shown to drive differences in both size at maturity and overall body size via phenotypic or genetic processes. For instance, elevated predation intensity from natural predators or commercial harvesting can drive declines in body size at reproductive maturity (e.g. Reznick et al. 1990; Jorgensen et al. 2007, but see Prowse et al. 2015 for an exception). Because predation tends to decrease with increasing latitude (e.g. Peterson et al. 2001; Freestone et al. 2011), latitudinal clines in predation pressure have also been correlated with a decrease in size at maturity and overall body size at decreasing latitudes (Manyak-Davis et al. 2013).

Regardless of the mechanism(s) influencing latitudinal patterns of life history, altered biotic and abiotic interactions resulting from climate change may impact traditional conceptions of ecogeographic patterns, which were first established by Bergmann (1847) more than a century and a half ago. In particular, theory suggests that range-expanding organisms may face selective pressures that are uniquely different from those experienced by individuals within the core of the species' range (Suarez and Tsutsui 2008; Burton et al. 2010; Phillips et al. 2010). Just as clines in biotic and abiotic interactions have the potential to drive ecogeographic patterns of body size and life history traits, habitat-specific differences in these factors may also influence these traits. For instance, temperature varies between both microhabitats and macrohabitats. If

climate change prompts range expansion into a novel habitat, as is the case with the species examined in this study, differences in temperature between habitat types may have the potential to alter broader geographic patterns of body size, as predicted by the temperature-size rule. Similarly, differences between habitat types in resource quality and availability may influence survival, growth rates, and fecundity. Individuals expanding their range and colonizing new areas also face unique selective environments that have the potential to influence life history evolution (Suarez and Tsutsui 2008; Burton et al. 2010). In addition to high levels of environmental uncertainty, range-expanding individuals experience predation pressure that differs from that of their core ranges, and individuals at the edge of a species' range are predicted to attain sexual maturity at a younger age or a smaller body size than their conspecifics in historical habitats (Burton et al. 2010).

Our study demonstrates that there is significant variation in key life history traits throughout the current geographic distribution of the mangrove tree crab *Aratus pisonii*. In accordance with traditional ecogeographic patterns of body size variation (i.e. Bergmann's Rule), intraspecific body size, including both size at maturity and average size of reproductive females, increased with latitude within historically inhabited mangrove habitats. However, this pattern reversed in mixed marsh and pure marsh sites at the northernmost latitudes of the species' distribution. Female size at maturity occurred at 14.4 mm CW in the representative mangrove habitat (27.55°N), while female size at maturity occurred at just 8.9 mm CW in the representative mixed marsh habitat (29.88°N). On average, gravid females in the marsh at the highest latitudes were just 60% as large as those in the mangroves at lower latitudes.

The pattern described here is an unusual contrast to previously described latitudinal clines in life history traits, and may be explained by differences in abiotic and/or biotic pressures in novel and historical habitats. For instance, the structural complexity of mangroves provides numerous shady microhabitats that are lacking in more structurally simplistic marsh habitats. Forest canopies maintain lower temperature maximums than contiguous forest gaps or grassy habitats (D'Odorico et al. 2013 and references therein), and the temperature profile of red mangrove trees taken in late summer demonstrates that the underside of red mangrove roots is 6.84° C cooler than the top of the roots (Chapperon and Seuront 2011). Thus, individuals with access to the shaded mangrove understory are likely exposed to reduced maximum temperatures than their conspecifics in less structurally complex salt marsh habitats. Consequently, after incorporating habitat-specific differences in temperature within its framework, the temperature-size rule would predict that individuals in the marsh reach maturity at a smaller size than those in the mangroves, while simultaneously predicting an increase in size at maturity with increasing latitude within mangrove habitats.

However, interpretation of the temperature-size rule in this scenario is inherently complicated by habitat-specific differences in resource quality and availability. Mangroves are the dominant vegetation in mangrove habitats, and although *A. pisonii* commonly consume small prey items such as insects and juvenile conspecifics, mangrove leaves are a core component of their diet in native habitats (Erickson et al. 2003). Conversely, mangrove leaves are scarce or entirely absent in novel marsh and mixed marsh sites, forcing individuals to consume an alternative to their native mangrove leaf diet. Additionally, previous work has demonstrated that the amount of animal material in

the diet of this highly omnivorous species is tightly linked to fitness and physiological condition (Riley et al. 2014b). Thus, habitat-specific differences in the availability of small prey items such as insects, juvenile conspecifics, or other crustaceans may influence the strength of the relationship between temperature and body size in these two habitats.

Additionally, our tethering trials indicated that predation pressure on *A. pisonii* was almost twice as high on large individuals than small individuals in the representative mixed marsh habitat. Conversely, there was no evidence of size-specific predation in the representative mangrove habitat. The size-specific predation discrepancy between large and small individuals in novel habitats could also drive the observed decrease in body size and size at reproduction (e.g. Reznick et al. 1990). Genetic analyses, which would provide insight into whether phenotypic differences were a result of plasticity, such as that predicted by the temperature-size rule, or microevolutionary changes, were beyond the scope of the current study. Therefore, because our data is consistent with both of the potential mechanisms explored here, we are currently unable to tease apart the relative influence of these two explanations.

Variation in size at maturity between habitats was also accompanied by differences in the population structure of reproductive females, which clearly contrasted between native and novel habitats. Specifically, the body size distribution of reproductive females was bimodal in the mangroves and unimodal in the mixed marsh habitat. These distributions suggest that two cohorts (each represented by a peak in body size distribution) are reproducing simultaneously in the population from the mangrove habitat, while in the marsh population, either a single cohort is reproducing or reduced growth

increments prevent the identification of discreet size/age classes. From our data, it is not evident whether differences in the size of ovigerous females reflect true differences in body size at a specific age or merely differences in body size at a particular life stage. Unfortunately, a reliable technique to directly determine age in crustaceans does not currently exist (Hartnoll 2001; Vogt 2012). A method of direct aging using growth rings in the eyestalks has recently been proposed for cold-water crustaceans (Kilada et al. 2012), but its validity has been questioned (Roer et al. 2014) and its utility in aging shorter lived, warm-water species such as *A. pisonii* requires further examination (Kilada et al. 2012).

In addition to the observed differences in population structure and female size at reproduction in mangrove and marsh habitats, fecundity increased significantly with maternal body size. Differences in the size of reproductive females in the two habitats therefore resulted in a higher mean nonstandardized fecundity (mean no. eggs/ individual) in native mangrove habitats than novel marsh habitats. Initial maternal investment in individual eggs, as measured by the mean egg volume and egg weight of recently extruded eggs, was not impacted by maternal habitat. However, offspring quality, as measured by larval starvation resistance, was improved in native mangrove habitats compared to novel salt marsh habitats. Because there was no relationship between egg volume or weight and maternal habitat, these differences in starvation resistance likely reflect differences in the quality, but not the quantity, of egg provisioning, which could result from habitat-wide differences in maternal physiological condition or previous reproductive investment. As climate change advances, these

differences in offspring quality may have enduring consequences for the success and persistence of this species in novel habitats.

We have demonstrated that a climate change-induced range expansion leading to a novel plant-animal interaction overrules latitudinal patterns of life history traits, potentially due to differences in predation pressure or environmental conditions in the native and novel habitats. As climate change advances, spatial mismatches between species distributions will continue to reshuffle biological communities, leading to novel communities and unique plant-animal interactions. An understanding of the potential for life history plasticity and/or evolution displayed by species engaged in novel plant-animal interactions, as well as the identification of potential mechanisms underlying these life history changes, is critical for the prediction of key demographic processes. A robust understanding of these demographic processes is essential for proper management of climate-induced novel ecosystems. This will not only aid in the preservation of biodiversity and the maintenance of ecosystem function, it may also benefit our understanding and utilization of global food supplies. Novel communities resulting from decoupled species interactions are already presenting serious challenges to marine policy makers and fisheries resource managers (Hoegh-Guldberg and Bruno 2010). Additionally, altered species distributions of both plant pests and pollinators are capable of severely impacting current agricultural practices (Bebber et al. 2013; González-Varo et al. 2013) and will likely continue to do so (e.g. Crespo-Perez et al. 2015). Thus, ecologists and resource managers should bear in mind the potential for climate change responses to impact both our traditional understanding of ecogeographic processes and

life history characteristics, as these may be important components of climate change mitigation strategies.

3.6 Acknowledgements

This work was partially supported by a SPARC Graduate Research Grant from the Office of the Vice President for Research at the University of South Carolina, a Smithsonian Predoctoral Fellowship, the Slocum-Lunz Foundation, and NSF grant # OCE-1129166.

3.7 Tables

Table 3.1. Description of sites where *A. pisonii* body size distribution surveys were conducted. Field surveys performed by the authors were supplemented by two sites from the literature: [1]Laguna de Tacarigua National Park, Venezuela (Diaz and Conde 1989) and [2]Port Royal Mangroves, Jamaica (Warner 1967).

Survey Site	**Habitat**	**Latitude (°N)**
Laguna de Tacarigua National Park, Venezuela[1]	Mangrove	10.87
Twin Cays, Belize	Mangrove	16.78
Port Royal Mangroves, Jamaica[2]	Mangrove	17.93
Kemp Channel	Mangrove	24.66
Keys Marine Lab	Mangrove	24.83
Biscayne Bay National Park	Mangrove	25.46
Hobe Sound National Wildlife Refuge	Mangrove	27.09
St. Lucie Inlet State Park	Mangrove	27.14
Avalon State Park	Mangrove	27.55
Sebastian Inlet State Park	Mangrove	27.85
Fort Matanzas	Mixed Marsh	29.73
Devil's Elbow	Mixed Marsh	29.75
Anastasia State Park	Mixed Marsh	29.88
Big Talbot Island State Park	Salt Marsh	30.51

3.8 Figures

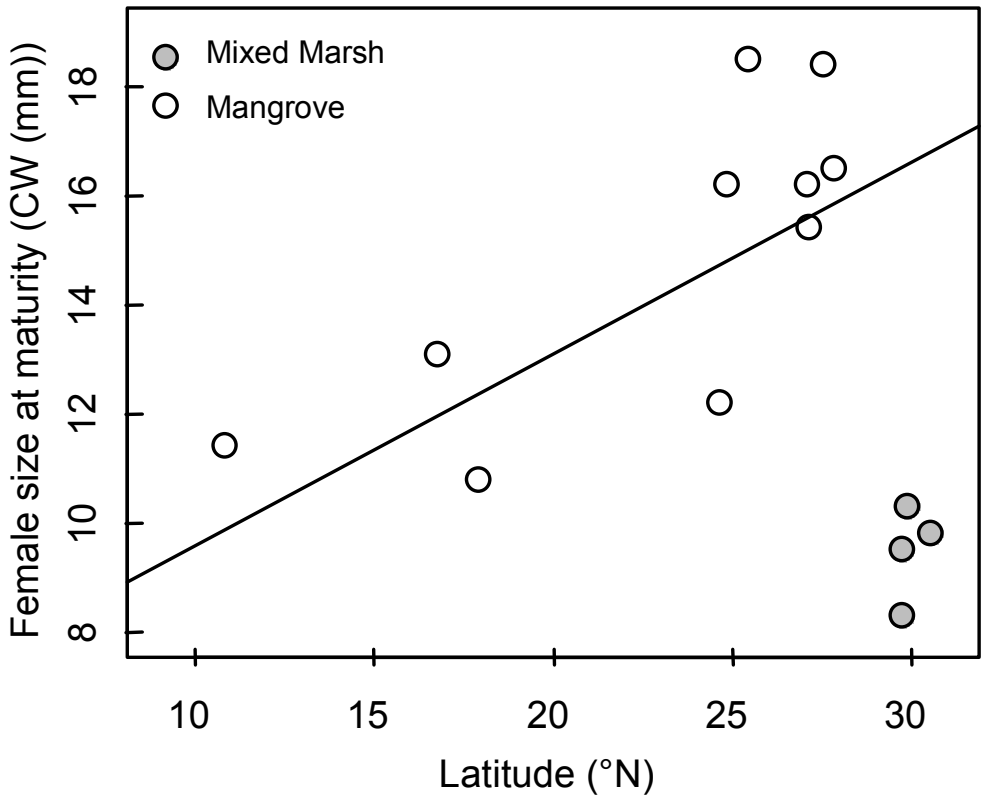

Figure 3.1. Latitudinal trends in female size at maturity in *A. pisonii* in both native and novel habitats.

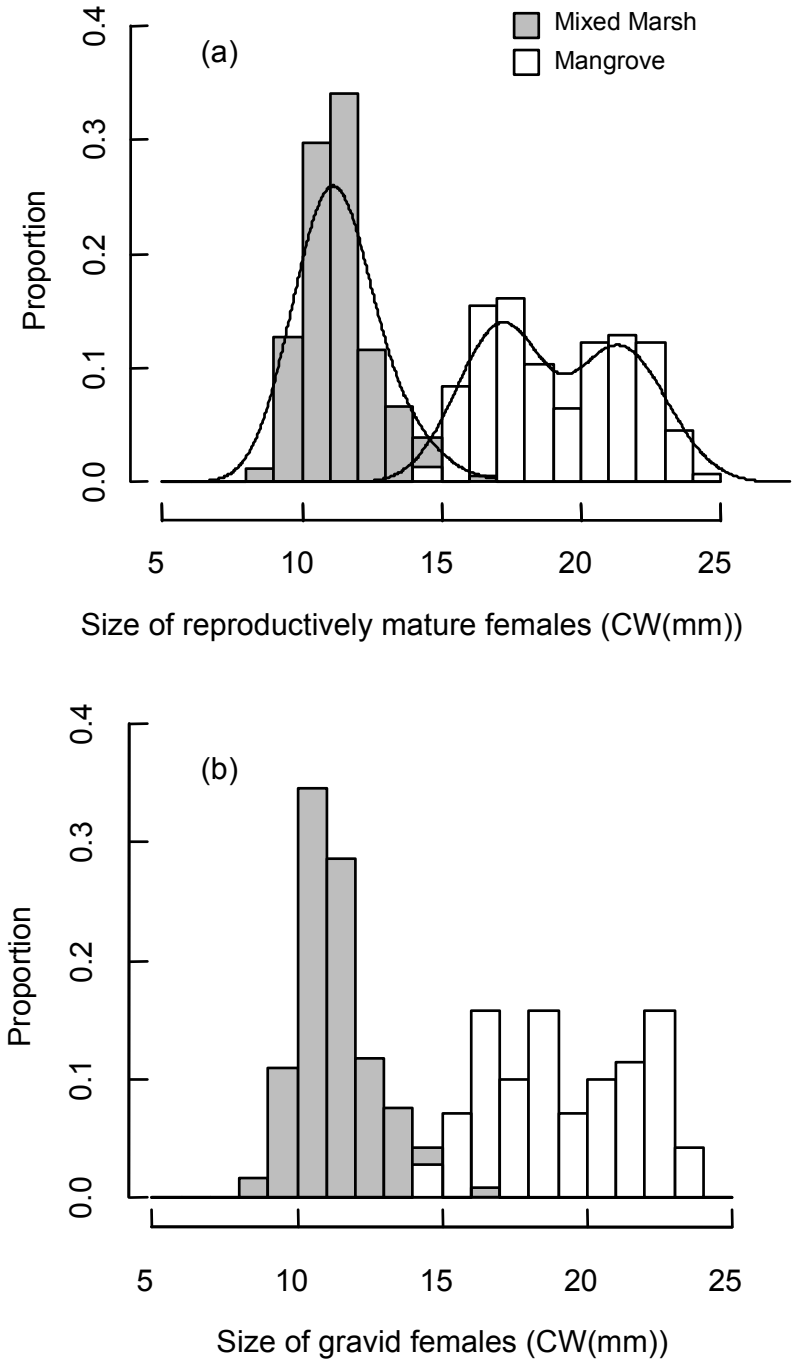

Figure 3.2. Body size distribution of all *A. pisonii* (a) females larger than the site-specific size at maturity and (b) gravid females and from Jun-2013 to Aug-2013. Density curves are included in (a) to highlight modality differences between habitats.

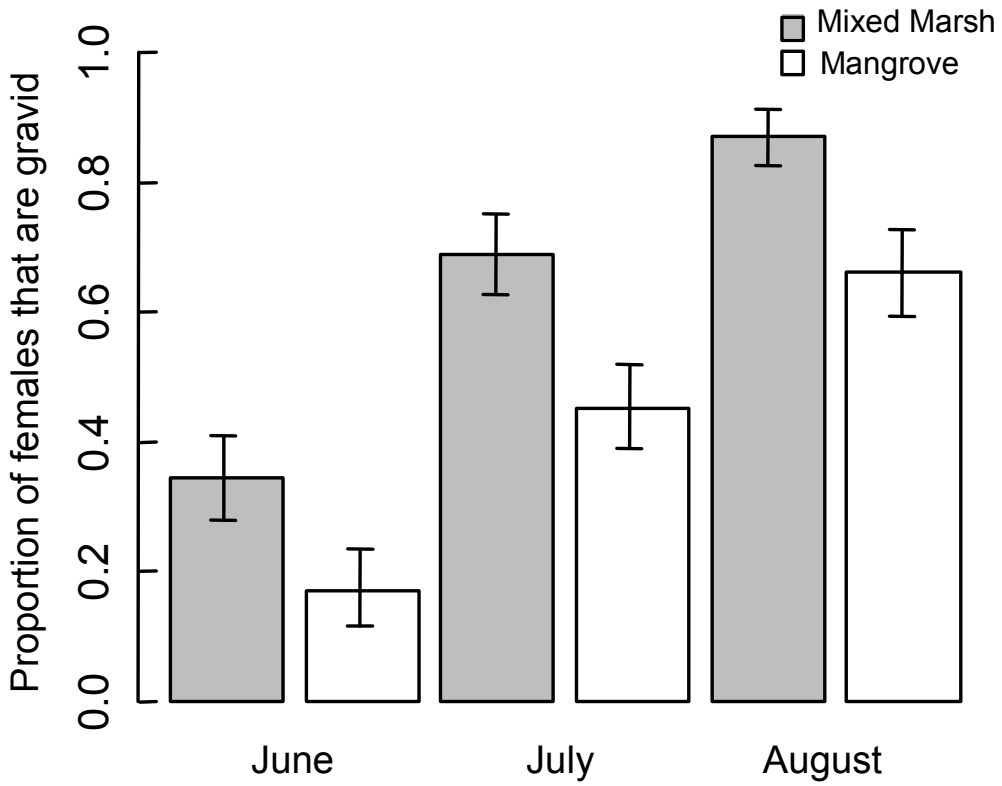

Figure 3.3. Proportion of gravid in each *A. pisonii* population throughout the survey period. Bars represent standard error.

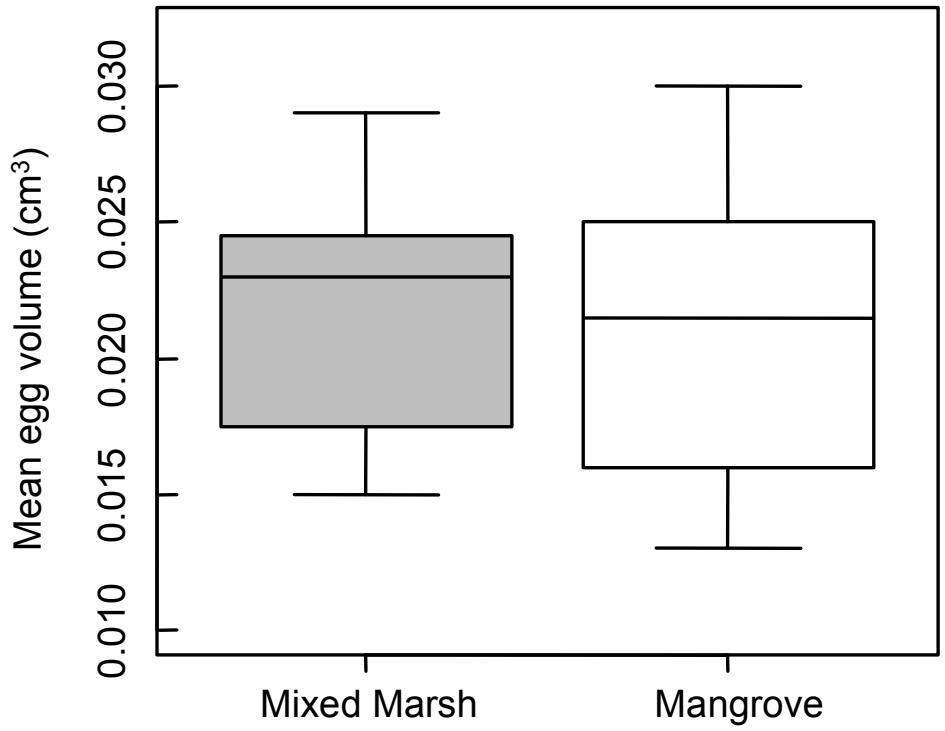

Figure 3.4. Mean *A. pisonii* egg volume (cm^3) of recently extruded, non-eyed eggs. Bars represent standard error.

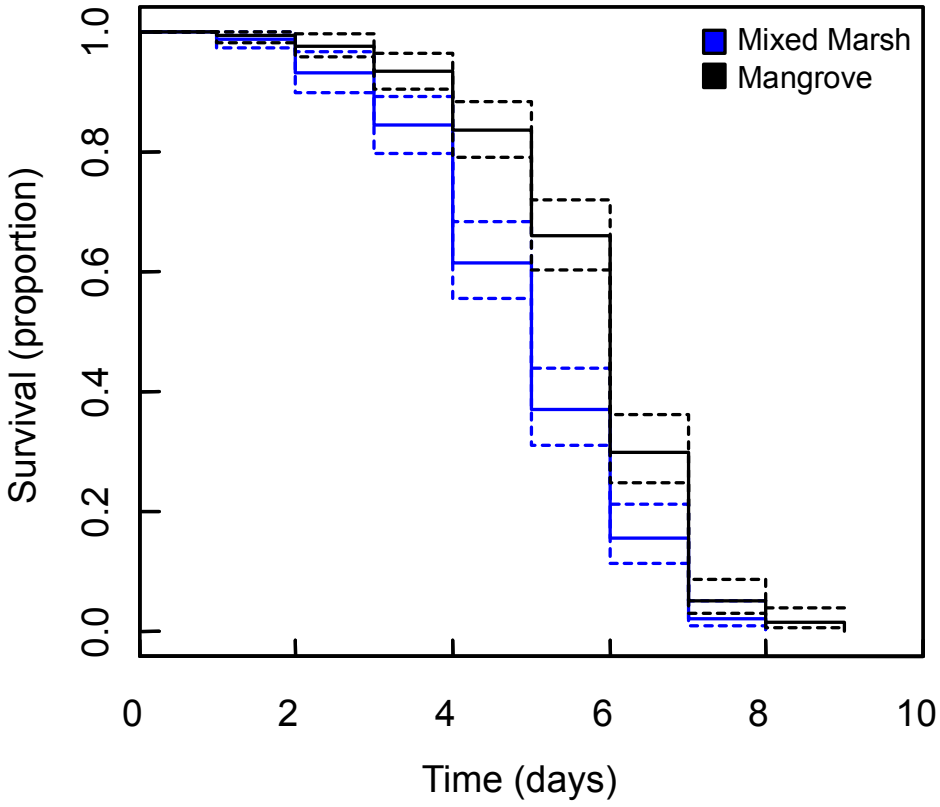

Figure 3.5. Kaplan-Meier curves showing *A. pisonii* larval survival (i.e., starvation resistance in days) as a function of maternal habitat. Solid lines represent mean survival and dashed lines represent 95% confidence intervals.

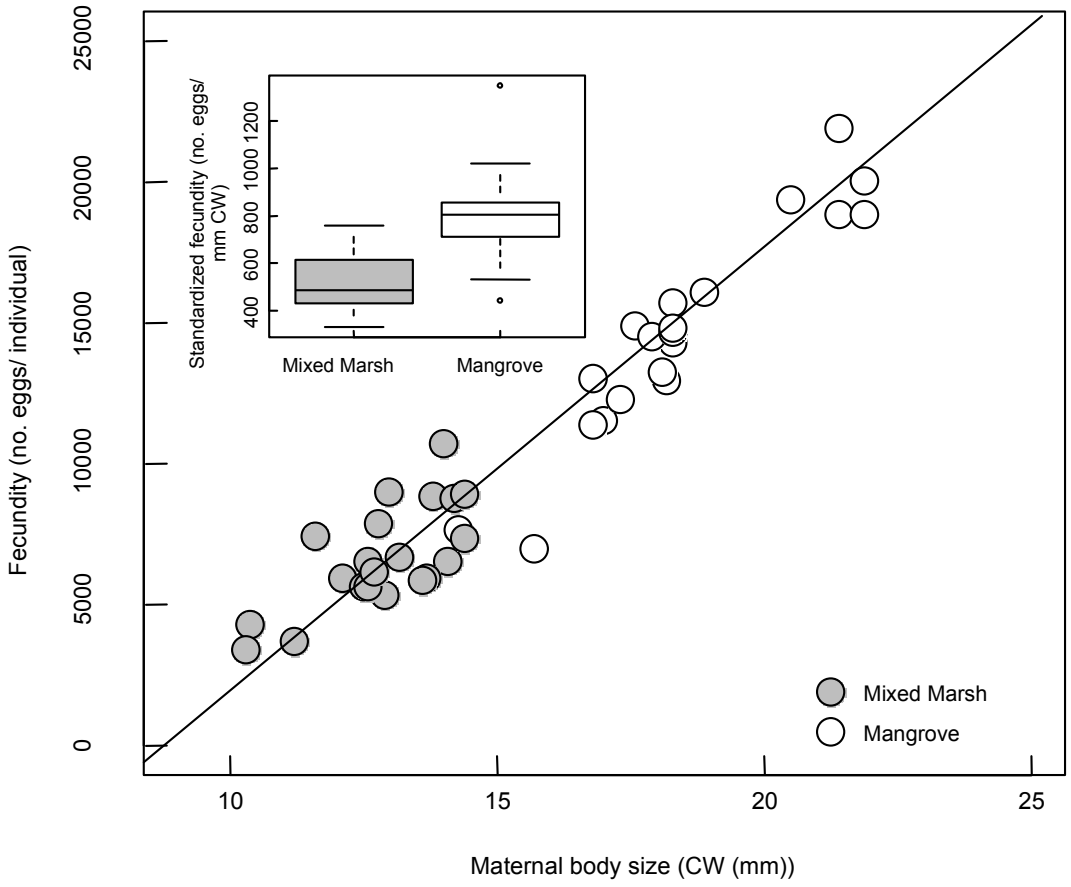

Figure 3.6. Relationship between maternal carapace width (mm) and fecundity (number of eggs) in *A. pisonii.* Inset shows a comparison of standardized fecundity (number of eggs per mm of CW) between habitats.

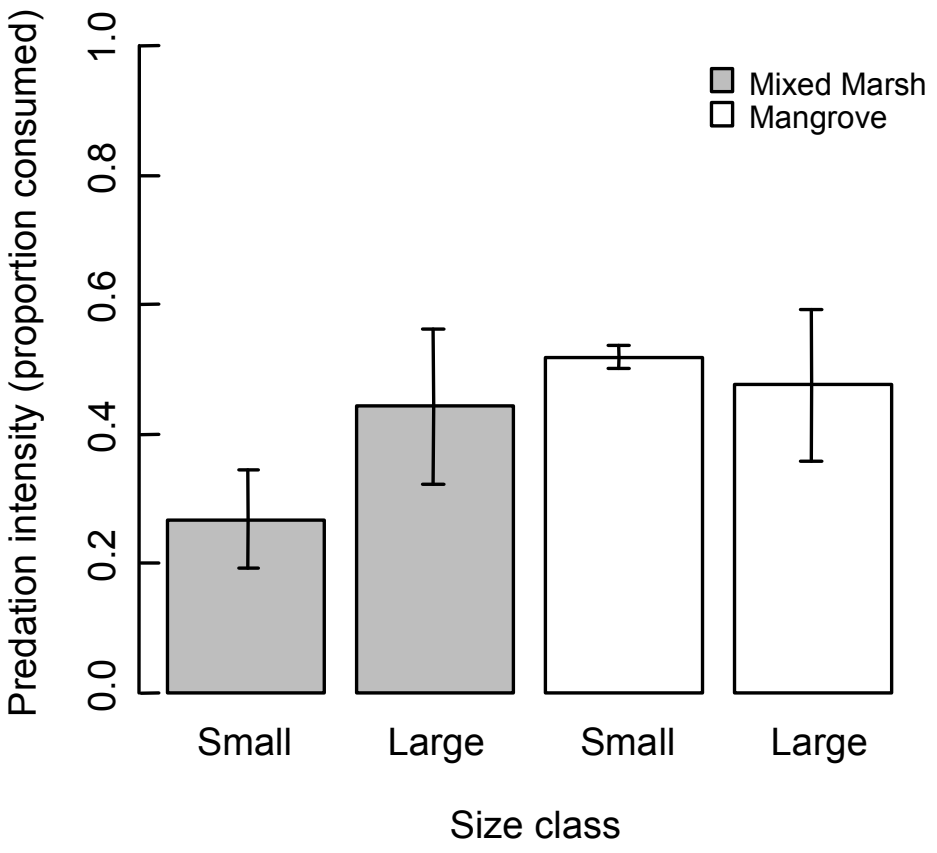

Figure 3.7. Size-specific predation pressure on individuals from native mangrove and novel mixed marsh sites. Bars represent standard error.

CHAPTER 4

GENETIC EVIDENCE SUGGESTS PHENOTYPIC PLASTICITY FACILITATES ALTERNATIVE LIFE HISTORY STRATEGY IN A CLIMATE CHANGE-INDUCED RANGE EXPANSION[3]

[3]Riley M., Rognstad R., Feller I. and B. Griffen. Genetic evidence suggests phenotypic plasticity facilitates alternative life history strategy in a climate change-induced range expansion. *In preparation.*

4.1 Introduction

Range expansions are a common response to changing climate trends, with a wide array of species shifting or expanding their distributions to include higher altitudes and more poleward latitudes (Chen et al. 2011; Poloczanska et al. 2013). Taxonomic differences in the timing and spatial response of organisms to climatic changes cause many species to alter their distributions at different speeds. These mismatches are restructuring fundamental ecological traits such as community composition and species interactions by creating novel ecosystems with unique community assemblages and plant-animal interactions (Parmesan et al. 2006, Lurgi et al. 2012).

Individuals that expand their range into novel habitats face selective pressures that are uniquely different from those experienced by individuals within the species' native habitat (Hardie and Hutchings 2010). For instance, theory suggests that individuals in the heart of a species' distribution, which are usually constrained by high levels of competition with members of their own species, should favor investment in growth prior to reproduction and the production of few, high quality offspring that are able to compete with conspecifics (Burton et al. 2010). Alternatively, individuals colonizing new areas often face high mortality risk from harsh environmental conditions and novel predation threats, as well as relaxed competition with conspecifics. These conditions favor investment in early reproduction (Burton et al. 2010, Phillips et al. 2011). Consequently, theory suggests that individuals at the edge of a species' range should attain sexual maturity at a younger age or a smaller body size than their conspecifics in more favorable habitats. Employing this life history strategy would facilitate the range expansion and

successful establishment of populations at the edge of a species' range (Burton et al. 2010, Phillips et al. 2011).

Although climate-induced range expansions have been widely documented, less research has focused on the life history traits associated with these geographic shifts. In particular, it is unclear whether these geographic shifts are generally facilitated by phenotypic plasticity in life history traits, or whether they are the result of underlying geographic patterns of genetic differentation (e.g. Krehenwinkel and Tautz 2013). In fact, there is limited evidence determining the molecular or plastic mechanisms for all climate change-induced phenotypic modifications (Merila and Hendry 2014). Because of ongoing global climate changes, there is considerable interest in separating the evolutionary and plastic contributions of species' responses to climate change (Gienapp et al. 2008), which is essential before general conclusions and predictions regarding species' responses to future climate scenarios can be made (Merila and Hendry 2014).

Here, we contribute to our understanding of this topic by investigating potential genetic differentiation underlying alternative life history strategies in the climate-induced range expansion of the mangrove tree crab *Aratus pisonii*. This widely distributed invertebrate species has recently expanded its range from historically native mangrove habitats into salt marsh vegetation (Riley et al. 2014a). Its range expansion has been accompanied by significant variation in key life history characteristics, in particular body size and size at maturity. Although the species conforms to the expectations of Bergmann's Rule in native mangrove habitats (body size and size at maturity increases with latitude), this pattern is reversed in novel marsh habitats at the highest latitudes of the species' range. Individuals in novel marsh habitats attain maturity at just 62% the size

of individuals in its native mangrove habitats, and ovigerous females in novel habitats are on average only 60% as large as those in native habitats (Riley et al. *in review*). However, it remains unclear whether this life history variation is the result of phenotypic plasticity, such as that predicted by the temperature-size rule (Horne et al. 2015), or whether there is underlying genetic differentiation between the two habitats that might promote rapid speciation in response to predation pressure (e.g. Reznick et al.1990, Walsh and Reznick 2009) or another selective agent.

Identifying geographic variation in genetic structure within a species' distribution can provide important insight into population connectivity and historical demographics. Understanding geographic patterns of genetic variation in *A. pisonii* will shed insight into the mechanisms underlying the phenotypic differences in native and novel habitats, thus highlighting the potential for current or future ecological speciation resulting from habitat-specific selection pressures. In order to further investigate this topic, we examined geographic variation in two mitochondrial loci (cytochrome oxidase subunit I and control region) from populations (used here to refer to individuals from the same locality) ranging from the center to the northern edge (21.35° latitudinal range) of *A. pisonii*'s distribution, including historically native and novel habitat types.

4.2 Methods

4.2.1 Specimen collection

We identified 11 distinct sampling sites within *A. pisonii*'s current distribution along the western Atlantic coast, where it is found from northern Brazil to Florida (Chace and Hobbs 1969). These sites are spread over 21.35° of latitude (>2300 km) and include

7 native mangrove sites and 4 novel marsh sites that span Central and North America (Table 1, Fig. 1). Sampling sites from the center to the northern edge of the species' range, including native and novel habitats, enabled us to effectively address broad phylogeographic trends as well as potential genetic differentiation between habitat types. At each locality, we randomly collected female *A. pisonii* (n=16-30 per site) above the site-specific size at maturity (established in Riley et al. *in review*) by hand. For three sites (PAN, STL, FTM), we collected whole specimens for transport to the laboratory, dried them completely in a drying oven, then removed a walking leg from each specimen and placed it in 95% ethanol within 6 months. For the remaining sites, we removed a walking leg immediately upon collection in the field, preserved it in 95% ethanol for subsequent DNA extraction and genetic analyses, and released the specimens at the location of capture.

4.2.2 Primer evaluation and design

We identified multiple sets of primers from the literature and evaluated their potential to amplify two regions of *A. pisonii*'s mitochondrial genome: cytochrome oxidase subunit I (COI) and the control region (CR). The use of these two molecular markers in combination allowed us to identify phylogeographic patterns at two distinct evolutionary time points.

We successfully used the primers mtd10 (5'-TTGATTTTTTGGTCA TCCAGAAGT-3') and C/N 2769 (5'-TTAAGTCCTAGAAAATG TTGRGGGA -3') (Gopurenko et al. 1999) to amplify fragments of COI. The PCR reactions consisted of 8.75 μL H_20, 2.5 μL 5X buffer, 0.75 μL $MgCl_2$, 0.25 μL dNTPs, 0.125 μL of each primer,

0.1 μL Taq, and 1 μL of DNA template. Thermocycling conditions for amplification of COI included an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles at 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72 °C for 5 minutes. We also successfully used the primers dlussaf1 (5'-GTATAACCGCGAATGCTGGCAC-3') and ileucar2 (5' – CCTTTTAAATCAGGCACTATA -3') (Oliveira-Neto et al. 2007) to amplify fragments of the control region. The PCR reactions consisted of 5 μL Apex Taq Master Mix Red, 0.25 μL of each primer, 3 μL of H_20, and 1.5 μL of DNA template. Thermocycling conditions for amplification of the control region included an initial denaturation step at 95°C for 5 minutes, followed by 40 cycles at 92°C for 30 seconds, 53°C for 30 seconds, and 68°C for 30 seconds, with a final extension at 68°C for 2 minutes.

For a select number of samples from varying sites, PCR product from the COI (n=8) and control region (n=5) primer sets was cleaned using an ExoSap protocol and sent to Functional Biosciences for bidirectional sequencing to ensure that they produced high-quality sequencing results. These sequencing efforts were successful for both primer sets. Due to the large size of the control region amplicon (~900 bp), we subsequently elected to redesign species-specific primers for this locus using the trimmed consensus sequence of the high-quality reads provided by initial sequencing with the primers dlussaf1 and ileucar2. We used Primer3 web v4.0.0 (Koressaar and Remm 2007, Untergrasser et al. 2012) to design primers with the following requirements: primer size of 18-26 base pairs, melting temperature between 50 and 62 C, and a 301-700 base pair product. This yielded 4 possible primer pairs, from which we elected to evaluate a primer set designed to yield a 485 bp fragment. We successfully amplified DNA using this

primer set: forward primer APMitoCtrl_L3 (5'-GTTTCAGCCAAAATTAAACA-3') and reverse primer APMitoCtrl_R3 (5'- TCAGCTAGAGTAAAAGTTCT-3'). This primer set was used in all subsequent PCR reactions for the control region locus. Thermocycling conditions included an initial denaturation step at 94°C for 3 minutes, followed by 40 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 5 minutes.

4.2.3 DNA extraction/ amplification and sequencing

We removed 500 mg of muscle tissue from the preserved walking leg of each specimen and extracted total genomic DNA using the Promega Wizard SV 96 Genomic DNA Purification System or the Qiagen DNeasy Blood and Tissue Kit. We then sent extracted DNA to Functional Biosciences for PCR following the optimized conditions detailed previously, PCR cleanup, and unidirectional sequencing of COI using the primer mtd10 and of the control region using the primer APMitoCtrl_R3. From the resulting sequences, 51 sequences (representing all unique control region haplotypes) were resequenced using the primer APMitoCtrl_L3 to address potential sequencing error and assess haplotype veracity. All 51 of these resequenced samples confirmed the original haplotypes.

4.2.4 Data analysis

Sequences were aligned using the MUSCLE (Edgar 2004) algorithm as implemented in MEGA6 (Tamura et al. 2013). Regions of poor sequencing quality were omitted, yielding 510 usable basepairs for the COI locus and 449 usable basepairs for the

control region locus. Alignments were imported into Arlequin (version 3.5, Excoffier and Lischer 2010), which was used to calculate standard diversity indices including the number of haplotypes, haplotype diversity, nucleotide diversity, and the average number of pairwise differences between haplotypes. Additionally, to assess genetic differentiation between sites, we calculated pairwise F_{ST} values, which were calculated with 10,000 permutations at a significance level of α=0.05. We applied a Bonferroni correction to correct for multiple comparisons, yielding an adjusted significance level of α= 0.0009.

Because the data violated the assumption of linearity for a Mantel test (Mantel 1967), we used a Mantel correlogram to examine patterns of isolation-by-distance. The Mantel correlogram is an extension of the Mantel test that examines the relationship between genetic and geographic distance across space, and is therefore appropriate for nonlinear data (Diniz-Filho et al. 2013). The Mantel correlogram was implemented in the "ecodist" package (Goslee and Urban 2007) in R with 5 distance classes and 10,000 replicate simulations. The F_{ST} matrix was used for genetic distances, and geographic distances between sites were calculated using latitude and longitude coordinates in the "fields" package (Fields Development Team) in R. Because all Florida mainland sites were located close to the open ocean (mean ± SE= 4.97 ± 1.39 km) and larvae may follow any number of pathways between the more geographically isolated sites depending on the currents, we elected to use the minimum geographic distance between sites. Because of the large geographic range covered in this study (21.35° latitude, >2300 km), this was unlikely to impact our ability to detect broad scale patterns of isolation-by-distance.

Additionally, we estimated genetic differentiation within and among populations with an analysis of molecular variance (AMOVA, Excoffier et al. 1992) using pairwise differences as a measure of divergence. Finally, we calculated Tajima's D for selective neutrality (Tajima 1989) with 10,000 permutations to investigate historical demography. A significantly negative Tajima's D-value, which indicates the presence of rare alleles at low frequencies, provides evidence of a recent population expansion or bottleneck event (Aris-Brosou and Excoffier 1996).

4.3 Results

4.3.1 Cytochrome Oxidase I

COI sequencing yielded 16 haplotypes, of which 10 were unique haplotypes (Table 2, Fig. 2). The average haplotype diversity was highest at the southernmost mangrove site, PAN (mean ± SD = 0.554 ± 0.052), which is located near the center of the species' range. There was a marginally significant decrease in haplotype diversity with increasing latitude (LM, mean ± SE = -0.016 ± 0.007, R^2=0.263, P=0.06). The lowest haplotype diversity occurred near the northernmost edge of the species distribution, in the marsh site ANA (mean ± SD = 0.000 ± 0.000), where only a single haplotype was present (Table 2, Fig. 2). Mean pairwise differences followed a similar trend (LM, mean ± SE = -0.016 ± 0.009, R^2=0.190, P=0.101). The highest mean pairwise differences occurred at the mangrove site STL (mean ± SD= 0.625 ± 0.517) and the southernmost mangrove site PAN (mean ± SD= 0.591 ± 0.490). They were lowest at the marsh sites ANA (mean ± SD = 0.000 ± 0.000) and SIS (mean ± SD= 0.143 ± 0.213) (Table 2). Likewise,

nucleotide diversity followed a trend of decreasing diversity with increasing latitude (LM, mean ± SE = $-3.18 \times 10^{-5} \pm 1.74 \times 10^{-5}$, R^2=0.188, P=0.102). Nucleotide diversity was highest at STL (mean ± SD = 0.001 ± 0.001) and PAN (mean ± SD = 0.001 ± 0.001), and nucleotide diversity was lowest at ANA (mean ± SD = 0.000 ± 0.000) and SIS (mean ± SD = 0.0003 ± 0.0005) (Table 2).

Pairwise comparison of F_{ST} demonstrated that there is significant genetic differentiation between many of the sampling sites. The two southernmost sites, Panama and Belize, were significantly genetically differentiated from each other and all 9 of the sites in Florida. Although the sites in Panama (PAN) and Belize (BZE) were significantly differentiated from each other, they were more closely related to each other (F_{ST}=0.282) than any other sites. They were both highly genetically dissimilar to all sites in Florida (PAN F_{ST} range=0.0.865to 0.939; BZE F_{ST} range= 0.894 to 0.966) (Table 3, Fig. 3). Additionally, the single Florida Keys site (CUR) was significantly genetically differentiated from the sites in Panama and Belize. CUR was not significantly genetically differentiated from the closest Florida mainland site (OLE) but it was significantly genetically differentiated from the rest of the Florida mainland sites (F_{ST} range= 0.294 to 0.675) (Table 3, Fig. 3). Within the eight Florida mainland sites, of which four were mangrove sites and four were marsh sites, there was no evidence of genetic differentiation based on pairwise comparisons of F_{ST} (P>0.009) (Table 3).

The Mantel correlogram demonstrated that this genetic differentiation (F_{ST}) follows a pattern of isolation by distance (Fig. 4). Specifically, populations separated by 236 km were highly genetically similar (Mantel r=0.698, P=0.0001), and genetic similarity decreased with increasing geographic distance (Fig. 5). Populations separated

by 709 km were slightly genetically similar (Mantel r=0.087, P=0.007), while those separated by 1181 km (Mantel r= -0.361, P=0.0001), 1654 km Mantel (Mantel r= -0.361, P=0.0001), and 2127 km (Mantel r= -0.374, P=0.0001) were genetically dissimilar.

Additionally, The AMOVA indicated that 81.23% of the genetic variation existed among populations, with 18.77% occurring within populations (F_{ST}=0.812, P<0.001) (Table 4). Significant negative Tajima's D values were calculated for the mangrove sites BZE (D= -1.89, P=0.005), STL (D= -1.93, P=0.01), and AVA (D= -1.51, P=0.041), as well as the marsh sites FTM (D= -1.51, P=0.041), SIS (D= -1.511, P=0.042), and BIG (D= -1.732, P=0.015) (Table 2), indicating the presence of rare alleles at low frequencies.

4.3.2 Control Region

Sequencing of the control region yielded a total of 66 haplotypes, of which 51 were unique haplotypes (Table 2, Fig. 66). The Florida Keys site CUR had the highest mean haplotype diversity (mean ± SD= 0.786 ± 0.054), followed closely by the Florida mangrove site STL (mean ± SD= 0.775 ± 0.088) and the southernmost site (PAN) (mean ± SD= 0.774 ± 0.071). There was a marginally significant decrease in haplotype diversity with increasing latitude (LM, mean ± SE = -0.009 ± 0.005, R^2=0.229, P=0.077), with the lowest haplotype diversity occurring near the northernmost edge of the species distribution, at the marsh site FTM (mean ± SD= 0.476 ± 0.155) (Table 2, Fig. 6). Mean pairwise differences decreased significantly with increasing latitude (LM, mean ± SE = -0.269 ± 0.045, R^2=0.777 P<0.001), with the most pairwise differences at the two southernmost mangrove sites, PAN (mean ± SD= 7.20 ± 3.52) and BZE (mean ± SD= 8.29 ± 3.97). The fewest pairwise differences occurred at the marsh sites FTM (mean ±

SD= 2.34 ± 1.35) and ANA (mean ± SD = 2.06 ± 1.19) (Table 2). Likewise, nucleotide diversity decreased significantly with increasing latitude (LM, mean ± SE = $-5.97 \times 10^{-4} \pm 1.08 \times 10^{-4}$, R^2=0.748, P<0.001). The highest nucleotide diversity occurred at the two southernmost mangrove sites, PAN (mean ± SD = 0.016 ± 0.009) and BZE (mean ± SD = 0.019 ± 0.010), and the lowest at the marsh sites FTM (mean ± SD = 0.006 ± 0.004) and ANA (mean ± SD = 0.005 ± 0.003) (Table 2).

Pairwise comparison of F_{ST} demonstrated that there is significant genetic differentiation between many of the sampling sites. The two southernmost sites, Panama and Belize, were significantly genetically differentiated from each other and all 9 of the sites in Florida. Additionally, the single Florida Keys site (CUR) was significantly genetically differentiated from the sites in Panama, Belize, as well as all of the Florida mainland sites (Table 3, Fig. 7). Belize and Panama were more closely related to each other (F_{ST}=0.361) than any of the Florida sites (PAN F_{ST} range=0.614 to 0.717; BZE F_{ST} range= 0.632 to 0.733) (Table 3, Fig. 7). Within the nine Florida sites, of which five were mangrove sites and four were marsh sites, there was no evidence of genetic differentiation based on pairwise comparisons of F_{ST} (P>0.009) (Table 3, Fig. 7).

The Mantel correlogram demonstrated that this genetic differentiation (F_{ST}) follows a pattern of isolation by distance (Fig. 8). Specifically, populations separated by 236 km were highly genetically similar (Mantel r=0.743, P=0.0001), and genetic similarity decreased with increasing geographic distance (Fig. 9). Populations separated by 709 km were slightly genetically similar (Mantel r=0.074, P=0.022), while those separated by 1181 km (Mantel r= -0.389, P=0.0001), 1654 km Mantel (Mantel r= -0.380, P=0.0001), and 2127 km (Mantel r= -0.379, P=0.0001) were genetically dissimilar.

The AMOVA indicated that 49.00% of the genetic variation existed among populations, with 51.00% occurring within populations (F_{ST}=0.490, P<0.001) (Table 4). Significant negative Tajima's D values were calculated for BZE (D= -1.61, P=0.039), PAN (D= -2.33, P=0.001), and all of the Florida sites except CUR and OLE (Table 2), indicating the presence of rare alleles at low frequencies at these localities.

4.4 Discussion

The mangrove tree crab *A. pisonii* displayed considerable genetic differentiation throughout the broad geographic range investigated in this study. Although differences in the partitioning of genetic variation (AMOVA) confirmed that the COI and control region loci were representative of different evolutionary time points, the phylogeographic patterns of genetic divergence identified here were consistent for both loci. Analysis of these mitochondrial loci demonstrated that *A. pisonii* populations sampled from native mangrove habitats in Panama, Belize, and the Florida Keys were distinct from each other. Additionally, populations from these three sites were highly genetically dissimilar from the eight sites on the Florida mainland, with the exception of the southernmost site on the Florida mainland, where the more variable control region was not genetically distinct from the Florida mainland. Significant F_{ST} values greater than 0.25 represent pronounced levels of genetic differentiation (Freeland et al. 2011); therefore, the F_{ST} values reported here (F_{ST} ranged from 0.282 to 0.966 for COI and 0.361 to 0.733 for the control region) indicate a marked level of genetic substructure that was previously unrecognized in this species.

The genetic differentiation demonstrated by both loci was characterized by a pattern of isolation-by-distance (Wright 1943). Populations separated by ~236 km were highly genetically similar, while those separated by ~1181 km were genetically dissimilar. Isolation-by-distance theory predicts that due to limited dispersal, the genetic distance between organisms increases with geographic distance, resulting in a clear geographic pattern of genetic structure (Wright 1943). Traditionally, the planktonic larval phase employed by many semi-terrestrial and marine organisms has been associated with large dispersal potential and high population connectivity that might obscure patterns of isolation-by-distance (e.g. Oliveira-Neto et al. 2007). However, more recent evidence suggests that a number of invertebrates with a planktonic phase demonstrate substantial levels of genetic substructure over a large spatial scale (e.g. Domingues et al. 2010). Over a broad geographic range, the genetic structure of *A. pisonii* was consistent with this pattern of isolation-by-distance, likely due to dispersal limitations that prevent gene flow between the more geographically isolated sites.

Intriguingly, the only consistent evidence of genetic differentiation among populations in Florida was between the Florida Keys and the Florida mainland. This differentiation is likely a result of a combination of historical vicariance and contemporary oceanic currents. Together, these factors have resulted in concordant patterns of genetic differentiation between populations in the Gulf of Mexico and Florida Atlantic coast for multiple taxa (Avise 1992). However, despite the phylogeographic patterns established for the species over a broad geographic range, there was no evidence of genetic differentiation within the Florida mainland sites, which included four native mangrove habitats and four novel marsh habitats. The lack of genetic substructure within

this region is particularly interesting considering the placement of the sampling sites within Florida. These sites span an established phylogeographic barrier near Cape Canaveral (Avise 1992, Pelc et al. 2009), with native mangrove habitats in a subtropical setting to the south and novel marsh habitats in a temperate setting to the north. The lack of genetic differentiation between sites is a strong indication of high gene flow between these two habitat types, which should restrict the potential for local adaptation in novel habitats (Gaston 2009). This in turn suggests that phenotypic plasticity, rather than genetic differentiation, underlies the strong reversal in ecogeographic life history trends associated with the species' range expansion into novel marsh habitats.

The mangrove tree crab has previously been described as notoriously plastic in regard to its body size and size at maturity (Conde et al. 1992). This plasticity appears to be facilitating its ongoing range expansion into novel salt marsh habitats, which are characterized by biotic and abiotic conditions distinct from those in their native mangrove habitats. As described previously, *A. pisonii*'s decrease in body size and size at maturity in marsh habitats at the northern edge of its range are consistent with patterns of size-selective predation pressure and the temperature-size rule (Riley et al. *in review*). Alternatively, these life history patterns are also consistent with theoretical life history predictions for range expansion scenarios, in which relaxed competition with conspecifics favors investment in reproduction at a younger age or smaller body size (Burton et al. 2010, Phillips et al. 2011). Thus, habitat-specific differences in population density may play a role in these life history patterns.

In addition to similar patterns of genetic differentiation, both loci demonstrated consistent trends for the molecular diversity indices investigated here. Haplotype

diversity, mean pairwise distances, and nucleotide diversity all decreased significantly or marginally ($P<0.001$ to $P=0.102$) as latitude increased for both COI and the control region. Our sampling locations from Panama to northern Florida spanned from the approximate center of the species' distribution to near the northern edge of its current range (Chace and Hobbs 1969, Riley et al. 2014a). Therefore, these trends correspond to increased genetic diversity at the center of *A. pisonii*'s range relative to its edge. This "central-margin" decline in diversity is generally seen in species whose density decreases from the center to the edge of its range (Vucetich and Waite 2003), and is supported in 64% of studies that have examined central-margin patterns of genetic diversity (Eckert et al. 2008). Genetic variation is the "raw material for evolution by natural selection" (Fisher 1930 in Hughes et al. 2008). Consequently, elevated genetic diversity is associated with increased evolutionary potential. Additionally, sufficient levels of genetic diversity within a population play an important role in disturbance responses (Hughes et al. 2008). Although the fitness consequences of genetic diversity should be interpreted with caution (Avise 2004), decreased levels of genetic diversity in novel habitats near the edge of *A. pisonii*'s range likely decrease the evolutionary potential of edge populations, as well as limit their ability to recover from disturbance events.

Unlike metrics of genetic differentiation and molecular diversity, COI and the control region yielded slightly different patterns for Tajima's D. These inconsistencies are likely reflective of historical demographics at slightly deeper (COI) and shallower (control region) evolutionary time points. For the COI locus, significant negative values of Tajima's D were calculated for the site in Belize as well as two mangrove sites and three marsh sites on the Florida mainland. For the control region locus, significant

negative values of Tajima's D were calculated for all sites except for the Florida Keys site and the southernmost mangrove site on the Florida mainland. Significant negative Tajima's D values indicate the presence of rare alleles at low frequencies, and provide evidence of recent population expansions or bottleneck events (Aris-Brosou and Excoffier 1996). These patterns could be the result of a number of past demographic events, such as population growth associated with a range expansion, or recovery from a disturbance episode. For instance, neotropical mangroves experience frequent disturbance episodes in the form of hurricanes, which can cause major structural damage and tree mortality (Baldwin et al. 2001 and references therein) that may be restricted to local areas. Alternatively, land-use changes such as coastal urbanization and wetlands impoundment can also have major impacts on coastal habitats (De Freese 1991). These disturbances may in turn affect the organisms residing in these habitats, such as *A. pisonii*.

Collectively, the data presented in this study confirms the presence of genetic substructure throughout *A. pisonii*'s range and demonstrates patterns of genetic differentiation and isolation-by-distance over a broad geographic scale. Additionally, it reveals a lack of genetic differentiation at the site of *A. pisoni'*s recent and ongoing range expansion into novel habitats. This suggests that phenotypic plasticity, rather than local adaptation or incipient speciation, is the mechanism underlying the life history changes associated with the species' range expansion. This work provides important empirical evidence of the mechanism underlying a climate change-induced range expansion, which is one of the most prevalent responses to recent global climate change (Chen et al. 2011; Poloczanska et al. 2013). Additionally, it highlights the potential importance of

phenotypic plasticity in facilitating species' responses to novel environmental conditions, particularly at the edge of their range. Like *A. pisonii,* the majority of species display decreased genetic diversity at the range edge relative to their center (Eckert et al. 2008). Because this may hinder their potential for local adaptation, phenotypic plasticity may provide large fitness benefits to highly plastic species under changing environmental conditions. Consequently, studies such as this are essential for understanding the relative roles of evolution and plasticity in species' responses to fluctuating environmental conditions, which will aid in an improved understanding of the impacts of current and future global climate changes (Gienapp et al. 2008, Merila and Hendry 2014).

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4.6 Tables

Table 4.1. Details of sites sampled in the study, including full name, habitat, and latitude/longitude.

Site	Full Site Name	Habitat	Latitude	Longitude
PAN	Dead Cow Creek, Panama	Mangrove	9.162	-82.152
BZE	Twin Cays, Belize	Mangrove	16.828	-88.101
CUR	Curry Hammock State Park, FL	Mangrove	24.744	-80.981
OLE	Oleta River State Park, FL	Mangrove	25.914	-80.131
STL	St. Lucie Inlet State Park	Mangrove	27.163	-80.168
AVA	Avalon State Park, FL	Mangrove	27.550	-80.327
SEB	Sebastian Inlet State Park, FL	Mangrove	27.851	-80.451
FTM	Ft. Matanzas North, FL	Marsh	29.727	-81.245
ANA	Anastasia State Park, FL	Marsh	29.873	-81.275
SIS	Sister's Creek, FL	Marsh	30.399	-81.461
BIG	Big Talbot Island State Park, FL	Marsh	30.511	-81.460

Table 4.2. COI and CR molecular diversity indices for each locality, including sample size (N), number of haplotypes (H), number of polymorphic sites (S), haplotype diversity (Hd), mean number of pairwise distances (K), nucleotide diversity (π), and Tajima's D.

	Locality	*N*	*H*	*S*	*Hd*	*K*	*π*	Tajima's *D*	Tajima's *D* *(p-val)*
COI	PAN	24	3	2	0.5543 ± 0.0525	0.590580 ± 0.490215	0.001163 ± 0.001076	0.22583	0.6863
	BZE	29	5	4	0.2611 ± 0.1063	0.275862 ± 0.306917	0.000543 ± 0.000672	**-1.88946**	0.0054
	CUR	29	2	1	0.4433 ± 0.0685	0.443350 ± 0.407074	0.000873 ± 0.000892	1.16806	0.9004
	OLE	29	2	1	0.3793 ± 0.0842	0.379310 ± 0.370256	0.000747 ± 0.000811	0.77169	0.8493
	STL	16	5	5	0.4500 ± 0.1507	0.625000 ± 0.517175	0.001233 ± 0.001143	**-1.9286**	0.0095
	AVA	27	3	2	0.1453 ± 0.0898	0.148148 ± 0.217096	0.000292 ± 0.000476	**-1.51197**	0.0406
	SEB	30	3	2	0.4667 ± 0.0873	0.498851 ± 0.437533	0.000984 ± 0.000960	-0.0243	0.4631
	FTM	27	3	2	0.1453 ± 0.0898	0.148148 ± 0.217096	0.000292 ± 0.000476	**-1.51197**	0.0404
	ANA	28	1	0	0.0000 ± 0.0000	0.000000 ± 0.000000	0.000000 ± 0.000000	0.0000	1
	SIS	28	3	2	0.1402 ± 0.0871	0.142857 ± 0.212616	0.000281 ± 0.000466	**-1.5106**	0.0416
	BIG	30	4	3	0.1931 ± 0.0951	0.200000 ± 0.255436	0.000394 ± 0.000560	**-1.73178**	0.0148
CR	PAN	20	8	39	0.7737 ± 0.0714	7.200000 ± 3.522370	0.016253 ± 0.008880	**-1.60825**	0.0388
	BZE	25	12	36	0.6967 ± 0.1047	8.286667 ± 3.972777	0.019227 ± 0.010272	**-2.33055**	0.0011
	CUR	28	8	16	0.7857 ± 0.0544	4.330688 ± 2.208054	0.010410 ± 0.005910	-0.22112	0.4558
	OLE	29	9	17	0.6527 ± 0.0972	3.807882 ± 1.973301	0.009088 ± 0.005242	-1.38338	0.0756
	STL	16	7	14	0.7750 ± 0.0876	3.341667 ± 1.809008	0.008033 ± 0.004871	**-1.63027**	0.0396
	AVA	29	9	18	0.5739 ± 0.1081	3.000000 ± 1.612575	0.007143 ± 0.004274	**-1.85666**	0.0151
	SEB	29	14	26	0.7685 ± 0.0824	4.625616 ± 2.336570	0.011040 ± 0.006207	**-1.88738**	0.0132
	FTM	15	5	13	0.4762 ± 0.1545	2.342857 ± 1.353773	0.005565 ± 0.003605	**-1.76624**	0.0249
	ANA	27	9	12	0.5128 ± 0.1180	2.062678 ± 1.192150	0.004911 ± 0.003161	**-2.0204**	0.0042
	SIS	26	9	14	0.5785 ± 0.1144	2.713846 ± 1.489169	0.006492 ± 0.003969	**-1.79652**	0.0192
	BIG	30	8	21	0.5908 ± 0.0998	2.912644 ± 1.571865	0.006935 ± 0.004165	**-2.2721**	0.0014

Table 4.3. Matrix showing pairwise F_{ST} comparisons for COI (above diagonal) and control region (below diagonal). Bold, italicized F_{ST} values in blue indicate significance after Bonferroni Correction (P<0.0009). Italicized values indicate significance at non-adjusted significance level (P<0.05).

	Mangrove							Marsh			
	PAN	BZE	CUR	OLE	STL	AVA	SEB	FTM	ANA	SIS	BIG
PAN		***0.28213***	***0.8645***	***0.8872***	***0.86986***	***0.91991***	***0.87417***	***0.91997***	***0.93877***	***0.92266***	***0.91662***
BZE	***0.36146***		***0.89359***	***0.91441***	***0.90552***	***0.94734***	***0.90028***	***0.94734***	***0.96551***	***0.94916***	***0.94215***
CUR	***0.61389***	***0.63155***		*0.31227*	***0.43135***	***0.58032***	***0.29432***	***0.58032***	***0.6745***	***0.61259***	***0.56917***
OLE	***0.6499***	***0.67837***	***0.22223***		0.05116	*0.11124*	-0.02424	*0.11124*	*0.2105*	*0.16383*	*0.11036*
STL	***0.63865***	***0.67078***	***0.35373***	0.04581		0.0049	0.02686	-0.00951	*0.03697*	0.02055	0.00626
AVA	***0.67692***	***0.70342***	***0.34416***	0.00017	0.02918		*0.08923*	-0.01887	0.00137	-0.0185	-0.01484
SEB	***0.62158***	***0.65634***	***0.19359***	-0.02296	0.04273	0.01017		0.07676	*0.16208*	*0.13288*	*0.09109*
FTM	***0.66713***	***0.68602***	***0.35827***	0.00188	0.06834	-0.02506	0.02197		0.00137	0.00002	-0.01484
ANA	***0.71717***	***0.73271***	***0.43343***	0.04201	*0.08294*	-0.01004	*0.05123*	-0.01791		0	-0.00235
SIS	***0.69442***	***0.7166***	***0.40818***	0.0371	0.0142	-0.00875	0.04414	0.00774	-0.0098		-0.0004
BIG	***0.69197***	***0.71541***	***0.37403***	0.01503	0.0372	-0.02142	0.02246	-0.01931	-0.01679	-0.01471	

Table 4.4. Analysis of molecular variance (AMOVA) comparing variation among and within populations for both COI and the control region (CR)

	Source of variation	df	Sum of squares	Variance components	% of variation	Fixation Index
COI	Among populations	10	175.874	0.64715	81.23	
	Within populations	286	42.772	0.14955	18.77	F_{ST}= 0.81229
	Total	296	218.646	0.79671	P=0.000	
CR	Among populations	10	502.781	1.94554	49.00	
	Within populations	263	532.46	2.02456	51.00	F_{ST}=0.49005
	Total	273	1035.241	3.9701	P=0.000	

4.7 Figures

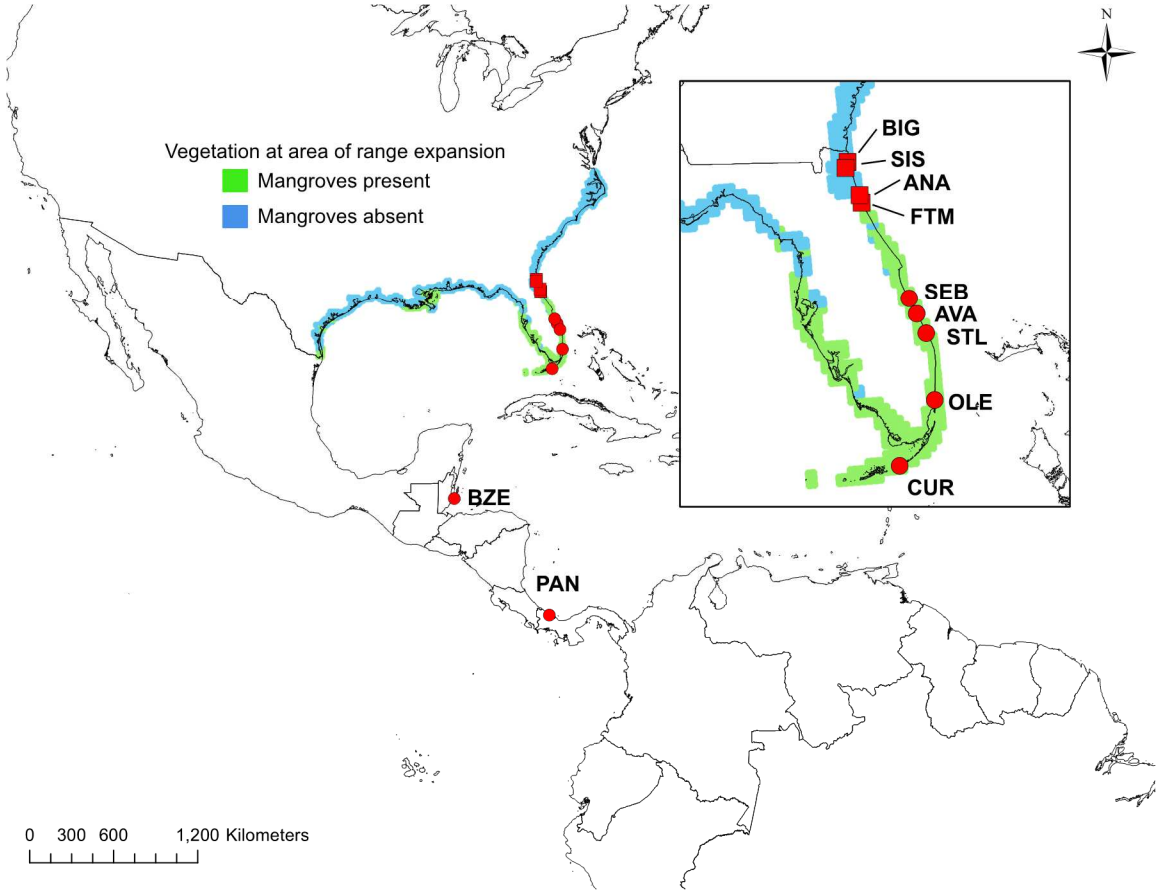

Figure 4.1. Map exhibiting the geographic distribution of the sampling sites, from the southernmost mangrove site near the center of *A. pisonii*'s range to the marsh sites near the edge of the species' current distribution. Square symbols represent mangrove habitats, and circle symbols represent marsh habitats.

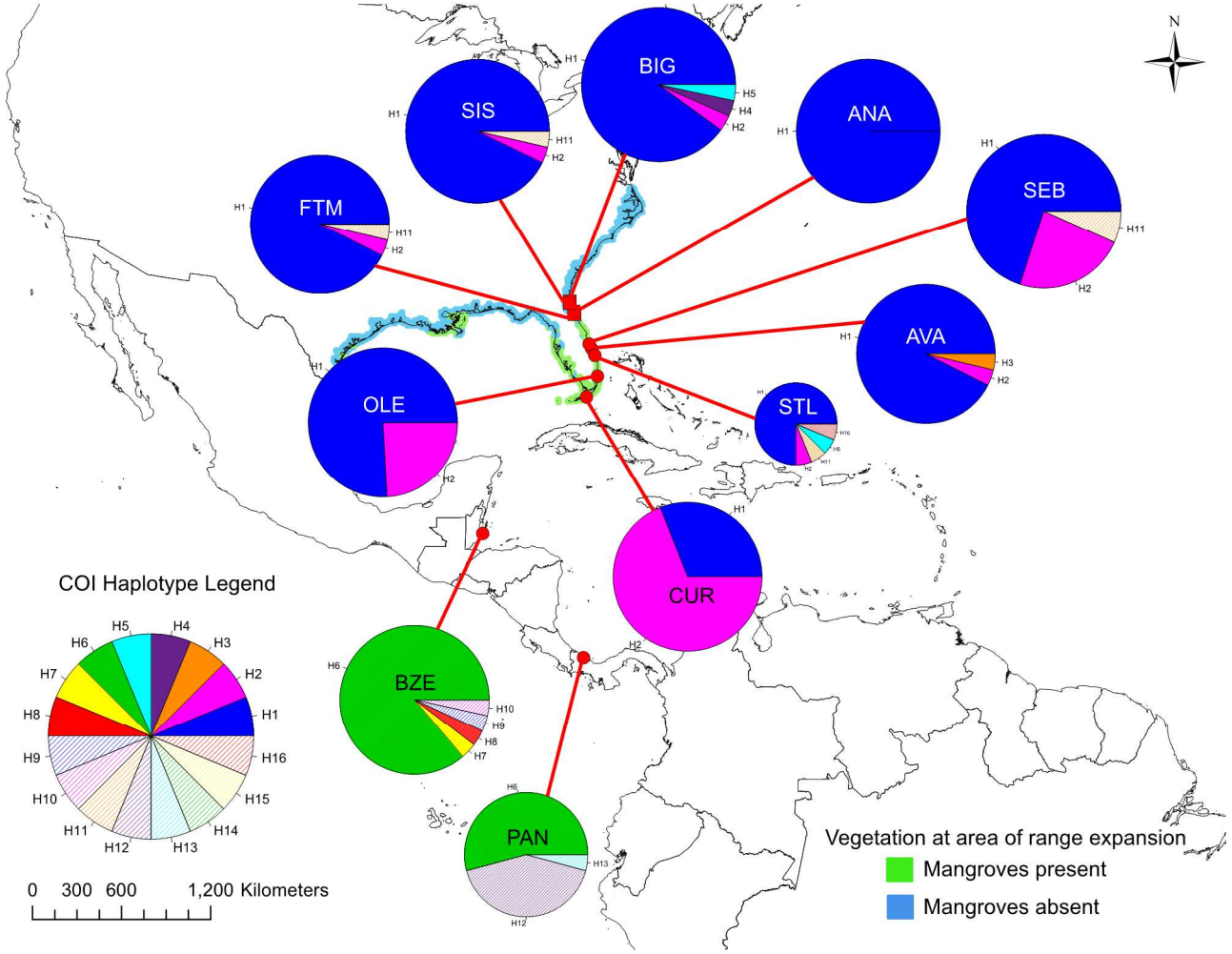

Figure 4.2. Relative haplotype frequencies for the COI locus arranged according to geographic location of sample sites. Circle diameter corresponds to sample size; haplotype legend represents a sample size of 40 individuals.

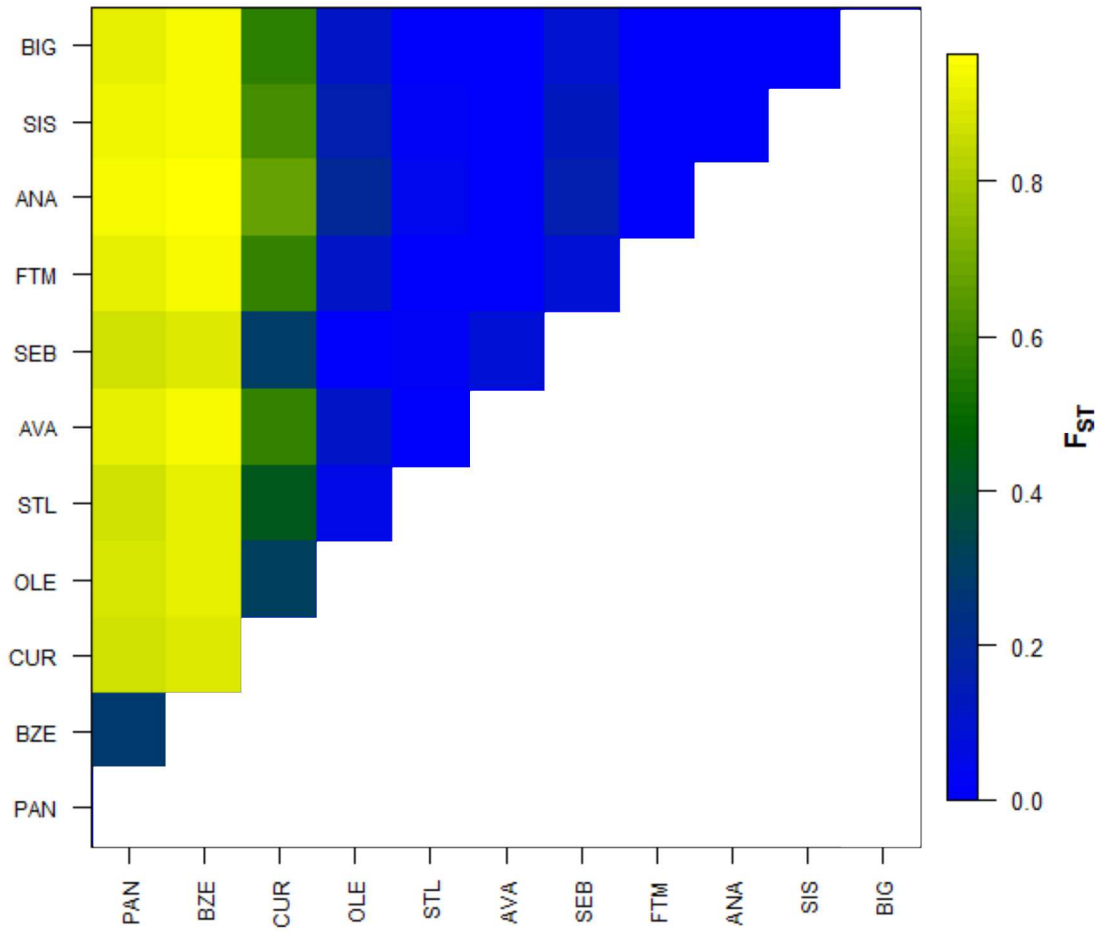

Figure 4.3. Heat map portraying relatedness of pairwise F_{ST} values for the COI locus.

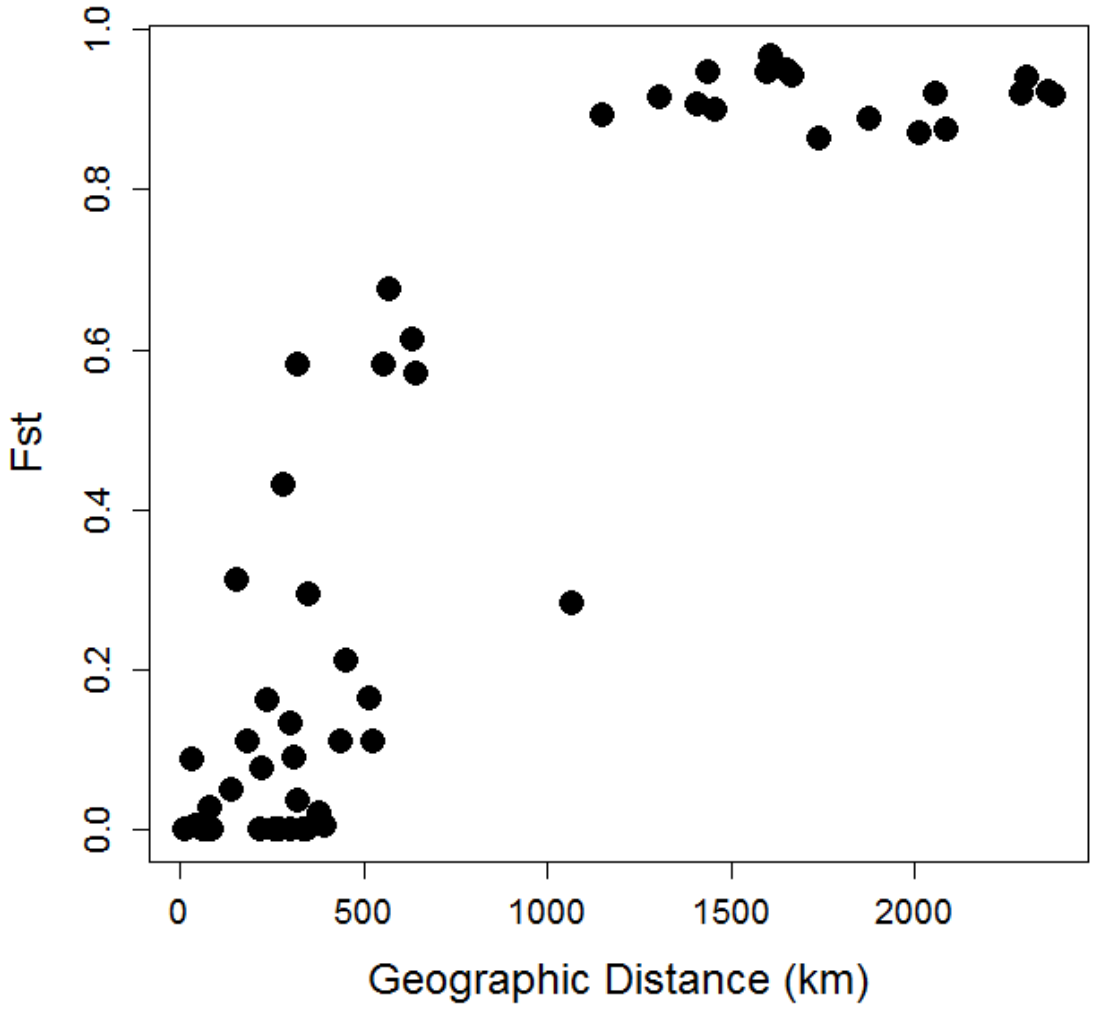

Figure 4.4. Relationship between genetic relatedness (F_{ST}) and geographic distance (km) for the COI locus.

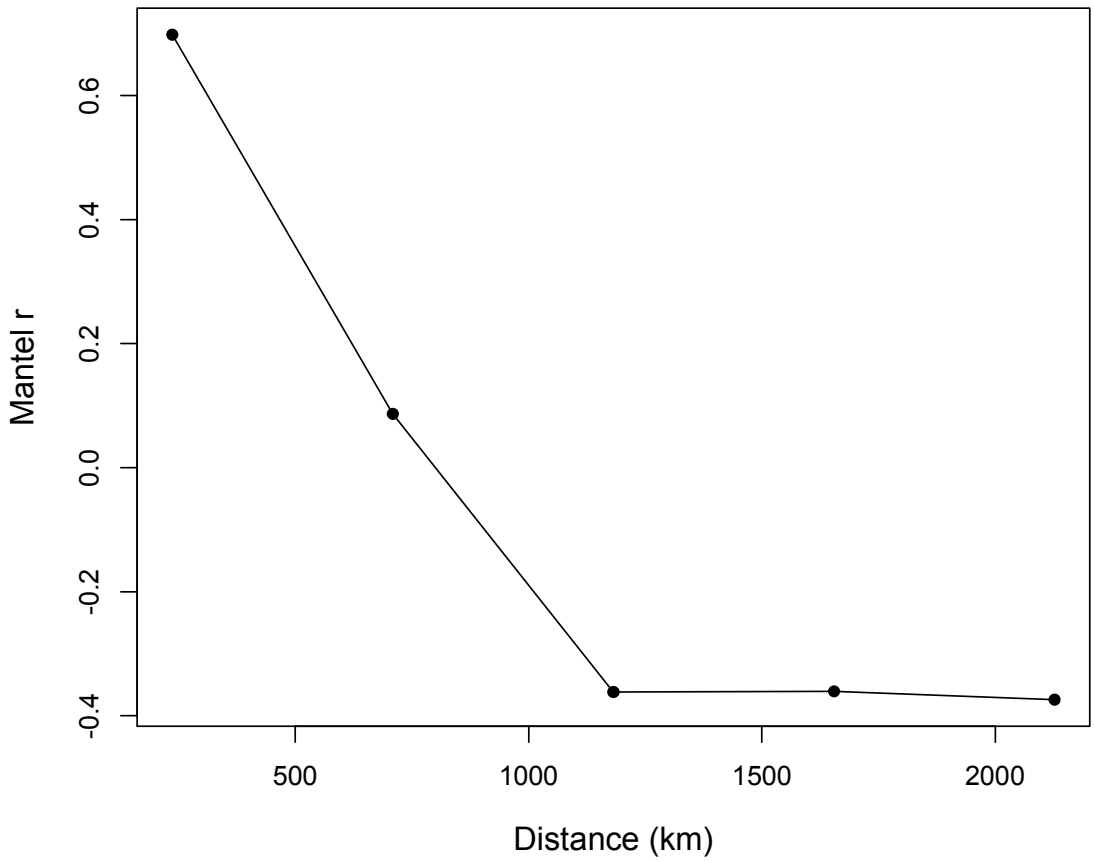

Figure 4.5. Mantel correlogram for COI locus showing a decrease in Mantel correlation (i.e. genetic similarity) with increasing geographic distance between sites.

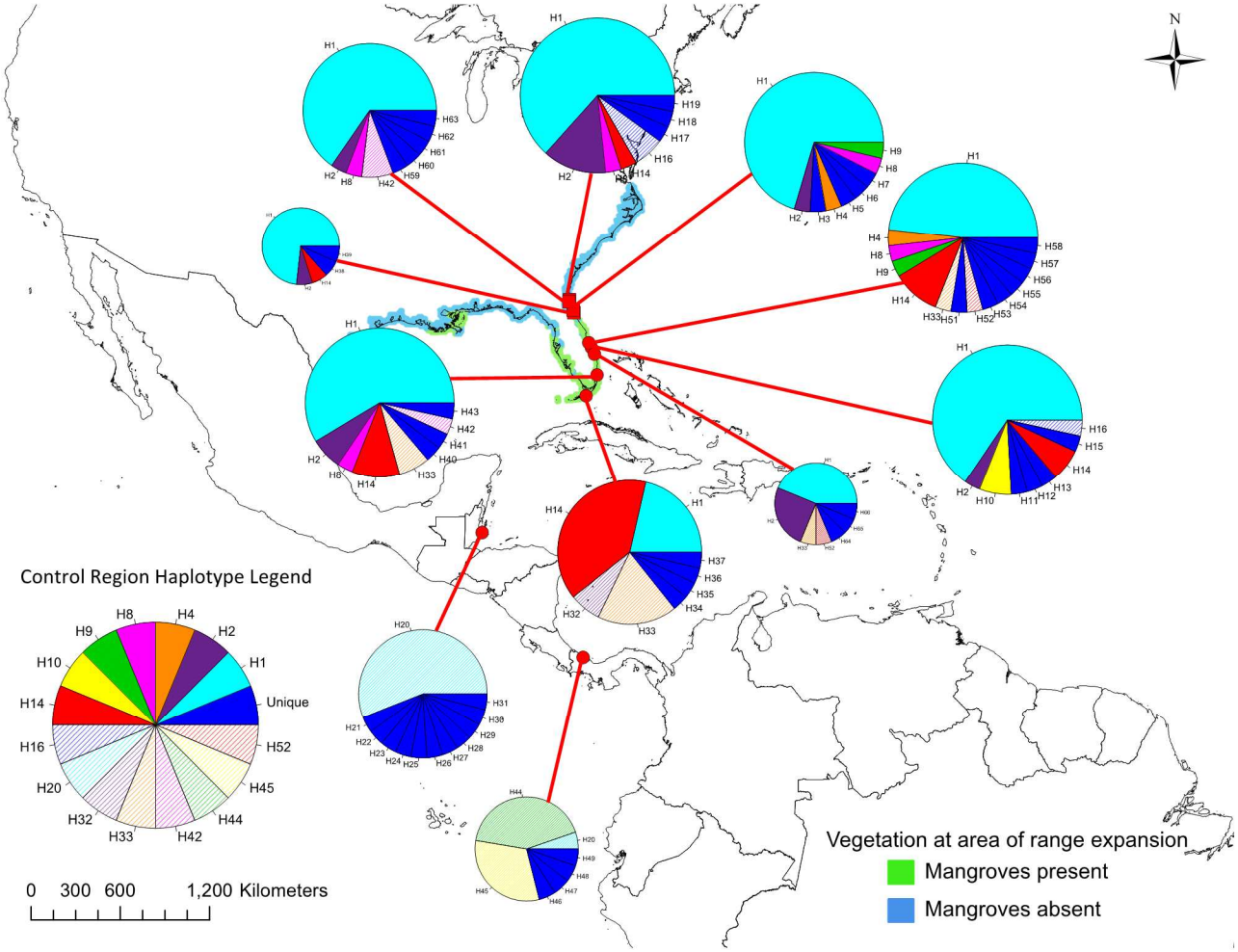

Figure 4.6 Relative haplotype frequencies for the control region locus arranged according to geographic location of sample sites. Circle diameter corresponds to sample size; haplotype legend represents a sample size of 40 individuals. Due to the high number of unique haplotypes (n=51), all unique haplotypes were assigned the same color and individually identified by text labels.

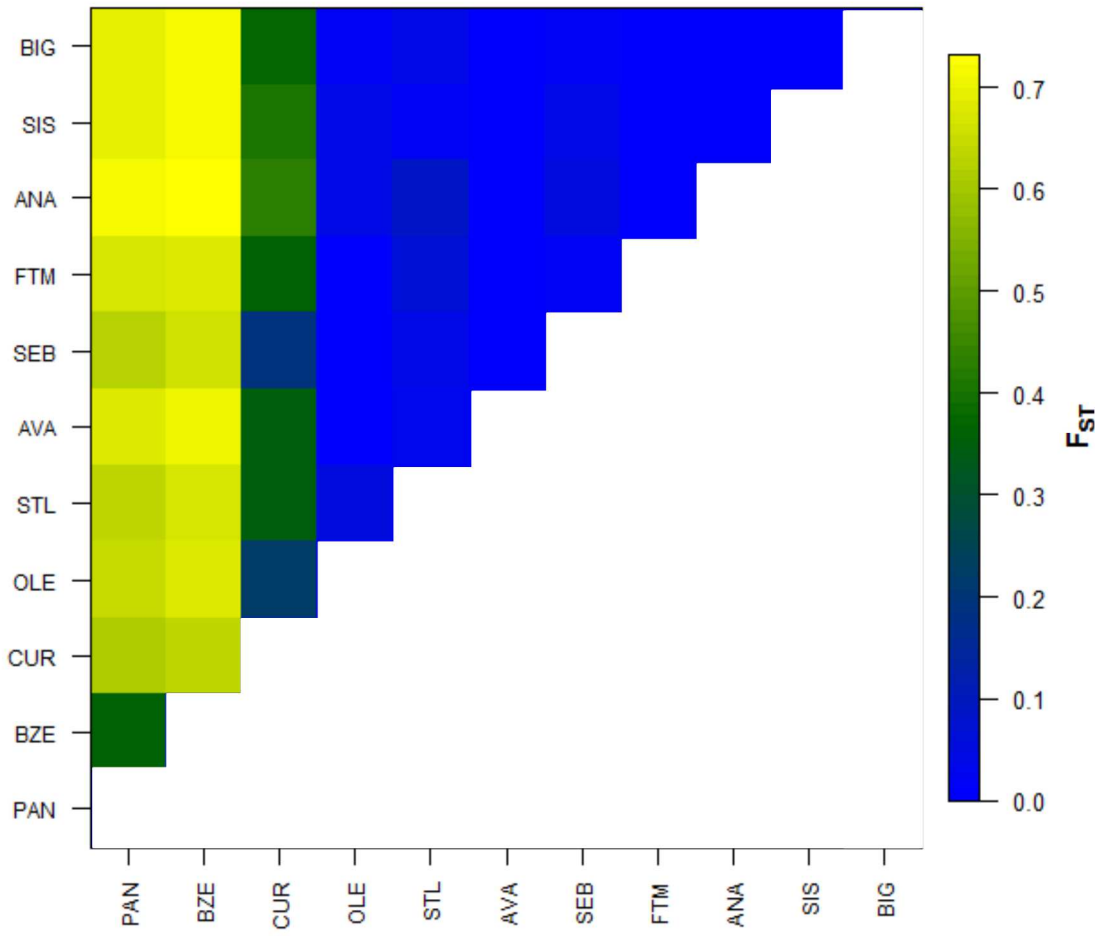

Figure 4.7. Heat map portraying relatedness of pairwise F_{ST} values for the control region locus.

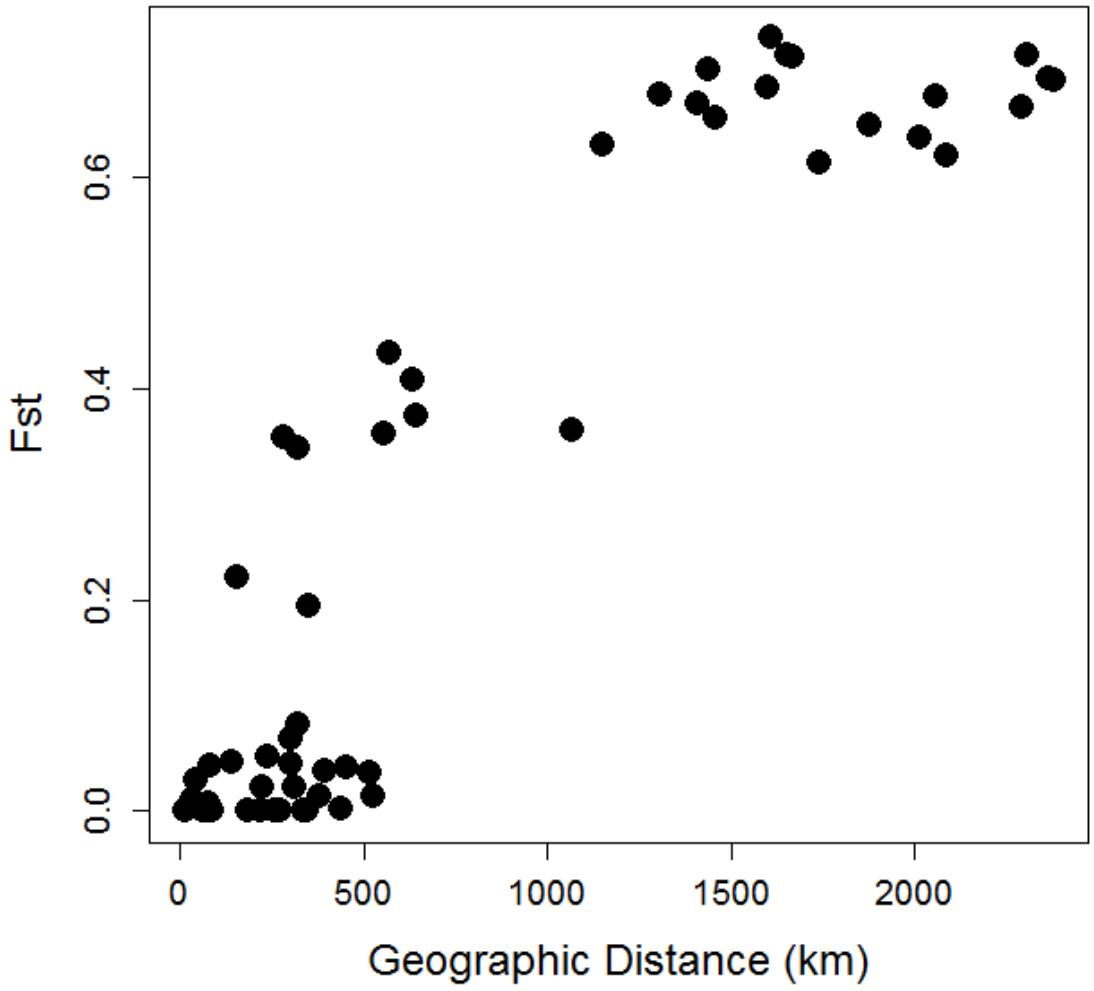

Figure 4.8. Relationship between genetic relatedness (F_{ST}) and geographic distance (km) for the control region locus.

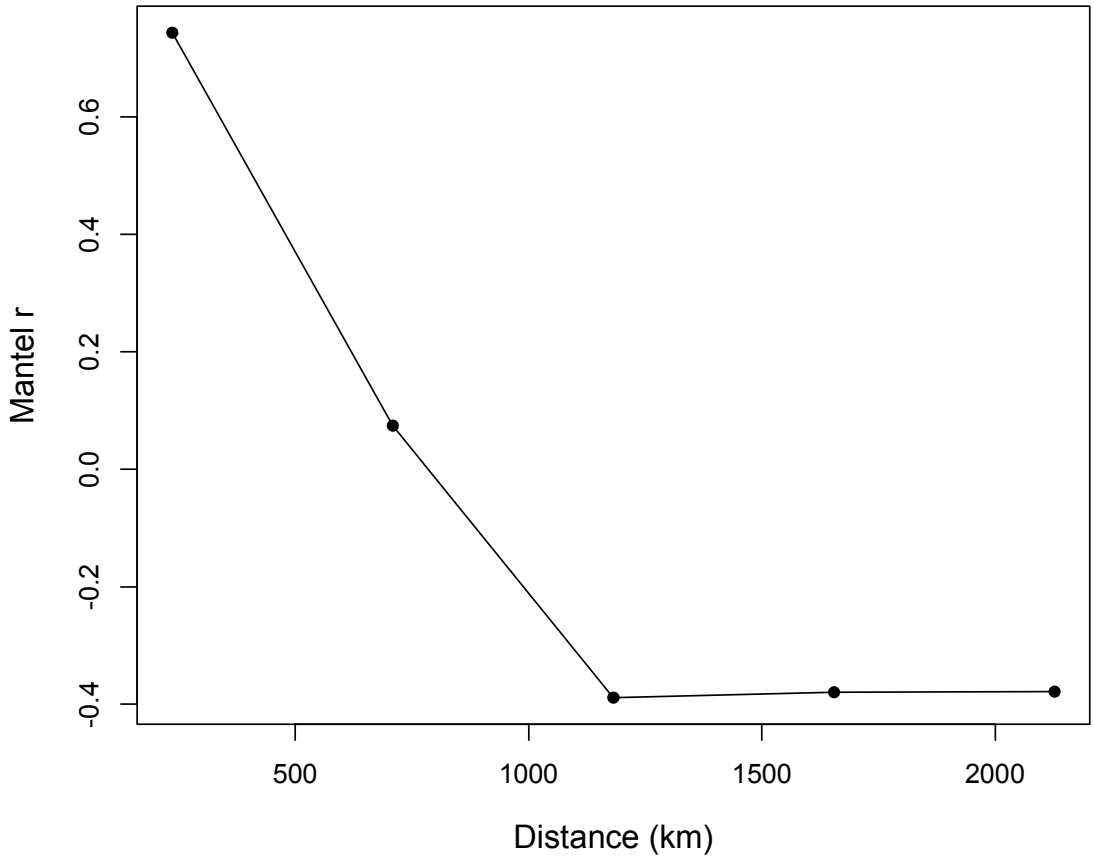

Figure 4.9. Mantel correlogram for control region locus showing a decrease in Mantel correlation (i.e. genetic similarity) with increasing geographic distance between sites.

CHAPTER 5

FITNESS-ASSOCIATED CONSEQUENCES OF AN OMNIVOROUS DIET FOR THE MANGROVE TREE CRAB *ARATUS PISONII*[4]

[4]Riley M, Vogel M, and B. Griffen. 2014. Fitness-associated consequences of an omnivorous diet for the mangrove tree crab *Aratus pisonii*. *Aquatic Biology* 20:35-43, DOI: 10.3354/ab00543.
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5.1 Abstract

Omnivory is a widespread trophic strategy with variable impacts on survival and reproduction, even within closely related taxonomic groups. In coastal ecosystems experiencing extensive environmental changes, many decapod crustaceans employ omnivorous feeding strategies. Because animals initially respond to environmental changes with behavioral modifications that can alter their foraging habits, a mechanistic understanding of how diet influences fitness is essential to predict the impact of future environmental changes on species fitness and population dynamics. We investigated the impact of an omnivorous diet on the consumption, survivorship, physiological condition, and reproductive effort of the mangrove tree crab *Aratus pisonii*, a major mangrove consumer. *A. pisonii* engaged in compensatory feeding on plant material; crabs consumed more food and digested it less efficiently as the plant material in their diet increased. Although there were relatively few deaths, survival appeared to be negatively impacted by large quantities of animal consumption and marginally negatively influenced by high plant consumption. Physiological condition improved due to consumption of both plant and animal material, while only consumption of animal material increased reproductive investment. These results demonstrate that the opportunistic inclusion of animal material in *A. pisonii*'s diet significantly improves physiological condition and reproductive effort, and suggests that vitellogenic individuals cannot fully compensate for a lack of animal material in their diet by increasing plant consumption. This study provides a mechanistic framework to understand how potential diet changes by omnivorous crabs such as *A. pisonii*, which are facing numerous changes in their environment, may impact their fitness and population dynamics.

5.2 Introduction

Omnivory is a widespread feeding strategy seen in a diverse range of taxa. Invertebrates such as insects (Coll and Guershon 2002) and crustaceans (e.g. Buck et al. 2003, Poon et al. 2010) commonly consume both plant and animal material. Vertebrate species, including lizards (Cooper and Vitt 2002), mammals (e.g. Home and Jhala 2009), birds (e.g. Carlisle et al. 2012), and fish (González-Bergonzoni et al. 2012), also exploit a combination of plant and animal food sources. These omnivorous feeding strategies can have variable impacts on survival, growth, and reproduction. This can be seen even within a single taxonomic group, such as crustaceans. For example, the generalist salt marsh crab *Armases cinereum* achieves higher growth rates on a mixed diet of plant and animal material than on monotypic diets of plant or animal material alone (Buck et al. 2003). The amphipod species *Ampithoe valida* and *Cymadusa compta* have significantly higher fecundity when consuming a mixture of algae and animal material than when consuming a diet of animal material alone. Conversely, the closely related amphipod *Gammarus mucronatus* has comparable growth and survival on an algal-only and mixed algal and animal diet, as well as equivalent fitness on exclusively algal or animal diets, and on diets that mix the two food types (Cruz-Rivera and Hay 2000a).

Due to the diversity of potential diet strategies and their variable impact on closely related species, it can be difficult to predict how changes in diet may influence growth and fitness. However, a mechanistic understanding of how diet influences these metrics is key in predicting how species and populations may respond to environmental changes. Organisms frequently respond to human-induced rapid environmental changes with behavioral modifications, including altered foraging, and this can lead to changes in

population dynamics (Tuomainen and Candolin 2011). For instance, the blue crab *Callinectes sapidus* in a contaminated estuary have reduced success capturing active prey items and consume more algae, sediment, and detritus than those from an uncontaminated reference site (Reichmuth et al. 2009). Additionally, the presence of the invasive Asian shore crab *Hemigrapsus sanguineus*, forces the European green crab *Carcinus maenas,* a previously established invader, to decrease its mussel consumption and increase its consumption of red algae, a less preferable food source (Griffen et al. 2008). This foraging shift to an algal-dominated diet is concurrent with reduced fecundity in *C. maenas*, and is likely a contributing factor to the regional replacement of *C. maenas* by *H. sanguineus* (Griffen et al. 2011).

Human-induced rapid environmental changes, such as habitat loss, exotic species introductions, human harvesting, pollution, and climate change, are widespread in coastal habitats (e.g. Valiela et al. 2001, Roessig et al. 2004, Molnar et al. 2008, Rabalais et al. 2009). Decapod crustaceans are ecologically and economically important components of these coastal systems. Crabs in these habitats can function as ecosystem engineers through their burrowing and bioturbation activities (Kristensen 2008), by mediating nutrient turnover and retention (e.g. Olaffson et al. 2002, Needham et al. 2011), by impacting primary production (e.g. Holdredge et al. 2010), and by driving mangrove tree recruitment patterns (Lindquist et al. 2009). Many of these ecological functions are associated with crab diet or foraging strategy. Few crabs engage in exclusively herbivorous or exclusively carnivorous trophic behavior, instead displaying a range of omnivorous feeding strategies. Predatory crabs commonly consume plant and algal material (e.g. Paul 1981, Ropes 1987, Kneib and Weeks 1990), and crabs that are

considered herbivorous actually incorporate large amounts of animal material in their diet (e.g. Dahdouh-Guebas et al. 1999, Samson et al. 2007).

The mangrove tree crab *Aratus pisonii* is one such omnivorous crustacean. This arboreal crab is abundant in neotropical mangrove systems (Beever et al. 1979, Diaz and Conde 1988, Erickson et al. 2003), where it is distinctive in that it forages on fresh leaf tissue in the canopy rather than leaf litter. *A. pisonii* is the primary herbivore of the red mangrove *Rhizophora mangle* (Feller and Chamberlain 2007, Feller et al. 2013) and its herbivory can account for over 40% of total leaf damage in monotypic red mangrove stands (Erickson et al. 2003). As a result, these fauna provide a key link between mangrove primary production and the detrital food web, and contribute to both nutrient and biomass outwelling from mangrove systems (Beever et al. 1979). Fresh red mangrove leaves compose an estimated 84% of the diet of adult crabs under natural settings (Erickson et al. 2003), but *A. pisonii* commonly supplement their plant diet with opportunistic scavenging and predation on insects, benthic infauna, and juvenile conspecifics (Warner 1967, Beever et al. 1979, Diaz and Conde 1988). Feeding assays indicate that like many crustaceans, these crabs preferentially consume animal material over plant material, including red mangrove leaves (Erickson et al. 2008).

We can conceive of three current and impending environmental changes that are likely to alter foraging strategies of *A. pisonii*. The first is ocean acidification. Crustaceans respond to acidic conditions by increasing calcification – in effect becoming bigger, stronger individuals (Ries et al. 2009). Predatory capabilities of crustaceans commonly scale with individual size and claw strength (Seed and Hughes 1995). We may therefore expect *A. pisonii* to incorporate more animal tissue in its diet as ocean

acidification progresses, though it remains uncertain the extent to which ocean acidification will enhance calcification of primarily terrestrial species, like *A. pisonii*, that spend limited time submerged. The second environmental change is chemical pollution. Chemical pollution reduces crab foraging capabilities, resulting in a diet shift away from predation (Reichmuth et al. 2009). The Indian River Lagoon on the eastern coast of Florida, where this study was conducted, currently experiences intense pollution (Qian et al. 2007). While public awareness of the effects of this pollution have focused primarily on mammals, birds, and reptiles, crustaceans may also experience negative impacts that could shift their diets away from animal consumption. The third environmental change that may alter diet is climate change. Many species are shifting their ranges in response to climate change (Hoegh-Guldberg and Bruno 2010), and these range shifts can at times force these species to utilize habitats that they otherwise would not choose to inhabit. Thus if *A. pisonii* expands its range northward beyond the presence of mangrove habitat, it would be forced in these areas to consume alternative diets because of the physical absence of its normal diet of mangrove leaves.

The goal of this study was to investigate the link between diet, physiological condition, and fitness in the mangrove tree crab *A. pisonii*. Should qualitative or quantitative foraging changes occur in this omnivorous mangrove crab in response to the current or future environmental changes highlighted above, understanding the link between diet and fitness will allow us to predict population implications of these diet changes. Thus, we performed a controlled diet experiment to examine the impact of diet quality (proportion of plant to animal tissue) and diet quantity on *A. pisonii* consumption, assimilation efficiency, physiological condition, and fitness (reproduction and

survivorship).

5.3 Methods

5.3.1 Experimental Design and Diet Preparation

We performed an experiment using a modified geometric analysis design (Simpson and Raubenheimer 1995) that orthogonally varied both diet quality (proportion of plant to animal tissue in the diet) and diet quantity (proportion of crab body weight offered). This design incorporated five levels of diet quality with different amounts of plant to animal material (100% : 0%, 75% : 25%, 50% : 50%, 25% : 75%, 0% : 100%) and four diet quantities (2%, 3%, 5%, and 11% of crab dry body weight per day). Crabs generally consume between 1.5 and 3% of their body weight per day (Emmerson and Mcgwynne 1992, Olafsson et al. 2002), so these diet quantities spanned a range from limited to abundant food conditions. Each combination of diet quality and quantity was replicated twice for a total sample size of 40 individuals. We were interested in the actual diet consumed, which cannot be controlled or replicated (i.e., individual diet choice would cause 10 crabs with identical food choices to consume 10 unique diets over the 6-week experiment, leading to 10 unique diet treatments rather than 10 replicates of the same treatment). However, the diet offered can be controlled and replicated, and can be used to maximize diet variation. We therefore elected to use a regression-type experimental design to maximize diet variation with our experimental treatments rather than increase replication of a more limited range of offered diets. The analyses described hereafter also reflect this strategy.

Red mangrove leaves, which account for the majority of *A. pisonii*'s natural diet (Erickson et al. 2003), served as the plant material in this study. House crickets (*Achetus*

domesticus) were offered for animal tissue. *A. pisonii* feed on insects, including crickets, in the field (Beever et al. 1979) and prefer *A. domesticus* to fresh plant and algal material in laboratory feeding assays (Erickson et al. 2008). In order to ensure that crabs consumed the desired proportion of plant to animal tissue, the two food types were mixed and embedded in an agar substrate in the five proportions of plant to animal material described previously. Freshly picked red mangrove leaves were collected from Avalon State Park in St. Lucie County, FL in June 2012. Live *A. domesticus* were obtained from commercial sources and euthanized. Both food types were then placed in a drying oven at 68° C until they reached a constant dry weight. They were ground separately into a fine powder using a Coffee-mate® coffee grinder and mixed to produce the five ratios of plant to animal tissue described above. They were then incorporated into an artificial agar diet by combining 2.8% agar, 4.6% food powder mixture, and 92.6% boiling DI water. This mixture was poured into 2.05 cm^3 plastic molds, which were placed in a drying oven at 68° C until they reached a constant weight and formed dried agar cubes.

5.3.2 Experimental Setup and Maintenance

Female, adult (carapace width 17-24 mm) *A. pisonii* were collected from Avalon State Park in St. Lucie County, FL in June 2012 and transported to the wet lab at the Smithsonian Marine Station at Fort Pierce, FL. Each crab was placed into an individual plastic aquarium (22.8 cm L x 15.2 cm W x 16.5 cm H) with a 400 mL glass finger bowl containing approximately 200-300 mL of filtered seawater (salinity ~31 ppt). They were maintained for 3 to 4 days without food to standardize hunger levels and clear their guts prior to the start of the experiment. Total dry weight of experimental crabs was estimated based on initial carapace width (CW) at the time of collection using a previously

established relationship between carapace width and dry weight (adj R^2=0.822, P<0.001, n=20, Riley unpublished data). This estimated dry weight was subsequently used to determine the precise amount of food to offer each crab.

The experiment consisted of a 3-day diet cycle that was repeated 14 times for a total experimental duration of 6 weeks. Diet cubes of the appropriate diet quality were shaved down to within ± 0.005 g of the assigned weight for each crab, and then offered to crabs for 48 hours. After that time, any remaining uneaten food was removed and seawater in finger bowls was refilled to its original volume to account for evaporative water loss. Crabs were then maintained without food for 24 hours to allow for digestion and fecal production, after which the contents of each experimental chamber were poured into a flask attached to a vacuum pump and rinsed through Whatman® qualitative filter paper (Grade 1, 11 μm particle size retention) to collect feces. After filtering, *A. pisonii* were offered fresh diet and a new feeding cycle was initiated. Uneaten food was dried at 68° C until it reached a constant dry weight to remove any humidity or water from the finger bowls that the cubes may have absorbed. Filter papers with collected feces were also dried at 68° C and weighed to quantify fecal production.

5.3.3 Post-experimental Sample Processing

At the conclusion of the experiment, crabs were frozen at -80° C and dissected within 3 months. In order to determine the relative physiological condition of individual crabs, the main energy storage organ, the hepatopancreas (O'Connor and Gilbert 1968, Parvathy 1971), was removed from the crab body. Similarly, the ovaries were isolated from the remainder of the crab body in order to determine reproductive investment of individual crabs. The hepatopancreas, ovaries, and remainder of the body were dried to a

constant weight at 68° C. Dry weight of the hepatopancreas as a proportion of the total body weight (i.e., the hepatosomatic index, HSI) was used as a mass-specific metric of investment in the main energy storage organ. Similarly, the dry weight of the ovaries as a proportion of the total body weight (i.e., the gonadosomatic index, GSI) was used as a mass-specific metric of reproductive investment.

5.3.4 Statistical Analyses

Mean daily food consumption (g) was calculated by averaging the dry weight of the food consumed (determined using the difference between initial and final food weight at each feeding period) during each 3-day feeding cycle. To eliminate any potential influence of starvation on diet consumption (see Cronin and Hay 1996) during the first feeding cycle, consumption values from the first feeding cycle were not included in this average. To standardize mean food consumption by crab body weight and convert it into daily consumption, the amount of food consumed during each feeding cycle was divided by three (due to the 3-day feeding cycles) and by the dry weight of the crab's body (g). Standardized mean daily plant and animal consumption were calculated by multiplying the standardized mean daily food consumption by the percentage of the diet (0, 25, 50, 75, or 100%) that was plant or animal material, respectively.

Assimilation efficiency was calculated using the amount of feces produced during each feeding period in the formula: [*total weight of food consumed (g) – total weight of feces produced (g)] / total weight of food consumed (g)*]. The resulting values were averaged and converted to percentages to calculate mean assimilation efficiency (% of food consumed that was assimilated) by individual crabs. Four of the experimental crabs consistently placed food pellets into the finger bowls of water, breaking down the food

pellets and making it difficult to distinguish uneaten food from disassociated feces. To avoid biasing our results by inadvertently mistaking food and feces, we eliminated these four crabs from the analyses.

The influence of diet quality and diet quantity treatments on standardized mean daily food consumption was determined using a general linear model with percentage of plant material in the diet and percentage of dry crab body weight offered per day as predictor variables. The influence of diet on assimilation efficiency was determined using a general linear model with mean daily plant consumption and mean daily animal consumption as continuous predictor variables.

The influence of diet on survivorship was determined using a logistic regression; occurrence of death was the response variable, and mean daily plant consumption and mean daily animal consumption were used as predictor variables. Although all crabs were included in our analyses of survivorship (n=36), our analyses of physiological condition and reproductive effort were restricted to crabs that survived the duration of the experiment (n=29). The influence of diet on physiological condition (approximated by the hepatosomatic index) was determined using a general linear model with mean daily plant consumption and mean daily animal consumption as continuous predictor variables. Due to the relatively high molting frequency of this species (every 53 days, Warner 1967), molting was also included as a categorical factor in this linear model. Similarly, the influence of diet on reproductive effort (approximated by the gonadosomatic index) was determined using a general linear model with mean daily plant consumption and mean daily animal consumption as continuous predictor variables. Zoeal release was included as a categorical factor in the model; due to year-round reproductive output in

this species (Warner 1967), 17 crabs were gravid at the start of the experiment and released zoeae 2 to 26 days after the experiment began (mean ± SD= 10 ± 6 days, n=17). Two models were developed and are presented for the gonadosomatic index: one that included all surviving crabs (n=29), and one in which the single crab in the experiment with late-stage developing ovaries was removed (n=28). This particular crab was maintained on a mixed diet of 0.25:0.75 plant to animal material and offered 8% of its body weight per day, and as a result of the advanced stage of its ovary development, its gonadosomatic index was very high relative to the remainder of the experimental crabs (i.e., it was in late-stage vitellogenesis at the conclusion of the experiment).

5.4 Results

A. pisonii increased their mean daily food consumption as the proportion of plant material in their diet increased, but only under the abundant food conditions represented by the highest diet quantity treatment (adj R^2=0.678, mean slope ± SE=4.23%±1%, t=4.23, P=0.003, Fig. 1). On average, crabs offered 2, 3, and 5% of their body weight consumed 1.68%, 2.86%, and 4.71% of their body weight daily, irrespective of the proportion of plant and animal material in their diet (Fig.1). In the 11% body weight treatment, crabs offered only animal material consumed an average of 5.10% of their body weight daily (Fig.1). When crabs in the same diet quantity treatment were offered a 50% : 50% mixture of plant: animal material and plant material alone, they increased their standardized mean daily consumption to 6.75% and 9.50%, respectively (Fig.1).

Assimilation efficiency (percentage of consumed food that was assimilated) decreased with daily plant consumption (Fig. 2A) and increased with daily animal

consumption (Fig. 2B) (adj R^2=0.675; plant, mean slope ± SE= -252% ± 52.4%, t= -4.80, P<0.001; animal, mean slope ± SE= 298% ± 90.1%, t=3.31, P=0.003). Specifically, *A. pisonii* assimilated 57.9% ± 0.227% (mean ± SE) of the exclusively animal diet, while individuals maintained on an all-plant diet assimilated 23.8% ± 0.314% of the food they consumed (Fig 2).

Crab survivorship decreased with daily animal consumption (mean ± SE= -42.1±19.9, z= -2.11, P=0.034) and was marginally negatively influenced by daily plant consumption (z= -1.88, P=0.060). Molting did not affect energy storage in the hepatopancreas (t= -0.789, P=0.437; Fig. 3), but *A. pisonii* physiological condition, as determined by the HSI, improved by 23.8% with each 1% of their body weight that crabs consumed in plant material each day (Fig. 3A) and increased by 148% with each 1% of their body weight that they consumed in animal material each day (Fig. 3B) (adj R^2=0.692; plant, mean slope ± SE=23.8%±10.2%, t=2.33, P=0.028; animal, mean slope ± SE=148%± 20.7%, t=7.17, P<0.001).

When all surviving crabs were included in the analysis to determine how diet influences reproductive effort, zoeal release had no impact on subsequent reproductive effort (t=-1.122, P=0.273). Investment in ovary development was also unaffected by plant consumption (t=0.632, P=0.533), but *A. pisonii* increased their reproductive effort by 51.7% with every 1% of their body weight that crabs consumed in animal material each day (adj R^2=0.353, mean slope ± SE= 51.7%± 12.9%, t=4.01, P<0.001). When the single crab with late-stage developing ovaries was removed, investment in ovary development remained unaffected by previous zoeal release (t= -0.078, P=0.938; Fig. 4) or plant consumption (t=0.173, P=0.864; Fig. 4A); however, without this single individual, the

effect of diet on reproductive effort became less extreme so that *A. pisonii* increased their reproductive effort by 31.8% with every 1% of their body weight that they consumed in animal material each day (adj R^2=0.392, mean slope ± SE= 31.8%±8.07%, t=3.94, P<0.001, Fig. 4B).

5.5 Discussion

In this study, we investigated the impact of diet quality (proportion of plant to animal material) and diet quantity on the feeding strategy, physiological condition, and fitness of the mangrove tree crab *A. pisonii*, an omnivorous crab common in neotropical mangroves. *A. pisonii* demonstrated strong evidence of compensatory feeding on plant material when maintained in abundant food conditions by increasing their food consumption as the plant material in their diet increased. This behavior is likely meant to compensate for the low nutritional quality of plant material, which contains high levels of indigestible material such as lignin and cellulose, as well as low levels of nitrogen and organic matter relative to animal tissue (Mattson 1980, Wolcott and O'Connor 1992, Linton and Greenaway 2007). Red mangrove leaves, the plant material used in this study, contain particularly high levels of condensed tannins (20-40% dry weight) as well as other secondary metabolites that are difficult to digest and may negatively impact consumer condition (Hernes et al. 2001, Erickson et al. 2004).

Despite the poor nutritional quality of plant material relative to animal material, many herbivorous crabs are extremely efficient at assimilating the plant tissue in their diet. For example, the mangrove crab *Neosarmatium meinerti* assimilates over 80% of the dry weight of fresh *Avicennia marina* mangrove leaves in its diet (Emmerson and

McGwynne 1992). In contrast, *A. pisonii* was relatively inefficient at assimilating plant material, and crabs maintained on a diet of all-plant material assimilated just 23.8% of the food they consumed. There is some indication in the literature that assimilation of senescent leaves is different than that of fresh leaves (e.g. Greenaway and Raghavan 1998). By necessity of our experimental design, we dried and ground fresh *R. mangle* leaves before incorporating them in the agar-based diet, which could have altered the crabs' ability to assimilate the plant material. However, the animal material was similarly processed and crabs confined to an all-animal diet assimilated 57.9% of the food they consumed, more than twice the amount assimilated by those on an all-plant diet. *A. pisonii*'s inefficient assimilation of plant material relative to animal material may partially explain the compensatory increase in crab food consumption that was seen as the amount of plant material in the diet increased.

Survivorship throughout the experiment was negatively impacted by daily animal consumption and marginally negatively influenced by plant consumption. However, there were relatively few mortality events (n=7), and this trend was likely driven by the death of two crabs with high daily animal consumption and a single crab with high daily plant consumption. Diet quality has been shown to have a mixed impact on the survivorship of other crustaceans, such as amphipods, whose survivorship on diets that mix high and low quality food sources varies widely depending on the species (Cruz-Rivera and Hay 2000a, 2000b), and diet quantity is inversely correlated with longevity in a variety of species (e.g. Lawler et al. 2008, Pietrzak et al. 2010). Alternatively, the inclusion of nitrogen-rich animal tissue in *A. pisonii*'s diet significantly improved *A. pisonii* reproductive investment and physiological condition. Nitrogen is an essential requirement

for crustacean growth (i.e. molting) (Skinner 1966, Chang 1995) and reproduction. Because egg production in some species of brachyuran crabs can equal or exceed 10% of dry body weight, the nitrogen needed for a single reproductive event can be considerable (Gifford 1962, Hines 1982, 1992). This may explain the demonstrated increase in *A. pisonii* reproductive effort (as measured by the gonadosomatic index) with daily animal consumption. Gonad biomass in crustaceans is directly proportional to the quantity of eggs produced (Hartnoll 2006, Griffen 2013), so this increase in gonad development should translate directly to increased fecundity and reproductive output in *A. pisonii*. Recent reproductive investment by crabs that were gravid upon collection from the field and subsequently released broods had no impact on their reproductive effort at the conclusion of this experiment. This is consistent with other reports for this species, in which brood size remained constant for females over several reproductive events (Leme 2006). Likewise, plant consumption had no impact on reproductive effort, providing evidence that crabs undergoing vitellogenesis cannot increase their consumption of plant material to compensate for a lack of animal material in their diet.

Both plant and animal consumption significantly improved physiological condition (as measured by the hepatosomatic index), although consumption of protein-rich animal material had a 6.24× stronger positive impact on investment in the hepatopancreas than consumption of plant material. The hepatopancreas is the primary energy storage organ for crustaceans, particularly for long-term storage. It serves as one of several storage sites for glycogen, a source of quick energy, and is the main storage site for lipids, which are longer-term energy stores (O'Connor and Gilbert 1968, Parvathy 1971, Chang 1995). Crabs increase lipid production as well as lipid storage in the

hepatopancreas prior to molting and previous studies have suggested that crabs rely on lipid stores in the hepatopancreas for molting (O'Connor and Gilbert 1968, Chang 1995). However, molting had no significant impact on the hepatosomatic index of *A. pisonii* and several crabs in this experiment maintained large hepatopancreas masses following molting. *A. pisonii*'s lack of a discernible decrease in hepatopancreas mass following molting suggests that this species may rely on alternative energy stores, such as those in muscle tissue, to finance the molting process (Parvathy 1971).

In conclusion, this study demonstrates that even for a largely herbivorous consumer such as the mangrove tree crab *A. pisonii*, the opportunistic consumption of animal material significantly improves physiological condition and reproductive effort. While crabs were able to improve their physiological condition by consuming both plant and animal material, only consumption of animal material increased reproductive investment. Although there have been no documented diet shifts by *A. pisonii* to date, a number of environmental factors, including ocean acidification, pollution, and climate change, have the potential to directly influence their foraging behavior (see Introduction). Because *A. pisonii* is an ecologically important consumer in neotropical mangrove systems, any changes in their population dynamics, either positive or negative, would also have important implications for the broader mangrove community (Feller et al. 2013). Additional work is needed to determine whether the mechanistic link established here between diet, physiology, and reproduction in *A. pisonii* applies to other species of omnivorous crabs that are ecologically and economically influential in coastal systems. In a rapidly changing environment in which species modify their behavior in response to

environmental changes, understanding this link is essential for predicting the total impact of current and future environmental conditions.

5.6 Acknowledgements

We gratefully acknowledge Dr. Ilka C. Feller for her advice and insight into the study system. This research was supported by NSF grant #OCE-1129166 and a Smithsonian Institution/ Link Foundation Graduate Fellowship. This is contribution #930 of the Smithsonian Marine Station at Fort Pierce, FL.

5.7 Figures

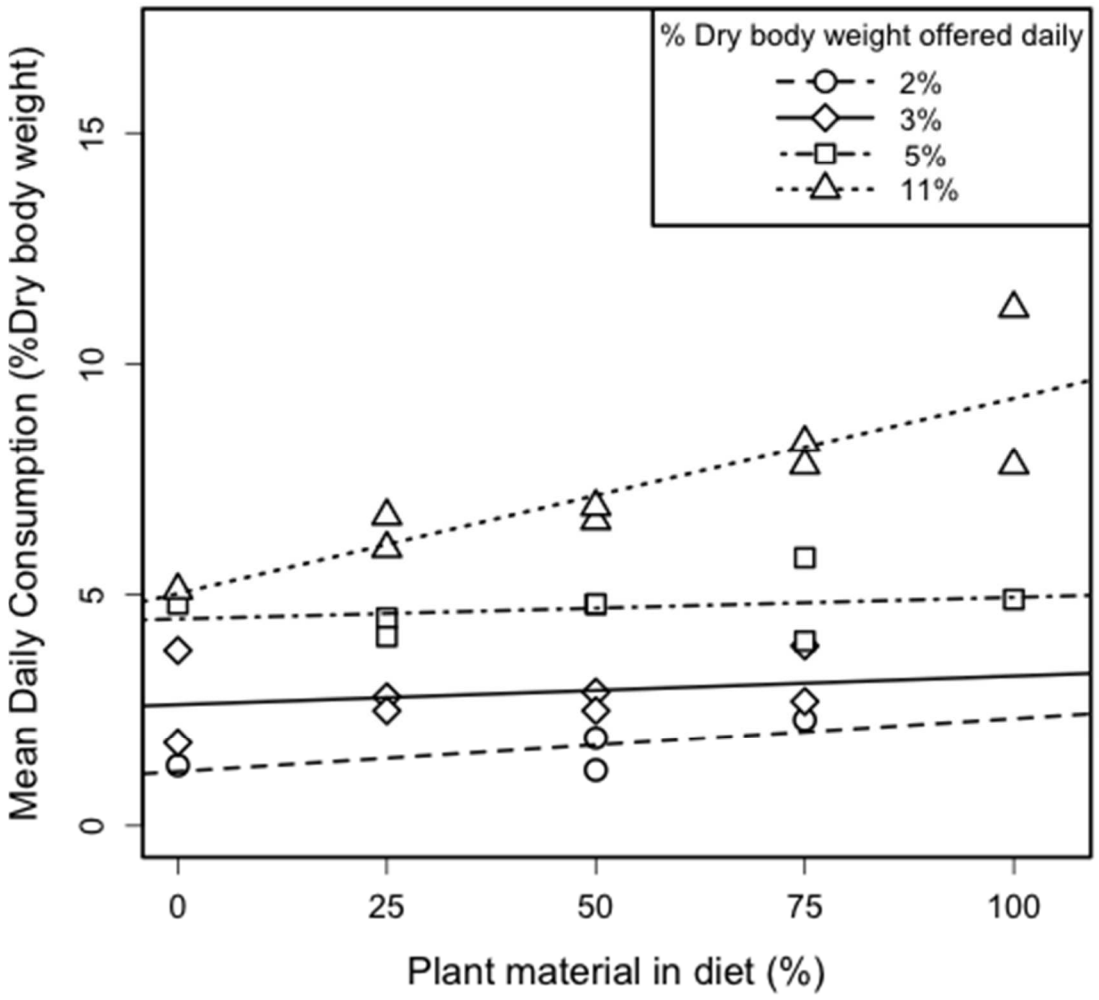

Figure 5.1. Changes in *Aratus pisonii* mean daily food consumption as a function of diet quality (percentage of plant material in diet) and quantity (percentage of crab dry body weight) offered.

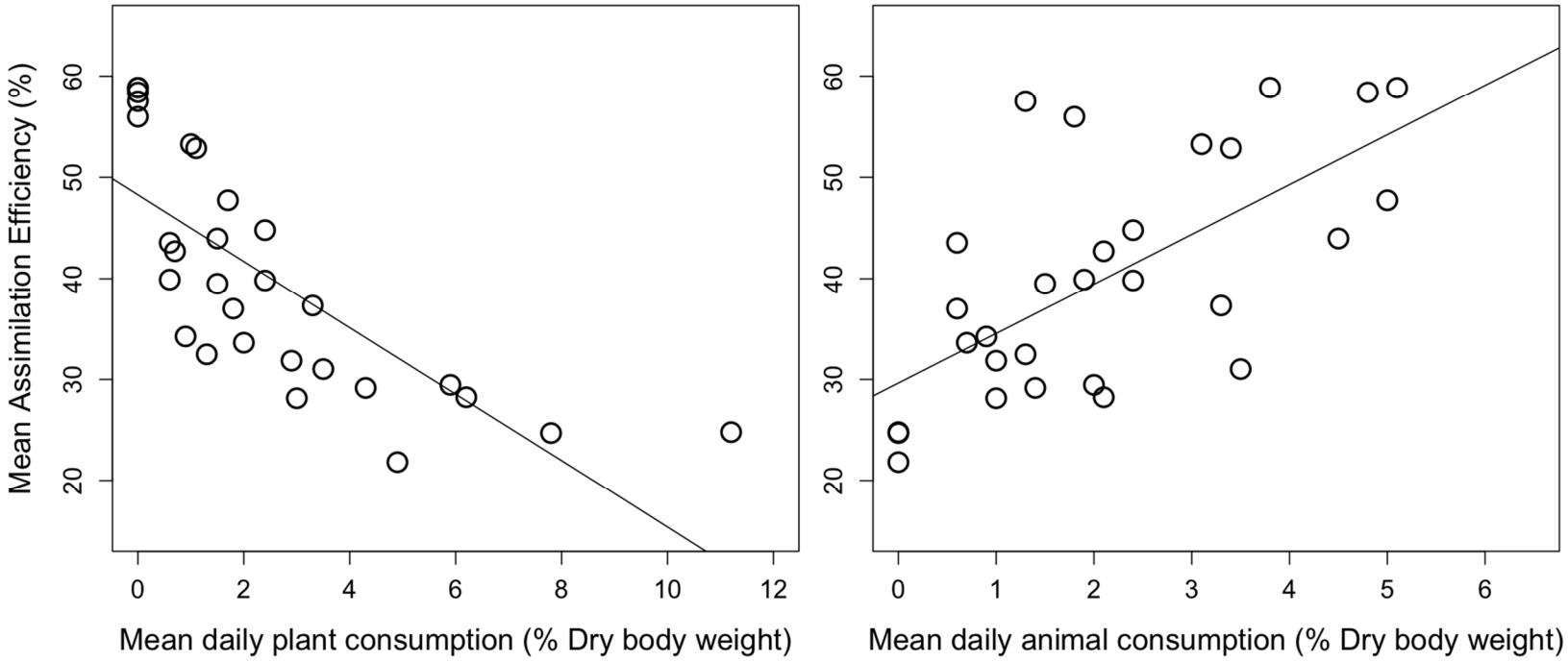

Figure 5.2. Relationship between mean daily plant (A) and animal (B) consumption and *Aratus pisonii* assimilation efficiency (%).

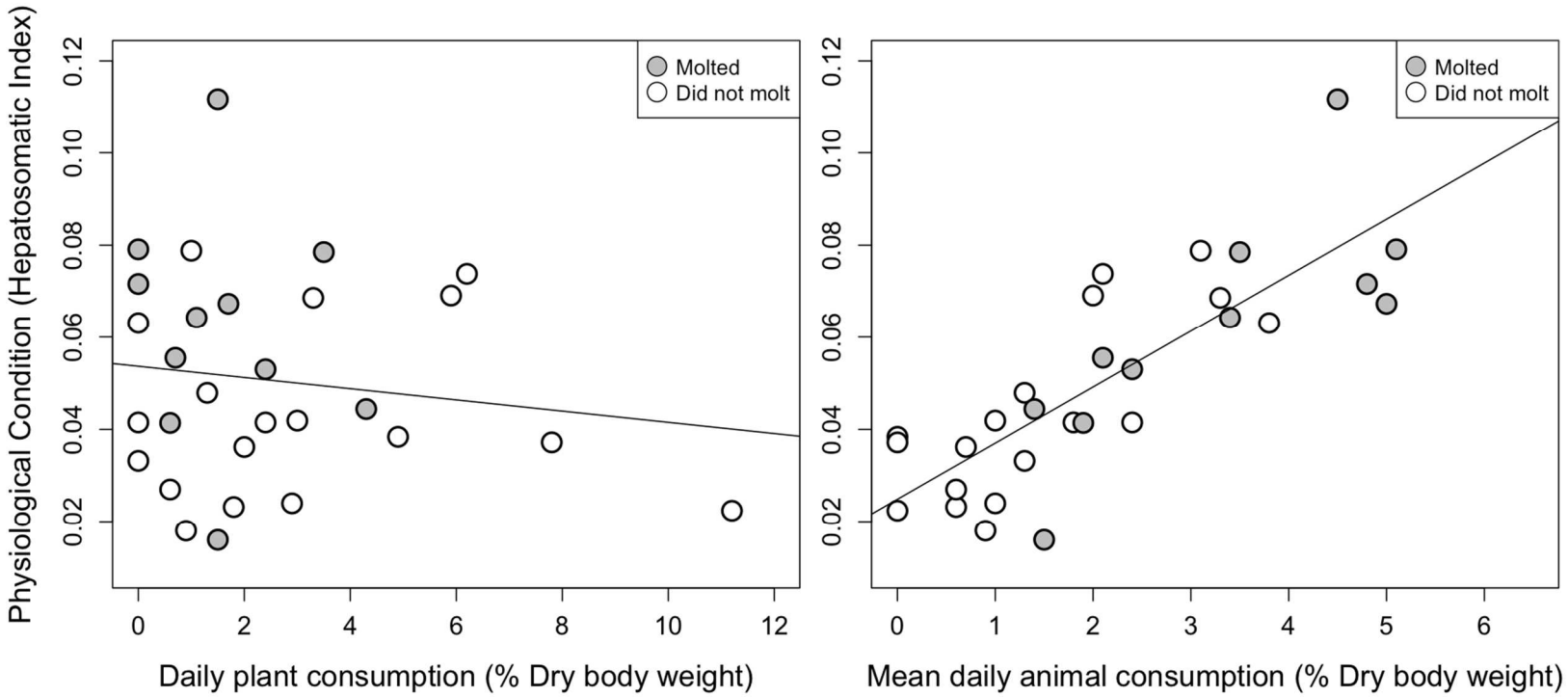

Figure 5.3. *Aratus pisonii* physiological condition (as measured by the hepatosomatic index) as a function of mean daily (A) plant and (B) animal consumption.

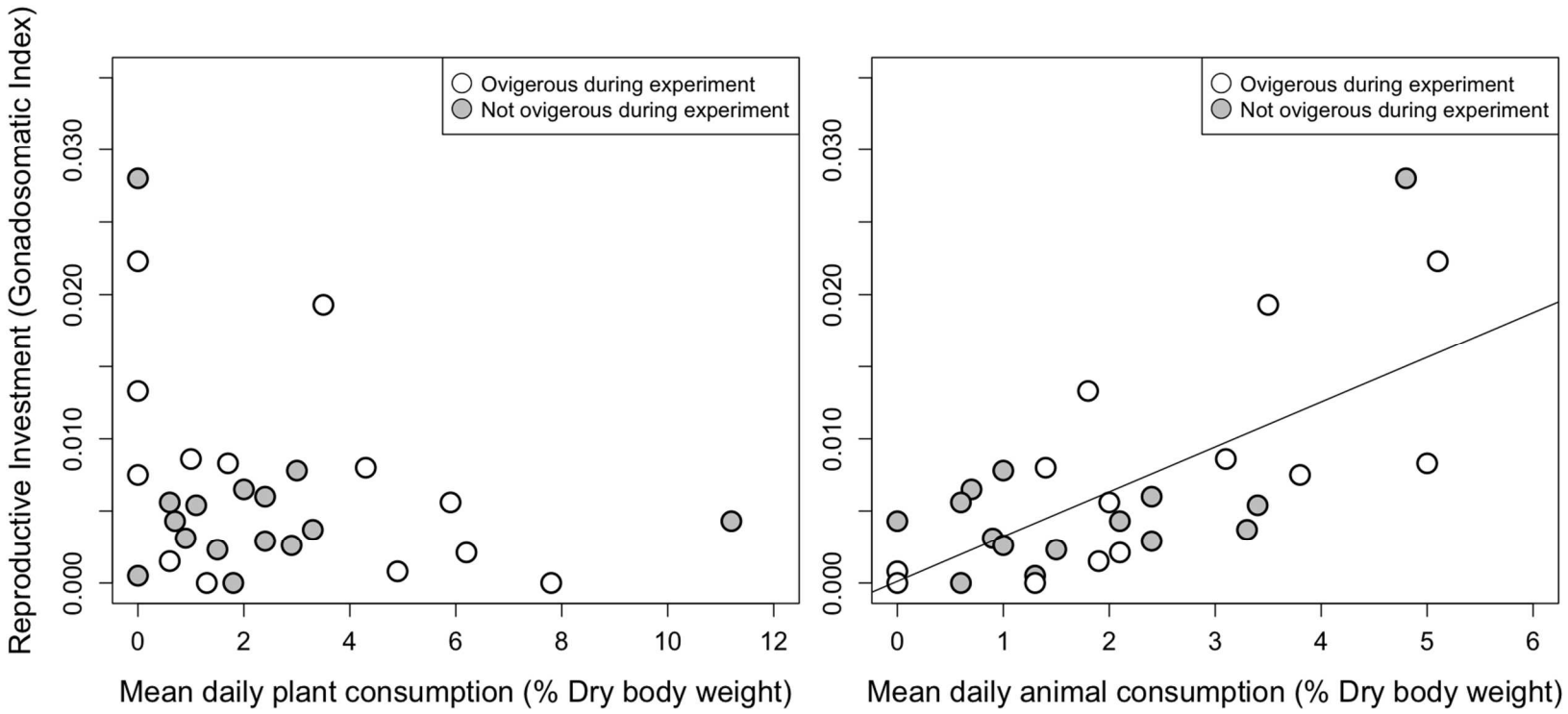

Figure 5.4. *Aratus pisonii* reproductive effort (as measured by the gonadosomatic index) as a function of mean daily (A) plant and (B) animal consumption.

CHAPTER 6

PAST NICHE CONSTRUCTION PROVIDES PREDATION REFUGES AND INTENSIFIES CURRENT PLANT-ANIMAL INTERACTIONS[5]

[5]Riley M., Feller I., and B. Griffen. Past niche construction provides predation refuges and intensifies current plant-animal interactions. *In preparation.*

6.1 Abstract

Niche construction, the process through which organisms modify their environments, is pervasive throughout nature. Species that engage in niche construction by physically altering their environment may leave behind evidence of their activities such as nests, burrows, and webs. This niche construction activity often has a subsequent impact on ensuing generations of the same species, as well as other members of the community, through an ecological inheritance. Here, we apply niche construction theory, which integrates both niche construction as an ecological process and ecological inheritance as a potential evolutionary process within a single comprehensive framework, to investigate the impact of past niche construction on the strength of current plant-animal interactions. We assessed the ecological inheritance of herbivore-constructed predation refuges by investigating spatial variation in the strength of current plant-herbivore relationships in a neotropical mangrove forest system. Past wood-boring activity impacted more than one-third of trees through the creation of predation refuges, and folivory was more than five orders of magnitude higher on trees influenced by niche-constructed refuges relative to those lacking predation refuges. Additionally, folivory decreased with increasing distance from predation refuges, emphasizing the influence of niche-constructed refuges on current plant-herbivore interactions. When considered within the framework of a species interaction network, our results demonstrate that niche construction has the potential to affect species in multiple trophic levels over various temporal scales. Thus, in addition to elucidating the magnitude of the ecological inheritance of past niche construction behavior, this work highlights the necessity of incorporating temporal variation in interaction networks to comprehensively understand

species interactions and community links.

6.2 Introduction

Niche construction- the process through which organisms modify the biotic and abiotic components of their environments through their metabolism, behavior, or choices- is widespread throughout nature (Odling-Smee et al. 2003, 2013). Many animal species engage in *niche construction* by physically altering their environment through the creation of artifacts such as nests, burrows, or webs (e.g. Jones et al. 1994 and references therein, Lill and Marquis 2003). These *artifacts* are physical abiotic resources created by niche construction activity (Odling-Smee et al. 2013). Other examples of niche construction include algal and plant metabolic activity, which modifies nutrient cycles, as well as fungal and bacterial decomposition of organic matter, which increases nutrient turnover (Odling-Smee et al. 2003, Meysman et al. 2006).

Niche construction by one species can impact other species by providing protection or provisions (positive niche construction), or by intensifying predation or competition (negative niche construction) (Odling-Smee et al. 2003). It can also modify natural selection on subsequent members of both the niche-constructing species and other members of the community (Odling-Smee et al. 2003, 2013; Silver and Di Paolo 2006). Thus, by altering selection pressure, niche construction, which is an ecological process, can impact evolutionary dynamics on a concurrent time scale (Odling-Smee et al. 2003, 2013). For instance, swallows and many species of cooperatively nesting birds physically alter their environment by constructing nests that enhance offspring survival and increase parental fitness. These nests are artifacts that can be reused. They are inherited by

offspring over multiple generations, thereby improving offspring fitness for generations after the niche construction activity itself (Hansell 1984, Skutch 1987).

The impact of niche construction by one species on subsequent generations of the same species, as well as other species in the community, is considered its *ecological inheritance*. Ecological inheritance is defined as the "legacy over time" of niche construction activities (Odling-Smee et al. 2013). *Niche construction theory* has developed as a comprehensive theory that integrates both niche construction (an ecological process) and ecological inheritance (a potential evolutionary process) into a single framework (Odling-Smee et al. 2003, 2013).

The application of niche construction theory can be useful when considering consumer-resource interactions and their subsequent impacts on the community (Laland and Boogert 2008). For instance, in predator-prey relationships, predators influence prey communities through both direct consumption and trait-mediated indirect effects. These non-consumptive predator effects include changes in prey phenotype, physiology, and behavior, such as predator avoidance via refuge use (Werner and Peacor 2003, Schmitz et al. 2004). Some niche construction activities have been shown to create artifacts that provide refuges for other organisms (e.g. Jones et al. 1994 and references therein, Lill and Marquis 2003, Pringle et al. 2008). Thus, any trophic cascades or broader ecosystem impacts sparked by predator avoidance behavior via the use of these refuges could be categorized as the ecological inheritance of past niche construction.

In instances where prey are herbivores, prey use of niche construction artifacts as central places from which to forage (i.e. central place foraging) could alter patterns of herbivory in their habitat. Because herbivory influences forest structure, nutrient

dynamics, and chemical cycling within a system (Schmitz et al. 2008 and references therein), this has the potential to result in a large ecological inheritance. The use of refuges by multiple prey could also lead to interference competition for nearby food resources between species with similar trophic niches.

Although the concept of niche construction is well established, the extent to which physical changes in the environment subsequently influence community interactions and ecosystem function (i.e. the strength of ecological inheritance) is still an active area of research. Additionally, incorporating ecological inheritance into our conceptual understanding of trophic dynamics has the potential to greatly advance our understanding of comprehensive species interactions. Sanders et al. (2014) have recently provided a framework for integrating physical modifications of the environment with food web studies and identified this topic as a research priority. Here, we contribute to an understanding of this topic by utilizing a mechanistic approach to investigate the ecological inheritance of past niche construction in the context of current plant-herbivore interactions.

In a system where past niche construction has created artifacts that serve as potential predation refuges, we (1) identified spatial patterns of folivory and (2) investigated potential mechanisms underlying variation in the intensity of this plant-animal interaction. We focused on the impact of (1) morphological tree characteristics and (2) the proximity of niche-constructed refuges on herbivory patterns. Additionally, the principal herbivore in this study was identified as an opportunistic omnivore, whose presence may deter other herbivores. Therefore, we examined how herbivory by each

species was influenced by the herbivory of other species to assess potential interference competition.

6.3 Methods

6.3.1 Description of study system and relevant herbivores

We conducted field surveys on the mangrove archipelago of Twin Cays, Belize (16°49'39.38" N, 88°6'3.20" W). Our surveys were specifically focused within one area of the island, Hidden Lake, which is an interior region chiefly composed of dwarf red mangrove trees (*Rhizophora mangle*) less than 1.5 m in height (Feller 1995). Many of these dwarf red mangroves are spatially isolated, and thus can be easily distinguished as separate trees, unlike denser growth along the mangrove fringe. Previous research has documented niche construction by the beetle *Elaphidion mimeticum* (Cerambycidae) and other wood-boring insects in this system (Feller 2002). These herbivores target the woody components of trees, girdling branches, creating light gaps, causing extensive loss of yield, and leaving trees riddled with small holes and hollows (Feller and Mathis 1997, Feller and McKee 1999, Feller 2002). The niche construction activity of these woodborers leaves behind tree holes as the result of their feeding. Insects and other arthropods have subsequently been observed inhabiting the hollows and galleries (i.e. tree holes) left behind in the wood from previous herbivory (Feller and Mathis 1997).

In addition to wood-boring insects, there are numerous other herbivores on the island that damage living mangrove leaves. Prior to conducting our formal survey, we observed leaf damage on *R. mangle* leaves from Hidden Lake, and collected leaves that provided representative examples of all observed herbivory (Fig. 1). We used

characteristic markings to assign herbivory to one of three prominent herbivores: (1) the mangrove tree crab *Aratus pisonii*, (2) the mangrove periwinkle snail *Littoraria angulifuera*, and (3) larvae of the bagworm moth *Oiketicus kirbii*. Herbivory by *A. pisonii* was characterized by irregularly shaped holes and scrapes on either side of the leaf (Fig. 1A). This damage was of variable size and occurred on both the edge and inner area of the leaf surface. Damage by *L. angulifuera* was characterized by distinctive half-moon-shaped scars on the leaf surface (Fig. 1B), while herbivory by larval *O. kirbii* was distinguished by spherical holes, which extended completely through leaf tissue (Fig. 1C).

6.3.2 Field surveys, sample collection, and leaf image analysis

We investigated spatial variation in herbivory, a potential ecological inheritance of niche construction by wood-boring insects, by establishing three random 10 m x 10 m plots. In each plot, we established the X, Y coordinates of each tree within the plot by determining the position of its central trunk. We measured morphological tree characteristics for each tree, including length (m, long axis of the tree), width (m, short axis of the tree), and height (m). Additionally, we categorized all trees into one of three categories: (1) no refuge, (2) adjacent to refuge, (3) refuge. Trees categorized as "no refuge" did not contain a niche-constructed tree hole that could serve as a potential refuge for herbivores and neither their trunk nor branches were in physical contact with a tree that contained a tree hole. Trees categorized as "adjacent to refuge" did not contain a tree hole, but were physically in contact with a tree that did contain a tree hole, and thus herbivores could move freely between the two trees. Trees categorized as "refuge"

contained a niche-constructed tree hole that could serve as a potential refuge from predators.

Within each of the three experimental plots, we randomly selected five trees from each of the three tree categories (except plot 3, in which there were only four "adjacent to refuge" trees). For each of these trees (n=44), we randomly collected 10 green leaves from a basal position on a twig (i.e. fourth or fifth leaf pair from the twig terminals). As the oldest leaves on the tree, basal green leaves were used to provide an estimate of maximum leaf herbivory damage. Because there were relatively few basal leaf pairs on the small dwarf trees, this sampling was designed to provide a comprehensive representation of the total herbivory present on the tree. Leaves were transported to the field station on Carrie Bow Cay and pinned flat. We then digitally photographed all 10 leaves from each tree in one frame, along with a size reference for use in subsequent leaf image analysis. Although the majority of leaf damage was visible on both sides of the leaf, both sides of the leaf were thoroughly examined for evidence of herbivory. Generally, leaves were photographed face-up. In a few instances, minor surface damage was only visible on the underside of the leaf. In this case the underside of the leaf was photographed so that the total leaf damage was captured in the photograph.

In order to analyze the proportion of leaf area damaged or removed by herbivory, we used the digital analysis software ImageJ 1.49u (U.S. National Institutes of Health). For each image, which included the 10 leaves collected from a single tree, we first set the scaling factor using the size reference. We outlined the total area of a single individual leaf to quantify total leaf area (cm^2), and then proceeded to outline each individual area (cm^2) on the leaf surface that showed evidence of complete herbivory (sections of leaf

completely removed) or damage from herbivory (partial removal of leaf, such as scraping or brown areas). For each of these instances of leaf damage, we used the recognizable leaf damage signatures of the three herbivores that damaged leaves in this study (see previous section for details) to categorize which was responsible for each piece of leaf damage. After determining the total area of all spots damaged by herbivory on a single leaf, we calculated the percentage of leaf area that was damaged or consumed by herbivory for each of the three herbivores, as well as the total percentage of leaf area damaged or consumed by all herbivores. We then repeated the process for the nine remaining leaves in the image, beginning with the outline of the next leaf's total leaf area. Once we had calculated the proportion of herbivory for all of the leaves collected from a single tree, we also calculated the mean total herbivory and herbivory by each herbivore for each particular tree.

6.3.3 Statistical analysis

First, we calculated the percentage of each tree category across all plots (mean ± SE) to determine the relative allocation of trees impacted by past niche construction in the system. Next, we investigated the impact of morphological tree characteristics, refuge availability, and plot identity on total herbivory of trees for which leaf herbivory was analyzed. We used a linear model with tree height, width, and length as continuous explanatory variables and refuge availability and plot identity as categorical explanatory variables. Because neither the morphological tree characteristics nor plot identity influenced herbivory, we simplified the model by removing these variables and running a separate linear model that focused solely on the impact of refuge availability on total herbivory.

Next, we calculated the minimum Euclidean distance between each tree for which leaf herbivory was calculated and the nearest tree in which a refuge was available. In determining the minimum distance, all trees were included, and thus the nearest tree in which a refuge was available was not necessarily a tree for which leaf herbivory had been calculated. We used these calculations to construct an exponential model investigating the impact of minimum distance to a tree with a refuge (m) on total herbivory (mean % of leaf area).

After investigating total herbivory patterns, we compared leaf damage by each of the three herbivores across all tree types with an ANOVA and post-hoc Tukey comparison to determine the relative contribution of each herbivore to the total amount of herbivory in the system. We also investigated the impact of refuge availability on herbivory by each of the three herbivores using three separate general linear models with mean herbivory by each herbivore as the response variable and refuge availability as the categorical explanatory variable. Finally, we investigated competitive exclusion amongst the three herbivores using three separate general linear models that looked at the effect of herbivory by two herbivores on herbivory by the third herbivore.

6.4 Results

Across all three plots, the majority of trees had no refuge (mean proportion ± SE= 0.624 ± 0.601). However, a substantial proportion of trees did have refuges (mean ± SE= 0.259 ±0.0 68) or were adjacent to trees with refuges (mean ± SE= 0.117 ± 0.0086) (Fig. 3). After non-significant variables were removed to simplify the model, the impact of refuge availability on herbivory was pronounced. There was an average increase in

herbivory of ~557% on trees adjacent to refuges (mean % leaf area damaged ± SE= 6.29 ± 0.352, P=0.003) and an average increase in herbivory of ~513% on trees with refuges (mean % leaf area damaged ± SE= 5.80 ± 2.39, P=0.006) relative to trees without refuges (mean % leaf area damaged ± SE= 1.13 ± 0.208)(Fig. 4). The minimum distance to the nearest tree with a refuge significantly impacted herbivory, with herbivory decreasing exponentially with increasing distance from a tree with a refuge (LM, mean % leaf area damaged ± SE= -0.388 ± 0.152, P=0.015, Fig. 5).

Of the total herbivory across all tree types (mean % leaf area damaged ± SE= 4.38 ± 0.738), the mangrove tree crab *A. pisonii* was responsible for significantly more leaf damage (mean % leaf area damaged ± SE= 4.34 ± 0.737) than either *L. angulifuera* (mean % leaf area damaged ± SE= 0.021 ± 0.028, P<0.001) or *O. kirbii* (mean % leaf area damaged ± SE= 0.023 ± 0.057, P<0.01) (Fig. 6).

Investigating the impact of refuge availability on the herbivory patterns of each individual herbivore demonstrated that herbivory by *A. pisonii* was significantly higher on trees with refuges (LM, P=0.006, mean % leaf area damaged ± SE= 5.75 ± 1.72) and trees adjacent to refuges (LM, P=0.003, mean % leaf area damaged ± SE= 6.27 ± 0.931) than those without them (mean % leaf area damaged ± SE= 1.12 ± 0.206). There was no difference in herbivory by *L. angulifuera* on trees with refuges (LM, P=0.164, mean % leaf area damaged ± SE= 0.041 ± 0.028) or those adjacent to refuges (LM, P=0.644, mean % leaf area damaged ± SE= 0.017 ± 0.009) compared to trees without them (mean % leaf area damaged ± SE= 0.005 ± 0.005). Similarly, there was no difference in herbivory by larval *O. kirbii* on trees with refuges (LM, P=0.928, mean % leaf area damaged ± SE= 0.022 ± 0.022) or those adjacent to refuges (LM, P=0.982, mean % leaf

area damaged ± SE= 0.046 ± 0.039) relative to trees without them (mean % leaf area damaged ± SE= 0.000 ± 0.000).

Finally, there was no evidence of interference competition. Herbivory by *A. pisonii* was not influenced by *L. angulifuera* herbivory (LM, P=0.630) or larval *O. kirbii* herbivory (LM, P=0.655). *Aratus pisonii* herbivory (LM, P=0.630) and larval *O. kirbii* herbivory (LM, P=0.167) did not influence *L. angulifuera* herbivory. Likewise, larval *O. kirbii* herbivory was not influenced by *A. pisonii* herbivory (LM, P=0.655) or *L. angulifuera* herbivory (LM, P=0.167).

6.5 Discussion

Here, we assessed the ecological inheritance of herbivore-constructed predation refuges by investigating the spatial nature of plant-herbivore relationships in a neotropical mangrove forest. We demonstrate that past niche construction by *E. mimeticum* wood-boring beetles results in an ecological inheritance that affects species in multiple trophic levels. Approximately 26% of trees had niche-constructed refuges while an additional ~12% of trees were adjacent to trees with refuges. Total herbivory was more than five orders of magnitude higher on both trees adjacent to refuges and trees with refuges than on those without tree holes. This pattern was driven by *A. pisonii,* whose herbivory was 513% higher on trees with niche-constructed refuges and 557% higher on trees adjacent to refuges. Additionally, leaf area damage decreased significantly with distance from a tree with a potential refuge, further highlighting the influence of past niche construction on the intensity of current plant-animal interactions. Because ~38% of trees either contained refuges or were adjacent to refuges, making it

easy for herbivores to traverse between them, niche construction altered herbivory patterns in almost half of the trees in the system.

In addition to herbivory, we also assessed whether interference competition among herbivores was another aspect of the ecological inheritance of refuge artifacts. However, despite the omnivorous tendencies of *A. pisonii* (Erickson et al. 2008), there was no evidence that it competitively excluded the other two herbivores in the system, or vice-versa. As a result, altered herbivory patterns appear to be the primary ecological inheritance of these niche-constructed refuges.

It is important to note that herbivory is a prominent plant- animal interaction, and although we did not explicitly address the evolutionary consequences of this ecological inheritance, the increase in herbivory demonstrated here is expected to have influential consequences. For instance, herbivory can be an important determinant of community structure and plant biodiversity, particularly in species-rich tropical and subtropical forests (e.g. Bagchi et al. 2014). Herbivores can directly harm plants through consumption of photosynthetic material (e.g. leaves) or reproductive structures (e.g. flowers), and can also indirectly impact plant fitness by altering floral traits and decreasing pollinator preference and efficiency (Mothershead and Marquis 2000). Additionally, herbivory can influence ecosystem function by altering feedback between plants and nutrient cycles, thereby modifying rates of decomposition and chemical cycling within a system (Schmitz et al. 2008 and references therein). In mangrove systems specifically, herbivory by wood-borers and folivores shapes forest structure, influences nutrient dynamics, and alters primary productivity (Feller 2002).

Additionally, the complex species interactions highlighted in this study are of broad significance, as they provide an interesting alternative to classic conceptions of trait-mediated interaction networks. Interaction networks integrate non-trophic and indirect links (e.g. trait-mediated indirect effects) with direct trophic interactions to elucidate the multiple facets of species interactions (Ohgushi 2005). These networks allow us to more effectively understand community structure and species interactions.

For instance, in classic examples of competition between herbivores, one herbivore has an indirect negative effect on another herbivore via competitive interference, while both herbivores have a direct negative effect on the plant through their consumption of plant material (Fig. 7A). Alternatively, one herbivore may have an indirect positive effect on another herbivore through facilitation, which has a corresponding indirect negative effect on plant fitness (Fig. 7A). However, the interaction network demonstrated in this study (Fig. 7B) is considerably more complex. Here, *E. mimeticum* beetles have an immediate direct negative effect on the mangrove through their wood-boring, as does the mangrove tree crab *A. pisonii* via its herbivory. Furthermore, *E. mimeticum* beetles have an indirect positive effect on *A. pisonii* via their niche construction (i.e. facilitation by providing a predation refuge). They also have a further indirect negative effect on mangroves via increased herbivory by *A. pisonii*. However, this indirect negative effect may not occur immediately, and it can persist long after the initial wood-boring activity is complete. Although most interaction networks assume that species interactions occur consistently through time, recent evidence suggests that incorporating temporal variation in our understanding of these networks is essential for accurately identifying species interactions (Poisot et al. 2015). The results

presented here further highlight the importance of incorporating temporal variables in interaction networks.

Collectively, we have demonstrated the extent to which a physical change in the environment (niche construction of predation refuge) subsequently influences community interactions via ecological inheritance. Past niche construction provides current predation refuges for the most influential herbivore in the system, thus modifying spatial herbivory patterns. Herbivory in this system affects forest structure, influences nutrient dynamics, and modifies primary productivity (Feller 2002). Although not explicitly examined, the consequences of these changes are expected to modify selection pressure on numerous species. Additionally, Sanders et al. (2014) recently highlighted the need to integrate the effects of physical environmental changes with concepts of trophic dynamics, which we have done here by applying niche construction theory. This knowledge will be useful in predicting the impacts of natural and anthropogenic disturbances on species interactions and community structure. Finally, we have incorporated direct effects, indirect effects, and temporal variation into a single interaction network to provide a comprehensive overview of the interactions in this community. Although most interaction networks ignore temporal factors, emerging evidence suggests that their inclusion is essential for accurately identifying species networks and community interactions (Poisot et al. 2015), and our results reinforce this conclusion. Therefore, this study contributes to a comprehensive understanding of the magnitude of ecological inheritance following niche construction, and also highlights the necessity of continually incorporating additional information in the design of species interaction networks.

6.6 Acknowledgements This work was partially supported by NSF grant # OCE-1129166 and an Elsie Taber Graduate Research Fellowship from the University of South Carolina.

6.7 Figures

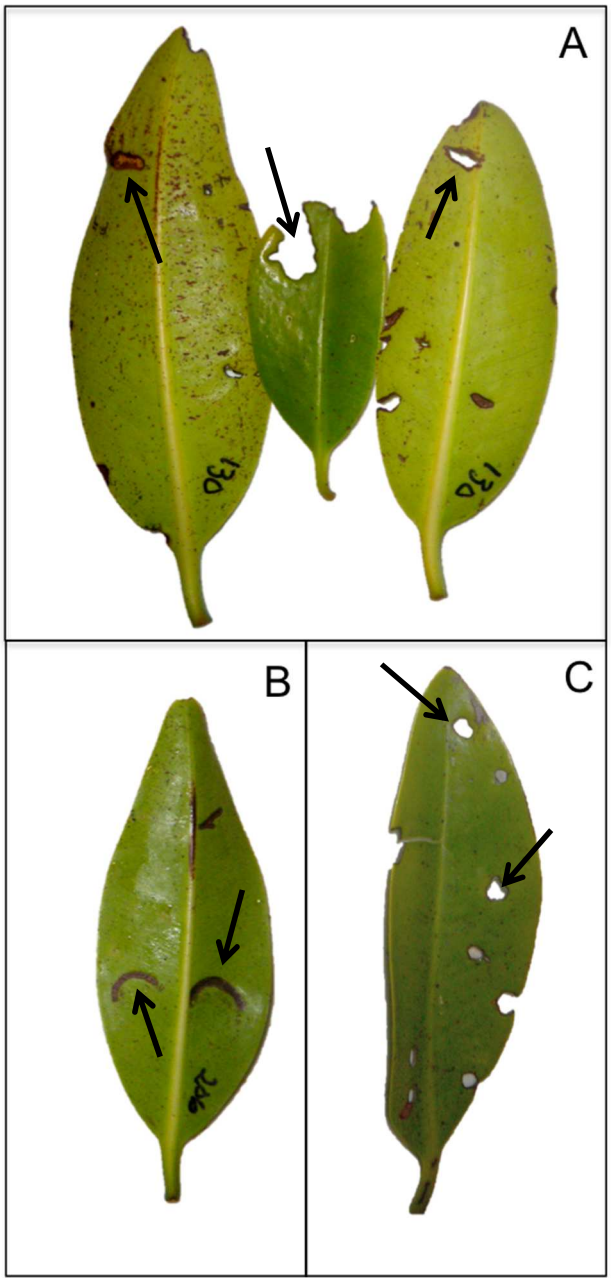

Figure 6.1. Characteristic examples of herbivory by the three herbivores relevant to this study: (A) the mangrove tree crab *A. pisonii*, (B) the mangrove periwinkle snail *L. angulifuera*, and (C) larvae of the bagworm moth *O. mangle*. Arrows indicate the location of select examples illustrating typical signatures of leaf damage.

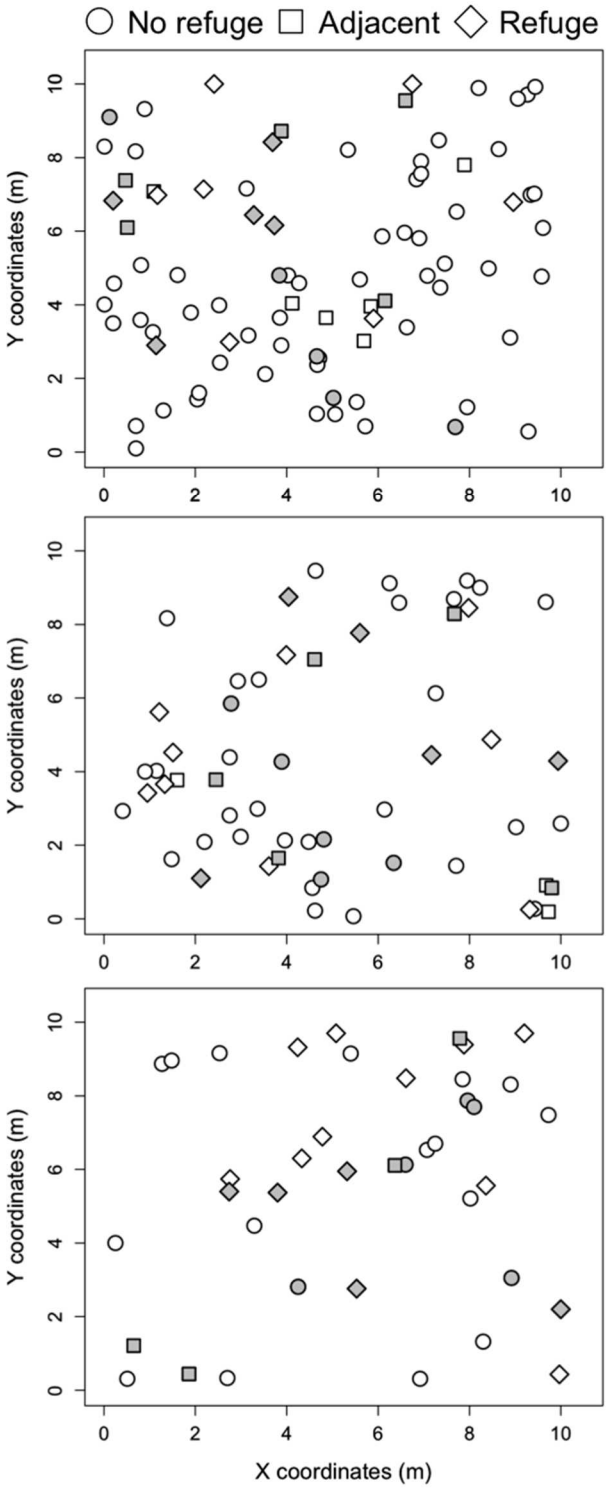

Figure 6.2. Spatial distributions of dwarf *R. mangle* trees in the three 10 m x 10 m plots established in this study. Closed symbols represent the trees for which leaves were randomly sampled for leaf image analysis.

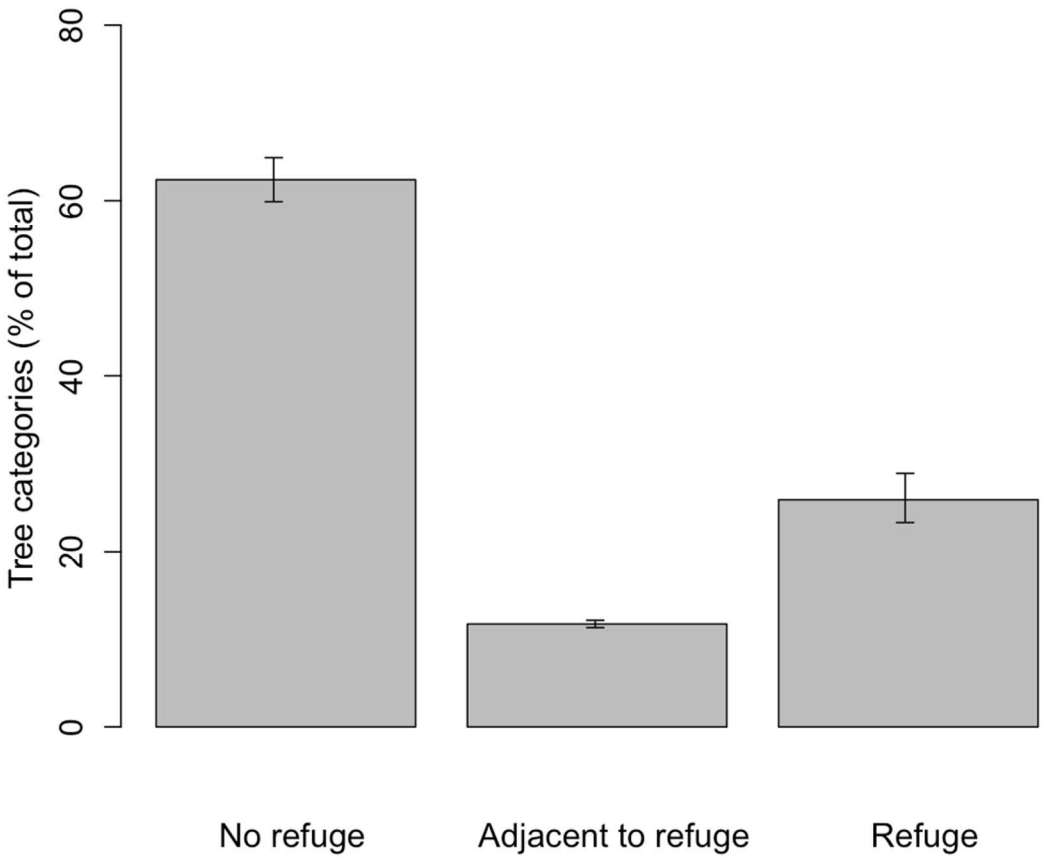

Figure 6.3. Relative distribution of tree categories across all plots (mean ± SE).

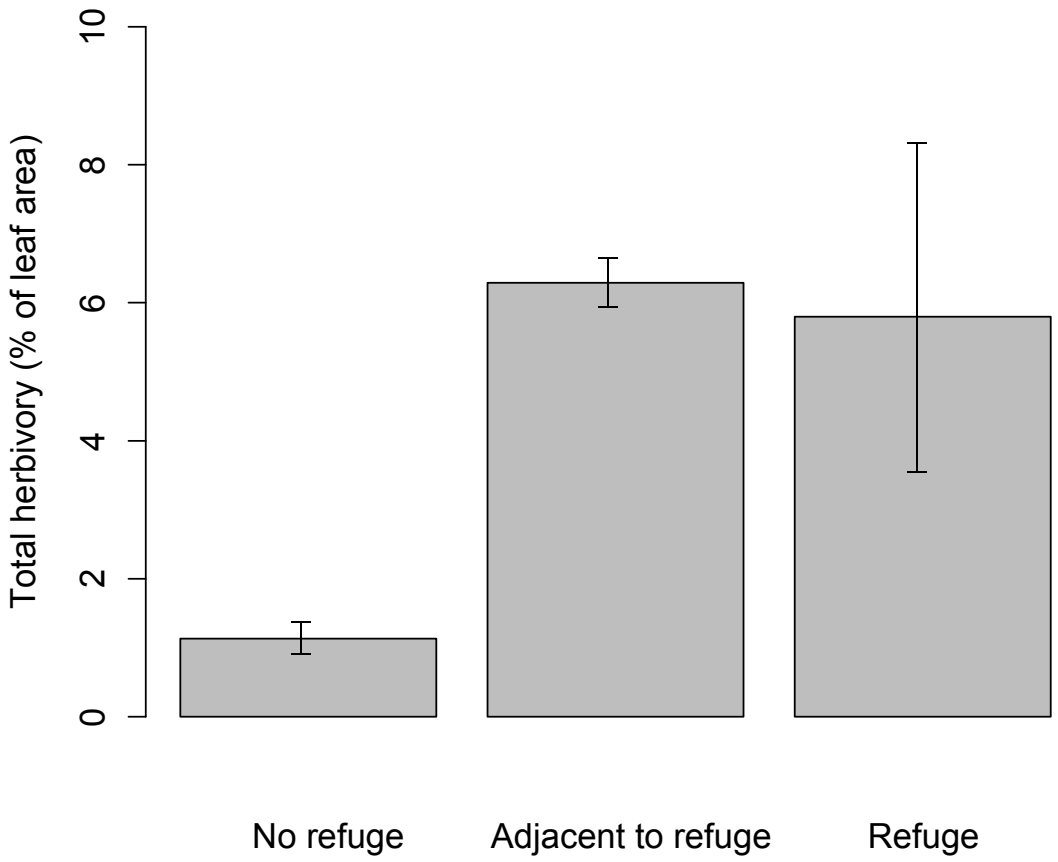

Figure 6.4. Total herbivory (mean % of leaf area ± SE) on *R. mangle* trees without refuges, adjacent to refuges, and with refuges.

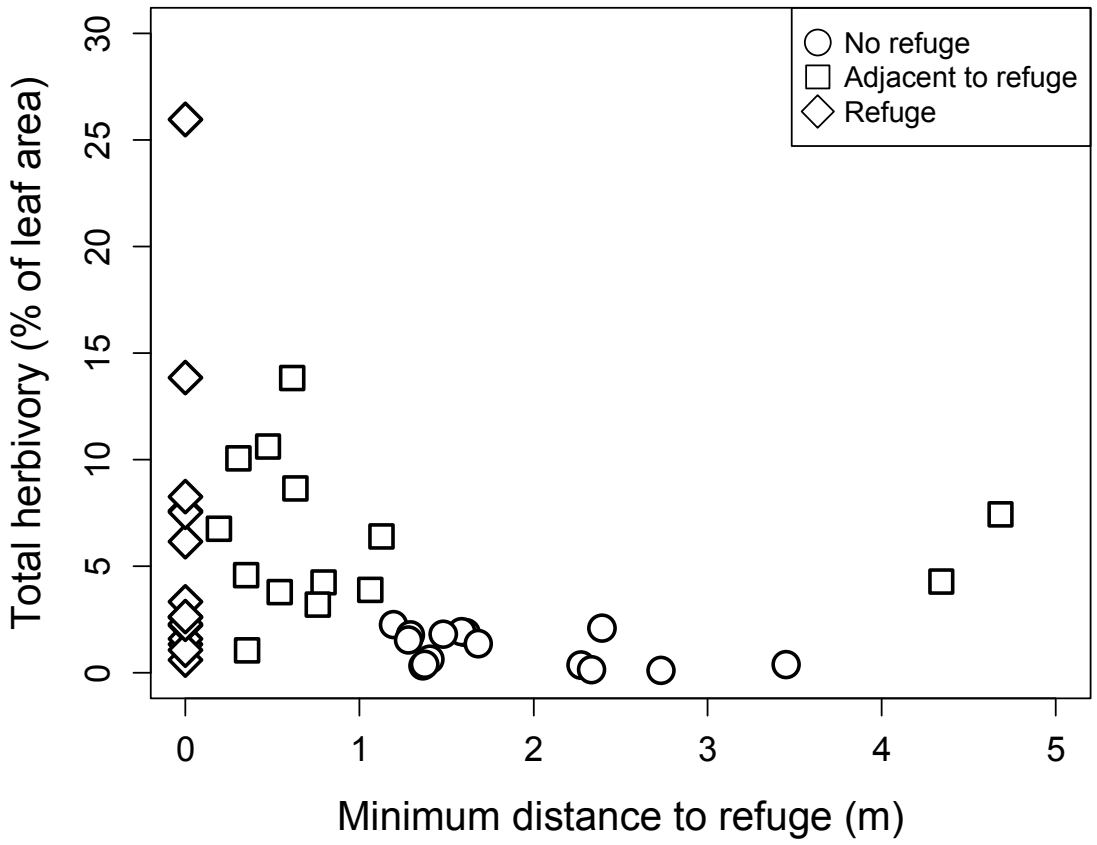

Figure 6.5. Impact of distance to refuge (m) on total herbivory (mean % of leaf area).

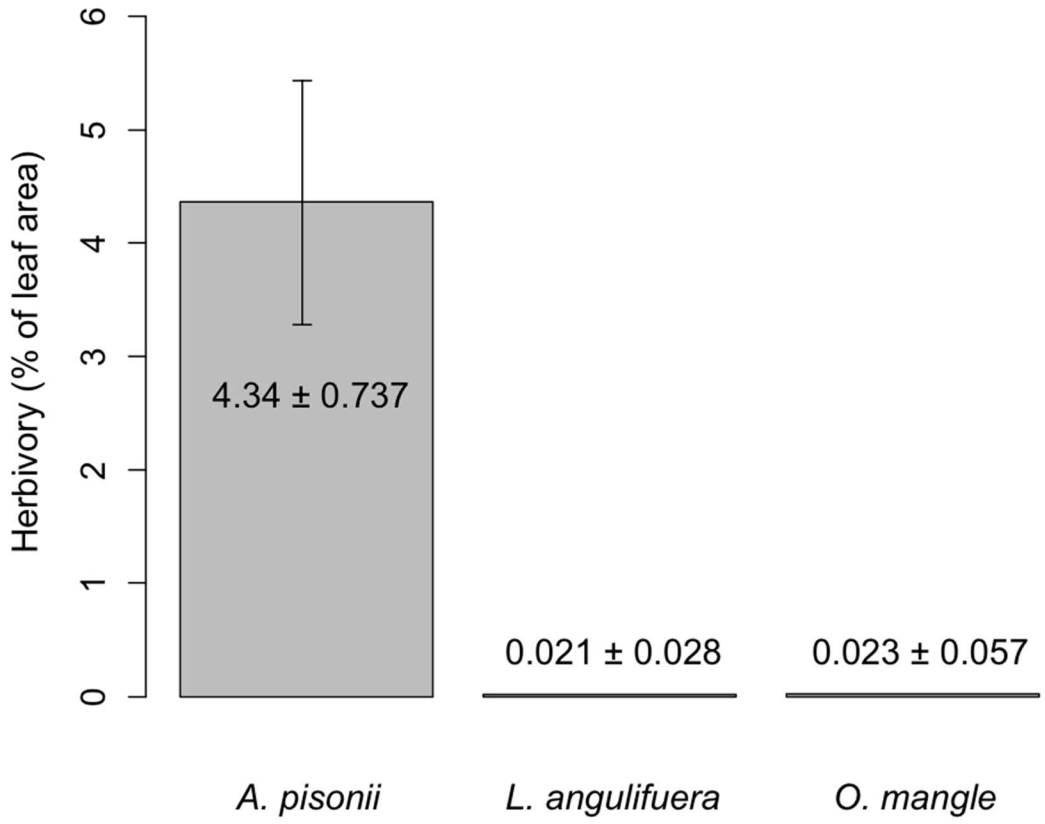

Figure 6.6. Herbivory contributed by each of the three herbivores. Herbivory values (mean % of leaf area ± SE) are indicated for each herbivore. Error bars are present but too small to be visible for *L. angulifuera* and *O. mangle*.

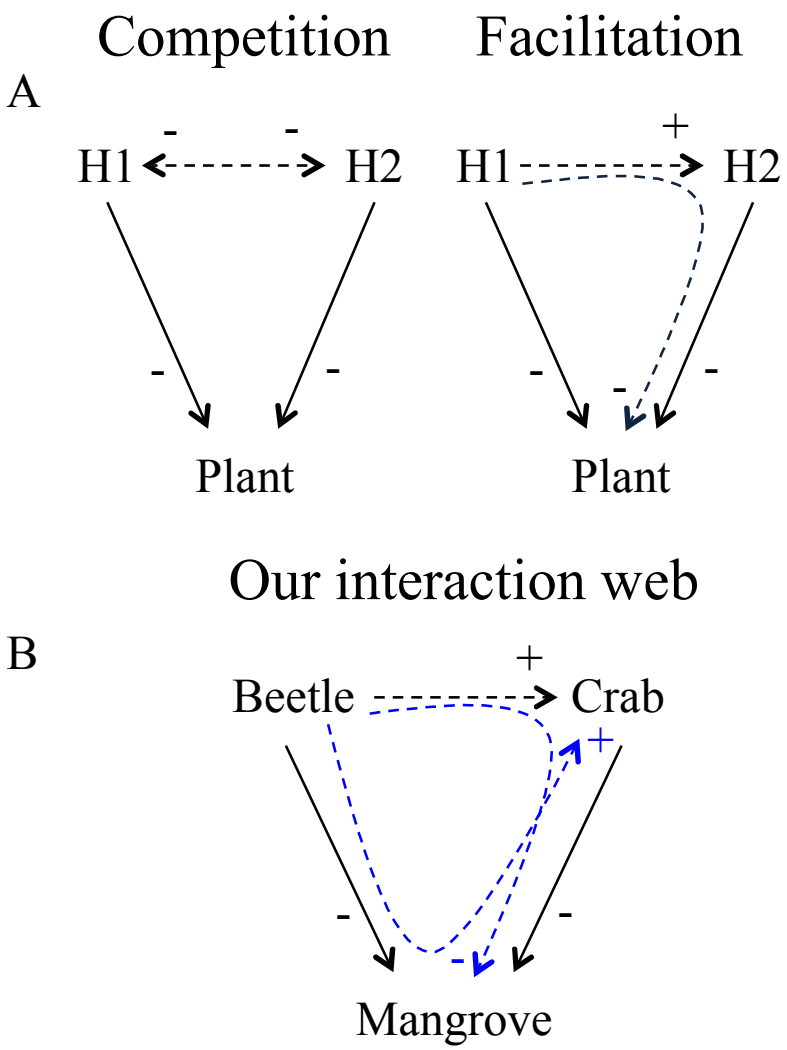

Figure 6.7. Interaction networks representing (A) the complex species interaction network established in this study and (B) classic competition and facilitation networks. Solid straight lines represent direct effect via consumption. Dashed straight lines represent direct effect via change in trait (e.g. foraging behavior). Curved dashed lines represent trait-mediated indirect effects. Black lines represent immediate effects, and blue lines represent future effects.

CHAPTER 7

GENERAL CONCLUSION

In this dissertation, we assessed species responses to climate change and environmental heterogeneity by addressing five key topics:

CHAPTER 2 described the climate change-induced range expansion of the mangrove tree crab *Aratus pisonii*. This species' range expansion has outpaced that of its historical mangrove habitat, leading to *A. pisonii*'s establishment in novel salt marsh habitats. Based on historical records of its range limit near the beginning of the 20th century, the species has expanded its range at a rate of ~62 km per decade, which is consistent with reports of the range expansion rates of other species with a planktonic larval phase (Poloczanska et al. 2013). Disrupted species interactions and novel communities such as the one described here may present serious challenges to policy makers and resource managers, and thus deserve particular attention as climate change advances (Hoegh-Guldberg and Bruno 2010).

CHAPTER 3 identified latitudinal trends in *A. pisonii*'s life history traits (body size and size at maturity). In concordance with traditional ecogeographic concepts (i.e. Bergmann's Rule; Ray 1960; Meiri and Dayan 2003), body size and size at maturity

increased with latitude within mangrove habitats. However, they decreased significantly in novel marsh habitats at the most poleward latitudes of the species' range. This reversal in latitudinal body size patterns was consistent with habitat-specific differences in both predation and temperature selection pressures. There were no habitat-specific differences in initial maternal investment (egg volume and weight), but fecundity was lower in novel habitats due to decreased maternal size at reproduction. Offspring quality (larval starvation resistance) was likewise lower in novel habitats relative to historical habitats. This chapter demonstrated that climate change responses (e.g. range expansions into novel habitats) have the potential to alter latitudinal trends of life history characteristics, resulting in potentially important fitness consequences relevant to their persistence in novel habitats.

CHAPTER 4 investigated phylogeographic variation in two mitochondrial markers (COI and control region) throughout a large part of *A. pisonii*'s range, including native mangrove and novel salt marsh habitats. Both loci provided evidence of significant genetic differentiation consistent with a pattern of isolation by distance (Wright 1943), with populations separated by ~236 km showing significant genetic similarity and those separated by more than ~1181 km showing significant genetic dissimilarity. Intriguingly, there was no evidence of genetic differentiation between mangrove and marsh sites on the Florida mainland, which span an established phylogeographic barrier (Avise 1992, Pelc et al. 2009). This lack of differentiation suggests the presence of high gene flow between mangrove and marsh habitats, and is a strong indication that the habitat-specific phenotypic differences described in CHAPTER 3 are the result of phenotypic plasticity rather than local adaptation. This chapter also examined geographic patterns of molecular

diversity, which tended to decrease from the southernmost sampling site (near the center of the species' range) to the northernmost sampling site (near the margin of the species' range). Collectively, this chapter revealed significant phylogeographic structure throughout a large portion of *A. pisonii*'s range, which was previously unrecognized in this species. It also provides evidence that phenotypic plasticity is the mechanism underlying altered life history traits associated with the species' range expansion, and highlights the potential importance of plasticity in facilitating species' responses to climate change.

CHAPTER 5 provided a mechanistic understanding of the impact of an omnivorous diet on the mangrove tree crab *A. pisonii*. Physiological condition improved with consumption of both plant and animal material, while only consumption of animal material increased reproductive investment. This demonstrates the importance of opportunistic inclusion of animal material in *A. pisonii*'s diet, and suggests that vitellogenic individuals cannot fully compensate for a lack of animal material in their diet by increasing plant consumption. Organisms often initially respond to environmental changes with behavioral changes that can alter their foraging habits (e.g. Reichmuth et al. 2009). Thus, a mechanistic understanding of how diet quality and quantity influences fitness is essential to predict the impact of future environmental changes on species fitness and population dynamics.

CHAPTER 6 assessed the ecological inheritance of herbivore-constructed predation refuges by investigating the spatial nature of plant-herbivore relationships in a neotropical mangrove forest. This chapter demonstrated that past niche construction (i.e. creation of tree holes), which affected approximately 38% of trees in the system,

facilitates central place foraging by the dominant herbivore, the mangrove tree crab *Aratus pisonii*. Total herbivory was an average of 556% higher on the trees adjacent to refuges and 513% higher on the trees with refuges than on those without tree holes, and herbivory decreased with increasing distance from these predation refuges. This study also integrated complex non-trophic and trophic species interactions by creating a comprehensive interaction web. This interaction web incorporates temporal variation, which was a key component of the indirect impact of predation refuges on mangroves. This chapter contributed toward a comprehensive understanding of the magnitude of ecological inheritance following niche construction, and also highlighted the necessity of continually incorporating additional information (e.g. temporal variation) in the design of species interaction networks.

Collectively, this dissertation provides important insights that further our understanding of species responses to global climate changes and environmental heterogeneity. It employed a multi-focal approach, including field sampling and surveys, laboratory-based physiological experiments, and genetic analyses, to address five primary topics. This dissertation documented the range expansion of the mangrove tree crab *Aratus pisonii* into novel habitats and utilized historical documentation of its range limit to estimate the rate of this range expansion (CHAPTER 2). It investigated latitudinal trends in key life history traits, and examined these life history trends and related fitness metrics within the context of the species' climate change-induced range expansion (CHAPTER 3). It also employed genetic analyses to investigate the relative roles of phenotypic plasticity and local adaptation in the phenotypic changes associated with its range expansion (CHAPTER 4). Additionally, it provided a mechanistic understanding of the impact of an

omnivorous diet on the mangrove tree crab *A. pisonii*, which is essential for predicting the impact of environmental changes on species fitness and population dynamics (CHAPTER 5). Finally, it investigated the ecological inheritance of past niche construction activities (i.e. habitat modification) on the intensity and spatial distribution of current plant-herbivore interactions (CHAPTER 6). This dissertation explored a number of ecological themes, and its results highlight the diverse impacts of various environmental changes on multiple levels of organization, from the genetic to the individual to the community level.

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APPENDIX A

PERMISSION LETTER FROM EDITOR FOR CHAPTER 2

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Megan Riley <riley.megan.e@gmail.com>

Copyright inquiry

Keith Goldfarb <keith.sena@eaglehill.us> Mon, May 18, 2015 at 9:18 AM
To: Megan Riley <riley.megan.e@gmail.com>

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Best,
Keith

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Keith Goldfarb, Editor-in-Chief
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APPENDIX B

CREATIVE COMMONS LICENSING AGREEMENT FOR CHAPTER 5

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