Summer 6-21-2023

Mating Burrows in the Fiddler Crab *Leptuca pugilator*: How a Key Resource is Contested, Constructed, and Shared

Zachary Michael Lane
University of Southern Mississippi

MATING BURROWS IN THE FIDDLER CRAB *LEPTUCA PUGILATOR*: HOW A
KEY RESOURCE IS CONTESTED, CONSTRUCTED, AND SHARED

by

Zachary M. Lane

A Dissertation
Submitted to the Graduate School,
the College of Arts and Sciences
and the School of Ocean Science and Engineering
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

Approved by:

Dr. M. Zachary Darnell, Committee Chair
Dr. Kelly M. Darnell
Dr. Eric Saillant
Dr. Jeffery Levinton

August 2023

THE UNIVERSITY OF
SOUTHERN
MISSISSIPPI.

ABSTRACT

The Atlantic sand fiddler crab, *Leptuca pugilator,* is found on sandy, vegetated beach across a large portion of the United States' Atlantic and Gulf of Mexico coasts and is iconic in regions where dense populations of the species occur for its charismatic courtship, competitive, and foraging behaviors. The social dynamics of the species are complex. Reproductively active males maintain mating territories in the dangerous heat of the intertidal zone, away from their food source at the water's edge, where they inhabit specialized mating burrows. These mating burrows are essential to successful female reproductive success, and when it is time to mate, females will move to the high intertidal to find a burrow-owning male to mate with. Males attract females with a species-specific waving display using their sexually dimorphic major claw to signal their availability. Following attraction, a female may approach a courting male and, provided the male's burrow is of adequate quality, the two will mate within. The female will then stay below ground in the safety of the terminal chamber of the burrow throughout the oviposition and incubation process until the time comes to release her larvae. The necessity of burrow ownership in mating for males creates a high demand for territory, and males interact with one another, sometime fighting, as each mating burrow is pass down from one owner to the next. In the following pages, I address several unanswered questions concerning the nature of *L. pugilator* social dynamics and behavior surrounding their most important resource: the mating burrow. In Chapter II, I attempt to discern what factors differentiate territory-owning males from others who do not or cannot own territory. Chapter III is an investigation of mating burrow structure and how it changes across time and space. In Chapter IV, I address the construction of new mating burrows which is a topic that has been left largely uninvestigated at the time of this writing. Finally, Chapter V attempts to unveil the social dynamics within burrow through paternity analysis of cohabitating males and females.

ACKNOWLEDGMENTS

My advisor, Dr. Zack Darnell, has played an integral role in this project. He has helped immensely by freely and openly offering his support from the first time we spoke on the phone, months before I started at The University of Southern Mississippi, until the very moment of this writing and, I imagine, will continue to offer his support until my degree is in-hand. My fellowship at the Gulf Coast Research Laboratory has been fruitful and fulfilling, in no small part due to the trust that Dr. Darnell has placed in me and the subsequent freedom this trust has granted me as a researcher. His input on the design and content of this project has elevated the final product into something I think we will both take pride in.

My committee members, Dr. Kelly Darnell, Dr. Eric Saillant, and Dr. Jeffery Levinton have also played integral roles. Each one is an excellent researcher in their own right. Dr. Darnell has invited me into her lab and home and allowed me to participate in some of the most important conservational work being done on the Gulf Coast. The time I spent surveying the seagrass beds of the Chandeleur Islands with her lab was particularly valuable. Dr. Saillant granted me access to the tools, space, and information needed to accomplish all of the molecular work associated with this project. Without his experience, I am not certain that the genetic analysis of Chapter V would have even been considered as a possibility. Dr. Levinton is what many scientists aspire to be: passionate, creative, and scholarly. The short periods of time we have had together have had a disproportionate influence on the direction of this project.

My colleagues, Dr. Warren Porter, Dr. Paul Mathewson, Talene Yeghissian, Candice Sheehan, Tami Hildahl, and Brooke Doucet are pillars of virtue and scientific

DEDICATION

This dissertation is dedicated to my mother, Judith K Lane (maiden name Harp).

All that remains is what was right and beautiful. I love you and miss you dearly.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

xiv

CHAPTER I – INTRODUCTION

The overarching goal of this project is to better understand how the Atlantic sand fiddler crab, *Leptuca pugilator* (Bosc 1802), competes for, shares, and constructs a key reproductive, thermal, and hydric resource: the mating burrow.

1.1 Background

Like all fiddler crabs, *L. pugilator* is sexually dimorphic with females having two small feeding claws and males having one small feeding claw and one greatly enlarged claw that is used in courtship display and male-male interactions (Crane, 1975). The major claw can comprise as much as 50% of a male's mass (Crane, 1975) and poses a significant energetic cost on due to its size (Allen and Levinton, 2007; Levinton and Judge, 1993) and the reduction in feeding efficiency that follows from having only one feeding claw (Miller, 1961; Weissburg, 1993, 1992). Sexual selection has favored the evolution of this greatly enlarged ornament/weapon due to the increased success that males with larger claw have in courtship (Christy, 1983; Jennions and Backwell, 1998; McLain and Pratt, 2007) and in the acquisition of mating territories (Hyatt and Salmon, 1978; Jennions and Backwell, 1996; Pratt and Mclain, 2002; Pratt et al., 2003).

Generally speaking, fiddler crab species fall into one of two categories in regard to their mating strategy: surface-mating species and burrow-mating species (Christy and Salmon, 1984; Crane, 1975; deRivera and Vehrencamp, 2001). In surface-mating species, which are more common in environments with ubiquitous nutrient and water availability (deRivera and Vehrencamp, 2001), males and females search each other out to mate on the surface of their habitat. Following copulation, the surface-mating pair will go their separate ways. Although this system benefits males by reducing the energetic

1

requirements of mating (e.g., performing courtship displays, disputing territories with other males), the fact that females undergo multiple bout mating can reduce the paternity contributions that each male has on any given egg clutch (Reaney et al., 2012).

Leptuca pugilator is a burrow-mating species. In burrow-mating species, one sex holds a small mating territory surrounding a mating burrow while the other sex actively wanders the habitat in search of territory-holding mates (deRivera and Vehrencamp, 2001), and in *L. pugilator*, it is the males that hold territory and the females who undergo mate searching (Christy, 1983, 1982). In *L. pugilator*, mating burrows are located in the high intertidal and low supratidal zones (Christy, 1982) away from the mixed-sex droves foraging at the water's edge (Crane, 1975; Reinsel and Rittschof, 1995; Robertson et al., 1980). In this species, mating burrows are complex structures comprised of a thin descending shaft and a relatively large terminal mating chamber (Christy, 1982).

In order to obtain a mating burrow, a territory seeking male must leave the foraging grounds in the low-intertidal and move to the high-intertidal mating area to obtain a burrow. The most common way of accomplishing this is through ritualized territorial disputes between burrow-owning and wandering males. The disputes begin when a wandering male approaches a territory resident and visually indicates that he would like to take over the residency of the mating burrow (Hyatt and Salmon, 1978). If the resident chooses to engage with the wanderer, the two will undergo a series of escalating interactions that move from initial visual signals to mutual contact of their major claws, to pushing with and interlocking their claws, and potentially to tossing one another from the territory (Hyatt and Salmon, 1978). In such disputes, the resident is at a distinct advantage (Hyatt and Salmon, 1978; Jennions and Backwell, 1996), and more

often than not retains his territory through his ability to defensively retreat into the burrow (Fayed et al., 2008). In some species this ownership advantage is strengthened through neighborhood dynamics and defensive coalitions (Backwell and Jennions, 2004; Booksmythe et al., 2010; Milner et al., 2011). Territorial disputes are frequently size assortative with wandering males choosing to engage with residents close to their own size, though smaller males are typically at a disadvantage when acquiring and defending territory (Hyatt and Salmon, 1978; Jennions and Backwell, 1996; Morrell et al., 2005; Pratt and Mclain, 2002)

Once in control of a mating burrow, males perform a species-specific claw-waving display to attract females to their territory (Crane, 1975; How et al., 2009; Pope, 2000). In general, females are attracted to males with large claws (Callander et al., 2012; Christy, 1983; Hyatt, 1977; McLain and Pratt, 2007; Oliveira and Custódio, 1998), likely because they are easier to see from a distance and effectively break the female's visual horizon when waving (Christy and Backwell, 1995; Land and Layne, 1995; Layne et al., 1997). Females also show preference for male displays that are performed rapidly (Callander et al., 2012; Milner et al., 2010; Sanches et al., 2017) or synchronously with surrounding males in certain species (Backwell et al., 1999; Kahn et al., 2014). The importance of claw size in mate attraction often presents large male with more opportunities to mate, but smaller males may still attract mates depending on their proximity to the female and other males, the preferences of the female, and the female's size (Backwell and Passmore, 1996; Booksmythe et al., 2008; deRivera, 2005; Greenspan, 1980; Reaney and Backwell, 2007; Reaney, 2009).

Courtship does not end after initial attraction. After approaching a waving male, females will inspect the male's mating burrow to ensure that it is of adequate quality, preferentially selecting burrows that fit their reproductive needs and body size (Backwell and Passmore, 1996; deRivera, 2005; Reaney and Backwell, 2007). In *L. pugilator*, if the male and his burrow are both of adequate quality, the female will descend the burrow followed by the male and the two will mate (Christy, 1983, 1982; Salmon and Hyatt, 1983). The female will remain underground to oviposit and incubate their eggs for ~14 days, after which she will emerge during a large amplitude night-time high tide to release her larvae (Christy, 1978). Proper timing of larval release decreases predation risk for the female and her offspring is important in female reproductive success (Christy, 1978; Morgan and Christy, 1995). By searching for mates at the appropriate time in the lunar cycle (Christy, 1983; Kerr et al., 2014; Salmon and Hyatt, 1983) and by undergoing larval incubation in the cool, stable environment of the mating burrow's terminal chamber, females are able to reliably release their larvae at the appropriate moment (Christy, 1982; deRivera, 2005; Reaney and Backwell, 2007). For this reason, it is rare for female *L. pugilator* to mate with a male that does not own a mating burrow, making the burrow a key reproductive resource for both sexes.

The location of the mating area in the high-intertidal has serious implications for reproductively active crabs of both sexes, but especially for territory-owning males. As previously mentioned, *L. pugilator*'s foraging grounds are located in the lowest portions of the low-intertidal zone, well away from the mating area (Reinsel and Rittschof, 1995; Robertson et al., 1980; Salmon and Hyatt, 1983). This means that crabs of both sexes must greatly reduce their nutritional intake for as long as they remain in the mating area,

leading to nutrient limitation in courting males (Allen and Levinton, 2014). In addition to the lack of available nutrients, the surface sediments of the high intertidal are dry (Allen and Levinton, 2014; Kim and Cho, 2003) and are subject to environmental temperature that can surpass *L. pugilator*'s critical thermal maximum (Allen and Levinton, 2014; Allen et al., 2012; Darnell et al., 2013; Munguia et al., 2017). Despite the fact that this species can change its pigmentation in response to environmental temperatures (Silbiger and Munguia, 2008; Wilkens and Fingerman, 1965) and the fact that its major claw seems to play a role in thermoregulation (Darnell and Munguia, 2011), the most direct solutions to thermal and hydric stress are to seek refuge in one's mating burrow (Darnell et al., 2020; Levinton et al., 2015; Smith and Miller, 1973) or vacate the mating area entirely. However, retreat in any form require the cessation of mating activity which imposes a direct cost to fitness (Darnell et al., 2020; Jennions et al., 2003; Reaney, 2007), and endurance rivalry between males results (Andersson, 1994; Darnell et al., 2013; Munguia et al., 2017). Regardless, the environmental stressors of the high intertidal along with the energetic costs of courtship (Matsumasa and Murai, 2005) and territory defense (Allen and Levinton, 2007) eventually take their toll, leaving males too weak to retain burrow residency for more than a few days (Christy, 1982; Salmon and Hyatt, 1983).

1.2 Chapter goals

In Chapter II, I attempt to discern what morphological, physiological, and behavioral differences exist between territory owning males and males that either cannot or will not hold territory. There have been many studies in the last century that have tried to accomplish similar goals, and most of their findings have been similar: larger, older males are more frequently found holding territory than smaller males, especially in higher

intertidal sites. This has led to many hypotheses that larger males are healthier, are more capable combatants, are able to defend their burrows for longer, or are more well suited to endure the stressors of the high intertidal than are small males. These hypotheses are built on the conception that territory ownership is determined by endurance, capability, and antagonistic competition between males, thus making territory holding males, and large males by association, appear as an elite class of extra-capable individuals. I believe that one rather simple way of testing these hypotheses that has not been fully utilized is to make direct comparisons between those males that hold territory and those males that do not. By defining a wide range of male quality metrics, randomly sampling within males of different groups, and making direct comparison between those groups I hope to bring a new perspective to this continuing discussion of what defines male fitness and competition in *L. pugilator*.

In Chapter III, I describe the structure of *L. pugilator*'s mating burrow and directly measure its thermal qualities. Though the behaviors surrounding mating burrows have been studied at great length, the burrow itself has been less vigorously investigated. I have come to see mating burrows as being nearly as integral to fiddler crab life history as our structures are to our own. Under that lens, continued study of the mating burrow itself is certainly warranted, and this chapter will serve to stimulate that topic of research for *L. pugilator* after a fairly long hiatus.

In Chapter IV, I attempt to measure how frequently *L. pugilator* constructs its mating burrows and how long it takes to do so. This information has not been reported in the literature to my knowledge, and its absence is somewhat befuddling. It is certainly useful to know, and I think the endeavor will be appreciated.

6

In Chapter V, I test paternity between cohabitating males and females. What happens below ground is extremely difficult to infer and keeping track of which individuals are where is next to impossible through observation alone. This investigation is an important first step in understanding how mating burrows are shared between males and females, and may have interesting implications regarding the potentially cooperative nature of breeding in *L. pugilator*.

CHAPTER II – MORPHOLOGICAL, PHYSIOLOGICAL, AND COMPETITIVE

CORRELATES OF TERRITORY OWNERSHIP IN *LEPTUCA PUGILATOR*

2.1 Introduction

A high-quality territory is critical for mating in many species (Best, 1977; Christy, 1983; Emlen and Oring, 1977). Because space for territories in preferred or optimal habitats is often limited, establishment of territories may be uncommon, and existing territories are frequently obtained through combat or competition and defended from intruders (Andersson, 1994). While a high-quality territory can be critical for attracting mates, there are often costs or consequences associated with holding that territory and defending it from intruders (Ord, 2021). First, there are energetic costs associated with territory defense (Marler et al., 1995). Second, maintaining and defending a mating territory may limit or preclude other necessary behaviors such as foraging (Allen and Levinton, 2014; Reinsel and Rittschof, 1995; Salmon and Hyatt, 1983). Third, if the mating territories occur in stressful environments (e.g., areas of high thermal stress), there may be stress effects that accumulate during territory ownership that gradually reduce the performance of the territory holder (Campagna and Le Boeuf, 1988). These costs and consequences of territory ownership may limit the amount of time a territory can be held and influence behavioral decisions concerning territory seeking, acquisition, and ownership.

Leptuca pugilator inhabits sandy shorelines along the Atlantic and eastern Gulf of Mexico of the U.S. and relies on territories in the high-intertidal zone for courtship and mating (Crane, 1975). These territories are centered on a specialized mating burrow, and courtship occurs on the sediment surface within the territory. These mating burrows and

8

the small territories which surround them are each maintained by individual males, who attempt to court receptive females into their territory by waving their large, sexually dimorphic major claw in a species-specific courtship display (Crane, 1975; Pope, 2009). The waving display serves to attract the attention of nearby females, who then assess the quality of the mating burrow before deciding whether or not to mate (Christy, 1983; deRivera, 2005; Reaney and Backwell, 2007). The specific microhabitat within the mating burrow is essential for reproduction in females (Christy, 1983; Reaney and Backwell, 2007). To increase the chance of survival for their larvae and themselves, females must time their reproductive cycle to coincide with a specific phase of the lunar cycle (night-time high and ebb tides) (Christy, 1978; Morgan and Christy, 1995; Pratt and Mclain, 2002; Salmon and Hyatt, 1983), and the microhabitat of the mating burrow provides an environment that is conducive to both oviposition and timely larval release (Reaney and Backwell, 2007).

Space is limited in the high intertidal mating habitat, which can easily contain hundreds of closely abutting territories (as many as 9-13 territories per m^2; pers. obs.) each with its male resident waving vigorously to attract any female that moves through the vicinity (Pratt and McLain, 2006). The limited space of the mating area means that not all males are able to hold territory at any given time, and this leads to inter-male competition for space. In addition to performing energetically demanding courtship displays, resident males must further expend their energetic resources to endure countless interactions with wandering males that would take their territories from them (Christy and Salmon, 1984; Hyatt and Salmon, 1979, 1978). These male-male interactions take the form of ritualized combat in which the resident male and the wandering male

communicate via visual displays and by touching, pushing, interlocking their claws, and, at times, lifting or throwing each other (Hyatt and Salmon, 1978). In general, larger males acquire territories at a higher rate than smaller males, apparently due to their size giving them the upper hand in territorial disputes (Christy, 1983; Hyatt and Salmon, 1978; Jennions and Backwell, 1996). Additionally, resident males, who can defensively retreat into their burrow or use their burrow for leverage during male-male interactions, have an ownership advantage over wandering males who must take an entirely offensive approach (Fayed et al., 2008; Hyatt and Salmon, 1978).

Males that are not courting within mating territories or wandering the high intertidal in search of mating territory can generally be found foraging along with non-reproductive females among the nutrient-rich surface sediment of the low intertidal zone (Crane, 1975). The wet sediment full of interstitial organic matter upon which *L. pugilator* feeds is not found homogenously throughout the intertidal zone but is concentrated in the lower reaches of the intertidal zone away from the high intertidal mating habitat (Reinsel and Rittschof, 1995; Robertson et al., 1980). The spatial heterogeneity of accessible nutrients and the restriction of courtship to the high intertidal zone makes foraging and courtship activities mutually exclusive for members of this species. This in turn creates a ubiquitous behavioral trade-off that must be balanced among all individuals: the decision to feed or breed.

For males, this trade-off limits behavior such that most males who are not actively mating or mate guarding can be broadly categorized as either courting, wandering, or foraging (hereafter termed 'behavioral states') (McLain et al., 2015). Courting males hold burrows gain access to mates, but are subject to starvation (Allen and Levinton, 2014;

Kim and Cho, 2003), intense energetic demands in the form of courtship displays and male-male interactions (Allen and Levinton, 2007; Matsumasa and Murai, 2005), and the dangerously high environmental temperature of the high intertidal zone with only their burrows to provide hydration and thermal refuge (Allen and Levinton, 2014; Allen et al., 2012; Darnell et al., 2013). Foraging crabs are afforded access to plentiful energetic resources, unlimited hydration, and low thermal stress, but are unable to mate (Allen and Levinton, 2014; Crane, 1975; McLain et al., 2015). Wandering crabs temporarily endure all the dangers and stressors of the high intertidal zone without access to mates or refuge but may acquire a mating territory as a result. If unable to acquire territory, wandering crabs must retreat away from the mating habitat or potentially face desiccation or exposure to fatal temperature extremes. All males move fluidly through these behavioral states over time.

The current study aims to determine what metrics of morphology, physiological condition, and performance determine the behavioral state chosen by male *L. pugilator*, and to investigate the impact of the lunar cycle and competition on these metrics. Hypotheses to be tested are as follows: (A) given the increased competitive advantage in territory acquisition and mate attraction held by larger crabs, larger crabs will be more likely than smaller males to be found courting, (B) physiological condition will be tied to behavioral mode with males in better condition being found more frequently among courting crabs and males in worse condition being found more frequently foraging or wandering, (C) courting males are likely to display increased ability to acquire territory and/or increased residence time at mating burrows compared to foraging and wandering males, (D) given the highly rhythmic reproductive cycle of fiddler crabs that is tied to the

lunar cycle, performance and physiology of males undergoing different behavioral modes will be tied to the lunar cycle in a way that logically follows the peaks and lulls of the female reproductive cycle, and (E) residence time at mating burrows is negatively impacted by the presence of wondering males.

2.2 Materials and methods

2.2.1 Field site and environmental data

The study was conducted in Panacea, Florida on the vegetated, sandy shoreline of Porter Island (30.0156, −84.3695) from 15 June–24 July 2021. The site is inhabited by a sizable population of *L. pugilator* with large, reproductively active males in the high intertidal waving at mate-searching females and protecting their mating territories from wandering males, and with non-reproductive males and females foraging together in the low intertidal during low tides. Males in this population can thus be divided into three groups base on their current behavioral mode (referred to as behavioral groups from here on): courting males (those maintaining a territory in the high-intertidal mating area and waving to attract females), foraging males (those males foraging in the low intertidal zone), and wandering males (males without a territory, wandering through the high-intertidal mating area).

Tides at the study location are semi-diurnal, and all observations, collections, and experimental replicates were made or began within 3 hours of low tide during daylight hours. During the 40-day study period, there was a full moon on days 10 (24 June) and 40 (24 July) and a new moon on day 26 (10 July). The number of days since the previous syzygy event (i.e., days since the previous new or full moon) was recorded throughout the study period. Additionally, a microclimate monitoring stations was deployed in a

representative area of the high intertidal breeding area and recorded sediment surface temperature, air temperature at a height of 6 cm, and air temperature at a height of 2 m above the sediment, every 5 min through the duration of the study.

2.2.2 Morphology and body temperature

Carapace width and claw length (measured as propodus length) were measured for all crabs collected for this study (n = 459). Additionally, body temperature was measured on the dorsal face of the carapace within 10 seconds of capture using an infrared thermometer for all crabs collected. Following collection, the relative claw length of each crab was calculated as the residuals of a linear regression of claw length against carapace width.

2.2.3 Physiological condition

To understand how immediate and short-term physiological condition differs between different behavioral groups, two measurements were recorded: claw-closing force and the hepatosomatic index (HSI). Every three days throughout the study period, five male crabs from each of the three behavioral groups (i.e., foraging, wandering, and courting crabs) were collected. Claw closing force was measured in the laboratory within 5 hrs of collection using a cantilever force meter much like that used by McLain et al. (2015). This meter consisted of two parallel plates attached to two arms which rested on an adjustable fulcrum and attached to a force sensor on the opposite side of the fulcrum. The force meter was connected via a cable to a digital meter which displayed the maximum force applied to the plates over the course of each individual measurement. Each crab was held by its carapace and positioned so that its claw was situated on the plates such that the distal end of the pollex touched the underside of the lower plate, and

the distal end of the dactyl contacted the top of the upper plate. Then, the focal crab was induced to close its claw resulting in a final reading of the focal crab's maximum claw-closing force in N. The claw-closing force was measured twice for each crab with a 5-minute rest between measurements and the higher of the two measurements was used in analyses. Following the claw-closing force measurements, a randomly selected subset of these crabs (3 per group per 3-day period) was frozen for later measurement of HSI.

At the time of HSI processing, each crab was thawed, dissected, and the hepatopancreas was removed. To control for variation in claw size among males, the major claw was removed at its base joint where the coxa attaches to the body and discarded. The hepatopancreas and the remainder of the crab were then dried at 60C for 48 hrs and weighed. Hepatosomatic index was calculated as the dry weight of the hepatopancreas divided by the total dry weight of the two samples (hepatopancreas + remainder of the crab).

2.2.4 Territory acquisition

To determine if male behavior and mating status were determined by individual ability or drive to secure a mating territory, we observed the combat of crabs from each of the three behavioral groups. Courting, wandering, and foraging males were captured and released into the mating area at least 2 m from their capture point. They were then followed at a distance of >3 m and their behaviors were recorded. Behavioral data included whether or not the focal crab initiated combat with a resident via physical contact, the escalation level of the combat, the duration of combat, the combat outcome, the species of the chosen opponent, the number of burrows checked before the end of the observation, and whether or not the focal crab initiated a second combat after the first.

Physical contact between the focal and resident crabs was used to establish the onset of combat because, despite the fact that non-physical signaling often occurs between residents and wanderers, non-physical signals are difficult to interpret and accurately observe and because these interactions rarely result in the exchange of territory between the combatants. Combat between two crabs was considered finished when one of the combatants moved 10 cm away from the contested burrow. Combat escalation was categorized into three groups: "pushing" in which the combatants made contact with their major claws but did not interlock them, "grappling" in which the combatants interlocked their claws, and "throwing" in which one combatant forcefully lifted or threw the other (Bolton et al., 2013; Pratt et al., 2003). The focal male was considered to have checked a burrow if they approached it and inserted at least one leg into its opening or else came into very close proximity to its owner eliciting a warning signal. Observations lasted ten minutes or until the focal crab secured a burrow, left the high intertidal, or initiated a second bout of combat. Observations in which the focal crab seemed to demonstrate a fear response behavior, by either quickly entering the nearest burrow or immediately running from the mating area into the shelter of dense vegetation or other cover at the trial onset, were discarded.

2.2.5 Territory residence time with and without competition

To determine if male behavior and mating status are determined by individual ability or drive to hold territory once in posseession of it, I measured the residence time of males from two of the behavioral groups. Courting and foraging males were captured, marked on the carapace with fingernail polish to be identifiable from a distance, and introduced to an empty burrow in the high intertidal mating area. Prior to beginning the

experiment, empty burrows were created by capturing and removing the original resident male of naturally constructed burrows, although a small number of burrows were artificially constructed by pressing a 19 mm diameter wooden dowel 25 cm into the sediment at a 45° angle. After introducing the focal male, a clear plastic fence (7 cm diameter, 7 cm high) was then placed around the burrow for 10 minutes to allow the focal male to become acclimated to its new burrow. At the onset of each crab's acclimation period, a PVC pipe with the burrow initiation time and date along with the crab ID was inserted into the sand nearby to the burrow, so that both the pipe and the burrow could be observed simultaneously using binoculars. The burrows were observed from a distance using binoculars three time each day: roughly three hours before low tide, within 15 minutes of low tide, and roughly three hours after low tide. The focal male was marked as present or absent during each of these observations. If a focal male was marked as absent during each observation for three days in a row or if a new resident had clearly moved into the focal burrow, the trial was ended.

To test the effects of competition from wandering males on residence time, two additional groups containing only males from the courting group were included in the residence time experiment. These groups utilized two types of experimental cages around each focal burrow: competitive exclusion cages and control cages. The competitive exclusion cages consisted of four sheets of stainless steel 0.5 cm mesh, cut and attached to one another to create a four-sided, outward-sloping fence shaped much like a trapezoidal prism with its smaller square face (25 cm L x 25 cm W) resting on the sediment around the focal burrow and its larger square face (38 cm L x 38 cm W) opening upward (Fig 2.1). The outer top edge (15 cm H) of the fence was lined with foil

tape. This outward-sloping design keeps wandering males out of the focal male's territory while allowing the focal male to easily abandon the territory at any time. The control cage was built identically to the competitive exclusion cage, but with large portions of its sides cut out (8 cm L x 9 cm H) to allow wandering crabs to enter the focal male's territory (Fig 1b). Besides the addition of the cages the crabs in these two caged groups were treated and monitored exactly as outlined above.

The design of the residence time experiment did not have the resolution to allow for the exact moment at which the focal male abandoned or was evicted from its burrow to be deduced, and instead gives two less accurate estimates of burrow tenure. The first estimate, which I refer to as minimum residence time from here on, is the time between the onset of the trial and the last observation in which the focal crab was known to be present at its burrow. The second estimate, referred to as maximum residence time from here on, is time between the onset of the trial and first observation in which the focal crab was absent from the burrow following the last observation in which the focal crab was known to be present.

2.2.6 General analytical approach

All statistical analyses were conducted in R 4.2.1 (R Core Team, 2021). The analytical approach used to test the hypotheses of the current study can be broken in four major parts: 1) the body temperature and morphology analyses, 2) the physiological condition analysis, 3) the territory acquisition analysis, and 4) the residence time analysis. Each of these analyses will be addressed in turn below, but to avoid repetition, the general statistical approaches used across the analyses is outlined here.

The most prevalent approach throughout the analyses is the use of univariate modeling (i.e., standard frequentist approaches), in which the effects of several independent variables on a single dependent variable are modeled mathematically. Most of the univariate models used in the current study fall into one of the following categories: (A) when dependent variable were continuous, linear fixed effect models (ANCOVA) were used and were created with the lm() model function of base R (R Core Team, 2021), or (B) when dependent variables were categorical, bi- and multinomial logistic fixed effects models (logistic regression) were used and were created with the multinom() model function of the nnet package (Venables and Ripley, 2003). When experimental design called for the inclusion of random effect variables, linear mixed effect model (analogous with nested ANCOVA) were used for continuous dependent variables using the lmer() model function from the lme4 package (Bates et al., 2015).

A majority of the univariate models were built with the following independent variables being initially considered for inclusion: behavioral group identity, number of days since the last syzygy event (referred to as lunar day from here on), carapace width, relative claw length, and daily averages of all of the recorded temperature variables (i.e., body temperature, air temperature at 2 m and 6 cm above the sediment, and surface sediment temperature). During analysis, behavioral group identity was treated as an unranked, categorical variable (i.e., nominal) and the morphological and temperature metrics were treated as standard continuous data. Lunar day was analyzed as circular data ranging from 0–15 days, and was included in the univariate models using the sine and cosine of the radian-transformed circular values to calculate two separate model coefficients according to the linear-circular regression approach (Zar, 1999).

18

When building models, multicollinearity (i.e., confoundedness) of the independent variables was addressed by searching for significant correlations that might exist between each independent variable and each other independent variable. In cases where statistically significant correlation existed between multiple independent variables, no more than one of those variables was used in the final model. Multicollinearity was a persistent issue, as all lunar and temperature variables were confounded due to the direct impact which lunar day has on the timing of low tide, the clear correlation which naturally exists at a given moment between various local environmental temperature metrics, and the subsequent impact which time of day has on both environmental temperatures and body temperature. Which of the confounded lunar and temperature variables to use in the final version of a given model was determined by the presence of a significant effect of the independent variables on the dependent variable when analyzed one at a time. This approach worked well in the current case, as no more than one of the confounded independent variables had a significant effect on any of the dependent variable at a time. In cases where none of the confounded lunar and temperature variables had a significant impact, lunar day was used in the final model and temperature data were excluded.

After assessing multicollinearity between independent variables and building the final model for each dependent variable, the model assumptions of residual normality and homoscedasticity were tested. If the assumptions were not met, the failed assumptions were rectified using a log-plus-one or square root transformation of the dependent variable. Once the assumptions were met, various R functions were to generate the ANOVA tables for the statistical models, and significance was determined using and

19

alpha threshold of 0.005. Specifically, ANOVA table were generated using base R anova() function for lm() models, the Anova(type = "III") function from the car package (Weisberg, 2019) for multinom() models, and the anova() function from the lmerTest package (Kuznetsova et al., 2017) for lmer() models. The post-hoc analyses used in the present study to decipher significant effect of categorical independent variables include Tukey's Honestly Significant Difference (HSD) test for continuous dependent variables and G test or pairwise G test with Bonferroni correction for categorical dependent variables.

2.2.6.1 Body temperature and morphological Analysis

For the analyses concerning body temperature, carapace width, and relative claw length as dependent variables, all data collected during all the experiments were pooled (n = 459). For these analyses, the identity of the experiment for which the data were initially collected (i.e., physiological condition, territory acquisition, or residence time) was included as a random variable. When analyzing body temperature variation among the crabs, the effects of behavioral group identity, lunar day, carapace width, relative claw size, and all two-way interactions were included as fixed model terms. Variation in carapace width and relative claw size were analyzed separately with behavioral group, lunar day, and all two-way interactions included as fixed model terms.

2.2.6.2 Physiological condition analysis

Both pinch force and HSI were analyzed using linear fixed effects models with behavioral group, lunar day, carapace width, relative claw length, and all two-way interactions which included behavioral group as fixed effects. To investigate the relatedness of pinch force and HSI, the Pearson's product moment correlation coefficient

was calculated, and significance of the correlation was determined using the t-distribution.

2.2.6.3 Territory acquisition analysis

In an attempt to simplify and visualize the relationships between the seven combat metrics collected during the territory acquisition experiment (i.e., combat initiation, combat escalation, combat duration, combat outcome, opponent species, number of burrows checked, and decision to fight again after first combat), a multivariate Factorial Analysis of Mixed Data (FAMD) was conducted (Pagès, 2015). This analysis combines the concepts of Principle Components Analysis (PCA) and Multiple Correspondence Analysis (MCA) to calculate the principal components (FAMD dimensions) of data containing both continuous and nominal variables in order to reduce the number of dimensions needed to capture the variation in the dataset. This analysis was first conducted using the entire territory acquisition dataset (n = 157) and all seven combat metrics, but it was found that roughly 46% of the variation in the full dataset was attributable to a single combat metric, combat initiation, with all those crabs which did not initiate a fight grouping extremely close to together along all principal component axes. This limited the usefulness of the initial FAMD for both data visualization and interpretation, so a second FAMD was conducted using only the data collected from crabs that initiated combat during their observation period (n = 57). In this second FAMD analysis, all combat metrics except combat initiation were included. To test my hypotheses concerning the effects of behavioral group and lunar cycle, the variation in FAMD dimensions one, two, and three of both completed FAMDs were modeled as a function of behavior group, lunar day, and crab morphology using linear fixed effect

models. Additionally, the relationships between pairs of combats metrics that were highlighted in the FAMD analysis were explicitly tested for significance using standard one-way ANOVAs or G-tests depending on the nature of the data, with post-hoc Tukey's HSD or pairwise G-tests with Bonferroni correction being utilized when significant relationships were found.

Though the FAMD approach proved fruitful for visualizing the various relationship that existed between the dependent combat metric variables, it was decided that a more in-depth look into the relationships between individual combat metrics and the other independent variables was needed to adequately address my hypotheses. The effects of behavioral group, morphology, and lunar day or temperature on the recorded combat metrics was determined using linear fixed effects models for the continuous combat metrics (i.e., number of burrows checked and combat duration), binominal logistic fixed effects models for the binomial combat metrics (i.e., combat initiation, combat outcome, opponent species, and decision to fight again after first combat), and multinomial logistic fixed effects models for multinominal combat metrics (i.e., escalation). Combat initiation was modeled using all of the data collected during the territory acquisition experiment (n = 157); combat escalation, combat outcome, combat duration, opponent species, and number of burrows checked were each modeled using only data from crabs which initiated combat (n = 57); and the decision to fight again after first combat was modeled using only those data collect from crabs which both initiated combat and lost (n = 39). The significant effects and post hoc analyses of these models were determined as outlined above.

2.2.6.4 Residence time analysis

Both maximum and minimum residence time were analyzed using linear mixed effects models with behavioral/cage group (i.e., four-level nominal data: foragers/no cage, courting crabs/no cage, courting crabs/control cage, and courting crabs/competitive exclusion cage), lunar day, carapace width, relative claw length, and all two-way interactions which included behavioral/cage group as fixed effects. Burrow type (i.e., naturally or artificially constructed) was included as a random effect.

2.3 Results

2.3.1 Morphology and body temperature

A total of 459 male crabs (courting = 196, foraging = 148, wandering = 115) were sampled across all experiments in the current study. Body temperatures did not vary consistently with behavioral group, carapace width, or relative claw length, though, as previously mentioned, body temperatures of all crabs fluctuated with lunar day (ANCOVA, sine of lunar day, F = 4.463, p = 0.035; Table 2.1A) according to the timing of low tide with higher body temperatures being recorded on lunar days 3–10 when day-time low tide occurred roughly midday (mean ± SD = 30.24 ±1.95 °C) and lower body temperatures being recorded on lunar days 11–2 when day-time low tides occurred toward the beginning and/or end of the day (26.31 ± 1.93 °C). There also was a significant interaction between behavioral group and lunar day on body temperature (ANCOVA, F = 9.187, p < 0.001; Table 2.1A) due to the fact that wandering crabs were hotter than foraging or courting crabs on days with mid-day low tides and colder than foraging or courting crabs on days with low tides in the morning/evening (Fig 2.2).

There was a small but significant difference in carapace width among the three behavioral groups (ANCOVA, F = 4.484, p = 0.012) with courting males having carapace widths that were roughly 0.7 mm larger on average than foraging and wandering males which did not differ statistically in carapace width according to Tukey HSD post hoc (courting = 17.6 ± 1.87 mm, foraging = 16.8 ± 1.82 mm, wandering = 17.0 ± 2.12 mm) (Table 2.1B). Carapace widths of the population did not vary with lunar day nor were there any significant two-way interaction terms (Table 2.1B).

Relative claw length did not differ between behavioral groups or across lunar day. However, there was a significant interaction between behavioral group and lunar day (ANCOVA, F = 4.468, p = 0.012; Table 2.1C) in which relative claw size of courting crabs peaked on lunar days 12–3 (0.19 ± 2.09 mm) and was at a minimum on lunar days 4–11 (–0.25 ± 1.94 mm), while a roughly opposite pattern is shown in foraging and wandering crabs whose relative claw length values peaked on lunar days 4–12 (foraging = 0.04 ± 2.20 mm; wandering = 0.07 ± 2.53 mm) and bottomed out on lunar days 13 - 3 (foraging = –0.24 ± 2.18 mm; wandering = –0.21 ± 2.24) (Fig 2.3).

2.3.2 Physiological condition

A total of 206 crabs were collected for the physiological condition analysis (courting = 70, foraging = 69, wandering = 67), with 124 of those being dissected for hepatosomatic index (courting = 41, foraging = 42, wandering = 41). Hepatosomatic index and pinch force were significantly and positively correlated for those crabs in which both measurements were taken (Pearson's product moment correlation, $r = 0.25$, $t_{(122)} = 2.90$, $p = 0.004$) (Fig. 2.4). The population mean HSI was 0.0368 ± 0.0192, and HSI values were log-transformed to meet ANCOVA model assumptions. Log-

transformed HSI values did not differ among behavioral groups but varied significantly with lunar day (ANCOVA; sine of lunar day, F = 7.860, p = 0.006; cosine of lunar day, F = 21.398, p < 0.001; Table 2.2A) with log-transformed HSI values peaking throughout the population on lunar days 14–5 (untransformed = 0.0456 ± 0.0208) and bottoming out on lunar days 6–13 (untransformed = 0.0289 ± 0.0134) (Fig 2.5). Log-transformed HSI and carapace width had a significant negative relationship (ANCOVA, F = 5.992, p = 0-.016; Table 2.2A). However, the significant interaction term between carapace width and behavioral group (ANCOVA, F = 4.436, p = 0.014; Table 2.2A) highlights that the negative relationship between HSI and carapace width only exists for wandering crabs, while no relationship exists between HSI and carapace width for courting or foraging crabs according to post hoc comparison of slopes (Fig 2.6).

Pinch force varied significantly among behavioral groups (ANCOVA. F = 9.698, p < 0.001; Table 2.2B) with courting crabs having a stronger pinch than foraging or wandering crabs whose pinch force did not differ statistically according to post hoc Tukey's HSD (courting = 831.26 ± 353.44 N, foraging = 678.49 ± 308.45 N, wandering = 591.60 ± 366.74 N). Pinch force also increased significantly with both relative claw length and carapace width (ANCOVA; carapace width, F = 5.751, p = 0.017; relative claw length, F = 14.275, p < 0.001; Table 2.2B) (Fig 2.7). Pinch force did not vary with lunar day alone, but there was a significant interaction between behavioral group and cosine of lunar day (ANCOVA, F = 3.910, p = 0.022; Table 2.2B) which indicated that wandering crabs had peak pinch force during lunar days 5–12 and decreased pinch force during lunar days 13–4, while no such lunar pattern existed for courting or foraging crabs (Fig. 2.8).

2.3.3 Territory acquisition

Of the 158 crabs that were observed during the territory acquisition experiment, 21 individuals either immediately fled the mating area (4 individuals) or immediately entered the nearest burrow and remined there for the rest of the observation (17 individuals); data from these crabs were discarded. Of the 137 remaining crabs which did not demonstrate a fear response, roughly 42% (58/137) initiated combat with a resident with the mean fight duration being 20.38 ± 16.97 s. Crabs which initiated combat chose to fight conspecifics in a majority of observations (51/58). Fights most commonly escalated to a maximum level of grappling (35/58) followed by pushing (19/58) and throwing (4/58), and focal crabs were more likely to win a contested burrow when fights culminated in a maximum escalation level of pushing (11/19 contests won) than when fight culminated in grappling (8/35 contests won) or throwing (0/4 contests won) (Table 2.3 A & B; G-test with post hoc pairwise G-test). Of the 58 crabs which initiated combat, roughly one third (19/58) gained control of the contested burrow, and a majority of the remaining 39 individuals which failed to acquire a burrow went on to initiate a second bout of combat after the first (28/39). On average, crabs investigated 3.42 ± 3.26 burrows before the end of the observation with those crabs which initiated combat checking more burrows (4.31 ± 4.05) than those which did not (2.77 ± 2.34) (Table 2.3A & B, Student's t-test).

When conducting FAMD on the entire territory acquisition dataset, FAMD dimensions 1–3 cumulatively accounted for 73% of the total variance in the data with dimension 1 accounting for 46% of the total variation, dimension 2 accounting for 16%, and dimension 3 accounting for 11%. The largest contributing variables for each of these

dimensions were combat initiation for dimension 1, contest outcome and escalation level for dimension 2, and escalation level and opponent species for dimension 3 (Fig. 2.9). There were no significant differences within any of these dimensions based on behavioral group, lunar day, or crab morphology metrics (Table 2.4A I-III). When conducting FAMD using only those crabs which initiated combat, FAMD dimensions 1–3 cumulatively accounted for 64% of the total variance in the data with dimension 1 accounting for 27% of the total variance, dimension 2 accounting for 19%, and dimension 3 accounting for 18%. The largest contributing variables for each of these dimensions were escalation level, contest outcome, and fight duration for dimension 1 and escalation level, number of burrows checked, and opponent species for both dimensions 2 and 3 (Fig. 2.10). Similar to above, there were no significant differences in individual loadings along any of these dimensions based on behavioral group, lunar day, or morphology metrics, except for individual loadings of dimension 3 which were significantly affected by lunar day (ANCOVA, cosine of lunar day, $F = 5.319$, $p = 0.026$; Table 2.4BI-III).

Crabs from different behavioral groups did not differ in their drive to initiate combat, their selection of conspecific opponents, the duration or escalation of their contests, the number of burrows they checked during observation, or their likelihood of winning contests for territory (Table 2.5AI & BI-V). The only metric which differed between behavioral groups was the decision to initiate a second bout of combat after the first (Binomial Logistic Regression, $Chi^2 = 9.401$, $p = 0.009$; Table 2.5CI). This significant model result was due to the relatively higher proportion of wandering crabs which initiated a second bout of combat (10/12 fought again after initial loss) compared

to the relatively smaller proportion of courting crabs which did so (5/11 fought again after initial loss). However, this difference between the two behavioral groups was ultimately insignificant in post hoc pairwise using Bonferroni multiple test correction (pairwise model coefficient comparison, z = 2.247, adjusted p = 0.074, unadjusted p = 0.025). The seven measured combat metrics largely did not vary with lunar day. Only combat outcome was significantly associated with lunar day (Binomial Logistic Regression, cosine of lunar day, Chi^2 = 5.674, p = 0.017; Table 2.5BI) with intruding crabs winning a higher proportion of contest during lunar days 13–3 (8/12 contest won; 66%) than the during the rest of the cycle (lunar days 4–12; 11/46 contests won; 24%) (Fig. 2.11). Morphology also had little impact on combat behavior and success. It was found that carapace width and body temperature were both positively related to number of burrows checked by focal crabs (ANCOVA; carapace width, F = 7.503, p = 0.000; body temperature, F = 11.284, p = 0.002; Table 2.5BV) (Fig. 2.12A & B), and that smaller individuals and individuals with higher relative claw lengths were more likely to undergo a second bout of combat beyond their first (Binomial Logistic Regression; carapace width, Chi^2 = 12.380, p < 0.001; relative claw length, Chi^2 = 14.551, p < 0.001; Table 2.5CI) (Fig. 2.13A & B).

2.3.4 Territory residence time with and without competition

Minimum residence time estimates averaged 11.79 ± 20.38 hrs (minimum = 0 hours, first quartile = 0.01 hours, third quartile = 22.78 hours, max = 99.03 hours) and maximum residence time estimates averaged 21.41 ± 22.86 hours (min = 0 hours, first quartile = 2.24 hours, third quartile = 24.98 hours, max = 99.97 hours) (n = 96) (Fig. 2.14). Residence time estimates were log-plus-one transformed to fit model assumptions.

28

Neither minimum nor maximum estimates were impacted by behavioral group, the presence or absence of experimental cages, or any measured morphological metrics (mixed effect ANCOVA; Table 2.6A & B). Both minimum residence time (mixed effects ANCOVA; cosine of lunar day, F = 7.956, p = 0.006; sine of lunar day, F = 6.349, p = 0.014; Table 2.6A) and maximum residence time (mixed effects ANCOVA; cosine of lunar day, F = 8.712, p = 0.004; sine of lunar day, F = 7.904, p = 0.006; Table 2.6B) estimates were significantly impacted by lunar day with both having peaks during lunar days 3–9 (minimum estimate = 18.65 ± 24.58 hrs, maximum estimate = 30.61 ± 25.71 hrs) and bottoming out during lunar days 10–2 (minimum estimate = 5.48 ± 12.88 hrs, maximum estimate = 12.93 ± 15.91 hrs) (Fig. 2.15). Maximum residence time estimates differed between natural and artificially constructed burrows with those crabs in artificially constructed burrows having shorter maximum estimates on average (natural burrows = 24. 13 ± 24.02 hrs, artificial burrows = 11.69 ± 14.89 hrs), and the burrow type random effect accounted for 23% of the variance not accounted for by the fixed effects in the minimum residence model (random effect variance = 0.63, residual plus random effect variance = 2.73) and 28% in the maximum residence model (random effect variance =1.83, residual plus random effect variance = 6.49).

2.4 Discussion

Mating territories are critical for many species, yet behavioral trade-offs occur when important life history activities such as foraging and mating are incompatible, forcing organisms to temporarily focus their attention on one behavior to the detriment of others. In *L. pugilator*, courtship and mating occur in mating territories in the thermally taxing high intertidal zone (Allen and Levinton, 2014; Allen et al., 2012; Darnell et al.,

2013) where courting males face starvation (Allen and Levinton, 2014; Kim and Cho, 2003) while undergoing energetically demanding courtship displays and defending their territories from competing males (Allen and Levinton, 2007; Matsumasa and Murai, 2005). Foraging, on the other hand, occurs in the low intertidal zone where nutrients and water are plentiful, but opportunities to mate are nonexistent (Allen and Levinton, 2014; McLain et al., 2015). Thus, male *L. pugilator* are forced to balance the trade-offs between feeding and breeding, switching between these behavioral modes to optimize their fitness. In the current study, I attempted to discern how morphology, physiological condition, and competitive performance impacts which males can be found courting, foraging, and wandering in search of territory throughout the lunar cycle.

2.4.1 Territory acquisition and residence time

The ability of individual males to acquire and retain territory was in no way correlated with their behavioral mode at time of collection. This is in opposition to not only the hypotheses of the current study concerning performance and behavioral mode, but to the more general hypothesis that combat prowess during territory acquisition and the ability to defend territories over relatively long periods are defining traits of males that gain access to mating territories in *L. pugilator*. Furthermore, the ability to acquire and retain territory was independent of both carapace width and relative claw length, making these results at odds with previous hypotheses that larger males are more capable combatants and territory holders than smaller males. Even one of the most fundamental hypotheses of male-male competition concerning *L. pugilator*, that intense physical competition with wandering males and forceful eviction of resident males is what generally leads the exchange of mating territories between males, is seemingly refuted in

30

the current study by the fact that residence time did not change when competitors were physically excluded from the territories of courting crabs.

The image of combat in this species, in which resident males ardently defend their territories for as long as physiologically possible against wandering males who use violence to forcefully take their territories from them, is replaced by something more benign: a scenario in which wandering males approach a resident and communicate, via ritualized displays and physical interaction, their desire to take over his territory, at which point the resident may engage with the wanderer and may or may not relinquish his burrow to the intruder depending on the information communicated and potentially his own desire to feed. This is supported in the current study by (A) the general lack of combat escalation recorded during the territory acquisition study (i.e., rarity of the combats escalating to the point of lifting or throwing), (B) the lack of performance differences between males across behavioral modes and sizes, (C) the ownership advantage of resident crabs seen in this study as well as several others, (D) the lack of influence of competition on residence time, and (E) the shorter-than-expected residence time of males overall. In other words, male 'combat' in the traditional sense was typically absent, the success of wandering males in acquiring territory was not tied to any clear metric of wandering male quality including size or previous success, and the exchange of territory appeared to be largely overseen by the current territory holder whose residence time was not significantly impacted by the presence of wandering competitors or previous experience holding territory. The physiological state of resident males at the time of territory exchange/abandonment was not known. Given the maximum recorded residence time of ~4 days and the average residence time of ~12–24 hrs recorded in the

current study, it seems likely that in most cases physiological condition does not absolutely necessitate retreat from the high intertidal to feed, but rather shifts behavior preference from courting to foraging in males well before a critically low energetic state is achieved.

The two viewpoints on male interactions outline in the previous paragraph (i.e., ritualize combat vs. ritualized communication) may seem to only differ semantically, but it is important that male-male interactions in this species and in others are not labeled as "combat" simply because they are physical in nature. Male-male interaction in *L. pugilator* can occasionally be violent, and although these extreme interactions are eye-catching and memorable, they are greatly outweighed by non-violent communication, negotiation, and physical touch.

This is not to say that there were no trends of interest present in the performance studies. Successful burrow acquisition was more common for focal crabs during lunar period of peak mating (i.e., on and around syzygy events), and this trend was reflected in the recorded residence times which were shorter during peak mating periods. This trend may represent differential nutrient availably throughout the lunar cycle (due to differing tidal amplitudes), but it is more likely that the higher rate of territory turnover associated with peak mating periods is due to the increased energetic costs of courtship displays, male-male interactions, and mating which are presumably associated with these periods. These increased energetic costs may lead residents to seek nutrients after a shorter residence period than during mating lulls when energetic costs are lower. Territory acquisition strategy was also impacted by temperature; it was seen that males which began combat acquisition with higher body temperatures investigated more burrows than

did males with lower starting temperature. This response likely reflects the warmer individuals' drive to seek thermal refuge. Additionally, differently sized males took slightly approaches to burrow acquisition, despite not differing in acquisition success. Larger crabs investigated more burrows per observation than did smaller crabs, while smaller crabs were more likely to engage in additional bouts of combat after suffering a loss. The cause and implications of these differences are unclear and warrant further investigation, but it should be noted that although both warmer males and larger male investigated more burrows, carapace width and body temperature were not correlated.

2.4.2 Morphology and physiological condition

Morphology was correlated with behavioral mode as expected. Carapace width differed significantly among behavioral modes with courting males typically having greater carapace widths than foraging or wandering males regardless of lunar day. This trend has previously been observed and has been hypothesized to be due to increased competitive ability possessed by large males to acquire territory following male-male interactions. This hypothesis has always been somewhat at odds with the generally size-assortative nature of male-male interactions (Hyatt and Salmon, 1978; Morrell et al., 2005) and given the lack of correlation in size and competitive performance reported above, it may be that this trend instead reflects a trade-off made by smaller crabs to allocate more resources to growth than courtship. The advantage of larger crabs in mate attraction is undeniable (Hyatt, 1977; McLain and Pratt, 2007; Oliveira and Custódio, 1998), and it therefore may be beneficial to lifetime reproductive activity to obtain a large size before shifting focus to courtship later in life.

Relative claw length differed between behavioral groups according to lunar day with courting crabs have larger relative claw lengths during the peak mating periods, but with foraging and wandering crabs having larger relative claw lengths during mating lulls. Relative claw length is static for individual males outside of the small changes that may follow a molt and the total loss of the major claw which can occur due to predation or violent bout of combat. With this being the case, the shift in relative claw lengths between behavior modes throughout the lunar cycle is likely indicative of behavioral shifts among individual crabs. Although larger males (i.e., those with greater carapace width) are able to court more frequently than smaller male throughout the lunar cycle, it appears that a secondary hierarchy exists among large crabs in which males with larger relative claw sizes are able to court during peak mating periods and those with smaller claws are left to court during mating lulls. Again, the data I have collected and reported here do not support the notion that greater relative claw length increases success in territory acquisition, though this is phenomenon calls for future, more direct studies of relative claw lengths impact on territory acquisition. Note that the morphological trends presented here, although significant, had substantial variation, and reproductive males of all sizes were recorded partaking in each behavioral mode throughout the lunar cycle.

The results concerning physiological condition on choice of behavioral mode are mixed in regard to their support of my initial hypothesis that males in better physiological condition would be more frequently found courting that foraging or wandering. No differences in HSI values were detected between behavioral groups, and instead, the population-wide HSI values fluctuated across all behavioral modes with the lunar cycle, peaking around periods of increased mating. The hepatopancreas serves several functions

34

in crustacean physiology, the most currently relevant of which is lipid storage (Gibson and Barker, 1979). The change in HSI values throughout the lunar cycle likely represents excess nutrients stored by males to prepare for, and subsequently used during, periods of peak mating. This interpretation suggests that males may partake in a general strategy of intense foraging during mating lulls and increases allocation of resources towards courtship, mating, and male-male interactions during mating peaks. Alternatively, the fluctuation in HSI reported here may simply reflect the physiological consequences of differing nutrient load within the high intertidal throughout the lunar cycle as tidal magnitude increases and decreases. HSI values were also significantly lower in large wandering males than in smaller wandering males. This would seem to point to territory acquisition having a greater acute cost for large males or towards older males investing less time/effort in nutrient acquisition between bouts of courting. Further study, tying HSI directly to relevant metrics of male ability and success, is warranted.

More in line with my initial hypotheses concerning physiological condition, pinch force was found to be greater in courting crabs than in foraging and wandering crabs. The increased pinch force found among courting crabs is an interesting trend, and although it is tempting to ascribe the increased likelihood of courtship behavior being observed in crabs with more powerful claws to an increased ability to succeed in territory acquisition, there is no data presented in the current study to directly support this. Correlation between claw closing force and success in combat have been previously reported (Lailvaux et al., 2009), but this study focused on difference in territory acquisition success between males with original and regenerated claws, not on the effect of differing pinch force within males with original claws. Given the positive correlation between

pinch force and carapace width reported in the current study and the fact that courting

crabs are generally larger than wandering or foraging crabs (this study and others), it is

simpler to attribute the increased pinch force of courting male to their increased size

rather than to a competitive advantage in territory acquisition provided by pinch force.

Studies in the field of how pinch force directly impact territory acquisition success of

males with non-regenerated (original) claws is warranted.

2.4.3 Conclusions

The results of the current study outline a potential shift in thinking that should be

considered in regard to male-male interactions over territory in *L. pugilator* and

potentially in other fiddler crab species. The idea that males are highly antagonistic, often

violent, and generally forceful with one another is not supported by these data, nor is this

idea well supported in previous literature outside of the traditional description of male-

male interactions as "combat" and its long-standing consideration as such (i.e., though

many studies are supposedly center on "combat", they do not provide fool-proof evidence

that the behaviors in question are truly combative in nature). Although, to the eyes of

many, male *L. pugilator* appear to fight each other (even the species name *pugilator,*

meaning "boxer", is reflective of this observation), actual violence between males is

relatively rare (Hyatt and Salmon, 1978) and the nature of their communication is far

from understood. The basic assumption of intense competition occurring between males

for territory ownership may even be worth reevaluating, a potential supported by the lack

of impact territory seeking crabs have on residence time. It is true that, generally

speaking, courting males are larger and have stronger pinch force than foraging and

wandering crabs, but these are trends, not rules, and their underlying causes remain undiscovered.

This study seeks to spark new hypotheses and future studies, not to discredit or replace current understanding. It is clear that social dynamics among males within this species are complex, and it is undeniable that they occasionally injure each and their interaction can escalate into actual combat. This escalation is usually centered around territoriality and drive to mate, which certainly points to some level of competition being involved in male-male dynamics, but the presence of these interactions does not imply that all interaction between males are adversarial. One hypothesis of interest not yet outlined is that, instead of being violent confrontations, many male-male interaction may serve to provide the current resident with information regarding his potential replacement's ability to maintain the burrow to the benefit of the mated females, and more importantly the current and previous residents' unborn offspring, which reside inside. There is little, if any, evidence that directly refutes this idea, and it could in part explain how residents, who seem to have considerable control over who replaces them, make the decision to pass on their territory.

Table 2.1 *Univariate linear fixed effects model results for body temperature (A),*

carapace width (B), and relative claw length (C)

Source	DOF	A. Body Temperature			
		SS	MS	F	p
Behavioral Group (BG)	2	0.691	0.3455	0.117	0.890
Cosine of Lunar Day (Cos)	1	5.702	5.702	1.926	0.166
Sine of Lunar Day (Sin)	1	13.213	13.213	4.463	**0.035**
Carapace Width (CW)	1	0.164	0.164	0.055	0.814
Relative Claw Length (RCL)	1	0.271	0.271	0.092	0.762
Significant Two-way Interactions					
BG:Sin	2	54.394	27.197	9.187	**<0.001**

Source	DOF	B. Carapace Width			
		SS	MS	F	p
Behavioral Group (BG)	2	31.924	15.962	4.484	**0.012**
Cosine of Lunar Day (Cos)	1	0.804	0.804	0.226	0.635
Sine of Lunar Day (Sin)	1	1.657	1.657	0.467	0.495
Carapace Width (CW)	1	-	-	-	-
Relative Claw Length (RCL)	1	-	-	-	-
Significant Two-way Interactions					
None	-	-	-	-	-

Source	DOF	C. Relative Claw Length			
		SS	MS	F	p
Behavioral Group (BG)	2	8.151	4.0755	0.807	0.447
Cosine of Lunar Day (Cos)	1	5.955	5.955	1.180	0.278
Sine of Lunar Day (Sin)	1	5.560	5.560	1.102	0.294
Carapace Width (CW)	1	-	-	-	-
Relative Claw Length (RCL)	1	-	-	-	-
Significant Two-way Interactions					
BG:Cos	2	45.102	22.551	4.468	**0.012**

Significant p-values are in bold type. Only significant two-way interactions are included (i.e., not all two-way interactions are included). Significant two-way interactions are written using shorthand labels indicated following main effect labels. For main effects, dashes represent those which were not included in particular models. For two-way interactions, dashes represent those which were insignificant or not included.

Table 2.2 *Univariate linear fixed effects model results for hepatosomatic index (A) and pinch force (B)*

Source	DOF	A. Hepatosomatic Index			
		SS	MS	F	p
Behavioral Group (BG)	2	0.896	0.448	2.233	0.112
Cosine of Lunar Day (Cos)	1	4.292	4.292	21.398	**<0.001**
Sine of Lunar Day (Sin)	1	1.577	1.577	7.860	**0.006**
Carapace Width (CW)	1	1.202	1.202	5.992	**0.016**
Relative Claw Length (RCL)	1	0.077	0.077	0.386	0.536
Significant Two-way Interactions					
BG:CW	2	1.779	8.890	4.436	**0.014**

Source	DOF	B. Pinch Force			
		SS	MS	F	p
Behavioral Group (BG)	2	2024294	1012147	9.698	**<0.001**
Cosine of Lunar Day (Cos)	1	120827	120827	1.158	0.283
Sine of Lunar Day (Sin)	1	8470	8470	0.081	0.776
Carapace Width (CW)	1	600193	600193	5.751	**0.017**
Relative Claw Length (RCL)	1	1489811	1489811	14.275	**<0.001**
Significant Two-way Interactions					
BG:Cos	2	816032	408016	3.910	**0.022**

Significant p-values are in bold type. Only significant two-way interactions are included (i.e., not all two-way interactions are included). Significant two-way interactions are written using shorthand labels indicated following main effect labels. Dashes represent two-way interactions that were not significant.

Table 2.3 *Test statistic matrix (A) and significance matrix (B) for territory acquisition dependent variables*

A. Test Statistic Matrix

	Initiation	Escalation	Outcome	Duration	Opponent sp.	Burrows Checked
Escalation	***					
Outcome	***	G = 9.87				
Duration	***	F = 2.49	t = 1.05			
Opponent sp.	***	G = 1.17	G = 2.24	t = 0.63		
Burrows Checked	t = 2.59	F = 0.39	t = 0.11	r = -0.14	t = 0.07	
Second Fight	***	G = 3.69	***	t = 0.25	G = 0.78	t = 0.39

B. Significance matrix

	Initiation	Escalation	Outcome	Duration	Opponent sp.	Burrows Checked
Escalation	***					
Outcome	***	**0.007**				
Duration	***	0.092	0.300			
Opponent sp.	***	0.556	0.134	0.546		
Burrows Checked	**0.011**	0.678	0.913	0.285	0.945	
Second Fight	***	0.192	***	0.807	0.376	0.697

Multiple types of tests were used to investigate the relationships between pairs of dependent combat variables depending on the naure of the variables. These tests were t-tests (indicated with t test statistic in (A)), ANOVA (indicated with F test statistic in (A)), G tests (indicated with G test statistics in (A)), and correlation tests (indicated with r test statistics in (A)). Significant p-values are in bold type. Three asterisks indicate that the relationship was not tested because it was not of interest (e.g., the relationship between combat outcome and initiation was not tested because crabs which chose not to initiate, had no combat outcome).

Table 2.4 *Univariate linear fixed effects model results using all crabs from the territory acquisition study (A) and only crabs that initiated combat (B) for FAMD dimensions 1-3*

| | | A. All crabs (n = 137) | | | | B. Only those which inititated (n = 58) | | | |
| | | AI. Dimension 1 | | | | BI. Dimension 1 | | | |
Source	DOF	SS	MS	F	p	SS	MS	F	p
Behavioral Group (BG)	2	19.37	9.685	2.041	0.134	0.369	0.185	0.095	0.909
Cosine of Lunar Day (Cos)	1	0.772	0.772	0.163	0.687	2.376	2.376	1.228	0.274
Sine of Lunar Day (Sin)	1	0.409	0.409	0.086	0.769	0.500	0.500	0.258	0.614
Carapace Width (CW)	1	2.219	2.219	0.468	0.495	0.536	0.536	0.277	0.601
Relative Claw Length (RCL)	1	0.151	0.151	0.032	0.859	0.030	0.030	0.015	0.902
Significant Two-way Interactions									
None	-	-	-	-	-	-	-	-	-

| | | AII. Dimension 2 | | | | BII. Dimension 2 | | | |
Source	DOF	SS	MS	F	p	SS	MS	F	p
Behavioral Group (BG)	2	0.789	0.3945	0.247	0.782	2.810	1.405	1.276	0.290
Cosine of Lunar Day (Cos)	1	4.009	4.009	2.508	0.116	1.473	1.473	1.337	0.254
Sine of Lunar Day (Sin)	1	0.018	0.018	0.012	0.915	0.001	0.001	0.001	0.993
Carapace Width (CW)	1	0.002	0.002	0.001	0.975	1.578	1.578	1.433	0.238
Relative Claw Length (RCL)	1	0.036	0.036	0.022	0.881	0.025	0.025	0.023	0.881
Significant Two-way Interactions									
None	-	-	-	-	-	-	-	-	-

| | | AIII. Dimension 3 | | | | BIII. Dimension 3 | | | |
Source	DOF	SS	MS	F	p	SS	MS	F	p
Behavioral Group (BG)	2	1.138	0.569	0.481	0.619	0.571	0.2855	0.258	0.774
Cosine of Lunar Day (Cos)	1	0.158	0.158	0.133	0.716	5.896	5.896	5.319	**0.026**
Sine of Lunar Day (Sin)	1	0.714	0.714	0.603	0.439	1.365	1.365	1.231	0.274
Carapace Width (CW)	1	0.277	0.277	0.234	0.629	0.806	0.806	0.727	0.399
Relative Claw Length (RCL)	1	0.343	0.343	0.29	0.591	1.756	1.756	1.584	0.215
Significant Two-way Interactions									
None	-	-	-	-	-	-	-	-	-

Significant p-values are in bold type. Only significant two-way interactions are included (i.e., not all two-way interactions are included). Significant two-way interactions are written using shorthand labels indicated following main effect labels. Dashes represent two-way interactions that were not significant.

Table 2.5 *Various univariate model results for territory acquisition independent variables using all crabs (A), only those which initiated combat (B), and only those which lost during combat(C).*

A. Models using all data (n = 137)

Source	DOF	I. Combat initiation (Binomial)	
		Chi^2	p
Behavioral Group (BG)	2	3.686	0.158
Cosine of Lunar Day (Cos)	1	0.671	0.413
Sine of Lunar Day (Sin)	1	0.099	0.753
Carapace Width (CW)	1	0.173	0.678
Relative Claw Length (RCL)	1	0.016	0.900
Body Temperature (BT)	1	-	-
Significant Two-way Interactions			
None	-	+	+

B. Models using males that initiated (n = 58)

Source	DOF	I. Combat outcome (Binomial Logistic)		II. Escalation (Trinomial Logistic)		III. Opponent sp. (Binomial Logistic)	
		Chi^2	p	Chi^2	p	Chi^2	p
Behavioral Group (BG)	2	0.355	0.837	1.784	0.775	0.549	0.760
Cosine of Lunar Day (Cos)	1	5.674	**0.017**	0.781	0.677	0.213	0.645
Sine of Lunar Day (Sin)	1	0.001	0.994	0.347	0.841	0.505	0.477
Carapace Width (CW)	1	1.983	0.159	0.202	0.904	1.064	0.302
Relative Claw Length (RCL)	1	0.693	0.693	0.496	0.781	1.114	0.291
Body Temperature (BT)	1	-	-	-	-	-	-
Significant Two-way Interactions							
None	-	+	+	+	+	+	+

Source	DOF	IV. Combat duration (Linear)				V. Number of burrows checked (Linear)			
		SS	MS	F	p	SS	MS	F	p
Behavioral Group (BG)	2	0.539	0.2695	0.471	0.628	1.527	0.7635	2.891	0.066
Cosine of Lunar Day (Cos)	1	-	-	-	-	-	-	-	-
Sine of Lunar Day (Sin)	1	-	-	-	-	-	-	-	-
Carapace Width (CW)	1	1.587	1.587	2.772	0.103	1.982	1.982	7.503	**0.009**
Relative Claw Length (RCL)	1	0.015	0.015	0.027	0.871	0.078	0.078	0.296	0.589
Body Temperature (BT)	1	1.817	1.817	3.174	0.082	2.981	2.981	11.284	**0.002**
Significant Two-way Interactions									
None	-	-	-	-	-	-	-	-	-

C. Models using males that lost (n = 39)

Source	DOF	I. Second fight (Binomial)	
		Chi^2	p
Behavioral Group (BG)	2	9.401	**0.009**
Cosine of Lunar Day (Cos)	1	2.641	0.104
Sine of Lunar Day (Sin)	1	0.119	0.730
Carapace Width (CW)	1	12.380	**<0.001**
Relative Claw Length (RCL)	1	14.551	**<0.001**
Body Temperature (BT)	1	-	-
Significant Two-way Interactions			
None	-	+	+

The models used varied and are indicated in parentheses below the combat variable labels. Significant p-values are in bold type. Only significant two-way interactions are included (i.e., not all two-way interactions are included). Significant two-way interactions are written using shorthand labels indicated following main effect labels. For main effects, dashes those which were not include. For two-way interactions, dashes represent those which were insignificant or not included. Pluses represent the absence of two-way interactions in the model.

Table 2.6 *Univariate linear mixed effects model results for minimum residence time (A)*

and maximum residence time (B)

Source	DOF	A. Minimum Residence Time			
		SS	MS	F	p
Behavioral/Cage Group (BCG)	3	5.764	1.921333	0.917	0.437
Cosine of Lunar Day (Cos)	1	16.667	16.667	7.956	**0.006**
Sine of Lunar Day (Sin)	1	13.300	13.3	6.349	**0.014**
Carapace Width (CW)	1	1.502	1.502	0.717	0.400
Relative Claw Length (RCL)	1	1.627	1.627	0.777	0.381
Significant Two-way Interactions					
None	-	-	-	-	-

Source	DOF	B. Maximum Residence Time			
		SS	MS	F	p
Behavioral/Cage Group (BCG)	3	5.472	1.824	1.447	0.236
Cosine of Lunar Day (Cos)	1	10.980	10.980	8.712	**0.004**
Sine of Lunar Day (Sin)	1	9.962	9.962	7.904	**0.006**
Carapace Width (CW)	1	2.362	2.362	1.874	0.175
Relative Claw Length (RCL)	1	1.624	1.624	1.288	0.260
Significant Two-way Interactions					
None	-	-	-	-	-

Significant p-values are in bold type. Only significant two-way interactions are included (i.e., not all two-way interactions are included). Significant two-way interactions are written using shorthand labels indicated following main effect labels. Dashes represent two-way interactions that were not significant.
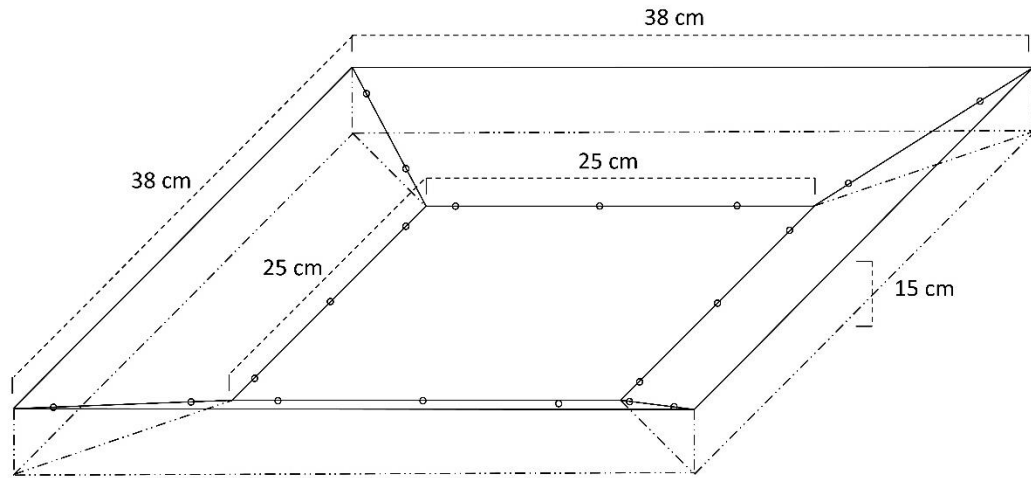
Figure 2.1 *Competitive exclusion device*

The competitive exclusion device serves to exclude intruding males from the focal male's mating territory while allowing the focal male to exist the territory at any point. The cage was placed such that the entrance to the focal male's burrow was at it center.
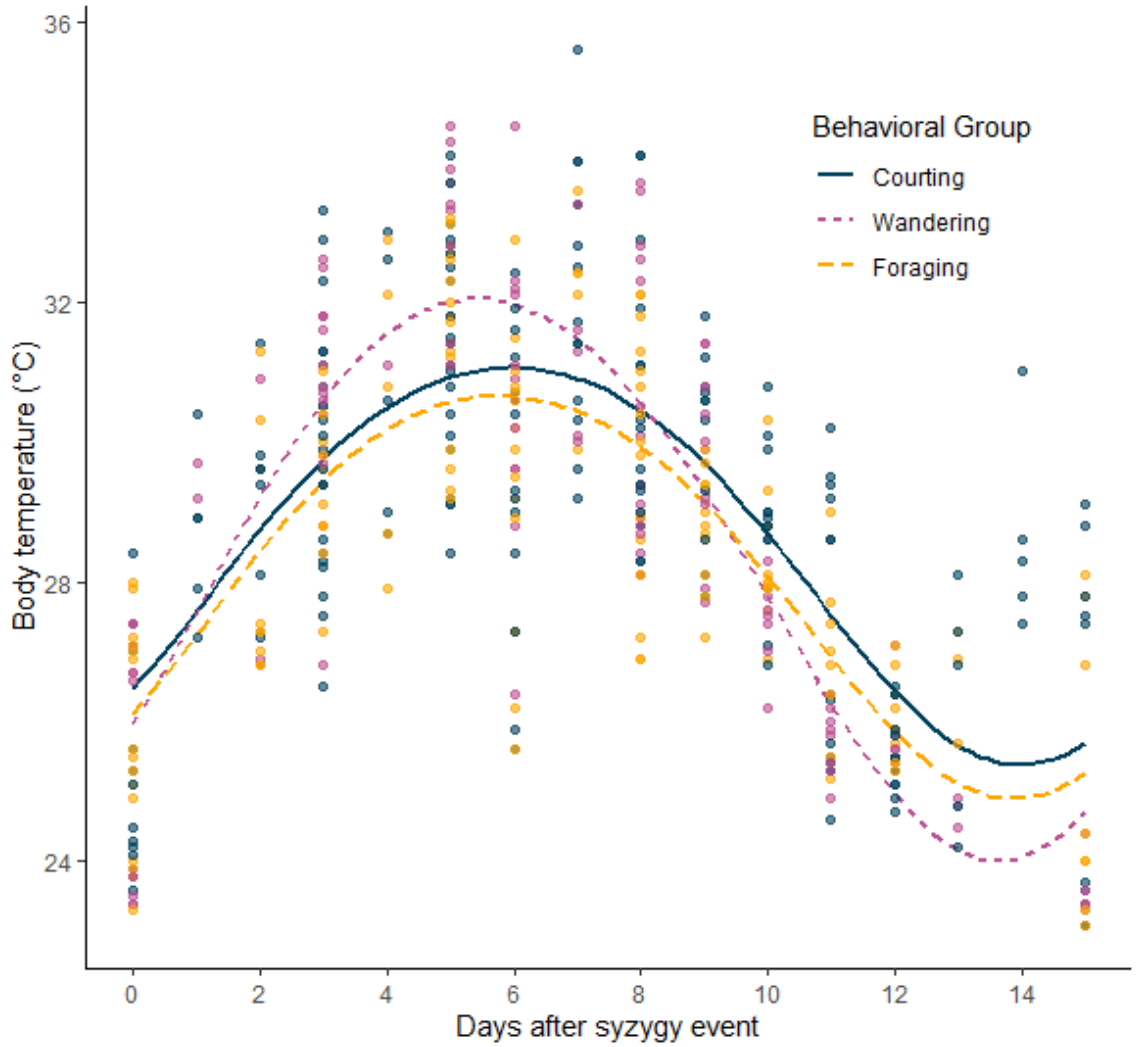
Figure 2.2 *Body temperature and the lunar cycle*

Body temperature measurements taken at time of collection across the lunar cycle for all crabs used in the current study are indicated with points (n = 459). Trend lines were significantly different from zero (ANCOVA, sine of lunar day, F = 4.463, p = 0.035) and from one another (ANCOVA, behavioral group: sine of lunar day interaction, F = 9.187, p < 0.001) according to univariate linear fixed effects model (Table 2.1A). Colors indicate behavioral group.
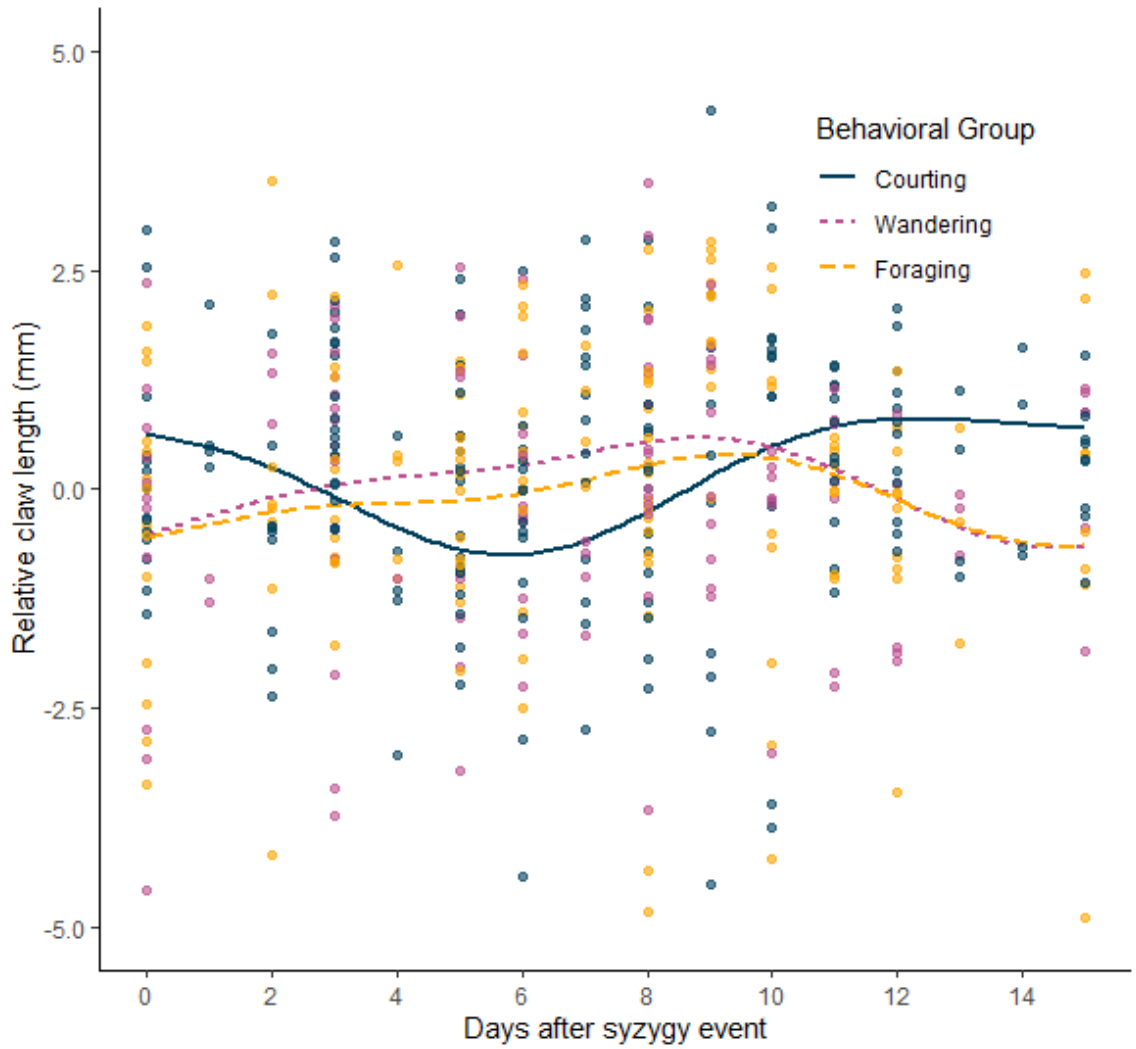
Figure 2.3 *Relative claw length and the lunar cycle*

Relative claw length measurements across the lunar cycle for all crabs used in the current study are indicated with points (n = 459).

Trend lines are significant different from one another (ANCOVA, behavioral group: cosine of lunar day interaction, F = 4.468, p = 0.012) according to univariate linear fixed effects model (Table 2.1C). Colors indicate behavioral group.
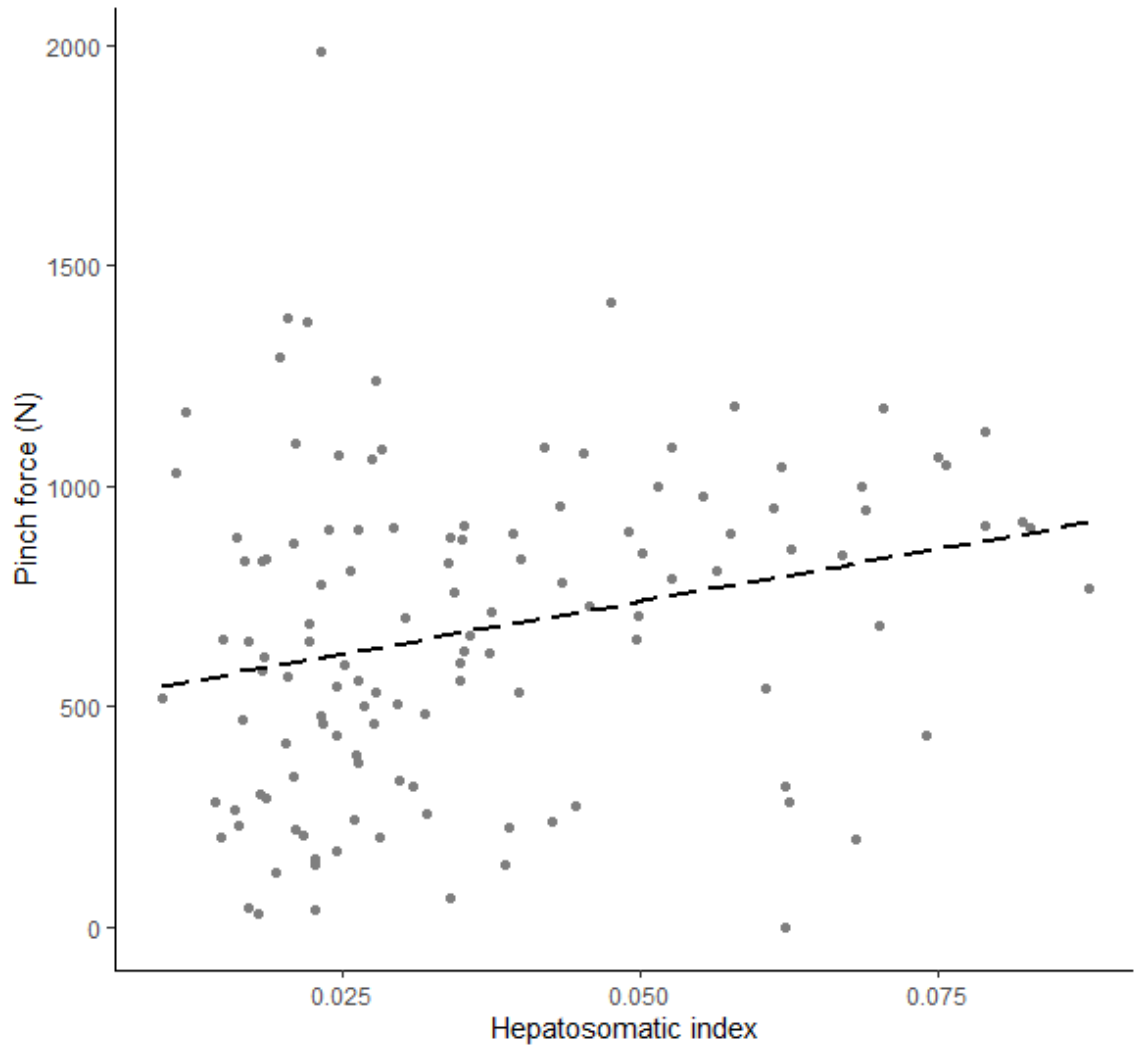
Figure 2.4 *Pinch force and hepatosomatic index*

Pinch force and hepatosomatic index measurement of each crab for which both metrics were measured are indicated with points (n = 124). The trend line demonstrates the relationship between the two variables which was found to be significant using correlation test (Pearson's product moment correlation, $r = 0.25$, $t_{(122)} = 2.90$, $p = 0.004$).
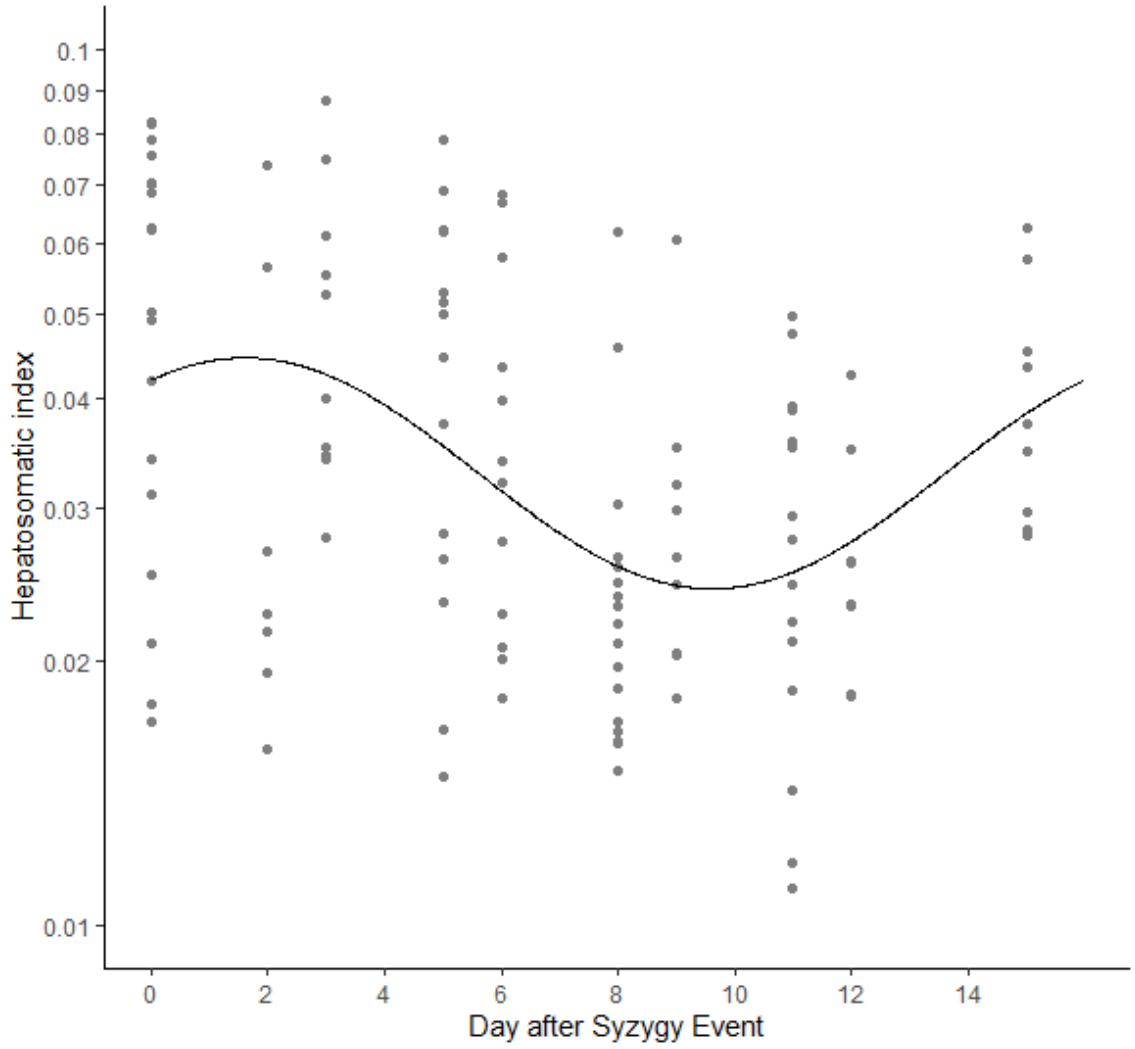
Figure 2.5 *Hepatosomatic index and the lunar cycle*

Hepatosomatic index measurements across the lunar cycle are indicated with points (n = 124). Trend line is significant (ANCOVA; sine of lunar day, F = 7.860, p = 0.006; cosine of lunar day, F = 21.398, p < 0.001) according to univariate linear fixed effects model (Table 2.2A).
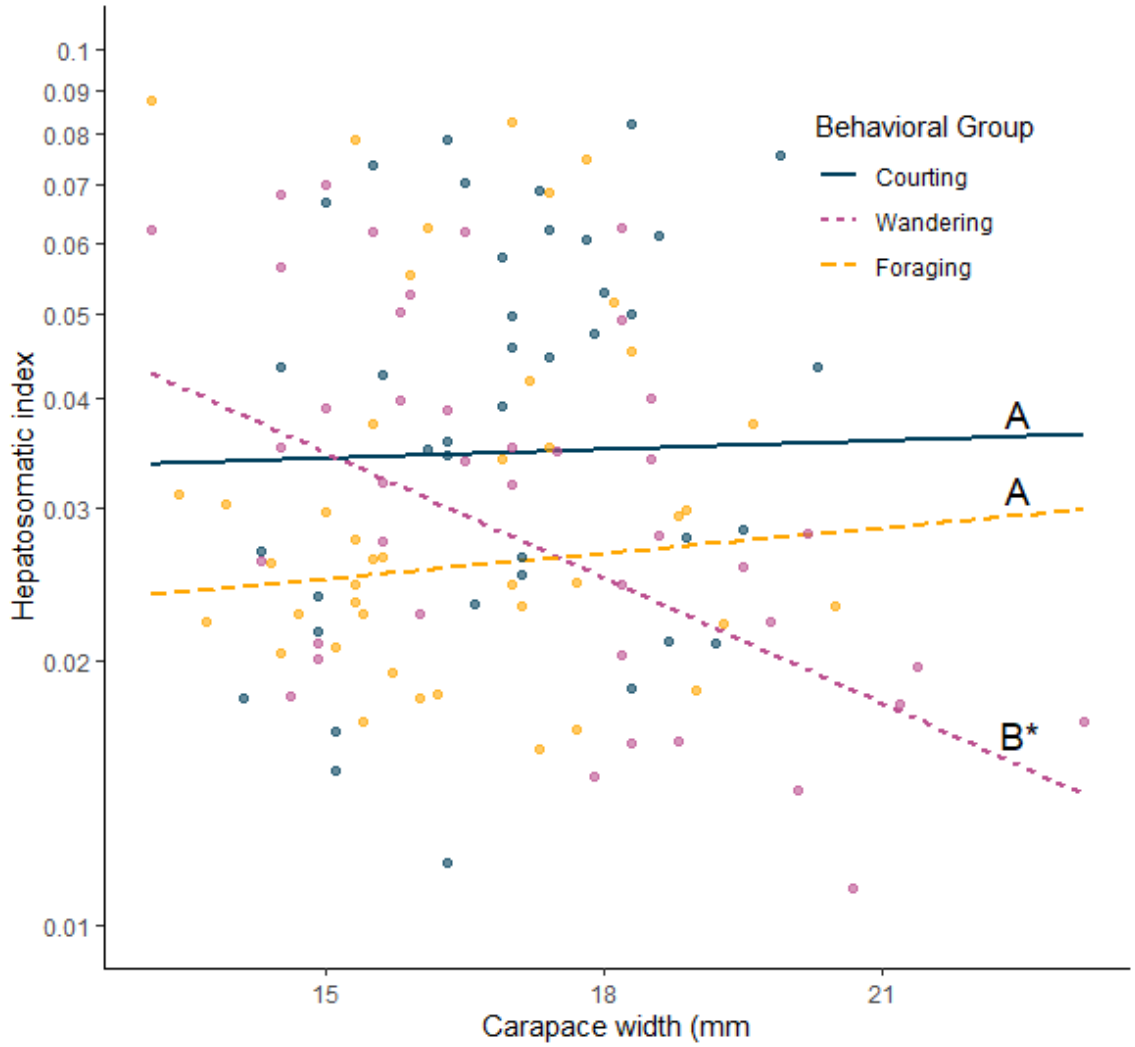
Figure 2.6 *Hepatosomatic index and carapace width*

Hepatosomatic index and carapace width are indicated with points (n = 459). Letters represent significant differences in slopes between groups (ANCOVA, behavioral group: carapace width interaction, F = 4.436, p = 0.014, post hoc comparison of model coefficients) and asterisks indicate slopes that are significantly different than zero (ANCOVA, carapace width, F = 5.992, p = 0.016, post hoc comparison of model coefficients) according to univariate linear fixed effects models (Table 2.2A). Colors indicate behavioral group.
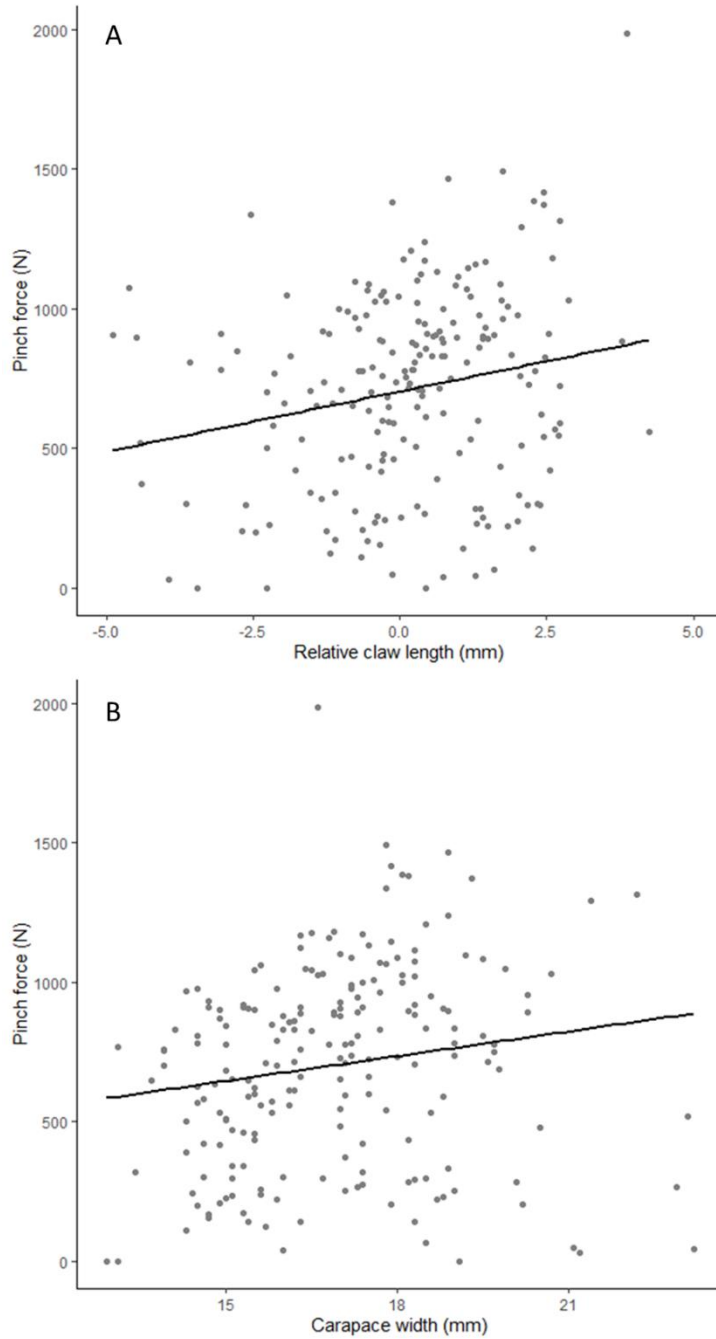
Figure 2.7 *Pinch force and relative claw length (A); Pinch force and carapace width (B)*

Pinch force and relative claw length (A) and pinch force and carapace width (B) are indicated with points (n = 206). Trend lines are significant (ANCOVA; relative claw length, F = 14.275, p < 0.001; carapace width, F = 5.571, p = 0.017) according to univariate linear fixed effects model (Table 2.2B).
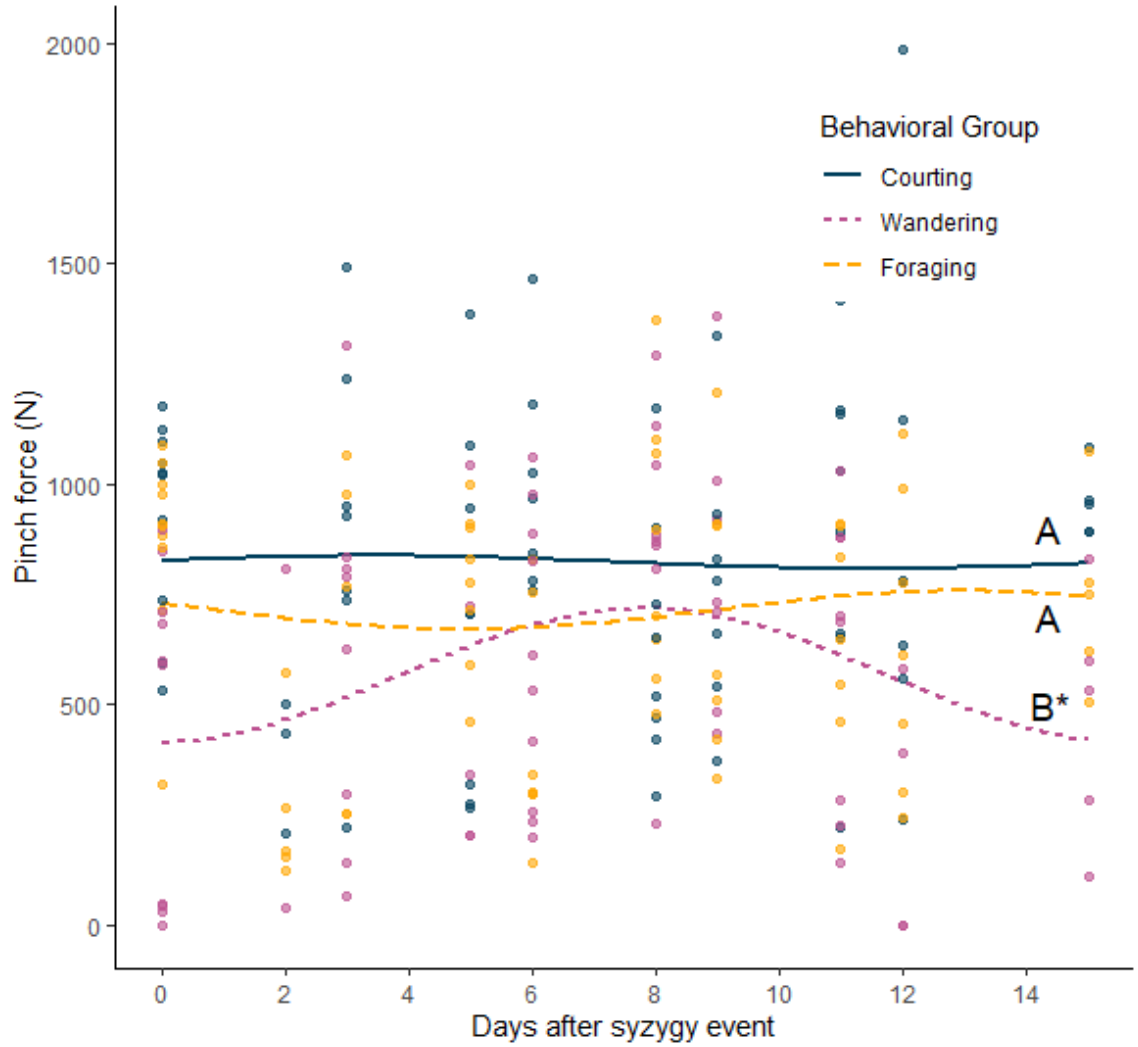
Figure 2.8 *Pinch force and the lunar cycle*

Pinch force measurements across the lunar cycle are indicated with points (n = 206). Letters represent significant differences in slopes between groups (ANCOVA, behavioral group: cosine of lunar day interaction, F = 3.910, p = 0.022, post hoc comparison of model coefficients) and asterisks indicate slopes that are significantly different than zero (post hoc comparison of model coefficients) according to univariate linear fixed effects models (Table 2.2B). Colors indicate behavioral group.
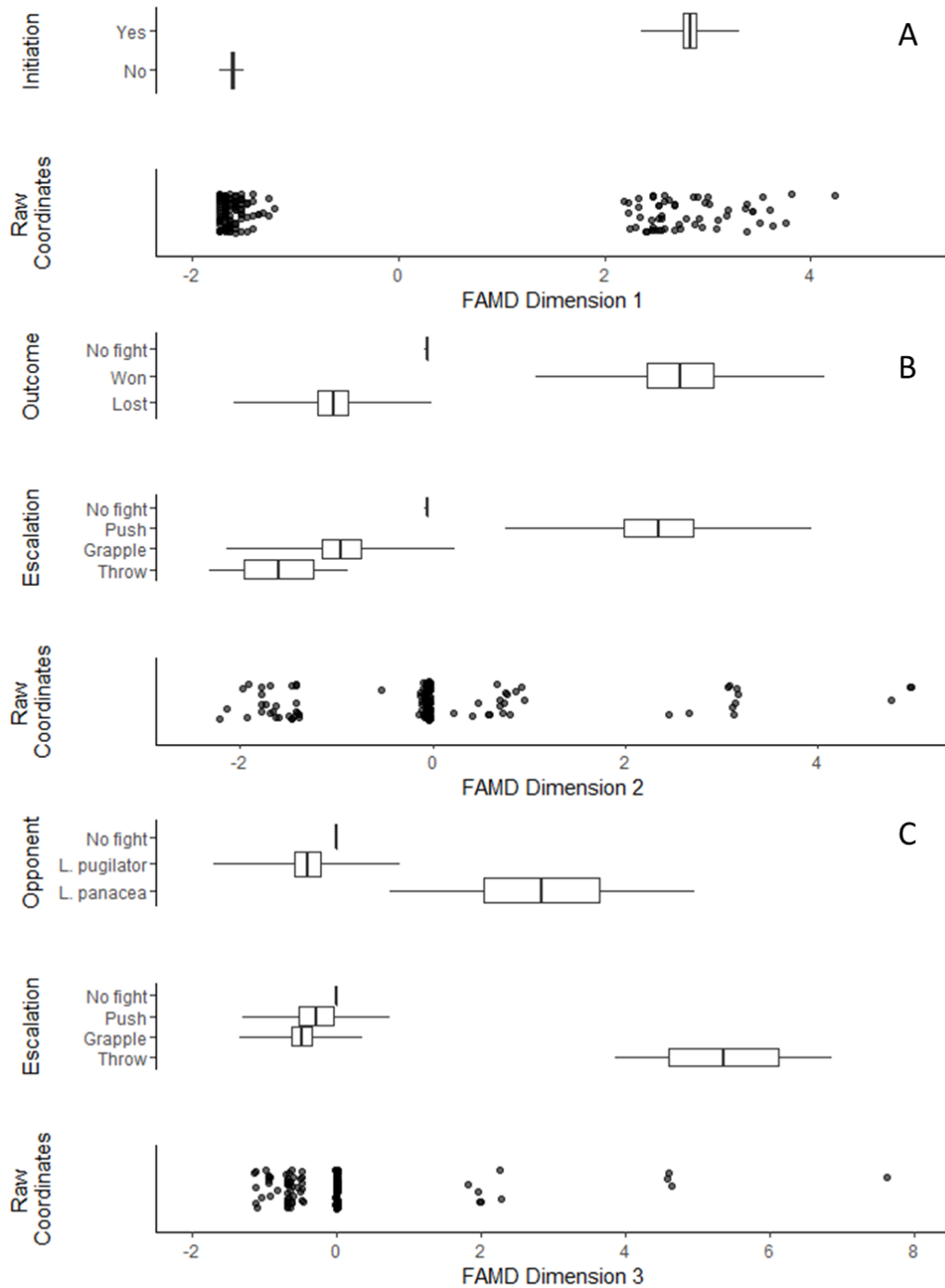
Figure 2.9 *FAMD results of all crabs*

Raw FAMD coordinates and the largest contributing factors for FAMD dimension 1 (A), dimension 2 (B) and dimension 3 (C) using all crabs from the territory acquisition study (n = 158). Points indicate the raw coordinates of each sample across the FAMD dimensions. Boxplots are used to show the mean (center line), standard error (box), and standard deviation (error bars) of the FAMD values of the values within each category of categorical continuous variables along the FAMD dimensions.
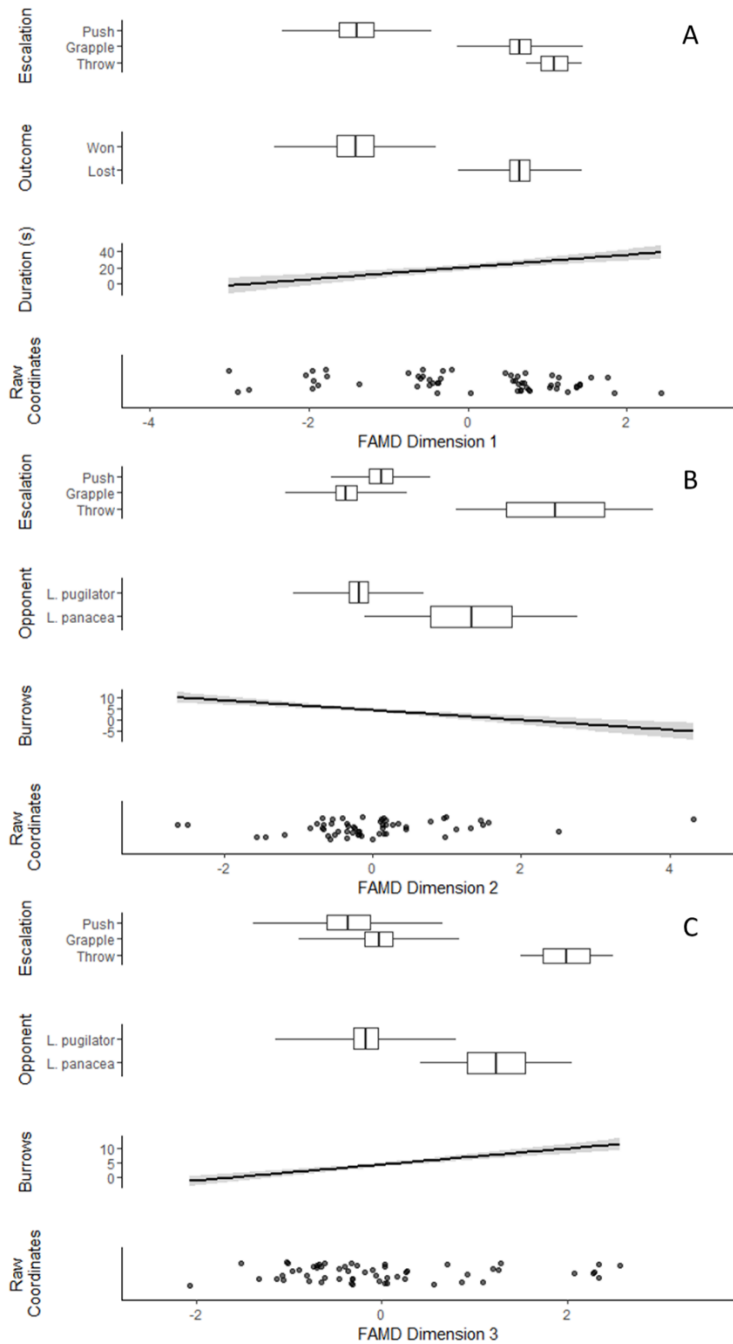
Figure 2.10 *FAMD results of crabs which initiated combat*

Raw FAMD coordinates and the largest contributing factors for FAMD dimension 1 (A), dimension 2 (B) and dimension 3 (C) using only crabs which initiated combat during the territory acquisition study (n = 58). Points indicate the raw coordinates of each sample across the FAMD dimensions. Boxplots are used to show the mean (center line), standard error (box), and standard deviation (error bars) of the FAMD values of the values within each category of categorical independent variables along the FAMD dimensions. Trend line indicate the correlation between FAMD values and continuous independent variables.
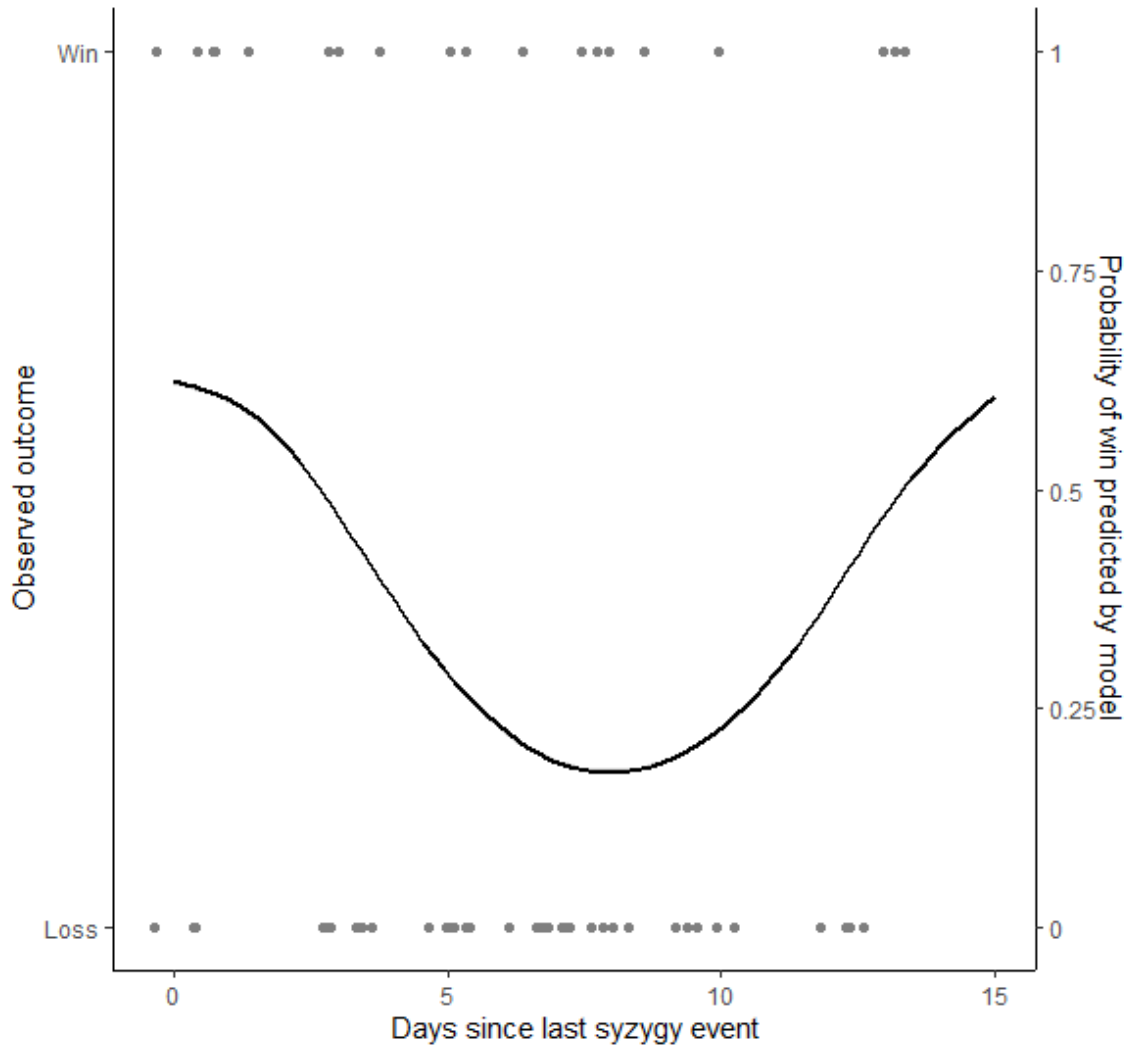
Figure 2.11 *Combat outcome and the lunar cycle*

Observed combat outcomes across the lunar cycle are indicated with points (n = 58). Trend line indicates the probability of observing a winning outcome across the lunar cycle and is significant according (binomial logistic regression, consine of lunar day, Chi^2 = 5.674, p = 0.017) to binomial logistic fixed effects model (Table 2.5I).
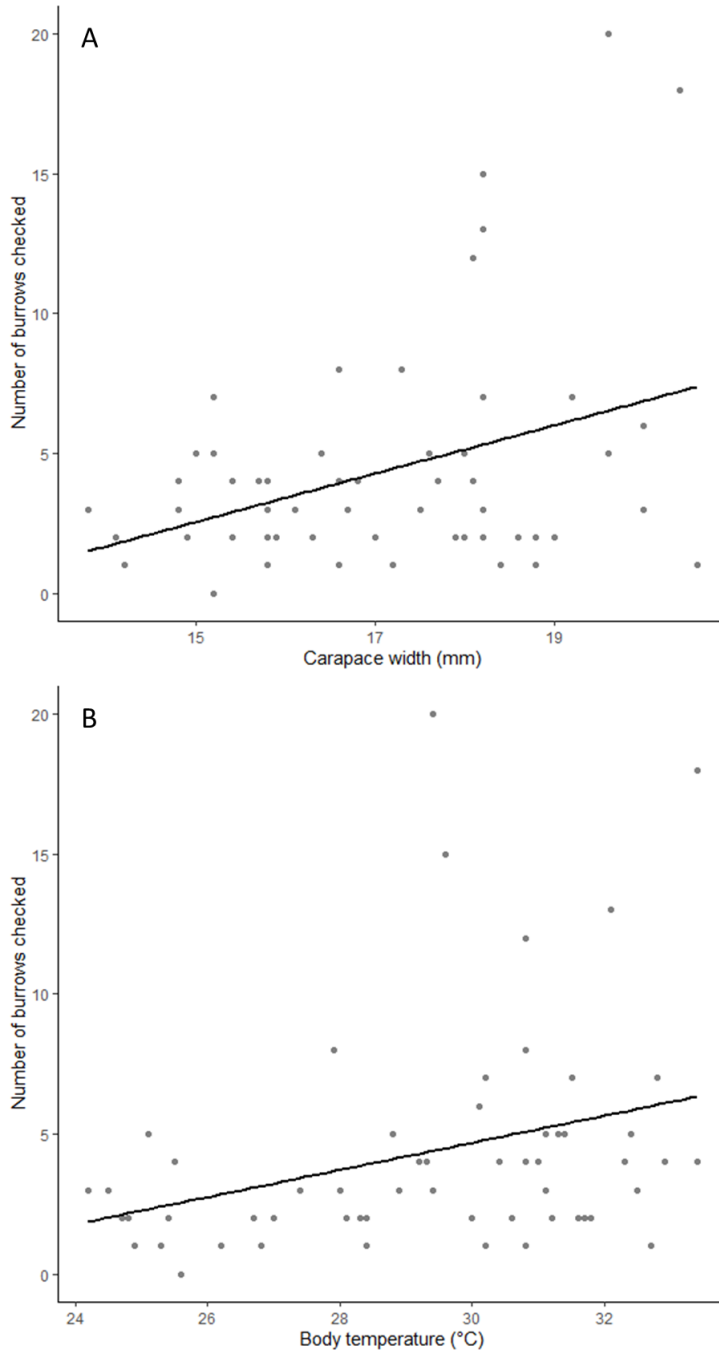
Figure 2.12 *Number of burrows checked and carapace width (A); Number of burrows checked and body temperature (B)*

The number of burrows checked during observation and measured carapace width of focal crabs (A) and the number of burrows checked during observation and the body temperature of focal crabs measured at the beginning of observation (B) are indicated with points (n = 58). Trend lines are significant (ANCOVA; carapace width, F = 7.503, p = 0.009; body temperature, F = 11.284, p = 0.002) according to univariate linear fixed effects model (Table 2.5BV).

55
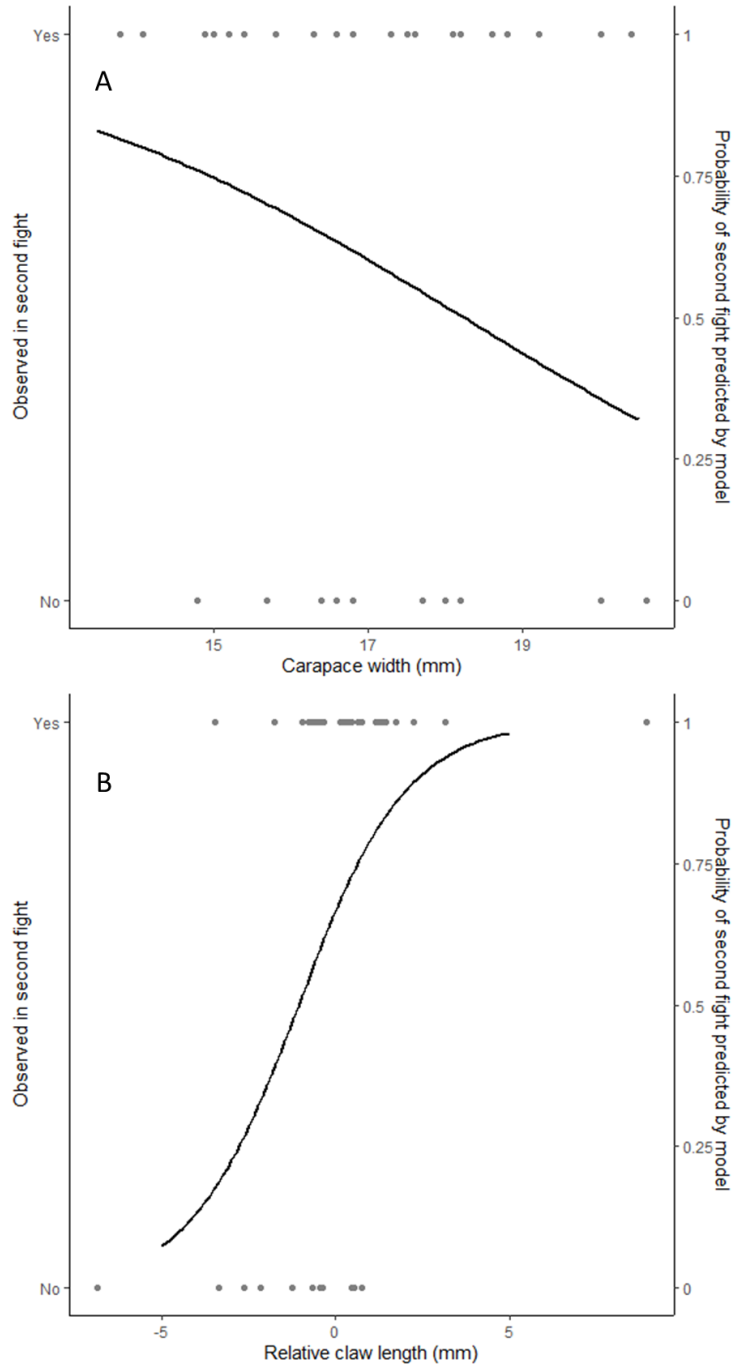
Figure 2.13 *Engagement in second combat and carapace width (A); Engagement in second combat and relative claw length (B)*

Focal crab's engagement in a second fight and carapace width (A) and focal crab's engagement in second a second fight and relative claw length (B) are indicated with points (n = 58). Trend lines indicate the probability of observing a second fight and are significant (binomial logistic regression; carapace width, F = 12.380, p < 0.001; relative claw length, F = 14.551, p < 0.001) according to binomial logistic fixed effects model (Table 2.5CI).
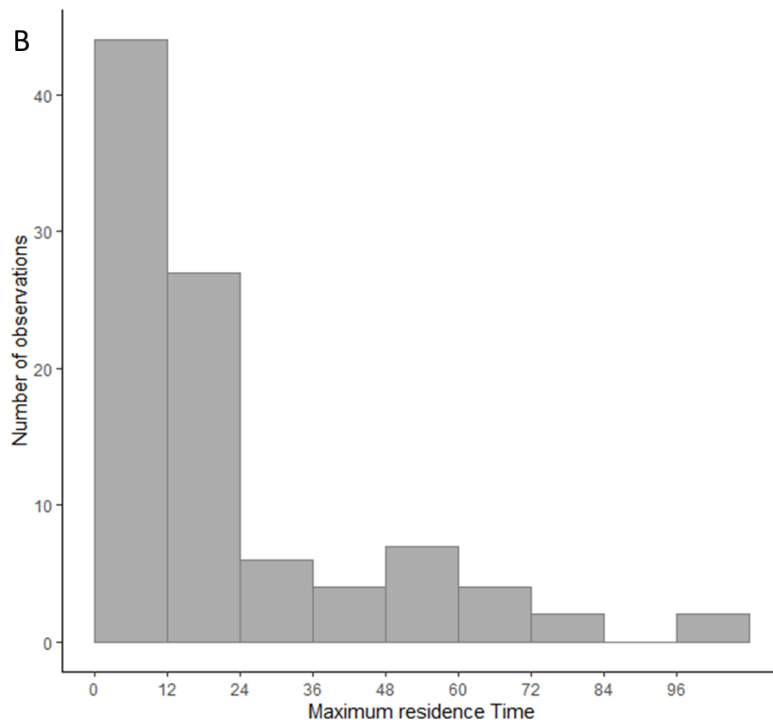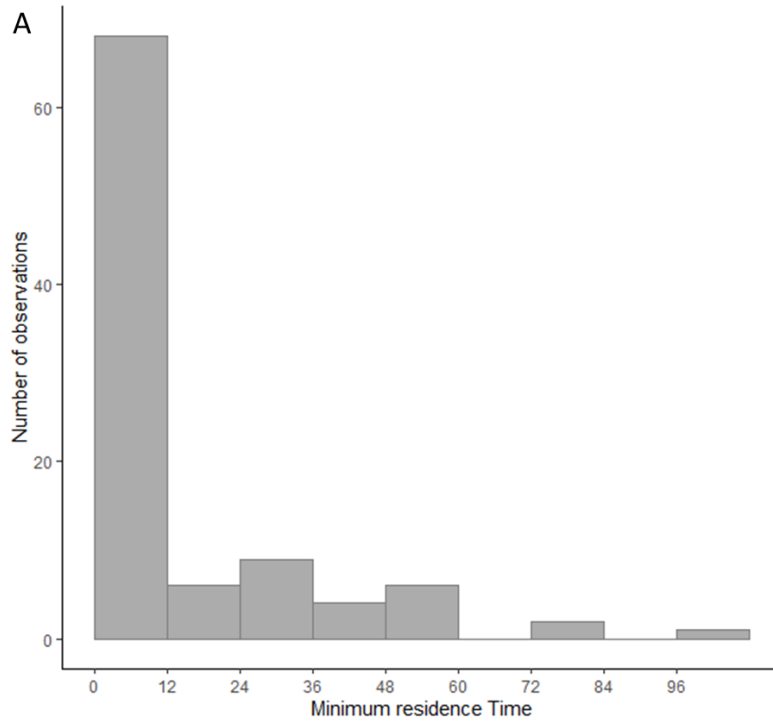
Figure 2.14 *Minimum residence time (A); Maximum residence time (B)*

The number of observations within 12-hour bins of minimum residence time (A) and maximum residence time (B) (n = 96).
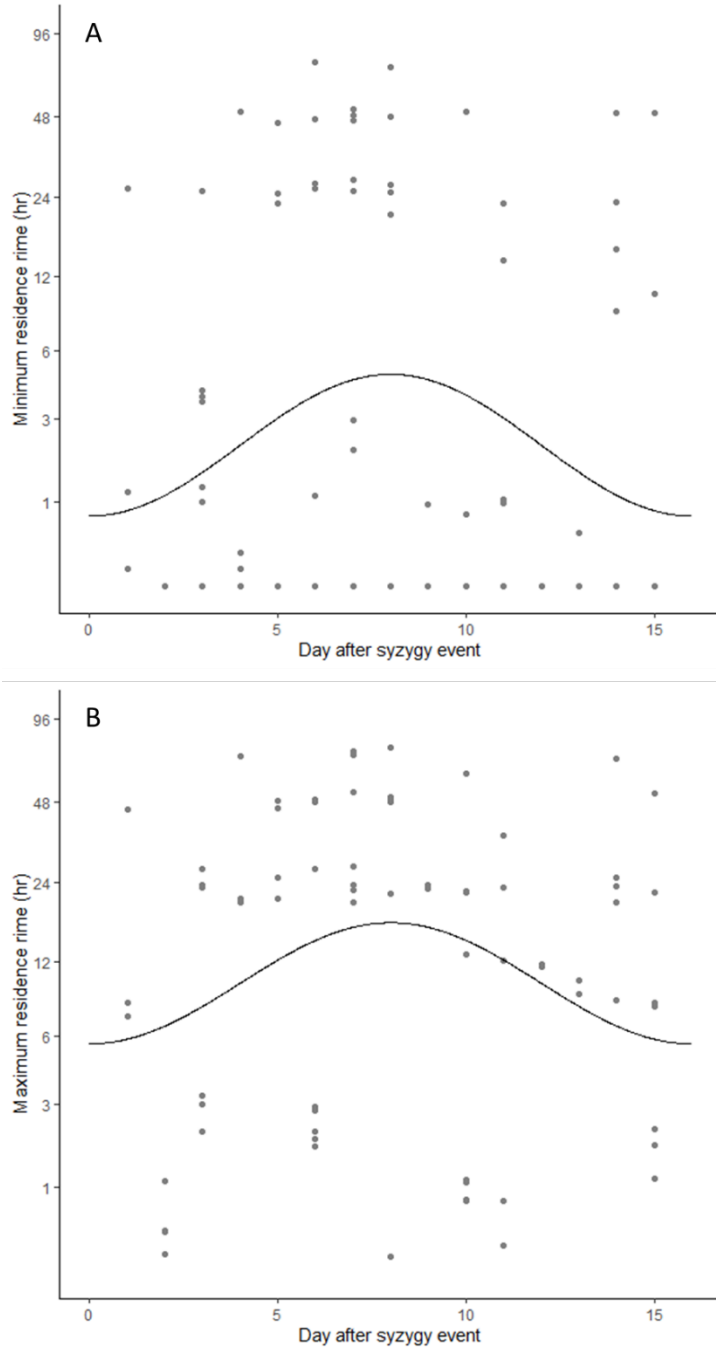
Figure 2.15 *Minimum residence time and the lunar cycle (A); Maximum residence time and the lunar cycle (B)*

Minimum residence time estimates across the lunar cycle (A) and maximum residence time estimate across the lunar cycle (B) are indicated with points (n = 96). Trend lines are significant for both minimum residence time (ANCOVA: cosine of lunar day, F = 7.956, p = 0.006; sine of lunar day, F = 6.349, p = 0.014) and maximum residence time (ANCOVA; cosine of lunar day, F = 8.712, p = 0.004; sine of lunar day, F = 7.904, p = 0.006) according to univariate linear mixed effects models (Table 2.6A & B).

58

CHAPTER III – STRUCTURE AND THERMAL BENEFIT OF MATING BURROWS

IN *LEPTUCA PUGILATOR* ACROSS TIME AND SPACE

3.1 Introduction

Broadly, the mating strategies of different fiddler crab species fall into one of two

categories: surface-mating and burrow-mating (Christy and Salmon, 1984; Crane, 1975;

deRivera and Vehrencamp, 2001). In surface-mating fiddler crabs, mating occurs

opportunistically as males and females move across the sediment surface environment

while foraging and/or overseeing their territories. In burrow-mating species, mating

occurs underground within mating burrows. *Leptuca pugilator* is a burrow mating fiddler

crab species, and mating in this species occurs within mating burrows owned and

maintained by individual males (Christy, 1982; Crane 1975). In this species, mating

burrows differ in size and structure from the temporary burrows used for protection

during high tides, with mating burrows being significantly deeper and terminating in a

mating chamber where mating, oviposition, and incubation occurs (Christy, 1982).

Unlike temporary burrows, mating burrows seem to persist through the tidal cycle due to

constant maintenance by their male resident (Christy, 1982).

The mating burrow is a critical structure for female reproductive success (Christy,

1983; Reaney and Backwell, 2007). For a female *L. pugilator*, the mating burrow

provides a thermally and physically stable environment where she can mate, oviposit, and

incubate her eggs over the course of the roughly 2-week period immediately following

courtship and mating (Christy, 1983, 1982). Like most brachyuran crabs, in order to

maximize larval survivorship and to reduce predation risk for their larva and themselves,

female *L. pugilator* release their larvae into the water column during nighttime high

spring tides, a feat which requires tight control of reproductive timing in regard to mating and incubation period (Christy, 1978; Morgan and Christy, 1995; Pratt and Mclain, 2002; Salmon and Hyatt, 1983). This tight control is thought to made possible in part by the stable environment of the mating burrow (Christy, 1982; Reaney and Backwell, 2007). It is rare for female *L. pugilator* to mate with a male that cannot provide her access to an adequate burrow, and, in turn, the restriction of mating access to only those males which own mating burrow makes these structures integral to male reproductive success as well.

In order to facilitate the creation of their specialized internal microhabitat and stability, mating burrows and the small mating territories which surround them are typically found in the high intertidal zone (Crane, 1975). Typically, the sediment of the high intertidal zone is low in nutrients and water content (Allen and Levinton, 2014; Kim and Cho, 2003) and is subject to temperatures that can be dangerously high for *L. pugilator* (Allen and Levinton, 2014; Allen et al., 2012; Darnell et al., 2013; Munguia et al., 2017). Since courtship, territory acquisition, and mate searching activities all occur on the high intertidal sediment surface, individuals participating in these activities must endure the associated nutritional, hydric, and thermal stressors. However, matting burrows provide both a refuge from high temperatures and access to water to their male and female residents (Darnell et al., 2020, 2013; Levinton et al., 2015; Munguia et al., 2017; Smith and Miller, 1973). Thus, mating burrows act as key thermal/hydric resources in addition to key reproductive resources. This especially true for courting males which must endure the stress of the high intertidal zone for prolonged periods in order to attract a mate, though the exact magnitude of the thermal benefits which mating burrow provides them is unknown.

In general, reproductively active males obtain their mating burrows and surrounding mating territory from the burrow's previous male resident. The exchange of ownership between two males is facilitated by a form of complex, ritualized communication that unique to male-male interactions concerning territory exchange (Christy and Salmon, 1984; Hyatt and Salmon, 1978). The burrow resident and the intruding male communicate via visual displays followed by physical contact including exploratory touching, pushing, and interlocking of their sexually dimorphic major claws, and although this communication can occasionally be prolonged and/or lead to more combative stages include lifting and throwing of the other male, it is far more often short and non-violent (Hyatt and Salmon, 1978). In a majority of cases, the current resident retains the territory, leaving the intruding crab to move on to continue his search for territory or retreat to the low intertidal to feed and rehydrate (Fayed et al., 2008; Hyatt and Salmon, 1978), but in certain scenarios, those in which the resident male relinquishes the territory or is forcefully evicted, the intruding male gains the territory and the previous owner moves on to other activities.

Once in control of a mating burrow and territory, courting males spend their time participating in male-male interaction over the territory, frequently retreating into their burrow to rehydrate and lower their body temperature, and attempting to attract a mate. Males attract females by waving the greatly enlarged major claw in a species-specific courtship display (Crane, 1975). This display communicates the presence and availability of a male and mating burrow. A female may then approach the displaying male who will then lead or attempt to herd her to the entrance of his burrow (Christy, 1983). Initial attraction does not ensure mating, and courtship is not final until the female examines the

quality of the burrow and decides if it suits her needs (deRivera, 2005; Reaney and

Backwell, 2007). How she makes this final decision is not well known but appears to be

tied to abiotic factors such as burrow temperature and shaft diameter. If the burrow is of

sufficient quality, the female will descend, followed by the male, and the two will mate.

After mating the female will remain underground until larval release and the male will

reemerge to court another female or exchange the territory with another male (Christy,

1982).

The importance of the mating burrow as a key reproductive and thermal/hydric

resource for both males and females makes an understanding of this structure imperative

to understanding the biology of *L. pugilator*. In the current study, I examined mating

burrow structure and thermal benefit directly. Specifically, I aimed to: A) provide a

complete description of mating burrow shape and size and how these metrics are tied to

the size of the resident male, with the hypothesis that male size and burrow morphology

are correlated, B) test the hypothesis that burrow structure differs among populations of

L. pugilator living under different thermal regimes, C) investigate how the lunar cycle is

tied to burrow structure under the hypothesis that if females seeking mates at different

times during the lunar cycle must adjust the timing of their larval release, then males may

alter the structure of their mating burrows to provide appropriate conditions in response,

and D) attempt to describe the thermal benefit that mating burrows offer their residents

throughout the day and how this thermal benefit may change with burrow size and/or

crab size. Additionally, I address a previously posed hypothesis (sensu Christy, 1982)

that mating burrow depth is tied to the depth of the water table throughout the daily tidal

cycle.

3.2 Materials and methods

3.2.1 Burrow Morphology

3.2.1.1 Study locations and environmental data

To better understand how mating burrows of *L. pugilator* vary structurally within populations and between populations with different climatic regimes, the burrows of actively courting males were cast and measured at three study sites during Summer 2019: the southern shore of Porter Island near Panacea, Florida (30.0156, −84.3695), the western shore of the Rachel Carson Reserve in Beaufort, North Carolina (34.7114, −76.6780), and West Meadow Creek in Stony Brook, New York (40.9349, −73.1444). All three of these sites consist of vegetated, sandy beaches inhabited by a sizable populations of *L. pugilator* with large, reproductively active males in the high intertidal waving at wandering females and protecting their mating territories from wandering males, and with non-reproductive males and females foraging together in the low intertidal during low tides. These sites were chosen because they span *L. pugilator*'s thermal range. Each site varies climatically with Panacea, FL having the highest temperatures (summer maximum = 32.7°C, winter minimum = 4.7°C, yearly average = 20.0°C); Stony Brook, NY having the lowest temperatures (summer maximum = 28.1°C, winter minimum = -4.4°C, yearly average = 11.6°C); and Beaufort, NC having intermediate temperatures (summer maximum = 30.0 °C, winter minimum = 2.8 °C, yearly average = 17.3°C) (Darnell and Darnell, 2018). Additional burrow measurements were taken from Panacea, FL during Summer 2021. Ground temperatures from each site were recorded once every five minutes during the day low tide using HOBO iButton data

loggers throughout the study. Tidal heights of each high and low tide relative to MLLW were recorded for Panacea, FL in 2021 using NOAA tidal prediction charts.

3.2.1.2 Casting mating burrows

Prior to casting burrows, resident males were captured using traps that allowed for removal of the resident without digging. These traps consisted of a stainless-steel mesh (6.35-mm mesh size) box with a hinged lid on its top and a circular chimney rising from its base into its center (Fig. 3.1). This trap was placed with the chimney over a mating burrow so that as the resident male exits the burrow, he climbs up the chimney and falls into the trap. Mating burrows were selected for study by observing a group of actively courting males from a distance, randomly selecting one, and approaching it, causing it to retreat to its burrow. Courting crabs utilize path integration to flee to their mating burrow when under threat of predation or capture (Zeil, 1998), allowing for relative certainty that the selected male was the current resident of whichever burrow it retreated to. A trap was then set over the entrance of the burrow and checked every 10–15 minutes. If, after an hour, a trap had not captured the resident, that trap was moved to a new mating burrow (as above). When a trap was successful in capturing the owner of the focal burrow, the crab was measured for carapace width (longest distance between the anterolateral carapace angles) and claw length (distance between the base and tip of the propodus) and then released.

Immediately prior to casting, the outermost diameter of the burrow entrance was measured using dial calipers. For the burrows cast in 2021, the beach slope at the mouth of the burrow was measured using a handheld pitch and slope locator. Additionally, the elevation of each burrow cast in 2021 was measured using a transit level and stadia rod

relative to NOAA NGS benchmark 8728258A and converted to elevations relative to MLLW. Burrows were then cast using Great Stuff™ Gaps and Cracks Insulating Foam Sealant (DuPont, Wilmington, DE). The plastic nozzle was inserted roughly 6 cm into the burrow and the space around the nozzle was covered by the researcher's gloved hand. The foam was then injected into the burrow until a significant change in pressure was felt, signifying that the foam had filled the entire burrow. The nozzle was then removed from the burrow and the foam was left to expand and harden for 1 hour. Preliminary observations indicate that, while the expansion of the foam is strong enough to push some of the foam out of the entrance of the burrow and onto the surrounding surface sediment, it is not strong enough to significantly alter the shape or size of the burrow.

Once the cast had set, it was excavated and taken to the laboratory for measurement. Note that wherever the foam touched sediment, a thin layer (1–5 mm thick) of sediment became adhered to the cast. Casts that were not completely covered in a sediment layer, indicating a cast which failed to fill the entirety of the burrow, were removed from the study. Additionally, two burrows were excluded from analyses as outliers, each of which was less than 6 cm in length and lacked a terminal chamber, suggesting a simple, temporary burrow and not a mating burrow (Christy, 1982). The two focal males associated with these burrows may have erroneously fled to a temporary burrow, rather than their mating burrows, or they may have been erroneously identified as courting males. Casts were allowed to rest in a dry environment for 2–8 hours prior to measurement. This drying period allowed for the excess sediment stuck to the cast to become firm and easily removed. The sediment was brushed by hand from the cast until

the sediment layer on the outside of the foam cast was as homogenously thin as possible without damaging the foam itself.

3.2.1.3 Morphological burrow measurements

The following morphological measurements were taken on each cast: burrow shaft diameter, terminal chamber diameter, burrow length, terminal chamber length, burrow depth, angle of initial descent, and burrow volume (Fig. 2.2). Mating burrows typically end in an enlarged terminal chamber where it is believed that mating and clutch incubation occur (Christy, 1982). The first step of cast measurement was to identify this portion of the burrow and to determine the threshold at which it began and where the shaft leading to it ended. Burrow shaft diameter and terminal chamber diameter were each measured using dial calipers. The shaft diameter (i.e., the diameter of the portion of the burrow leading to the terminal chamber) was measured at 3 randomly selected points along the shaft portion of the cast, and those three points were then averaged for a final representative value for the cast as a whole. Terminal chamber diameter was measured and averaged for each burrow in the same way as the shaft diameter except the measurements were made of the terminal chamber portion of the cast. Burrow length was measured using a flexible measuring tape from the burrow entrance to the tip of the terminal chamber, following the path of the burrow. Burrow length was measured along the outside edge of each cast three times per burrow: once along the upward facing edge of the cast, once along the downward facing edge, and once along one of the cast's sideways facing edges. These three measurements were averaged within each burrow cast for a final representative measurement. Terminal chamber length was measured in ImageJ software using photos of the burrow casts, which included a meter stick for scale.

66

Burrow depth is the linear distance from the burrow entrance to the tip of the terminal chamber, measured along the vertical plane perpendicular to the horizontal plane of the surface sediment, which was directly observable as the flat, bottom edge of the foam overflow portion of the cast. The angle of initial descent was measured in ImageJ software using photos of the burrow casts taken specifically for this purpose, and is the minimum angle formed between the plane of the surface sediment and a line running centrally through the first 6 cm of the burrow shaft. Burrow volume was measured via liquid displacement after removing the foam overflow portion of the cast.

In addition to the measurements described above, which were collected on all casts, the overall direction of descent relative to the water line was also measured and the absolute depth was calculated as the beach height relative to MLLW of the burrow entrance minus the measured depth relative to the surface on casts collected from Panacea, FL in 2021. The overall direction of descent was measured in ImageJ software using photos of the burrow casts taken specifically for this purpose, and is the direction, in degrees, that the burrow descends into the sediment relative to the water's edge when viewed from directly overhead. Direction values of 0° indicate a burrow that descends directly towards the water's edge and a value of 180° indicates a burrow which descent directly away from the water edge. To ensure accurate measurement of the direction of decent following cast excavation, a reference arrow, pointing directly towards the water's edge, was drawn on top of the foam overflow prior to excavation using a permanent marker.

3.2.1.4 Correcting for cast sediment layer error in burrow cast measurements

Each burrow cast had a thin layer of sediment stuck to its outer surface. This thin layer introduced significant error to certain morphological measurements (burrow shaft diameter, terminal chamber diameter, and burrow volume) which ideally describe only the empty space of the burrow and should not include any of the surrounding sediment.

The thickness of the sediment layer was measured using dial calipers. Each burrow cast was haphazardly broken along cross-sectional planes into seven pieces, creating six cross-sectional faces on which to measure the thickness of the sediment layer. The sediment layer thickness was then measured at a single, randomly selected point at the edge of each cross-sectional face. The six measurements were then averaged for each cast to give a single value per cast which was then used to correct the morphological measurements for that cast.

To correct burrow shaft diameter and terminal chamber diameter measurements, twice the average thickness of the sediment layer was subtracted from the measured diameter values for each cast. To correct burrow volume measurements, it was necessary to calculate the volume of the sediment layer. Annulus (i.e., ring) geometry defines the volume of an annular prism as:

$$V = \pi(R_O^2 - R_I^2)L$$

where V is the volume of the annular prism, R_O is the radius from the center of the annulus to its outside edge, R_I is the radius from the center of the annulus to its inside edge, and L is the length of the prism. This was used to derive the following equation which roughly models the volume of the sediment layer which forms an irregular annular prism around the burrow cast:

$$V_S = \pi \left(\left(\left(\frac{D_{Sm}}{2} \right)^2 + \left(\frac{D_{Sc}}{2} \right)^2 \right) (L_B - L_T) + \left(\left(\frac{D_{Tm}}{2} \right)^2 + \left(\frac{D_{Tc}}{2} \right)^2 \right) L_T \right)$$

where V_S is the volume of the sediment layer, D_{Sm} and D_{Sc} are the measured and corrected shaft diameter values, D_{Tm} and D_{Tc} are the measured and corrected terminal chamber diameter values, and L_B and L_T are the burrow length and terminal chamber length, respectively. After calculating the estimated volume of the sediment layer, it was subtracted from the measured burrow volume to calculate a corrected burrow volume. It should be noted that this volume correction is imperfect. The annular prism used to model the sediment layer is perfectly smooth, straight, has a static thickness throughout, and transitions immediately between the thinner shaft portion and wider terminal chamber portion. This is in contrast to the actual sediment layer, which is bumpy, curves with the burrow, has variable thickness, and transitions gradually in diameter from shaft to chamber. However, the total volume of the sediment layer is not trivial relative to the total burrow volume, and therefore deriving a correction estimate using the perfect annular prism as a model is likely to yield values which are closer to reality than the uncorrected values.

3.2.2 Thermal benefit of mating burrows

To better understand the magnitude of the thermal benefits provided by mating burrows to the crabs which inhabit them, males were tethered and held either inside of or immediately outside of a mating burrow, and the changes in their body temperatures over time were recorded. All trials were conducted during low tides while males were actively courting, and each trial utilized a pair of size-matched males with one being held inside a mating burrow and the other being held outside on the surface.

To begin each trial, an actively courting, burrow-owning male was identified from a distance and then captured. A cup was then placed upside-down on his burrow to mark it temporarily and to keep any wandering males from taking up residence. If, in capturing the male, the burrow was significantly damaged, the male was released and the trial discarded. Then, a visually-size-matched male was selected and captured from elsewhere in the high intertidal mating area, and both males were then measured for carapace width and claw length. They were then placed into separate, shaded cups containing 4 cm of seawater. Here, they were each attached to their tethers (comprised of monofilament line and wooden skewers; 30 cm tether for the original resident and 5 cm tether for size-matched male) and held for 10 min to standardize their body temperatures and ensure full hydration. During this 10 min period, temperature data were collected from the marked burrow. Using a digital thermometer and thermocouple probe, the air temperature at 1 cm above the sediment and at 6–10 cm inside the burrow was recorded, as was the sediment temperature of the surface near the burrow entrance and at 6–10 cm inside the burrow.

Following the 10 min body temperature standardization/hydration period, the starting body temperature of each male was recorded via infrared thermometer, and each was tethered according to its experimental group. The original burrow resident was always tethered and held within its burrow. This was accomplished by introducing the burrow resident to his burrow, allowing him to enter of his own accord, placing a 10 cm × 10 cm section of steel 0.5 cm mesh over the burrow entrance to prevent his leaving the burrow, and staking his tether nearby. The other crab was always held on the surface by placing a 10 x 10 cm section of steel 0.5 cm mesh near the mouth experimental burrow, covering that mesh in a small amount of sediment from the surrounding area, and then

70

staking the crab in such a way that it could not leave the mesh area, preventing the crab

from burrowing to escape the heat of the surface. Each crab was held under its respective

treatment conditions for 10 min, after which its final body temperature was taken, and it

was released. In the case of the crab held within the burrow, the monofilament line to

which it was tethered was used to pull it out of the burrow in order to measure its body

temperature.

3.2.3 Data analyses

3.2.3.1 Burrow morphology

All statistical analyses were conducted in R 4.2.1 (R Core Team, 2021). In order

to understand the relationships that exist between measured burrow morphometrics, a

correlation matrix was calculated, and a Principal Components Analysis (PCA) was

conducted for all burrows collected in 2019 and 2021 with all burrow morphometrics

shared across the two sampling years included (i.e., depth; total and terminal chamber

length; opening, shaft, and terminal chamber diameter; volume; and descent angle). PCA

is often used to separate groups in multidimensional space, but can also be used to

simplify complex datasets containing many dependent variables (such as the current

dataset which has many morphological burrow metrics) to potentially reduce the number

of statistical test needed (e.g., a single hypothesis test using PC values from a highly

descriptive dimension instead of many hypothesis test on every dependent variable used

to create that PC dimension). The second approach (data simplification) is utilized here

with principal component values from dimensions 1–3 being used as dependent variables

in the hypothesis tests outlined in the following paragraph. A second correlation matrix

and PCA were conducted using only sampled collected in Panacea, FL in 2021 so that the

71

additional burrow parameters recorded in that year (i.e., beach height, absolute depth, beach slope, and overall direction of descent) could be included in the analyses along with those already mentioned in the first correlation/PCA analyses. However, the principal components from the 2021 PCA were not used in subsequent analyses due to the reduced sample size of the 2021 PCA and redundant results compared to the original 2019/2021 PCA.

Multiple univariate linear fixed effects models were built using each of the recorded burrow morphometrics as well as principal components 1–3 from the first PCA as dependent variables. Univariate linear fixed effects models are the underlying framework of common frequentist hypothesis tests, such as ANOVA, ANCOVA, and other types of regression. Study site, the number of days since the last syzygy event, crab carapace width and relative claw size, and daily average ground surface temperature were included in the models either together or separately (see below) as independent variables to test for significant effects on burrow morphometrics and principal components. Study site was included in the models as a categorical variable, and carapace width, relative claw size, and ground temperature were included as standard continuous data. The relative claw length of each crab was calculated as the residuals of a linear regression of claw length against carapace width, and surface temperatures were taken from ibutton temperature loggers deployed in the field throughout the study. The number of days since the last syzygy event was analyzed as circular data ranging from 0–15 days, and was included in the univariate models using the sine and cosine of the radian-transformed circular values to calculate two separate model coefficients according to the linear-circular regression approach (Zar, 1999). After building each model, the assumptions of

residual normality and homoscedasticity were tested, and the statistical significance of

each independent variable was calculated using the base R anova() function to perform

standard F-statistic ANOVA significance tests. This is the long way of saying that I

conducted ANCOVA analyses for all burrow morphometrics and PC values from PCA

dimensions 1-3 using lunar day, carapace width, relative claw length, and ground

temperature as main effects.

There was complex collinearity (i.e., confoundedness) among the independent

variables. Most of these confoundedness issues arose due to natural differences that

existed between crabs at the different study sites or were due to logistical issues in

sampling each site throughout the lunar cycle. Due to restraints in time, I was unable to

sample the Beaufort, NC and Stony Brook, NY sites evenly throughout the syzygy cycle,

resulting in NC being sampled only in the first half and NY being sampled only in the

second half of the cycle. However, Panacea, FL was sampled evenly throughout the

syzygy cycle. Therefore, to test the effect of lunar day on burrow morphometrics, only

burrows from Panacea, FL were used. These models included days since the last syzygy

event as their independent variable of interest and included carapace width and relative

claw size as covariates.

Another significant collinearity issue was that crabs from the different sites were

of very different sizes. Particularly, crabs from Beaufort, NC were significantly smaller

than crabs from Panacea, FL and Stony Brook, NY which were statistically the same size

(ANOVA; $F = 48.3$; $p < 0.0001$; Tukey's HSD post hoc). Crab size can naturally be

expected to have an impact on most burrow morphometrics, so this confoundedness

posed a significant issue. Ultimately, after trying and failing to account for the issue with

nested analyses, mixed effects models, and data standardization, I decided to exclude

samples from Beaufort, NC from analyses of study site effects, and instead only consider

burrows from Panacea, FL and Stony Brook, NY when investigating site differences.

Since crabs in Panacea, FL and Stony Brook, NY were the same size, there was no

confoundedness between site and size when analyzing only these two sites. Therefore, to

detect inter-site differences between burrow morphometrics, only burrows from Panacea,

FL and Stony Brook, NY were used, and these models included site identity as their

independent variable of interest and included carapace width, relative claw size, and

ground temperature as covariates.

Once the potential effects of day relative to syzygy and site were considered, the

effect of carapace size, relative claw length, and ground surface temperature were

analyzed using all burrow morphology data collected throughout the study. These models

included each of these three independent variables and all two-way interaction terms

between them. The conclusions drawn from these models were informed by results of the

previous lunar day and site identity analyses but did not actually include either of these

variables. Additionally, the extra burrow morphometrics collected in Panacea, FL in 2021

were analyzed separately using models which included carapace width, relative claw size,

number of days since syzygy, and all two-way interactions included.

3.2.3.2 Burrow depth and tidal amplitude

To test the hypothesis that absolute depth of mating burrows (i.e., the elevation of

the terminal end of the burrow above MLLW) is in some way correlated to the elevation

of the water table within beach sediment throughout the tidal cycle, the absolute depth of

the mating burrows observed in Panacea, FL during 2021 was used in an additional

74

analysis along with tidal data. The depth of the water table was not directly measured in Panacea, FL in 2021, but it has been shown previously that the elevation of the water table is strongly correlated with tidal height in areas of beach within 39 m of the ocean (Raubenheimer and Guza, 1999). The total beach width at the Panacea, FL site is well within this limit even at low tide (est. beach wide 12-17 m), so tidal height was used as a proxy of water table elevation. The correlation between absolute burrow depth and the recorded tidal heights were tested using Pearson's product-moment correlation coefficients to calculate significance. Additionally, correlations between absolute burrow depth and the daily maximum tidal amplitude (i.e., daily higher high tide height minus daily lower low tide height) as well as the daily difference in high tide height (i.e., daily higher high tide height minus daily lower high tide height) were tested similarly.

3.2.3.3 Thermal benefit of mating burrows

In order to understand how air temperature, sediment temperature, and crab temperature differed inside and outside of mating burrows throughout the day, three univariate linear fixed effects models were built, one for each temperature metric. All of the models included location of sample (i.e., inside of or outside of burrow) as a categorical variable, burrow entrance diameter as a standard continuous variable, and time of day as a circular variable using the linear-circular regression approach outlined above (Zar, 1999) with the average time of July sunrise in Panacea, FL (~6:40 am) set at 0 radians and average time of sunset (~8:40 pm) set at 2π radians. The model for crab temperature included carapace width and relative claw width as well as those outlined above. Each model included all two-way interaction terms. This analysis is synonymous with performing ANCOVA for sediment temperature, air temperature, and crab body

temperature using location (inside or outside of burrow), burrow entrance diameter, and time of day as main effects with the addition of carapace width and relative claw size as main effect in the body temperature analysis.

3.3 Results

3.3.1 Burrow morphology across latitude

Across the 83 burrows cast and measured, all burrow size metrics (i.e., burrow length, terminal chamber length, burrow depth, entrance diameter, shaft diameter, terminal chamber diameter, and burrow volume) were significantly and positively correlated with one another, meaning that as any single burrow size metric increased all others had the tendency to do so as well (Table 3.1A & B). Angle of decent was not significantly correlated with any other dependent burrow metrics. The full principal components analysis (n = 83) further highlighted the correlation between burrow size metrics, with all the burrow size metrics heavily loading to the same side of PC1 which accounted for 50% of the total variance in the data. Cumulatively, PC1–3 accounted for 78.6% of the variance in the dataset with PC1 being largely a metric of general burrow size and PC2 and PC3 being largely shaped by angle of descent (Fig. 3.3A & B). Additionally, PC2 seems to represent the balance between diameter measurements and length measurements in determining burrow volume, with burrows of a given volume achieving that volume by being either long or wide (Fig 3.3A), and PC3 seems to represent some sort of complex relationship between chamber diameter and the other morphometrics. Averages of all burrow metrics, resident morphology measurements, and abiotic environmental data can be seen in Table 2.2 and most will not be reported in the text.

Burrow metrics of mating burrows cast in Panacea, FL taken throughout the lunar cycle in 2019 and 2021 showed no evidence of lunar impacts on mating burrow shape (n = 42) (Table 3.3AI-XII).

When comparing burrows from Panacea, FL (n = 42) and Stony Brook, NY (n = 19), it was found that burrow length (ANCOVA, F = 4.521, p = 0.039; Table 3.4AII) and shaft diameter (ANCOVA, F = 8.270, p = 0.006; Table 3.4AVII) differed significantly between sites. Independent of crab size, burrow length was greater in NY (mean ± SD, NY = 34.4 ± 8.31 cm, FL = 30.3 ± 6.92 cm) and shaft diameter was greater in FL (NY = 17.8 ± 2.50 mm, FL = 19.4 ± 2.15 mm). Although burrow depth was not significantly correlated to relative claw length in either FL or NY, the regression line relating relative claw length to burrow depth had significantly different slopes in each location (ANCOVA, site: relative claw length interaction, F = 5.577, p = 0.022; Table 3.4AI), being more positive in FL and more negative in NY despite neither being significantly differ than a slope of zero (Fig. 3.4). A significant negative relationship between ground temperature and angle of descent was found in this analysis (ANCOVA, F = 8.976, p = 0.004; Table 3.4AVIII). However, the significant interaction term between ground temperature and angle of descent (ANCOVA, F = 6.974, p = 0.011; Table 3.4AVIII) along with a post hoc comparison of model coefficients highlights that the negative relationship between temperature and angle only exists in NY, while no relationship exists between temperature and angle in FL (Fig. 3.5).

The study-wide analysis, utilizing all sampled burrows (n = 83) and excluding site and lunar day as independent variables, indicates a significant, positive impact of crab carapace width on all burrow size metrics (i.e., burrow length, terminal chamber length,

77

burrow depth, entrance diameter, shaft diameter, terminal chamber diameter, and burrow

volume) (ANCOVAs; all $F \geq 11.266$, all $p < 0.001$; Table 3.5AI-VII) (Fig. 3.6AI-VII), as

well as all principal components (ANCOVAs; all $F \geq 5.763$, all $p \leq 0.019$; Table 3.6AI-

III) (Fig. 6BI-III). The study-wide results also indicate a positive relationship between

relative claw length and burrow shaft diameter (ANCOVA, $F = 5.972$, $p = 0.017$; Table

3.5AV) (Fig 3.6AVIII). Using the full dataset, a negative relationship between ground

temperature and the angle of descent was found (ANCOVA, $F = 6.746$, $p = 0.011$; Table

3.5AVIII), but, as above in the FL vs NY analysis, this overall trend between ground

temperature and angle of descent was due to a highly significant negative relationship

between these variables in NY specifically, and the trend does not exist in FL or NC (post

hoc comparison of model coefficients). Additionally, a significant interaction between

carapace width and ground temperature exists for burrow length (ANCOVA, $F = 7.641$, $p = 0.004$; Table 3.5AII) in which burrow length tends to increase with increased ground

temperature in smaller crabs but does not increase with ground temperature in larger

crabs (Fig 3.7).

The additional burrow metrics collected only in 2021 (i.e., beach height, absolute

depth, beach slope, and direction of descent) did not significantly vary with any measured

independent variables (Table 3.3AIX-XII). Beach height was found to be significantly

and positively correlated with several other dependent variables including burrow depth,

length, volume, and absolute depth for those burrows in which beach height was

measured (n = 17) (Table 3.2). Overall direction of descent and beach slope were not

correlated with any other dependent metrics of burrow morphology (Table 3.2). The

average beach height of the burrows in Panacea, FL was 131.98 ± 7.64 cm above

78

MLLW, which was above of the upper reaches of intertidal zone of the Panacea, FL location based on the highest amplitude tides recorded on days that burrows were cast (lower low tidal height = 5.9 ± 12.7 cm above MLLW; higher high tidal height = 109.2 ± 7.7 cm above MLLW). All but one of the recorded beach heights were higher than the height of higher amplitude high tide on the day they were measured (average height relative to higher high tide = 17.95 ± 11.94 cm). The average direction of descent relative to the waterline was 105.33° ± 58.02° (n = 17). The distribution of the measured directions of descent was not significantly different from a uniform distribution between 0° and 180° (Komogorov-Smimov test; D = 0.256; p-value = 0.182), nor did the observed number of values within each 45° bin (i.e., 0-45°, 45-90°, 90-135°, and 135-180°) differ from expected number of values for each bin given a random distribution (i.e., 25% of observations falling into each bin) (Chi-squared test, X^2 = 3.436, df = 3, p-value = 0.329).

3.3.2 Burrow depth and tidal amplitude

Absolute burrow depth was not significantly correlated with any tidal metrics according to Pearson's product moment correlation tests. Absolute burrow depth averaged 115.04 ± 6.47 cm above MLLW. Based on the absolute depth measurements, roughly half (9/17) of the measured burrows would have had some portion of their length inundated during the higher high tide on the day they were measured (average absolute depth relative to higher high tide = 1.01 ± 10.88 cm), but all absolute depths were above the reach of the lower high tide (average absolute depth relative to lower high tide = 16.97 ± 7.60 cm).

3.3.3 Thermal benefit

Air temperatures were significantly higher outside of the burrows than inside (outside = 34.6 ± 2.75 °C, inside = 32.2 ± 2.53 °C) (ANCOVA, F = 26.039, p < 0.001), and peaked during the middle of the day (ANCOVA; sine of time of day, F = 42.368, p < 0.001; cosine of time of day, F = 30.526, p < 0.001) (Table 3.7AI) (Fig. 3.8A). Sediment surface temperatures were significantly higher outside of the burrows (outside = 38.1 ± 6.18 °C, inside 30.9 ± 2.45 °C) (ANCOVA, F = 49.324, p < 0.001), peaked during the middle of the day (ANCOVA; sine of time of day, F = 26.280, p < 0.001; cosine of time of day, F = 17.525, p < 0.001), and had a significant interaction term between location and time of day (ANCOVA, F = 5.960, p = 0.029), indicating that the sediment temperatures inside the burrow are lower and more stable throughout the day than the sediment temperatures outside of the burrow which fluctuate more widely (Table 3.7AII) (Fig 3.8B). Crab body temperatures were higher outside of the burrow (outside = 34.9 ± 3.32 °C, inside = 30.0 ± 1.83 °C) (ANCOVA, F = 102.755, p < 0.001), peaked during the middle of the day (ANCOVA; sine of time of day, F = 16.600, p < 0.001; cosine time of day, F = 38.448, p < 0.001), and were lower and more stable inside the burrow (i.e., significant interaction between location and time of day) (ANCOVA, F = 12.939, p = 0.002) (Table 3.7BI) (Fig 3.8C). There was also a significant effect of relative claw size, with a negative relationship between relative claw size and body temperature (i.e., larger claw relative to carapace width = lower body temperature) (ANCOVA, F = 5.470, p = 0.032) and a significant interaction between relative claw size and carapace width (ANCOVA, F = 5.207, p = 0.036), indicating that this relationship between claw size and body temperature was greatest in larger crabs (Table 3.7BI) (Fig. 9AI-II). The significant

impact of relative claw length as a main effect was largely shaped by a singular crab that was particularly cold at the end of the experiment duration and had a particularly large claw for its size. Excluding this individual from analysis removes the significance of relative claw size as a main effect (ANCOVA, F = 1.764, p = 0.203) but retains the interaction term on crab body temperature (ANCOVA, F = 4.939, p = 0.041) (Fig. 9BI-II).

3.4 Discussion

The mating burrows of *L. pugilator* are key reproductive, thermal, and hydric resources for both males and females and are the center point of almost all individual-level social interaction in the species. An understanding of these abiotic structures is imperative to understand the biology of *L. pugilator*. Although many studies have investigated the social dynamics and behaviors associated with mating burrows as well as the abiotic stressors of the high intertidal mating area in which they are found, fewer studies have directly investigated the structures themselves. The current study was designed to provide more detailed information about the typical size and shape of *L. pugilator* mating burrows, measure the thermal benefit they provide, and to test several hypotheses concerning how the general structure may change with resident size, local temperature, climate, and the lunar cycle.

3.4.1 General burrow structure

Burrows typically descend at roughly a 45° angle into the sediment, descending in an apparently random direction relative to the waterline. The mating chambers of most burrows comprise roughly 40% of their total length and have a diameter that is roughly 50% larger than that of the burrow's main shaft. All metrics of burrow size (i.e., depth,

total length, chamber length, entrance diameter, shaft diameter, chamber diameter, and total volume) were positively correlated with one another, meaning that a burrow which is larger in one aspect is likely to be larger in all others.

Though difficult to describe quantitatively, it bears mentioning that the mating burrow took on a variety of different general shapes that are not well reflected in the recorded metrics. All mating burrows begin with a shaft which descends to a terminal mating chamber, but while some burrows descend linearly at a relatively constant angle (Fig. 3.10A) other spiral downward (Fig. 3.10B) or descend at a relatively constant angle before leveling out abruptly at depth usually at the transition from shaft to mating chamber (Fig. 3.10C). The underlying cause of this variation is unknown but may be due to abiotic factors such as changes in the sediment composition with depth, biotic factors such as female preference, or random chance/individual level differences in burrow construction among males. Although most burrows could be placed squarely into one of the three designations mentioned above (i.e., linear, spiraling, or angular), some burrows were more chaotic and difficult to describe (Fig. 3.10D).

Mating burrows are often described, in this study and many others, as being found in the high intertidal zone. However, the beach heights recorded in the current study suggest that this may not always be the case. In Panacea, FL the average beach height of mating burrow entrances was beyond the reach of the high tide, and it would be more accurate to describe their location as the low supratidal zone. However, it may be unwise to attempt to generalize the location of the mating area relative to tidal influence across different sites. Beach heights were only measured in Panacea, FL where the mating habitat was supratidal, but in both Beaufort, NC and Stony Brook, NY it was seemingly

quite clear that a majority of mating burrow were well within the intertidal zone with their entrances being submerged during high tide.

3.4.2 Burrow structure and male morphology

The single best predictor of burrow size found in the current study is the carapace width of the male resident, with larger male residents typically inhabiting burrows that are larger across all metrics, supporting the hypothesis that burrow size and male size are linked. For measures of burrow diameter (i.e., entrance, shaft, and chamber diameter), it is tempting to attribute this correlation simply to the fact that, in order to move throughout their burrows, male residents must construct them to have proper clearance for their bodies which they likely accomplish by moving the minimum amount of sediment necessary. This is the simplest explanation and, as such, should be considered the most likely to be true; however, it has been shown that female preference is based, in part, on burrow diameter, with females tending to choose burrows that allow for their size. With this female preference in mind, smaller males could potentially benefit, through access to a wider size range of females, by widening the diameter of their burrow's entrance and shaft. However, this is not reflected in the data. The fact that smaller males apparently "opt-out" of the wider range of mates they might access by increasing their burrow diameter to fit the females they court, rather than themselves, points to some counter-selecting force that restricts them from doing so. One such force may be male-male competition. Selection may favor males who construct burrows with shaft diameters that are tightly tied to their own size because such burrows are likely easier to defend from intruding males. A tightly fitted burrow would allow the resident male to quickly and fully obstruct the shaft of the burrow to discourage entry into the

burrow's deeper reaches by unwelcome individuals (i.e., as long as the resident descends in front of a territory seeking male or an individual seeking refuge from predation, the resident can block them and maintain control of the burrow). A tightly fitted burrow shaft may be important in maintaining the well-documented ownership advantage of residents over intruding crabs. It is not possible in the current study to determine the exact clearance preferred by a resident male since both of the recorded metrics of crab morphology (carapace width and claw length) are lateral measurements, and, given that fiddler crabs descent their burrows laterally, axial measurements would be needed to determine clearance. However, the burrow shaft has a diameter that is clearly insufficient for two crabs to occupy the same portion, while the mating chamber evidently has space for the mating pair.

It is more difficult to hypothesize why metrics of burrow length and depth (e.g., depth, total length, and chamber length), which have less to do with a resident's ability to traverse and defend its burrow, were also correlated with male size. It is likely that length and depth have an impact on the microclimate of the terminal mating chamber, so the correlation may reflect a male's drive to construct a burrow that is appropriate for the females he is most likely to court or would have the greatest benefit in courting. In general, larger males are more likely to attract females of all sizes (McLain and Pratt, 2007). Selection for smaller males, whose smaller burrows offer increased incubation temperatures, has been reported in an Australian species, *Austruca mjoebergi,* in which mate searching females that become reproductively active later in the mating cycle select smaller male because the increased internal temperatures of their burrows reduce the females' incubation time (Reaney and Backwell, 2007). It would be beneficial to

84

measure the abiotic parameters in the mating chamber of burrows of differing depths and lengths. It is unlikely that the humidity of the air within the burrow changes much with depth (Warren Porter, pers. comm.), but it may be that deeper burrows provide a cooler environment with wetter sediment in their mating chambers.

3.4.3 Burrow structure and local temperature/climate

Local temperature and climatic differences had an impact on burrow size and shape. In the study-wide analysis, it was seen that smaller males tend to dig deeper burrows when ground temperatures are higher. As mentioned above, the reason that smaller crabs dig shorter burrows on average is unknown, but this response to temperature most likely represents an attempt to create a cooler environment in the mating chamber of their burrows for themselves and/or the females they court.

Burrow structure differed between northern and southern populations of *L. pugilator* in NY and FL, respectively. The diameters of burrow shafts in FL were larger than those in NY independent of resident size. Previous studies have shown that burrows with wider diameters tend to be cooler than those with narrower diameters (Reaney and Backwell, 2007). The differences in burrow diameter between NY and FL may then reflect a population level adaptation to climatic differences in temperature, with the FL population having increased burrow diameters to account for increased environmental temperatures. Following this logic, it is somewhat counterintuitive that burrow length was greater in NY than in FL, as an increase in burrow length could be expected to decrease the internal temperature of the burrow. The longer burrows found in NY may be an adaptation to the higher amplitude tides of this region compared to FL (~1 m in FL and ~2m in NY). Finally, males in NY constructed burrows with shallower angles of

descent when local temperatures were high, a trend that was not seen in FL or NC alone. The reason for this is unclear. It may be that burrows with shallower angles of descent help in some way to ameliorate thermal stress, but if this were the case, one would expect to see a similar trend at the other sites. It seems likely that this phenomenon is tied to some unmeasured difference in abiotic factors between the sites.

3.4.4 Burrow structure and the lunar cycle

The hypothesis that males augment the size, shape, or location of their burrows throughout the mating cycle to account for changes in the reproductive needs of mate-seeking females had no support in the current study. No metrics of burrow morphology or location changed with the lunar day, and this held true for males of all sizes. It is likely that the slight differences in female reproductive need throughout the mating cycle is either not significant enough or too complex to provide adequate levels of selection to promote this behavior.

3.4.5 Burrow depth and tidal amplitude

The importance of physical stability within the mating chamber has long been a topic of debate. Christy (1982) hypothesized that mating chamber stability was of the utmost importance for females during oviposition due to the potential that a burrow collapse would lead to loss of eggs. He hypothesized that males built their burrow in such a way that their mating chambers would not be inundated by high tide or by the water table in order to reduce the likelihood of burrow collapse, and that males competed for high territory to elevate themselves above the hydrological dangers. This hypothesis was made based on the observations that (A) physical disturbance during oviposition causes egg loss in the laboratory for other brachyuran crabs, (B) mating burrows higher up the

86

beach on which he conducted his study had a higher proportion of large residents, and (C) that males abandon lower elevation burrows during particularly high tides. This hypothesis runs on the assumptions that (A) inundation by the water table causes burrow collapse, (B) the disturbance cause by this collapse is sufficient to cause egg loss, and (C) that large male has a competitive advantage in territory acquisition that allow them access to the most preferential territories. Interestingly, Christy's study was also conducted on a population of *L. pugilator* located on the Gulf Coast of FL (Cayo Pelau; near Fort Meyers) and is one of the only studies to explicitly describe the mating habitat of the study population as supratidal.

For many, the presence of populations across *L. pugilator*'s range with distinctly intertidal mating habitats may be enough to discredit Christy's hypothesis or any of the assumptions under which the hypothesis was made. The existence of intertidal mating habitat alone refutes the hypothesis that males construct their burrow such that the mating chambers are above the high tide and the water table. Intertidal mating habitat also seems to refute either the assumption that inundation of the mating chamber causes chamber collapse or the assumption that the collapse causes significant egg loss in females, though both assumptions are difficult to directly assess. However, Christy's study population has remained distinct in the literature as being supratidal, and his study is one of the only previous studies to describe mating burrows in *L. pugilator* or to posit hypotheses about their construction. That being the case and given that the similarities between his study population and the Panacea, FL population of the current study, it seems appropriate to address his hypothesis directly.

87

In the current study, the hypothesis that males construct their burrows to a depth that is in some way related to the height of the tide or the water table has mixed support. The relative height of burrows within the high intertidal zone was positively correlated with burrow depth and length in those burrows collected during 2021 from Panacea, FL for which beach height was recorded (n = 17). These results might be interpreted as meaning that males at higher beach elevations dig longer, deeper burrows in order to position their mating chamber relative to the water table. Additionally, the average absolute depth of the mating chambers in the current study is remarkable close to the average height of the higher amplitude daily high tide (average absolute depth relative to higher high tide = 1.01 ± 10.88 cm), which also points to males terminating their burrows at a depth that is in some way determined by the height of the water table. However, under the assumption that the height of the tide and the height of the water table are close to the same in the intertidal zone of sandy beaches, it was found that more than half of the measured burrows (9/17) were inundated to some extent by the water table during high tide on the day they were measured. Also, beach height, depth, and absolute depth of burrows were not correlated with any tidal metrics from the days on which the burrows were collected. These results seem to indicate that males are not accounting for tidal influence and inundation when setting the depth of their mating chambers over short periods of time. Further study of how tidal inundation affects burrow structure and female reproductive success as well as the environmental factors that differ between intertidally mating and supratidally mating populations are needed.

3.4.6 Thermal benefit of mating burrows

As expected, mating burrows offer a significant thermal benefit to their residents. The air, sediment, and crab body temperatures recorded were lower inside of mating burrows throughout the day, and both sediment and body temperatures were more stable within the burrow. By remaining inside their burrows, male residents experience body temperatures that were roughly 5 °C lower than their counterparts on the surface over the course of ten minutes. The thermal benefit provides by mating burrows is likely to be most important on days when low tide occurs from midday through mid afternoon. During midday low tides, crabs outside the burrow experience body temperatures dangerously close to or, in the unfortunate case of one focal male, in excess of their critical thermal maximum, while those crabs inside the burrow experience body temperature well with their healthy range. The difference in body temperatures between males inside of and outside of mating burrows is much smaller during low tide that occur in the early morning or evening, making the mating burrow less valuable as a thermal resource during these periods.

Relative claw size was negatively related to body temperature of crabs in the current study which supports previous finding that between male claw size negatively impacts body temperature (i.e., large claws are useful for heat mitigation) (Darnell and Munguia, 2011). However, when removing a single relatively cool, small male with a very large claw for its size, this overall trend was no longer present. However, this lack of a trend on an experiment-wide level does not necessarily mean that certain males are not thermally benefitted from having a large claw. It was seen both with and without the abovementioned outlying male that larger claw sizes seem to reduce body temperature to

a greater effect in large males than in small males. Many previous studies (Christy, 1983; Hyatt, 1977; Jennions and Backwell, 1996; Pratt and Mclain, 2002) have shown that larger males are generally more successful reproductively, and this additional benefit of their large claw may play some role in their success.

This study showed no significant impact of burrow entrance diameter on internal air and sediment temperatures despite the fact that previous studies have found correlation between diameter and internal temperature of burrows (Reaney and Backwell, 2007). The design of this experiment was focused primarily on the magnitude of thermal benefit throughout the day, not on the effects of burrow diameter (i.e., burrow diameter was not controlled for), so this lack of result should likely be attributed experimental design.

3.4.7 Conclusion

The mating burrows of *L. pugilator* are complex structures that serve many functions. Despite their paramount importance in determining individual fitness and shaping social interactions, little research into their structure and construction has been conducted to date. This study serves as a modern baseline along this line of questioning. Here I have described in greater detail the general shape of mating burrows and how this is related to resident morphology, showed the potential for difference in burrow structure to occur within and between populations based abiotic factors, investigated the influence of the lunar cycle on structure, clearly measured the magnitude of thermal benefit which mating burrow offer, and added some modern insight to a long-unaddressed hypothesis concerning the connection between mating burrow construction and tidal inundation. Yet, much work remains to be done. Some portion of our understanding of the biology of *L.*

pugilator still depends on yet untested assumptions of their mating burrows (e.g., that larger males hold territories at higher beach elevations because higher beach elevations provide a more suitable subterranean habitat; that female reproductive success is tied to burrow stability; etc.). One such assumption that bears mention is that the mating burrows of *L. pugilator* persist from one low tide to the next. As of this writing, I do not believe that there is any concrete evidence that mating burrows do not collapse on their residents during every high tide, especially in intertidal populations. The implications of daily burrow collapse in shaping neighborhood dynamics and determining energetic costs of burrow ownership are potentially serious, and I believe that this avenue of study may be fruitful.

Table 3.1 *Correlation matrix (A) and significance matrix (B) for burrow morphometrics*

A. Correlation Matrix

	Depth	Length	Chamber Length	Opening Diameter	Shaft Diameter	Chamber Diameter	Volume	Descent Angle	Beach Height	Beach Slope	Direction to Water
Length	0.731										
Chamber Length	0.447	0.470									
Opening Diameter	0.284	0.320	0.476								
Shaft Diameter	0.403	0.338	0.400	0.750							
Chamber Diameter	0.242	0.338	0.364	0.543	0.477						
Volume	0.592	0.750	0.576	0.614	0.606	0.635					
Descent Angle	0.147	-0.134	0.034	-0.160	-0.064	-0.262	-0.163				
Beach Height	0.544	0.483	-0.283	0.101	-0.050	0.036	0.494	0.125			
Beach Slope	0.389	0.285	-0.311	-0.311	-0.209	-0.235	0.072	-0.146	0.461		
Direction to Water	-0.026	-0.271	-0.040	-0.355	0.113	0.220	-0.212	0.400	0.041	0.026	
Absolute Depth	-0.168	-0.020	-0.390	0.172	-0.158	0.204	0.272	0.014	0.736	0.227	0.069

B. significance Matrix

	Depth	Length	Chamber Length	Opening Diameter	Shaft Diameter	Chamber Diameter	Volume	Descent Angle	Beach Height	Beach Slope	Direction to Water
Length	**< 0.001**										
Chamber Length	**< 0.001**	**< 0.001**									
Opening Diameter	**0.005**	**0.002**	**< 0.001**								
Shaft Diameter	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**							
Chamber Diameter	**0.014**	**0.001**	**< 0.001**	**< 0.001**	**< 0.001**						
Volume	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**					
Descent Angle	0.093	0.886	0.380	0.926	0.718	0.992	0.930				
Beach Height	**0.012**	**0.025**	0.864	0.350	0.575	0.446	**0.022**	0.316			
Beach Slope	0.061	0.143	0.887	0.894	0.790	0.818	0.392	0.712	**0.031**		
Direction to Water	0.540	0.854	0.560	0.919	0.333	0.198	0.793	0.056	0.348	0.431	
Absolute Depth	0.740	0.531	0.939	0.254	0.727	0.216	0.146	0.479	**0.004**	0.190	0.396

When possible, all data were used to calculate correlation coefficients in (A) and p-values in (B) (n = 83). However, for each relationship which included one of the additional burrow metrics collected in 2021 a smaller number of samples had to be used (n = 17), and these calculations with fewer data points are indicated by the grey coloration. Significant p-values are in bold type.

Table 3.2 *Descriptive statistics of resident morphology, environmental data, and mating burrow structure metrics for each site and the total pooled dataset*

Metric (units)	Panacea, FL n = 42	Beaufort, NC n = 21	Stony Brook, NY n = 20	Pooled n = 83
Resident Morphology				
Carapace Width (mm)	18.69 ± 2.10	14.38 ± 1.47	18.68 ± 1.05	17.56 ± 2.56
Claw Length (mm)	30.83 ± 4.95	19.50 ± 3.29	29.36 ± 2.81	27.49 ± 6.31
Environmental				
Max High Tide (cm to MLLW)	109.22 ± 7.70	104.36 ± 3.33	224.03 ± 12.68	135.65 ± 50.83
Max Low Tide (cm to MLLW)	5.88 ± 12.66	9.72 ± 2.07	16.15 ± 11.87	9.33 ± 11.46
Tidal Amplitude (cm)	103.34 ± 19.85	94.63 ± 5.32	207.87 ± 24.54	126.33 ± 49.93
Difference in Daily High Tides (cm)	15.17 ± 7.17	18.58 ± 3.96	31.24 ± 3.68	19.90 ± 8.72
Ground Temperature (°C)	31.39 ± 2.74	30.58 ± 0.08	30.09 ± 2.69	30.88 ± 2.4
Mating Burrow Structure				
Angle of Descent (°)	48.68 ± 12.53	49.22 ± 13.57	47.88 ± 14.74	48.63 ± 13.19
Entrance Diameter (mm)	15.39 ± 1.68	12.08 ± 1.29	14.95 ± 0.92	14.44 ± 1.99
Shaft Diameter (mm)	19.35 ± 2.15	14.10 ± 1.83	17.75 ± 2.44	17.64 ± 3.04
Chamber Diameter (mm)	27.20 ± 3.37	22.97 ± 3.31	26.50 ± 2.66	25.96 ± 3.63
Total Length (cm)	30.26 ± 6.92	25.62 ± 5.55	34.17 ± 8.15	30.03 ± 7.49
Chamber Length (cm)	13.31 ± 2.46	9.82 ± 1.84	12.47 ± 4.74	12.22 ± 3.34
Depth (cm)	19.59 ± 5.66	14.74 ± 3.98	19.32 ± 6.55	18.30 ± 5.85
Volume (ml)	104.84 ± 34.80	54.63 ± 16.38	102.01 ± 44.23	91.45 ± 39.88
Additional Mating Burrow Metrics (2021)	n = 17			
Elevation (cm to MLLW)	131.98 ± 7.65	-	-	-
Absolute Depth (cm to MLLW)	115.04 ± 6.47	-	-	-
Beach Slope (°)	10.06 ± 3.55	-	-	-
Direction of Descent (°)	105.33 ± 58.02	-	-	-

The mean ± standard deviation for many of the metrics of interest from the current study separated by site as well as pooled and with sample sizes indicated .

93

Table 3.3 *Univariate linear fixed effects model results for Florida burrow metrics with lunar day included as main effects along with carapace width and relative claw length*

A. Lunar effects models using FL crabs		I. Burrow Depth (n = 42)				II. Burrow Length (n = 42)			
Source	DOF	SS	MS	F	p	SS	MS	F	p
Carapace Width (CW)	1	131.735	131.735	4.913	**0.035**	312.311	312.311	9.309	**0.005**
Relative Claw Length (RCL)	1	101.971	101.971	3.803	0.061	138.262	138.262	4.121	0.052
Sine of Lunar Day (Sin)	1	26.164	26.164	0.976	0.332	52.298	52.298	1.559	0.222
Cosine of Lunar Day (Cos)	1	33.830	33.830	1.262	0.271	114.245	114.245	3.405	0.076
Significant Two-way Interactions									
RCL:Sin	1	-	-	-	-	144.273	144.273	4.300	**0.047**

		III. Chamber Length (n = 42)				IV. Opening Diameter (n = 42)			
Source	DOF	SS	MS	F	p	SS	MS	F	p
Carapace Width (CW)	1	74.168	74.168	14.287	**<0.001**	60.999	60.999	71.126	**<0.001**
Relative Claw Length (RCL)	1	5.179	5.179	0.998	0.326	0.011	0.011	0.013	0.912
Sine of Lunar Day (Sin)	1	5.851	5.851	1.127	0.297	3.235	3.235	3.772	0.062
Cosine of Lunar Day (Cos)	1	0.008	0.008	0.002	0.969	0.925	0.925	1.079	0.308
Significant Two-way Interactions									
None	-	-	-	-	-	-	-	-	-

		V. Shaft Diameter (n = 42)				VI. Chamber Diameter (n = 42)			
Source	DOF	SS	MS	F	p	SS	MS	F	p
Carapace Width (CW)	1	75.200	75.200	38.081	**<0.001**	139.777	139.777	19.620	**<0.001**
Relative Claw Length (RCL)	1	17.571	17.571	8.898	**0.006**	6.456	6.456	0.906	0.349
Sine of Lunar Day (Sin)	1	4.394	4.394	2.225	0.147	12.209	12.209	0.866	0.360
Cosine of Lunar Day (Cos)	1	0.009	0.009	0.005	0.946	12.209	12.209	1.714	0.201
Significant Two-way Interactions									
CW:Cos	-	8.528	8.528	4.319	**0.047**	-	-	-	-

		VII. Burrow Volume (n = 42)				VIII. Angle of Initial Descent (n = 42)			
Source	DOF	SS	MS	F	p	SS	MS	F	p
Carapace Width (CW)	1	18634.092	18634.092	26.461	**<0.001**	399.548	399.548	2.668	0.114
Relative Claw Length (RCL)	1	1720.176	1720.176	2.443	0.129	0.105	0.105	0.001	0.979
Sine of Lunar Day (Sin)	1	0.089	0.089	0.001	0.991	119.869	119.869	0.800	0.379
Cosine of Lunar Day (Cos)	1	571.489	571.489	0.812	0.375	19.973	19.973	0.133	0.718
Significant Two-way Interactions									
None	-	-	-	-	-	-	-	-	-

		IX. Beach Height (n = 17)				X. Beach Slope (n = 17)			
Source	DOF	SS	MS	F	p	SS	MS	F	p
Carapace Width (CW)	1	1.306	1.306	0.015	0.909	0.035	0.035	0.002	0.969
Relative Claw Length (RCL)	1	5.748	5.748	0.067	0.812	28.789	28.789	1.482	0.311
Sine of Lunar Day (Sin)	1	7.061	7.061	0.083	0.792	8.700	8.700	0.448	0.551
Cosine of Lunar Day (Cos)	1	15.211	15.211	0.178	0.701	0.225	0.225	0.012	0.921
Significant Two-way Interactions									
None	-	-	-	-	-	-	-	-	-

		XI. Descent Direction to Water (n = 17)				XII. Absolute Depth (n = 17)			
Source	DOF	SS	MS	F	p	SS	MS	F	p
Carapace Width (CW)	1	1342.255	1342.255	0.327	0.607	5.462	5.462	0.084	0.791
Relative Claw Length (RCL)	1	1583.556	1583.556	0.386	0.578	101.937	101.937	1.562	0.300
Sine of Lunar Day (Sin)	1	27.321	27.321	0.007	0.940	25.115	25.115	0.385	0.579
Cosine of Lunar Day (Cos)	1	384.908	384.908	0.094	0.779	2.997	2.997	0.046	0.844
Significant Two-way Interactions									
None	-	-	-	-	-	-	-	-	-

Significant p-values are in bold type. Only significant two-way interactions are included (i.e., not all two-way interactions are included). Significant two-way interactions are written using shorthand labels indicated following main effect labels. Dashes represent two-way interactions that were not significant.

Table 3.4 *Univariate linear fixed effects model results for Florida and New York burrow metrics with site included as a main effect along with carapace width, relative claw length, and ground temperature*

A. Site effects models using FL & NY crabs (n = 61)

Source	DOF	I. Burrow Depth SS	MS	F	p	II. Burrow Length SS	MS	F	p
Site (ST)	1	2.348	2.348	0.069	0.793	192.480	192.480	4.521	**0.039**
Carapace Width (CW)	1	84.320	84.320	2.491	0.121	240.739	240.739	5.655	**0.022**
Relative Claw Length (RCL)	1	17.007	17.007	0.502	0.482	97.786	97.786	2.297	0.136
Ground Temperature (GT)	1	2.960	2.960	0.087	0.769	160.423	160.423	3.768	0.058
Significant Two-way Interactions									
ST:RCL	1	188.772	188.772	5.577	**0.022**	-	-	-	-
CW:GT	1	-	-	-	-	513.499	513.499	12.062	**0.001**

Source	DOF	III. Chamber Length SS	MS	F	p	IV. Opening Diameter SS	MS	F	p
Site (ST)	1	3.420	3.420	0.298	0.587	1.256	1.256	1.600	0.212
Carapace Width (CW)	1	70.430	70.430	6.147	**0.017**	67.458	67.458	85.986	**<0.001**
Relative Claw Length (RCL)	1	5.304	5.304	0.463	0.500	1.110	1.110	1.415	0.240
Ground Temperature (GT)	1	0.247	0.247	0.022	0.884	0.133	0.133	0.169	0.683
Significant Two-way Interactions									
None	-	-	-	-	-	-	-	-	-

Source	DOF	V. Shaft Diameter SS	MS	F	p	VI. Chamber Diameter SS	MS	F	p
Site (ST)	1	28.945	28.945	8.270	**0.006**	12.216	12.216	1.726	0.195
Carapace Width (CW)	1	80.520	80.520	23.007	**<0.001**	140.361	140.361	19.836	**<0.001**
Relative Claw Length (RCL)	1	6.177	6.177	1.765	0.190	3.094	3.094	0.437	0.512
Ground Temperature (GT)	1	14.789	14.789	4.226	**0.045**	0.008	0.008	0.001	0.973
Significant Two-way Interactions									
None	1	-	-	-	-	-	-	-	-

Source	DOF	VII. Burrow Volume SS	MS	F	p	VIII. Angle of Initial Descent SS	MS	F	p
Site (ST)	1	79.924	79.924	0.078	0.782	25.241	25.241	0.173	0.680
Carapace Width (CW)	1	20161.937	20161.937	19.552	**<0.001**	268.893	268.893	1.841	0.181
Relative Claw Length (RCL)	1	143.797	143.797	0.139	0.711	48.238	48.238	0.330	0.568
Ground Temperature (GT)	1	720.753	720.753	0.699	0.407	1311.126	1311.126	8.976	**0.004**
Significant Two-way Interactions									
ST:GT	1	-	-	-	-	1018.802	1018.802	6.974	**0.011**

Significant p-values are in bold type. Only significant two-way interactions are included (i.e., not all two-way interactions are included). Significant two-way interactions are written using shorthand labels indicated following main effect labels. Dashes represent two-way interactions that were not significant.

Table 3.5 *Univariate linear fixed effects model results for Florida, North Carolina, and New York burrow metrics with carapace width, relative claw length, and ground temperature included as main effects*

A. Morphological effects models using all crabs (n = 81)

Source	DOF	I. Burrow Depth SS	MS	F	p	II. Burrow Length SS	MS	F	p
Carapace Width (CW)	1	363.185	363.185	11.266	**0.001**	669.171	669.171	14.457	**<0.001**
Relative Claw Length (RCL)	1	38.669	38.669	1.200	0.277	12.788	12.788	0.176	0.601
Ground Temperature (GT)	1	2.410	2.410	0.075	0.785	84.601	84.601	1.827	0.181
Significant Two-way Interactions									
CW:GT	1	-	-	-	-	399.949	399.949	7.641	**0.004**

Source	DOF	III. Chamber Length SS	MS	F	p	IV. Opening Diameter SS	MS	F	p
Carapace Width (CW)	1	219.605	219.605	25.706	**<0.001**	237.88	237.88	339.427	**<0.001**
Relative Claw Length (RCL)	1	6.527	6.527	0.764	0.385	2.672	2.672	3.813	0.055
Ground Temperature (GT)	1	0.787	0.787	0.092	0.762	0.193	0.193	0.276	0.601
Significant Two-way Interactions									
None	-	-	-	-	-	-	-	-	-

Source	DOF	V. Shaft Diameter SS	MS	F	p	VI. Chamber Diameter SS	MS	F	p
Carapace Width (CW)	1	428.193	428.193	116.512	**<0.001**	418.735	418.735	54.605	**<0.001**
Relative Claw Length (RCL)	1	21.948	21.948	5.972	**0.017**	9.864	9.864	1.286	0.261
Ground Temperature (GT)	1	7.860	7.860	2.139	0.148	0.545	0.545	0.071	0.791
Significant Two-way Interactions									
None	-	-	-	-	-	-	-	-	-

Source	DOF	VII. Burrow Volume SS	MS	F	p	VIII. Angle of Initial Descent SS	MS	F	p
Carapace Width (CW)	1	57868.758	57868.758	67.798	**<0.001**	374.513	374.513	2.241	0.139
Relative Claw Length (RCL)	1	173.073	173.073	0.203	0.654	0.151	0.151	0.001	0.976
Ground Temperature (GT)	1	846.598	846.598	0.992	0.323	1127.549	1127.549	6.746	**0.011**
Significant Two-way Interactions									
None	-	-	-	-	-	-	-	-	-

Significant p-values are in bold type. Only significant two-way interactions are included (i.e., not all two-way interactions are included). Significant two-way interactions are written using shorthand labels indicated following main effect labels. Dashes represent two-way interactions that were not significant.

Table 3.6 *Univariate linear fixed effects model results PC values for PCA dimensions 1-3*

taking from the 2019/2021 PCA analysis

A. Models of PC values for PCA dimensions 1-3

I. PC1

Source	DOF	SS	MS	F	p
Carapace Width (CW)	1	15.982	15.982	134.202	**<0.001**
Relative Claw Length (RCL)	1	0.360	0.360	3.039	0.086
Sine of Lunar Day (Sin)	1	0.074	0.074	0.627	0.431
Cosine of Lunar Day (Cos)	1	0.051	0.051	0.427	0.516
Significant Two-way Interactions					
None	-	-	-	-	-

II. PC2

Source	DOF	SS	MS	F	p
Carapace Width (CW)	1	2.066	2.066	8.303	**0.005**
Relative Claw Length (RCL)	1	0.001	0.001	0.001	0.998
Sine of Lunar Day (Sin)	1	0.749	0.749	3.008	0.087
Cosine of Lunar Day (Cos)	1	0.001	0.001	0.005	0.944
Significant Two-way Interactions					
RCL:Sin	1	1.617	1.617	4.219	**0.044**

III. PC3

Source	DOF	SS	MS	F	p
Carapace Width (CW)	1	1.853	1.853	5.763	**0.019**
Relative Claw Length (RCL)	1	0.125	0.125	0.388	0.535
Sine of Lunar Day (Sin)	1	0.488	0.488	1.519	0.333
Cosine of Lunar Day (Cos)	1	0.622	0.622	1.933	0.168
Significant Two-way Interactions					
None	-	-	-	-	-

Significant p-values are in bold type. Only significant two-way interactions are included (i.e., not all two-way interactions are included). Significant two-way interactions are written using shorthand labels indicated following main effect labels. Dashes represent two-way interactions that were not significant.

Table 3.7 *Univariate linear fixed effects model results for environmental burrow temperatures (A) and crab body temperature collected at the end of the ten-minute experimental period (B)*

A. Environmental Temperature Models (n = 42) — I. Air Temperature

Source	DOF	SS	MS	F	p
Location (LO)	1	65.025	65.025	26.039	**<0.001**
Sin of Time of Day (Sin)	1	105.804	105.804	42.368	**<0.001**
Cosine of Time of Day (Cos)	1	76.230	76.230	30.526	**<0.001**
Burrow Diameter (BD)	1	3.899	3.899	1.561	0.222
Significant Two-way Interactions					
None	-	-	-	-	-

II. Sediment Temperature

Source	DOF	SS	MS	F	p
Location (LO)	1	528.529	528.530	49.324	**<0.001**
Sin of Time of Day (Sin)	1	281.602	281.600	26.280	**<0.001**
Cosine of Time of Day (Cos)	1	187.784	187.784	17.525	**<0.001**
Burrow Diameter (BD)	1	11.843	11.843	1.105	0.302
Significant Two-way Interactions					
LO:Cos	1	63.864	63.864	5.960	**0.029**

B. Crab Temperature Models (n = 42) — I. Crab Ending Temperature

Source	DOF	SS	MS	F	p
Location (LO)	1	253.012	253.012	102.755	**<0.001**
Sin of Time of Day (Sin)	1	40.874	40.874	16.600	**<0.001**
Cosine of Time of Day (Cos)	1	94.672	94.672	38.448	**<0.001**
Burrow Diameter (BD)	1	0.344	0.344	0.140	0.713
Carapace Width (CW)	1	0.671	0.671	0.272	0.608
Relative Claw Length (RCL)	1	13.469	13.469	5.470	**0.032**
Significant Two-way Interactions					
LO:Cos	1	31.858	31.858	12.939	**0.002**
CW:RCL	1	12.820	12.820	5.207	**0.036**

Significant p-values are in bold type. Only significant two-way interactions are included (i.e., not all two-way interactions are included). Significant two-way interactions are written using shorthand labels indicated following main effect labels. Dashes represent two-way interactions that were not significant.
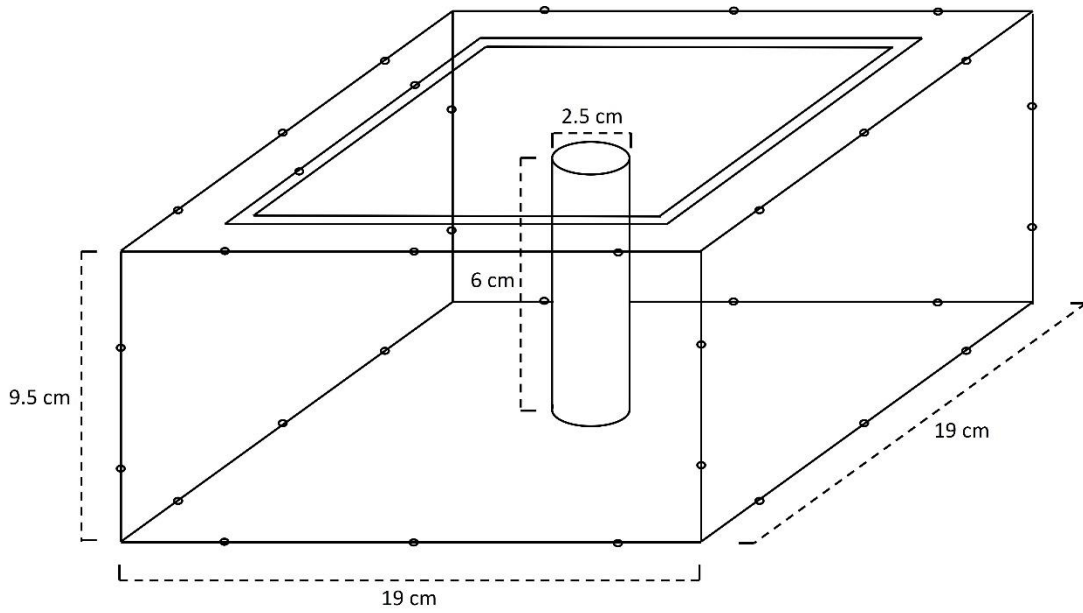
Figure 3.1 *Crab trap*

The trap was designed so that, when positioned with the central chimney directly over a burrow's entrance, the resident male would climb out of the chimney and fall into the trap when attempt to leave his burrow to court. This design allowed for resident males to be removed from their burrows with minimal destruction of the burrow itself.
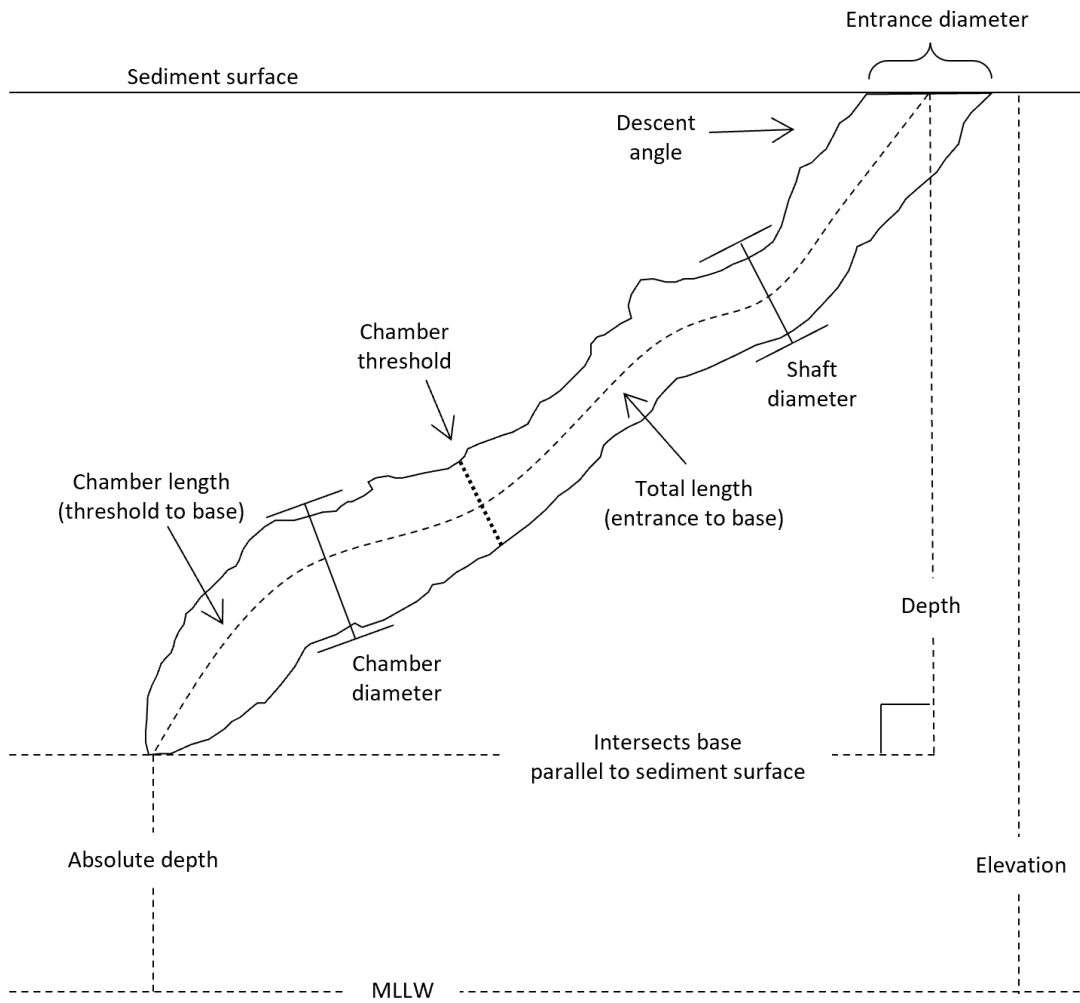
Figure 3.2 *Measured burrow metrics not including beach slope and direction of descent relative to waterline*
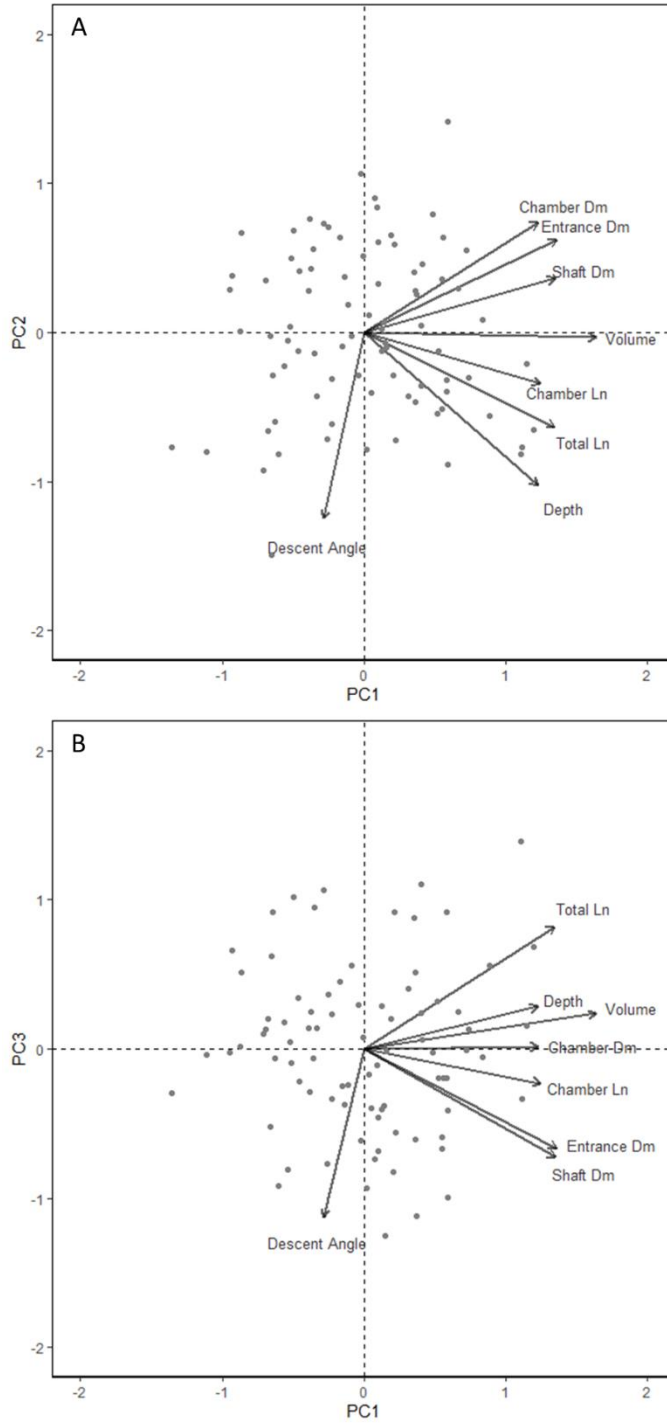
Figure 3.3 *Results of burrow metric PCA using all collected burrows for PC1 and PC2 (A) and PC1 and PC3 (B)*

Values of PC1 and PC2 (A) and PC1 and PC3 (B) for all collected burrows are indicated with point (n = 82). Arrows represent the

loadings of each burrow metric included in the PCA with their length representing the magnitude of their influence within the PCA

space and are labeled according to the metric they represent.
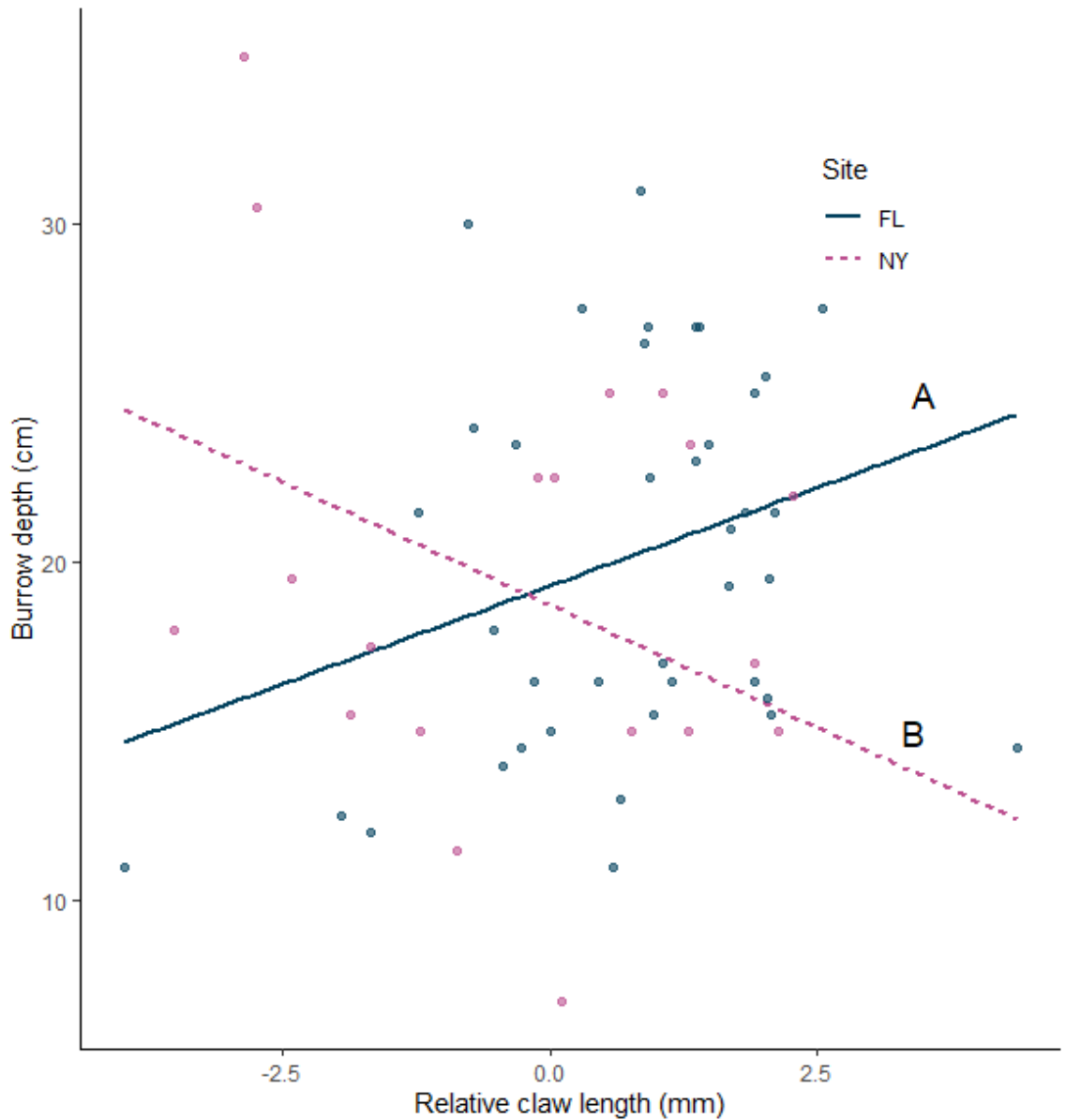
Figure 3.4 *Burrow depth and relative claw length*

Burrow depth and relative claw length are indicated with points (n = 82). Letters represent significant differences in slopes between groups (ANCOVA, site: relative claw length interaction, F = 5.577, p = 0.022) according to the univariate linear fixed effects model (Table 3.4AI). Neither trend line in the current figure is significantly different than zero. Colors indicate the site.
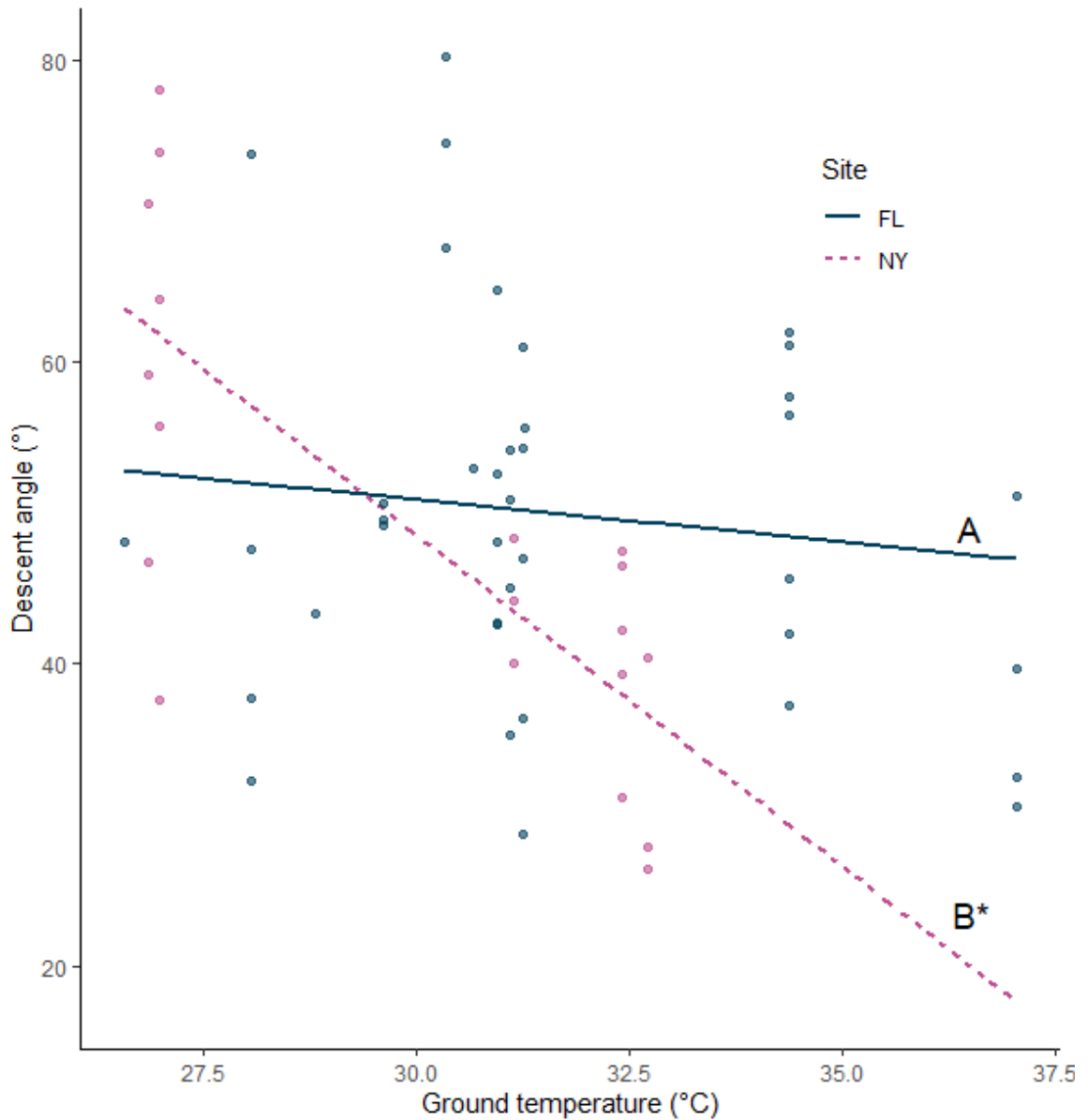
Figure 3.5 *Descent angle and ground temperature*

Descent angle and ground temperature are indicated with points (n = 82). Letters represent significant differences in slopes between groups (ANCOVA, site: ground temperature interaction, F = 6.974, p = 0.011, post hoc comparison of model coefficients) and asterisks indicate slopes that are significantly different than zero (ANCOVA, ground temperature, F = 8.976, p = 0.004, post hoc comparison of model coefficients) according to univariate linear fixed effects models (Table 3.4AVIII). Colors indicate the site.
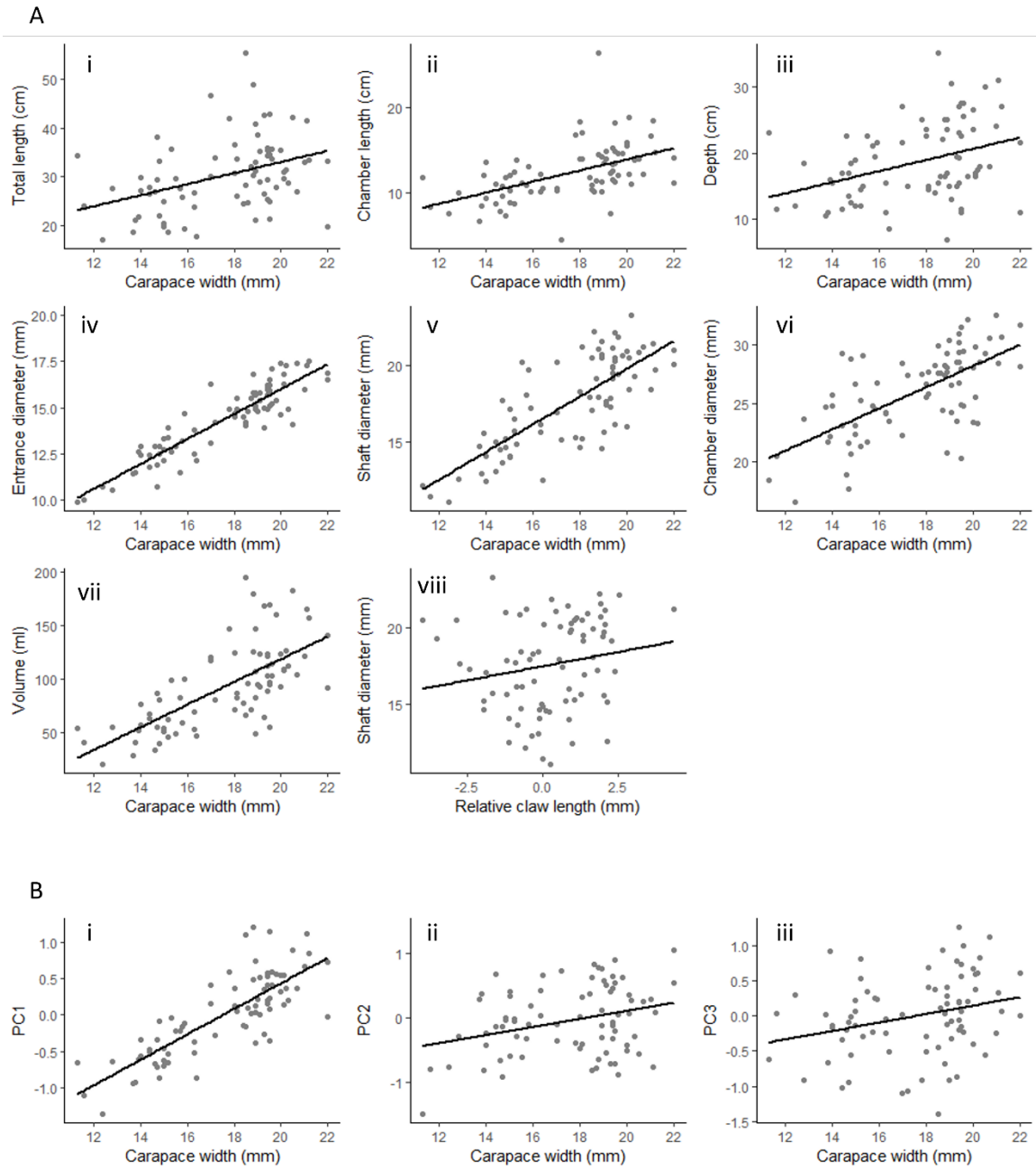
Figure 3.6 *Burrow metrics and crab morphological metrics (A); PCA dimensions 1-3 and crab morphological metric (B)*

Burrow metrics and crab morphological metrics (A) and PCA dimensions 1-3 and crab morphological metrics (B) are indicated with points (n = 82). Trend lines are significant (ANCOVAs, all p < 0.05) according to univariate linear fixed effects models (Table 3.5AI-VII & Table 3.6AI-III).
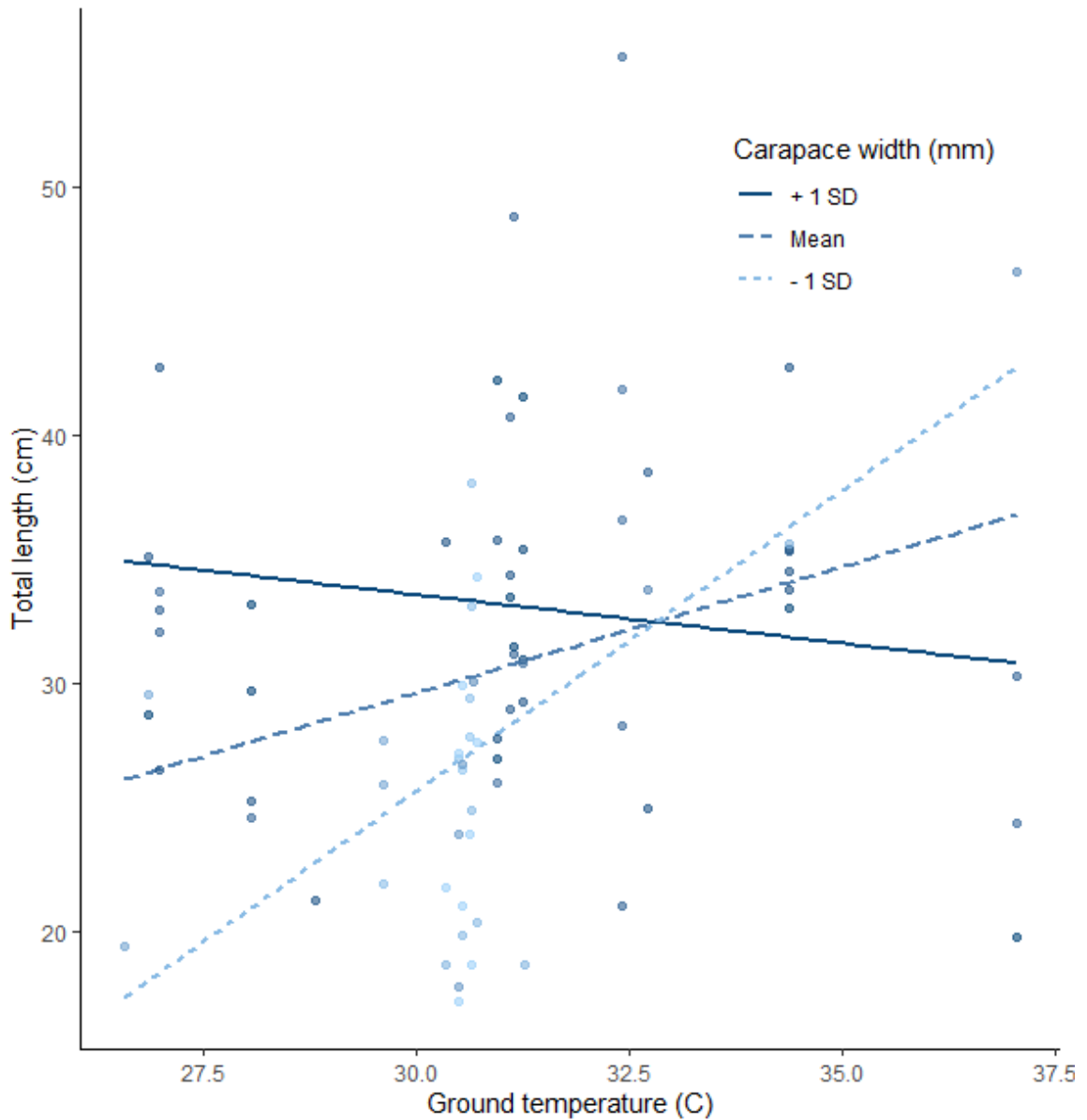
Figure 3.7 *Burrow total length and ground temperature*

Burrow total lengths and ground temperatures are indicated with points (n = 82). The trend lines represent the interaction between ground temperature and carapace width which is significant (ANCOVA, F = 7.641, p = 0.004) according to the univariate linear fixed effects model (Table 3.5AII). Colors represent carapace width with darker blues indicating larger carapace widths.
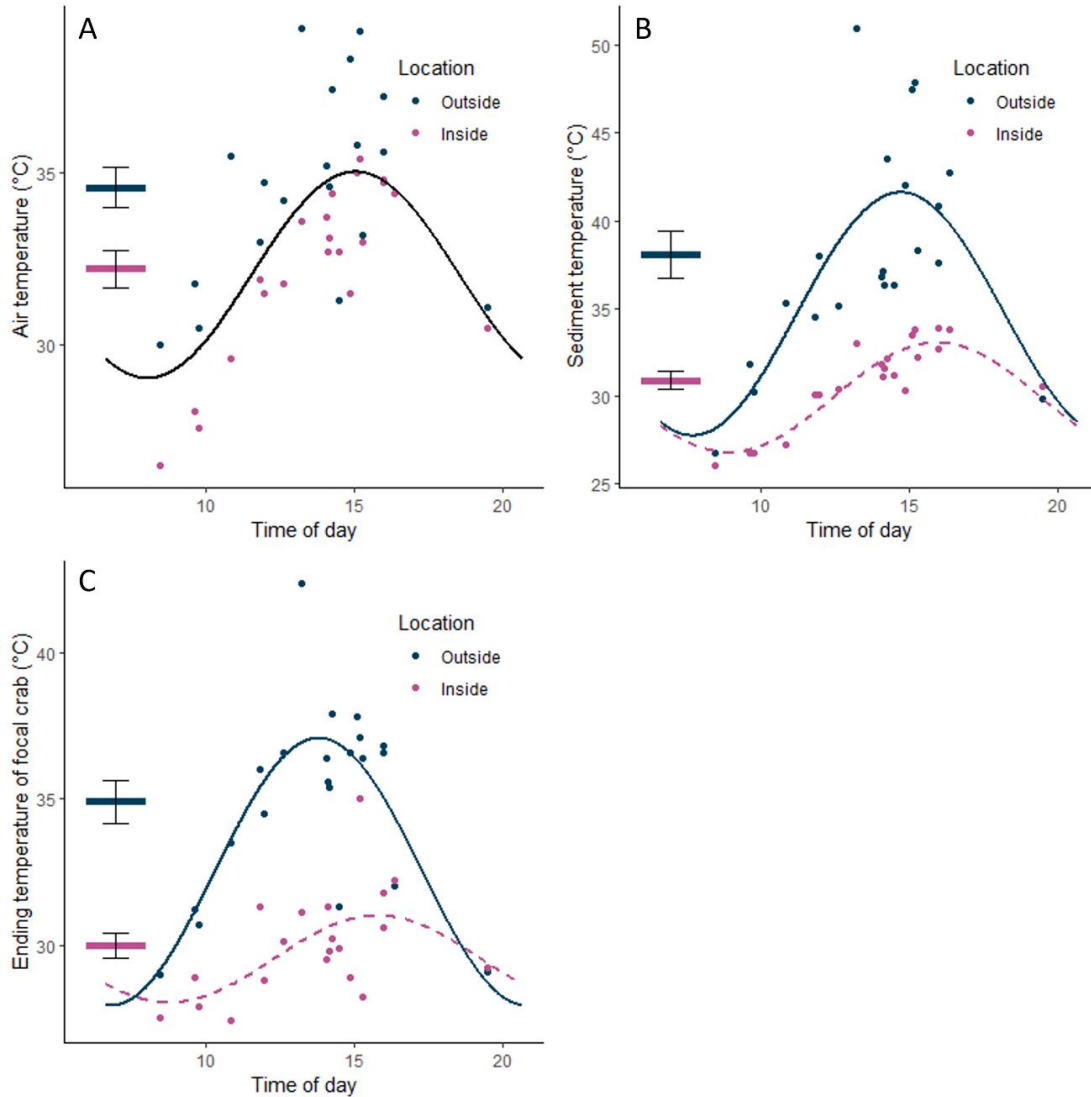
Figure 3.8 *Air temperatures inside and outside of the burrow throughout the day (A); Sediment temperatures inside and outside of the burrow throughout the day (B); Body temperatures of crabs recorded at the end of the ten-minute experimental period throughout the day (C)*

Air temperatures throughout the day (A), sediment temperature throughout the day (B), and body temperatures of crabs recorded at the end of the ten-minute experimental period throughout the day (C) are indicated with points (n = 42). All trend lines are significant (ANCOVAs, time of day main effects, all F ≥ 16.600, all p < 0.001; Table 3.7AI-II & BI) according to the univariate linear fixed effects models, and when two trend lines are present it is because they different significantly from one another (ANCOVAs; location: cosine of time of day interactions; Graph B, F = 5.960, p = 0.029' Graph C, F = 12.939, p = 0.002; Table 3.7AII & BI) . Color represents the location (i.e., inside or outside of the burrow) that each measurement was taken. Horizontal bars and error bars represent the mean temperature ± standard error in each location and are significantly (ANCOVA, site main effects, all F ≥26.039, all p < 0.001) different from one another in all cases according to the models (Table 3.7AI-II & BI).
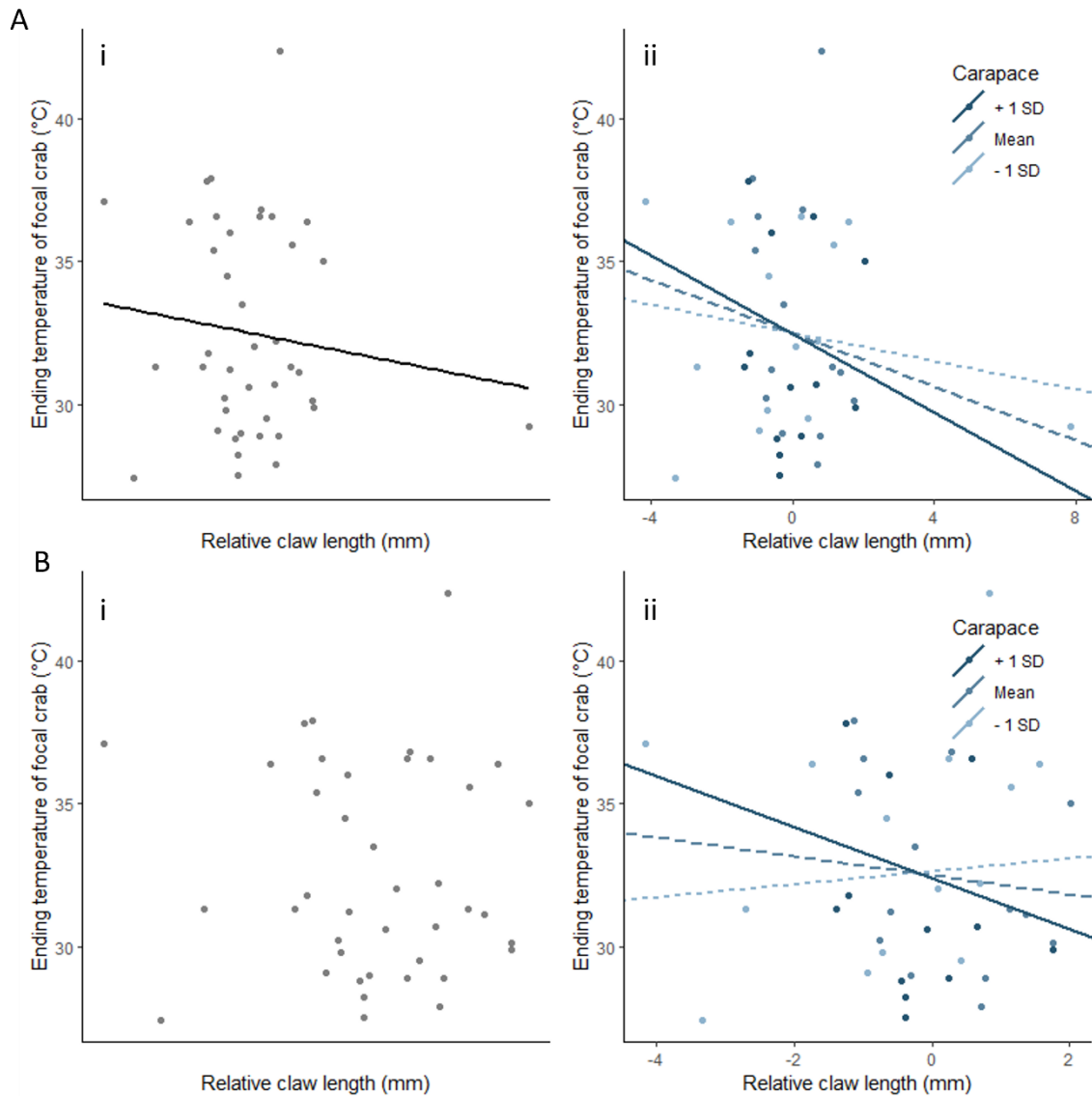
Figure 3.9 *Body temperatures recorded at the end of the ten-minute experimental period and relative claw lengths of all crabs (A); Body temperatures recorded at the end of the ten-minute experimental period and relative claw lengths excluding outlining sample (B)*

Body temperatures recorded at the end of the ten-minute experimental period and relative claw lengths of all crabs (n = 42) (A) and body temperatures recorded at the end of the ten-minute experimental period and relative claw lengths excluding outlying sample (n = 41) (B) are indicated with points. In AI, the trend line is significant (ANCOVA, F = 5.470, p = 0.032) according to the univariate linear fixed effects model (Table 3.7BI), whereas the lack of trend line in BI represents a lack of significance without the outlying sample (ANCOVA, F = 1.764, p = 0.203). In AII and BII, the trend lines represent the interaction between relative claw length and carapace width which is significant (ANCOVAs, Graph AII, F = 5.207, p = 0.036; Graph BII, F = 4.939, p = 0.041) according to the univariate linear fixed effects model both with and without the outlying sample.
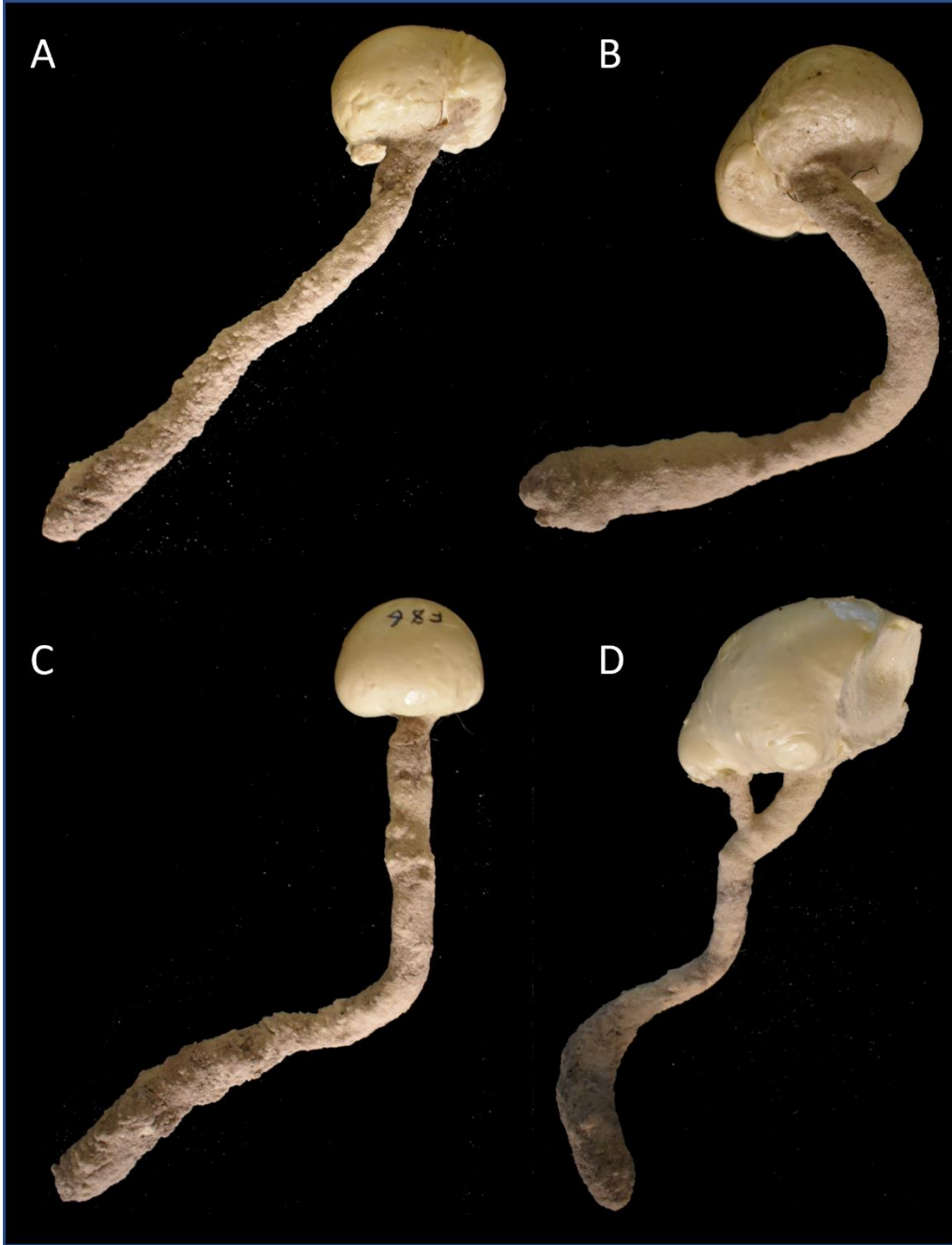
Figure 3.10 *Linear burrows (A); Spiral burrows (B); Angular burrows (C); Chaotic burrows (D)*

Casts are not to scale relative to one another. The "mushroom cap" is not a part of the burrow but is the foam overflow portion of the

cast mentioned in the text.

CHAPTER IV – RECOLONIZATION AND BURROW BUILDING IN *LEPTUCA*

PUGILATOR

4.1 Introduction

Leptuca pugilator is a burrow-mating fiddler crab species, and, as such, many

aspects of its biology revolve around a single resource, the mating burrow. Mating

burrows are relatively complex structures (see Chapter III) that extend deep into the

sandy sediments of *L. pugilator*'s vegetated beach habit. Mating burrows terminate at

depth with a mating chamber in which mating, oviposition, and incubation occur

(Christy, 1982). The cool, stable, subterranean environment of the mating chamber plays

a key role in female reproductive success by offering protection from predation

throughout the period between mating and larval release and by providing the proper

thermal and hydric conditions that allow females to time the release of their larvae to

coincide with nighttime spring high tides (Christy, 1983, 1982; Morgan and Christy,

1995; Reaney and Backwell, 2007; Salmon and Hyatt, 1983). Mating burrows thus

coincide as a key resource for courting males and brooding females alike.

In order to achieve the internal conditions required for successful female

reproduction, mating burrow are located in the highest portions of the intertidal zone

(Christy, 1982; Crane, 1975). This portion of *L. pugilator*'s habitat is dry, subject to

environmental temperature near and beyond the critical thermal maximum of the species,

and low in available nutrients (Allen and Levinton, 2014; Allen et al., 2012; Darnell et

al., 2013; Kim and Cho, 2003; Munguia et al., 2017). For burrow-owning males, who

hold their burrows for relatively long periods and who must endure the stressors of the

high intertidal zone in order to court, these stressors would likely be lethal if not for

access to their mating burrows. Their burrows provide them with refuge from the heat as well as access to water, making the burrow a valuable thermal and hydric resource in addition to their role in reproduction (Darnell et al., 2020, 2013; Levinton et al., 2015; Munguia et al., 2017; Smith and Miller, 1973).

Burrow ownership for males is temporary, usually lasting 12-24 hours in the population observed in the current study (see Chapter II). Burrow ownership typically ends following an instance of male-male communication in which a wondering male in search of territory approaches a resident, indicates to that resident that he is seeking burrow ownership, and the two interact with one another via visual displays and physical contact to reconcile the issue (Christy and Salmon, 1984; Hyatt and Salmon, 1978). Male interactions over territory are highly ritualized, typically flowing through a series of higher intensity modes starting with visual displays and then escalating from mutual contact using the outside of their major claws, to pushing against one another, to the interlocking of their major claws (Hyatt and Salmon, 1978). Occasionally, these interactions over territory end in violent behavior such as lifting or throwing, and males may potentially be injured in the process. The nature of the information communicated between males is not clear, and, although observations of prolonged and violent interactions between males have led many to hypothesize that the interactions are form of highly competitive and antagonistic combat, the fact remains that a majority of male interactions are short and terminate without much escalation (Hyatt and Salmon, 1978). Male-male interactions generally result in one of two outcomes. In most cases the resident will keep the burrow due to his ownership advantage (i.e., his option to retreat to his burrow and/or utilize it to his mechanical advantage in escalated interactions) (Fayed

et al., 2008), but in some smaller proportion of instances the wanderer will be granted ownership either by willing concession of the territory by the resident or by the forceful eviction of the resident by the wanderer.

The construction of a new burrow appears to be an infrequent means of gaining a mating site, but there is little information available in the literature about the energetic costs, time requirements, or frequency at which it occurs. It is widely accepted that burrow construction must happen from time to time, especially in the case of range expansion, but that the construction of new burrows is a less prevalent route of obtaining a burrow than is obtaining one through male interaction. This suggests that burrow construction is either significantly more costly in terms of time or energy investment that is burrow acquisition, or that some other factor, such as space limitation in the high intertidal mating area, reduces the effectiveness of this approach compared to the more common route of displacing resident males.

Here, I attempt to give baseline measurements of how long burrow construction takes to complete and how frequently burrow construction occurs when there is space available for new burrows within high quality mating areas. Additionally, I test the hypotheses that (A) given its apparent rarity, the time to construct a burrow will be greater than that required to acquire an existing burrow through interaction with a burrow-owning male, (B) burrow construction is likely to be more prevalent during peak mating periods of the lunar cycle, and (C) the physical disturbance caused by the incoming and outgoing tide will make burrow construction more prevalent during certain periods of the daily tidal cycle.

4.2 Materials and methods

4.2.1 Field site and environmental data

The study was conducted in Panacea, Florida on the vegetated, sandy shoreline of Porter Island (30.0156, -84.3695) from 15 June–24 July, 2021. The site is inhabited by a sizable population of *L. pugilator* with large, reproductively active males in the high intertidal waving at mate-searching females and protecting their mating territories from wandering males, and with non-reproductive males and females foraging together in the low intertidal during low tides. Tides at the study location are semi-diurnal, and all observations and experimental replicates were made or began within 3 hours of low tide during daylight hours. During the 40-day study period, there was a full moon on days 10 (24 June) and 40 (24 July) and a new moon on day 26 (10 July). The number of days since the previous syzygy event (i.e., days since the previous new or full moon) was recorded throughout the study period.

4.2.2 Establishment of recolonization plots

In order to record the rate at which *L. pugilator* constructs new mating burrows, plots of inhabited mating habitat where isolated, the resident crabs within the plots were removed and their burrows destroyed, and the empty plots (herein referred to as recolonization plots) were then observed as new residents moved in and constructed burrows. The recolonization plots were established in groups of three every seven days throughout the study period beginning on 15 June 2021, with a total of 15 plots being established in over five cohorts.

Establishment of a recolonization plot took three days. On the first day, a suitable 1-m x 1-m section of mating habitat was identified and surrounded with a 1-m high

plastic mesh (1/4" mesh size) fence that extended ~20 cm into the sediment. The top edge of the fence was lined on both sides with a strip of aluminum tape to ensure that no crabs could climb in. Once the fence was in place, an effort was made to remove all the crabs living inside of the encloser by hand. This effort necessarily disturbed the sediment such that all the burrows in the enclosure were completely destroyed. Throughout the rest of the day, the plot was occasionally observed and there was any sign that crabs remained within the enclosure following the initial removal effort, they were dug up and removed throughout the remainder of the first day. The enclosure was left overnight so that the empty plot was subject to tidal inundation like the rest of the mating habitat.

On the second day, the enclosure was disturbed only if there were signs that crabs remained within; these crabs were removed with as little disturbance of the sediment as possible. The enclosure was left overnight once more and was removed from the plot roughly three hours before the daytime low tide on the third day, producing an uninhabited and burrow-free plot of mating habitat surrounded on all sides by actively mating crabs.

4.2.3 Observation of recolonization and burrow building

Each plot was observed from a distance with binoculars a minimum of three times per day: once 2-3 hours before low tide, once within 30 minutes of low tide, and once more 2-3 hours after low tide. Each observation lasted roughly two minutes or less, and during this time the total number of *L. pugilator* and the number of actively waving *L. pugilator* in the plot were recorded. In some plots, *L. pugilator*'s sister species, *L. panacea*, were also present and their numbers were recorded in the same way.

After each observation, the plot under observation was slowly approached. When approached, the *L. pugilator* within the plot either ran away if they were simply wandering in the area or fled into their burrows if they were residents. This fear response was closely monitored during the approach, and all the individuals which fled to burrows situated within the plot were noted as plot residents. The burrows of these residents were then marked with a labeled dowel rod if they had not already been marked during a previous observation. The time and date that each burrow was initially observed was recorded after placing the labeled dowel rod. This time point acted as a rough estimate of when construction of the burrow had first begun. A burrow was considered to be fully constructed when its resident was observed courting at the burrow entrance. When this occurred, the time and date were recorded as a rough estimate of when construction of the burrow had ended. Observation of recolonization plots ceased once the density of resident crabs in the plot reflected that of the surrounding mating habitat.

4.2.4 Data analyses

To determine the population of residents within each plot, only the number of waving *L. pugilator* were considered, and the population of the plot at any given time was considered to be the maximum number of waving crabs that had been previously observed within the plot during a single observation. The result is a population estimate for each plot that began at zero on the first observation of the plot and increased over time until observations of the plot were concluded. This allows for the maximum population size and the number of days to reach maximum population size after the appearance of the first resident to be measured for each plot, and for the plot population growth rate per day, calculated as the quotient of these two metrics, to be calculated.

To test the effect of lunar day on population increase within the plots, the lunar day associated with each instance of population growth (i.e., days on which the population estimate of the plot increased) was recorded and a Rayleigh's test of circular uniformity was conducted using the lunar day of the growth events as circular temporal variable (lunar day range: 0–15; 0 on days of syzygy events). The impact of *L. panacea* presence was tested using a simple linear regression of maximum *L. pugilator* population size against maximum *L. panacea* population size.

The time required to construct each mating burrow could not be determined exactly due to the nature of these observations; the initiation and completion of burrow construction were not directly observed. To account for this, two estimates of burrow construction time were calculated: a minimum estimate and a maximum estimate. The minimum estimate of construction time was calculated as the difference in hours between when a burrow belonging to a resident *L. pugilator* was first observed and when the resident crab was seen courting outside its entrance. The maximum estimate was calculated as the difference in hours between the observation immediately prior to the first observation of the burrow and when the resident crab was seen courting outside its entrance. To test the effect of lunar day on burrow building, the lunar day associated with the completion of each burrow was recorded and a Rayleigh's test of circular uniformity was conducted using the lunar days as circular temporal variable. Additionally, a Komogorov-Smirnov test was conducted to determine if burrows were completed uniformly throughout the daily low tide period.

4.3 Results

Of the 15 plots, four were removed from analysis because no *L. pugilator* took up residence within their boundaries. Each of these four plots were observed through multiple syzygy events (mean = 20 days), and their lack of population growth is likely due to the localized destruction of the surrounding mating area following a massive deposition of seagrass wrack. On average, *L. pugilator* began to take up residence in the plots 2.36 ± 2.42 days (mean $\pm$ SD, n = 11) after their establishment, growing to a maximum population size of 4.73 ± 2.61 crabs per 1 m^2 plot over the course of 6.09 ± 4.98 days for an average population growth rate of 1.28 ± 0.87 crabs per day per m^2 (n = 11 plots). The maximum population size of *L. pugilator* was independent of maximum *L. panacea* population size (Linear regression: n = 11, F = 0.113; *P* = 0.745). Instances of population growth were not uniform throughout the lunar cycle (Rayleigh's test of circular uniformity; mean resultant length = 0.551; p-value < 0.001) but were instead centered 4.79 ± 2.78 days after syzygy events (n = 22 instances of population growth).

The time that is required to construct mating burrows in *L. pugilator* appears to be less than the standard amount of time between observation of the plots (~2.5 hours). This is made clear by the fact that a large majority burrows were initially observed with their residents already courting at their entrance (52 of 64 observed burrows), or, in other words, were entirely constructed between immediately sequential observations. Of the 52 observations which fall into this category, 31 of them were built between sequential observations taken during a single low tide and have maximum estimates of construction time that are less than 3 hours (2.52 ± 0.29 hrs) with the lowest maximum estimate of any burrow being 1.75 hours. The remaining 21 burrows that were built between immediately

116

sequential observations were built between the final observation of one day-time low-tide

and the first observation of the following day-time low-tide. This would seem to indicate

that these 21 burrows were likely constructed at night. Though a majority of burrows

were constructed between immediately sequential observations, some burrows were built

across several sequential observations (n = 12). These twelve burrows had a mean

minimum estimate of construction time equal to 42.06 ± 34.43 hr, with the largest

minimum estimate being 135 hours. Burrows were not constructed uniformly throughout

the lunar cycle (Rayleigh's test of circular uniformity; mean resultant length = 0.506; p-

value < 0.001) but were mostly constructed 3.72 ± 2.95 days after syzygy events (Fig. 1).

Also, burrows were not constructed uniformly throughout the day-time low tide

(Komogorov-Smimov test; D = 0.274; p-value < 0.001) with most burrows being

completed before the day-time maximum low-tide during tidal recession or potentially at

night (mean = -0.47 ± 1.38 hr relative to maximum low-tide) (Fig 2). It was uncommon

(n = 5) for burrows to be completed during the rising tide occurring 1–3 hours after

maximum low tide.

4.4 Discussion

      Mating burrows are a key reproductive resource for male and female *L. pugilator*.

These complex structures are owned by courting males and provide an appropriate

environment for females to successfully oviposit and incubate their young (Christy,

1982). Mating burrows are typically found in the high intertidal zone, and burrow-owning

males utilize their burrows as a refuge from the stressful high intertidal environment

(Crane, 1975; Darnell et al., 2020). Although courting males typically obtain existing

burrows as they are passed from one male to the next via male interaction, this method of

burrow acquisition has drawbacks in the form of high energetic costs, the danger of potential combat, and a relatively low success rate for incoming males (Allen and Levinton, 2007; Fayed et al., 2008; Hyatt and Salmon, 1978; Matsumasa and Murai, 2005). However, other avenues of burrow acquisition exist including the construction of new mating burrows. Though burrow construction simply must occur, it is infrequently studied or observed and very little information is available on the topic. In the current study, I have attempted to observe the phenomenon and test some hypotheses concerning it.

Although a precise estimate of burrow construction time was ultimately not possible given the nature and frequency of my observations, it is safe to say that a majority of burrows require less than 2.5 hrs to complete from start to finish. It is my opinion that the true mean construction time is likely less than this. The shortest recorded construction time was 1.75 hrs, and I believe that this single value is likely to be the closest to the true mean. Future studies of construction time should utilize video to make constant observations to produce a more accurate estimate. Regardless of sampling issues, this over-estimate produced by this study is much lower than what was expected at the onset of the project. It is surprising to me, given the importance of burrow ownership for males and the difficulties associated with obtain them through male interaction, that the total time investment required to construct a new burrow is only a portion of a single low tide. The finding that burrow construction typically occurs during the ebb and slack tide and rarely occurs during flood tide suggests that tidal inundation prohibits or interferes with the construction process.

The many observations recorded in the literature suggesting that burrow construction is rare in occurrence compared to other forms of burrow acquisition seem to be correct. Per square meter of experimentally cleared mating habitat, roughly one male per day chose to construct a burrow. The exact number of males attempting to acquire mating burrows via male interaction per square meter is not known for my field site, but it is significantly more than one per day. Despite being relatively rare, burrow construction was significantly more common during peak mating periods. This should not be surprising given the increased value of burrow ownership and increased male-male competition during these periods. During peak mating periods, burrow construction is more likely to have a reproductive payoff than during mating lulls.

The hypothesis that burrow construction is rare because it requires a greater investment of time or energy than does burrow acquisition via male interaction has mixed support in the current study. On one hand, a burrow-searching male can potentially secure a burrow after interacting with only a single burrow-owning male. This single interaction could take only a few seconds to complete, allowing the burrow-searching male to potentially secure burrow ownership in a matter of moments. Compared to an example such as this, burrow construction requires an extensive amount of time and effort. However, burrow acquisition via male interaction is often far more complicated and energetically demanding than what has been described above, and it is likely that is some cases more time is lost in searching for a burrow than would be lost in constructing a new one. The rarity of burrow construction may be some form of bet-hedging in which males opt into the uncertain but potentially extremely low-cost behavior of searching for burrows rather than endeavoring to undergo the more certain but higher cost behavior of

burrow construction. It may be a better strategy over a lifetime to frequently make relatively quick and low energy attempts to obtain an existing burrow on the off-chance of success, retreating to the low intertidal to forage after a failure, than to commit the time and effort required to construct a burrow even though ownership would be ensured for a time following construction. In this way, males would be able to maximize their available energy stores and to commit more of their energy directly toward growth and reproductive activities. Size has been shown to be important in male mating success for *L. pugilator* (Christy, 1983; Hyatt, 1977; Jennions and Backwell, 1996; Pratt and Mclain, 2002) so the energy lost to burrow construction over a lifetime would likely be better spent in obtaining a larger size. Perhaps a better hypothesis is that burrow construction is rare because the energy spent constructing a burrow does not directly benefit reproductive success or growth. Another alternative hypothesis is that burrow construction is rare because newly made burrows are physically inferior is some way to burrow which have existed for period of time.

In conclusion, mating burrow construction in *L. pugilator* is a relatively short, energetically expensive, and infrequently utilized behavior in areas of existing mating habitat. However, there is much more to be learned. The change in frequency of burrow construction over the lunar cycle and the avoidance of construction during flood tide indicates that there are times when this behavior is more preferable than at other times, which in turn may indicate that there are circumstances under which burrow construction may be advantageous. Additionally, the short-term permanence of mating burrow (i.e., their tendency to persist through the mating period and to be handed from one male to the next) makes their construction of particular interest. While a male which obtains an

existing burrow will benefit only himself, a male which constructs a burrow benefits males beyond himself by adding a mating territory to the existing number to be passed down. It may be that burrow construction results when there is space to allow a larger proportion of the population to mate. Finally, the limited number of males which construct matting burrows makes raises the question of whether or not these males are different in some way from males that do not construct burrows. It might be expected that male who are less likely to obtain a burrow via male interaction, such as small males or males with regenerated claws, might be more likely to construct burrows. When burrow construction is most advantageous, how males who construct burrow are similar to each other and different from other crabs, and how the construction of burrows effects the competitive dynamics of the mating area are all topics of interest for future studies.
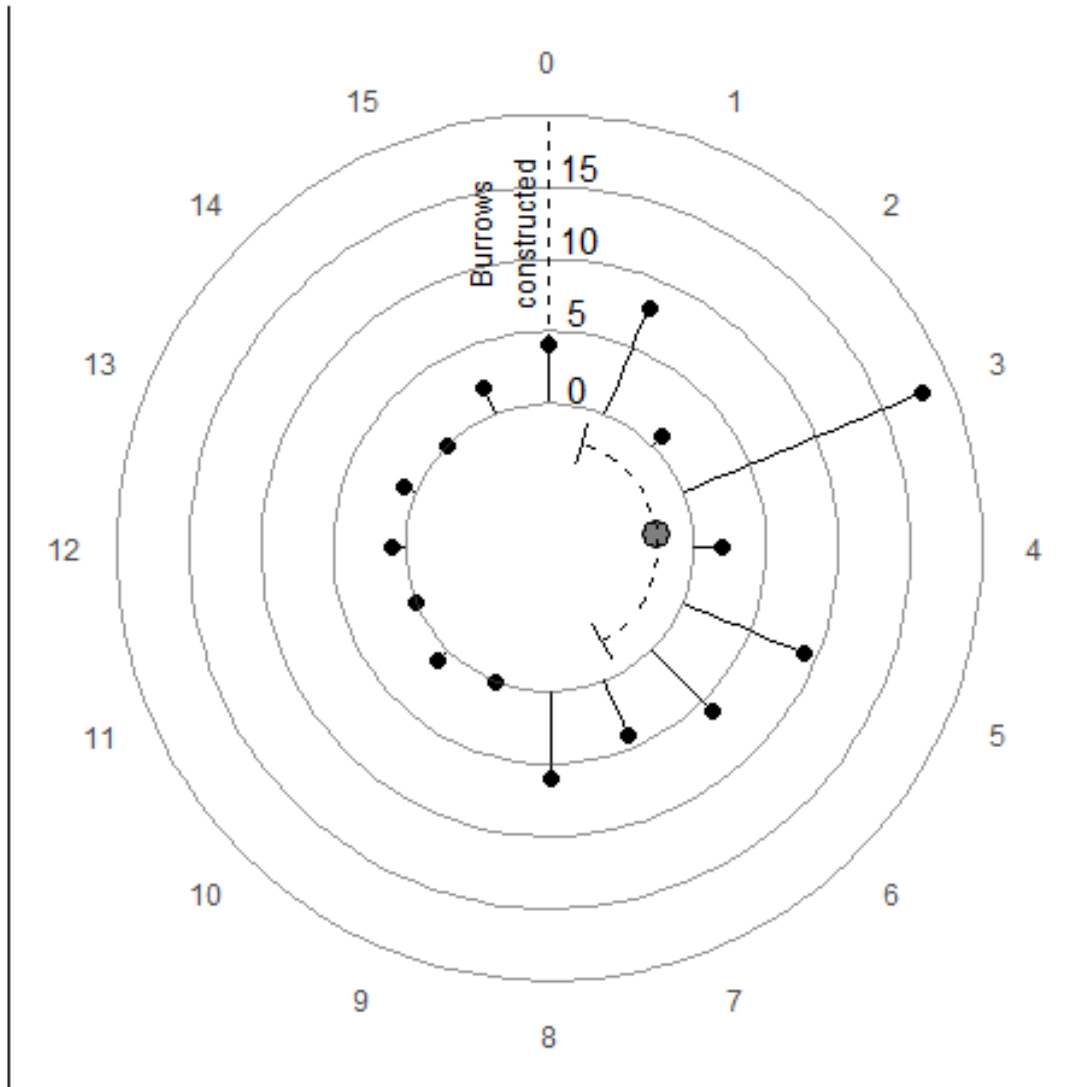
Figure 4.1 *Circular histogram of burrow construction throughout the lunar cycle*

A circular histogram of burrow construction throughout the lunar cycle. The radial axis shows the number of burrows who's construction was completed on each day throughout the semilunar cycle. The polar axis shows the number of days since the precious syzygy event. The black lines and points should be interpreted much like the bars of a standard histogram, with their height representing the number of observations made within each lunar day. The grey dot and curved error bar in the middle of the plot shows the circular mean ± standard deviation. The distribution is not-uniform according to Rayleigh's test of circular uniformity (mean resultant length = 0.506; p-value < 0.001)
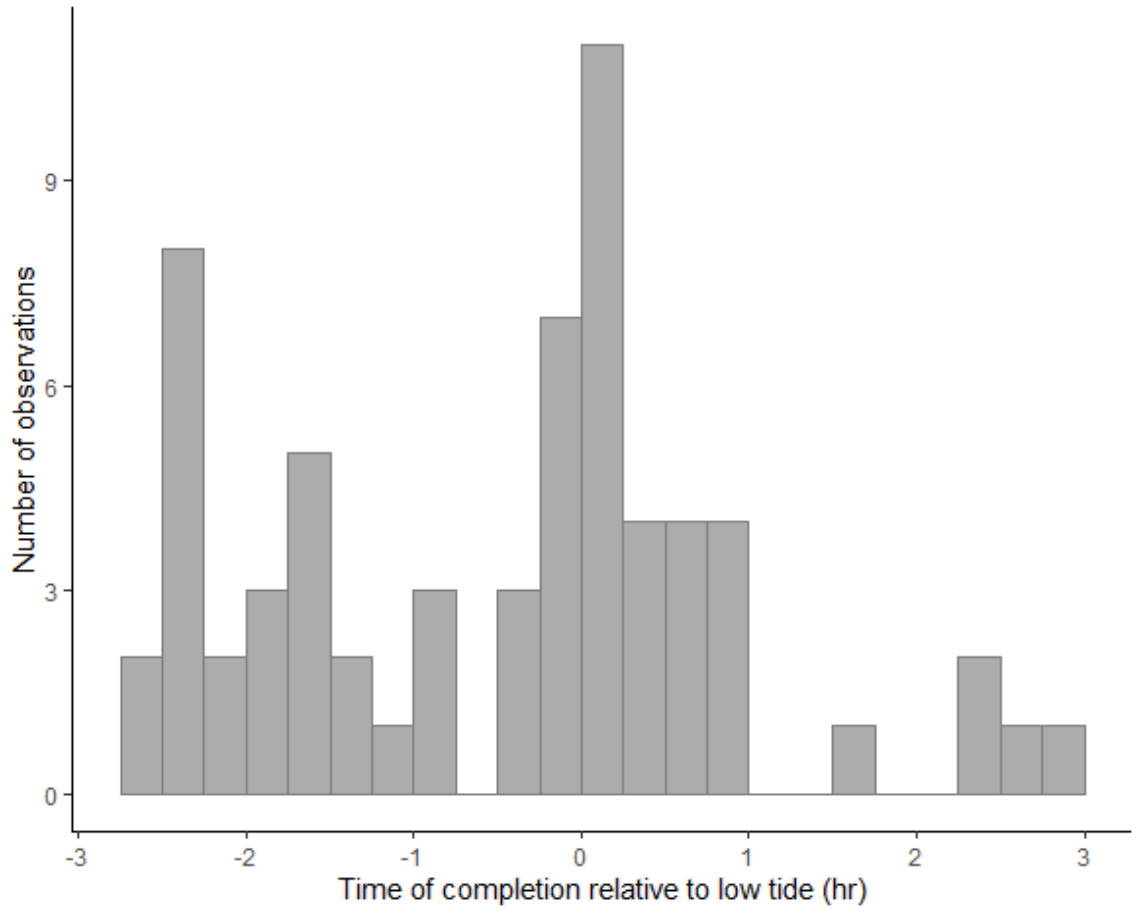
Figure 4.2 *Histogram of burrow construction throughout the period surrounding day-time low tide*

A histogram of burrow construction relative to maximum low tide throughout the period surrounding the day-time low tide. Bar height indicates the number of observations with observations being binned in 15 minute increments.

CHAPTER V – PATERNITY ANALYSIS OF COHABITATING CRABS TO ASSESS

THE POTENTIAL OF COOPERATIVE BREEDING IN *LEPTUCA PUGILATOR*

5.1 Introduction

Dugatkin et al. (1998) describes cooperation between organisms by saying that

"cooperation is an outcome that – despite potential costs to individuals – is 'good'

(measured by some appropriate fitness measure) for the members of a group of two or

more individuals and whose achievement requires some sort of collective action".

Cooperation can be achieved in several different ways. One such way is reciprocity, in

which one individual act cooperatively with another for as long as they, too, act

cooperatively (Fischer, 1984; Hart and Hart, 1992; Mooring and Hart, 1995). Another

way cooperation can occur is through group selection which describes a scenario under

which individuals may band together to increase their competitive fitness against other

such groups during confrontations, such in chimpanzee raids and pleometrosis, thus

creating cooperation within each group (Goodall, 1986; Rissing et al., 1989). In both

reciprocity and group selection, there exists the opportunity for one or more members

acting in cooperation to cheat, thus enhancing their fitness compared to their fellow

cooperators, and the potential of beneficial cheating within cooperative groups is

commonly referred to as the prisoner's dilemma (Axelrod and Hamilton, 1981; Trivers,

1971). However, there exists a third cooperative mode, known as by-product mutualism,

in which cheating has no benefit or may even be detrimental (Brown, 1983; Dugatkin and

Reeve, 1998). Under by-product mutualism, individuals may benefit one another as a by-

product of behaving strictly in their own self-interest (Clements and Stephens, 1995;

Davies and Houston, 1981). By-product mutualism has been shown to exist when

individuals face a "common enemy" such as harsh environmental conditions (De Jaegher and Hoyer, 2016; Mesterton-Gibbons and Dugatkin, 1992).

The Atlantic sand fiddler crab, *Leptuca pugilator*, is found living intertidally on sandy, vegetated beaches along the Atlantic and Gulf of Mexico coasts of the USA (Crane, 1975). Like all fiddler crabs, males of this species have a single, enlarged major claw that they use in waving courtship displays to attract females and in territorial disputes with other males (Crane, 1975). *Leptuca pugilator* is active during the period around each daily low tide and feeds on the organic matter of wet sediment found at the water's edge (Crane, 1975; Reinsel and Rittschof, 1995; Robertson et al., 1980). However, in order to mate, males must acquire and hold mating territory, centered on a specialize mating burrow, located in the highest reaches of the high intertidal zone away from their foraging grounds (Christy, 1982; Salmon and Hyatt, 1983).

To obtain a mating territory, foraging males must leave the low intertidal to wander through the high intertidal mating area in search of a roughly sized-matched territory resident from whom he might acquire a mating burrow (Hyatt and Salmon, 1978; Jennions and Backwell, 1996; Morrell et al., 2005). This is typically accomplished through ritualized male-male interactions in which the wandering male will approach a territory resident, visually indicate to him that he would like to take the territory, and then, if the resident engages, the two will proceed through a series of escalating behaviors (Hyatt and Salmon, 1978). These behaviors typically begin with visual signaling from both parties and then proceed through a standard series of physical interaction that intensify from mutual touching of their major claw, to pushing, to interlocking their major claws, and occasionally to violent acts such as lifting and throwing (Hyatt and

Salmon, 1978). Although violence between males does occur, most territorial disputes end quickly and with little escalation, and when violence does occur it is often performed by the resident rather than the intruder (Hyatt and Salmon, 1978). In territorial disputes, the resident, who can defensively retreat into his mating burrow or use the burrow entrance for leverage, is at a distinct advantage, winning the dispute far more often than not (Fayed et al., 2008; Hyatt and Salmon, 1978; Jennions and Backwell, 1996). However, on occasion the intruder will be granted the territory, and although this may occur following the violent and forceful eviction of the previous resident, the territory is often conceded with little to no confrontation.

Once in possession of a mating burrow, males must attract a female to their territory by performing a species-specific waving display with their major claw (Crane, 1975; How et al., 2009; Pope, 2000). Following successful attraction, the female will investigate the quality of the burrow (Backwell and Passmore, 1996; deRivera, 2005; Reaney and Backwell, 2007), and, if it is sufficient, the two will descend to the burrow's terminal chamber to mate (Christy, 1983, 1982; Salmon and Hyatt, 1983). After copulation, the male will return to the surface to attract additional mates or return to the low-intertidal foraging grounds once his burrow is taken over by an intruder. Meanwhile, the female will remain below ground to oviposit and incubate her eggs over the course of roughly 14 days, after which she will emerge during a large amplitude night-time high tide to release her larvae and return to the low intertidal to forage (Christy, 1978; Morgan and Christy, 1995).

Territory ownership is physiologically stressful for males. While in the high-intertidal mating area, resident males are simultaneously faced with low available

126

nutrients (Allen and Levinton, 2014; Kim and Cho, 2003) and energetically costly

behaviors in the form of both courtship displays (Matsumasa and Murai, 2005) and male-

male interactions (Allen and Levinton, 2007; Levinton and Allen, 2005), leading to

nutrient limitation (i.e., starvation) (Allen and Levinton, 2014). Additionally, the extreme

temperatures of the high intertidal are often near to or in excess of *L. pugilator*'s critical

thermal maximum (Allen and Levinton, 2014; Allen et al., 2012; Darnell et al., 2013;

Munguia et al., 2017), and although resident can take refuge from the heat within their

burrows (Darnell et al., 2020; Smith and Miller, 1973), doing so costs them potential

mating opportunities (Darnell et al., 2020; Reaney, 2007). The reproductive cost of

refuge use encourages males to repeatedly push their thermal limits before retreating

from the heat (Darnell et al., 2020). At some point, usually after less than 24 hr and rarely

beyond 2-3 days (See Chapter II) (Christy, 1982; Salmon and Hyatt, 1983), the effects of

nutrient limitation and heat stress weaken territory-holding males to the point that they

can no longer defend their burrows and they are either evicted or willingly

concede/abandon the territory to return to the low-intertidal foraging grounds.

It has been suggested that males play an important role in female reproductive

success post-copulation by maintaining the structure and integrity of their mating

burrows which, in turn, protects the resident females from the egg loss that would occur

if the burrow were to collapse (Christy, 1983, 1982). However, the discrepancy between

male and female residence time (roughly 24 hours in males and 14 days in females)

would seem to indicate that few males, if any, are physiologically capable of maintaining

their burrows for long enough to effectively protect their own offspring from this fate.

Interestingly, mating burrows are inhabited by courting males continuously, even during

mating lulls, and intruding crabs have not been observed evicting resident females after acquiring a burrow. It may then be that male *L. pugilator* participate in a system of cooperative breeding fueled by by-product mutualism in the face of their "common enemy", extreme environmental conditions. In this scenario, all parties act selfishly and all parties benefit. Physiologically exhausted resident males are given respite from the stressors of the high intertidal zone with assurance that the intruding male will maintain the burrow which contains his offspring for their own reproductive benefit.

Here I attempt to take the first steps into testing this hypothesis of indirect benefit by determining the paternity status of resident males over the broods of the females they cohabitate with. If a male deserts the mating burrow in 1-2 days, the male occupying the burrow at the time of female emergence and larval release cannot be the father of the offspring of the emerging female. If territory owning males frequently lack paternity over the broods of cohabitating females, it will lend initial support to this hypothesis of sequential maintence of mating burrows. If not, and paternity is common, then cooperative breeding in this species is unlikely and our understanding of their mating system flawed.

5.2 Materials and methods

5.2.1 Sample collection

To assess the paternity status of resident males over the offspring of cohabitating ovigerous females, the mating burrows of actively courting males from a single population in Panacea, FL were carefully excavated. Courting males were first observed and selected from a distance and then slowly approached until they retreated into their burrows. Once they had entered the burrow and the burrow could be identified, the

128

burrow was excavated. Excavation was accomplished by hand. By inserting a finger into the burrow entrance, scraping away the sediment surface beneath the base of the inserted hand, and then probing further into the burrow as necessary, it was possible to isolate the burrow from those surrounding it and prevent the escape of the resident crabs until the mating chamber had been accessed. Once the burrow had been excavated all the way to the mating chamber, the inserted digit could be removed and the resident crabs were easily collected as they fled from the now-exposed chamber. In cases where the male was residing alone or with a non-ovigerous female(s), the crabs were released. In cases where the male was residing with an ovigerous female(s), the resident crabs were all collected alive, placed in separate, labelled holding containers with roughly 0.5 cm of local seawater.

Following collection, the crabs were returned to the lab where the males were immediately sacrificed via freezing, dismantled (i.e., legs, claws, and cephalothorax removed from abdomen) to enhance preservation, preserved in 99% ethanol in a 20 mL glass vial, and labeled to reference the females they were found cohabitating with. The females were each kept alive in separate small holding containers with roughly 0.5 cm of local seawater. The females were held and monitored until they released their larvae (usually after 1-6 days in captivity). Throughout this period, they were kept within a screen porch near the study site, the water in their containers was completely replaced once per day, and they were fed a small amount of TetraMin® Tropical Flake fish food (Tetra Inc., Blacksberg, VA, USA) once daily. Once they had released their larvae, the females were removed their container and sacrificed/preserved/labeled in the same way as the males. The larvae left in the container were then strained from the seawater, rinsed

from the strainer into a 20 mL glass vial, preserved in 99% ethanol, and labeled to reference the female that had produced them. The ethanol used to preserve each individually contained specimen was replaced regularly until the time of DNA extraction.

5.2.2 Library preparation and sequencing

Genomic DNA of individual adult crabs was extracted using NucleoSpin® Insect DNA extraction kits (Takara Bio Inc., Kusatsu, Shiga, Japan) which utilize physical lysis via steel bead disruption and DNA isolation via mini spin columns with silica-membrane technology. Each larval brood was subsampled 10 times, combining 10 larvae per subsample. This was done, in part, because the DNA of a single larva was of insufficient quality for further preparation but also to increase the number of larvae per brood that could be included in the paternity analysis. Genomic DNA of larval samples was extracted using Mag-Bind® Blood & Tissue DNA extraction kits (Omega Bio-Tek Inc., Norcross, GA, USA) which utilize chemical lysis via enzymatic digestion and DNA isolation via reversible nucleic acid-binding magnetic particles. DNA concentration for each sample was determined using an Invitrogen^{tm} Quibit^{tm} 4 fluorometer (ThermoFisher Scientific Inc., Waltham, MA, USA) and the quality of the extracted DNA was evaluated using agarose electrophoresis. Each sample was diluted so that concentrations were no higher than 50 ng/μl, and final concentrations ranged from 37.8 – 50.0 ng/μl in adult samples and from 22.9–50.0 ng/μl in larval samples. Double-digest RAD-Tag (ddRAD) sequencing libraries were prepared from each sample using protocols modified from Baird et al. (2008) and Peterson et al. (2012). Each library was created from 0.298-0.65 μg of genomic DNA and were simultaneously digested with restriction enzymes Sau3AI (3.75 units; New England Biolabs Inc., Ipswitch, MA, USA) and SPEI (7.5 units; New

England Biolabs Inc.) for 1.5 hr at 37°C. The process was then inactivated for 15 min at 65°C and the digested fragments were ligated to sample-specific Illumina adapters for 30 min at 16°C using T4 Ligase (400 units; New England Biolabs Inc.). Each adapter pair included a unique 6 bp barcode in order to identify sequencing reads that originated from specific samples as well as a degenerate 8 bp unique molecular identifier (UMI) in order to remove PCR duplicates during sequence read processing (Schweyen et al., 2014). Ligation was terminated through 5-fold dilution with ddH20, and the products of the ligation were purified and size selected (minimum 250 bp) using Agencourt AMPure XP magnetic beads (Beckman Coulter Inc., Brea, CA, USA). The beads were applied at a 0.65x ratio and unbound DNA was removed via a double wash using 70% ethanol. This cleansed DNA product was amplified via PCR in 25μl reactions with 5 pmol of forward and reverse primers and 12.5 μl of 2X Master Mix (New England Biolabs Inc.) containing Taq Polymerase, dNTPs, MgCl2, and 2X buffer. The thermocycle was programed to undergo initial denaturation (3 min at 95°C; 1 cycle); 30 cycles of denaturation at 30 s at 95°C, annealing for 30 s at 55°C, elongation 30 s at 72°C; and a final extension for 7 min at 72°C. The pooled library was cleansed using Agencourt AMPure XP magnetic beads (Beckman Coulter Inc.) as described above and size selected (300-500 bp) using a PippinPrep 2% agarose gel cartridge (Sage Science Inc. Beverly, MA, USA). The final pooled library was cleaned one last time with Agencourt AMPure XP magnetic beads (Beckman Coulter Inc.) before it was submitted to the University of Colorado, Denver School of Medicine (Aurora, CO, USA) for 2x125 paired-end sequencing on the Illumina HiSeq2000 platform.

5.2.3 Bioinformatics analysis of sequencing data and SNP recovery

Illumina reads were initially quality-checked to reduce base-calling errors by removing portions with Phred-scores lower than 30. The remaining sequences were then filtered to exclude sequences with forward or reverse reads that were shorter than 75 bp. The sequence data were then screened for PCR duplicates using the UMI portion of the adapters ligated in the previous section. PCR duplicates negatively impact down-stream sequencing by artificially inflating confidence and homozygosity (Andrews et al., 2014), and one of the duplicates from each pair was therefore removed.

Quality-checked Illumina reads were processed using the dDocent pipeline (Puritz et al., 2014). In order to find the optimal dDocent parameters, test assemblies were created using K1 values (within-individual coverage) ranging from 2-6, K2 values (among-individual coverage) ranging from 2-9, and C values (cluster similarity for merging) ranging from 0.8 – 0.95. The final parameters used were as follows: K1 = 2, K2 = 2, C = 0.95. This process involves read assembly using RAINBOW to create reference sequence clusters (Chong et al., 2012) and cluster merging to create a assembly of unique contiguous sequences (contigs) using CD-HIT (Fu et al., 2012; Li and Godzik, 2006). The resulting *de novo* reference assembly, created using all adult and larval samples, was comprised of 406,246 sequences mapped to 9,861 contigs containing 307,760 allelic sites such as Single Nucleotide Polymorphisms (SNPs), insertion/deletions (INDELS), Multi-nucleotide Polymorphisms (MNPs), and other complex events (e.g., composite insertions and substitutions) identified using FREEBAYES (Garrison and Marth, 2012) using the parameters A = 1, B = 3, O = 5. Multi nucleotide variants were decomposed in allelic primitives resulting in 90,414 sites being retained for further evaluation.

The remaining allelic sites were filtered using VCFtools (Danecek et al., 2011) and dDocent filters. Filtering began by removing INDELs and limiting the remaining SNP sites to those that were bi-allelic. Sites that were present in less than 50% of samples were then removed as were sites with minor allele counts less than 3 and quality scores less than 30. The dataset was further filtered so that only SNPs with within-individual read depth greater than or equal to 10 and those with average read depths greater than or equal to 15 were kept. Sites that were mapped to both forward and reverse reads were removed as were sites showing excessively high or low depth. The thresholds for allelic balance at heterozygous loci was set to 10%, as opposed to the default of 25%, to account for the fact that larval samples were pooled. The SNPs of the remaining samples were limited to only those present in at least 95% of samples and those that were in Hardy-Weinberg equilibrium.

5.2.4 Paternity analysis

Paternity analyses were completed using Cervus (Kalinowski et al., 2007; Marshall et al., 1998) which utilizes a likelihood ratio approach to assign offspring samples to parents. Simulated parent/offspring trios and unrelated trios are used to determine threshold LOD (Logarithm of the Odds; i.e., likelihood) scores and comparing the calculated score to a threshold score determined by the simulation (Slate et al., 2000). The likelihood approach used by Cervus accounts for potential genotyping errors when determining parentage which reduces the impact that such errors might have when comparing many loci, such as when using SNPs. Initially a maternity analysis was conducted between the collected females and their associate larval subsample to ensure efficacy of the analysis. The paternity analysis was conducted both without the true

mother included to ensure a conservative approach and maintain comparability between tests (including the true mother decreases the number of alleles that could logically come from any potential father). Father-offspring relationships were simulated along with unrelated duos for 10,000 simulations. The genotyping success rate was set at an estimate average of 90% and an estimated minimum of 80% and the genotyping error rate was set at an estimated 1%. The simulated duos were given 100 genotypes for simulation.

5.3 Results

A total of twelve burrows containing ovigerous females were successfully excavated. Of these, nine contained a single male and single ovigerous female, two contained a single male and two ovigerous females, and one contained two males and three ovigerous females. In this last case, it is likely that a second burrow was accidentally breached during excavation or that a wandering male fled into the burrow as I approach, but regardless, I tested paternity within the breached burrow across all three broods and using both males as potential fathers so the impact of inferred breach should be immaterial in testing the hypothesis.

However, SNP filtering resulted in the loss of several low-quality parental and larval genomes for final analysis (4/16 female and 12/160 larval genome lost). In the end, paternity was tested in all 12 burrows. In instances where the genome of the mother was retained, she was confidently and accurately assigned as the mother of all of her associated larval samples except for one mother who was only assigned in 8/10 larval samples (Table 5.1; Burrow 12, ♀3). In six of the twelve burrows, the resident male completely lacked paternity over all larval samples taken from the broods of cohabiting females (Table 5.1; Burrows 2, 3, 4, 5, 10, and 11). In those six burrows with residents

whose paternity was supported (Table 5.1; Burrows 1, 6, 7, 8, 9, and 12), none appeared to have full paternity over the brood with the highest paternity assignment being 8/10 larval samples assigned. In the case of the potentially breached burrow mentioned above a single male had partial paternity over all three broods while the other male had zero paternity over all three broods (Table 5.1; Burrow 12, ♂1 has partial paternity, ♂2 has no paternity).

5.4 Conclusions

By-product mutualism is a cooperative mode in which unrelated individuals act collectively to achieve mutually increased individual fitness, compared to their fitness in isolation, by acting strictly according to their own self-interests (Clements and Stephens, 1995; Davies and Houston, 1981; Dugatkin and Reeve, 1998). This form of cooperation is particularly impervious to cheating (Brown, 1983) and seems to appear when individuals share a "common enemy" such as harsh environmental conditions (De Jaegher and Hoyer, 2016; Mesterton-Gibbons and Dugatkin, 1992).

In order to protect the reproductive success of females with whom they have mated, and therefore their own mating success, male *L. pugilator* must maintain the structural integrity of the mating burrow in which they and their mate reside (Christy, 1983, 1982). However, burrow owning males must endure the low nutrient content (Allen and Levinton, 2014), extreme temperatures (Allen and Levinton, 2014; Darnell et al., 2013; Munguia et al., 2017), and high energetic cost (Allen and Levinton, 2007; Levinton and Allen, 2005; Matsumasa and Murai, 2005) associated with the high intertidal mating environment in order to continue to hold ownership of the mating burrow containing their offspring. These stressor typically results in a relatively shorter male tenure (1-3 days)

135

than female tenure (~14 days) at the mating burrow (Christy, 1982, 1978; Salmon and Hyatt, 1983) (see Chapter 2). The male's inability to maintain the structure of the mating burrow for the entirety of his mate's incubation period might otherwise drastically reduce the fitness of both individuals if it weren't for the fact that a new resident male will take over burrow maintenance duties in his stead, most likely at the very moment the original owner can no longer do so.

This is selfish on the part of the new resident who needs the burrow for his own reproductive success and who, in some cases, may have forcefully evicted the previous resident himself, but regardless of how ownership is obtained, the new resident effectively protects the offspring of the previous owner all while acting in accordance with his own self-interests. The original owner, too, seems to act selfishly in many cases by handing over the territory and abandoning his young in favor of replenishing his own energy stores by foraging in the low-intertidal. In the absence of competitors to concede territory ownership, resident males typically abandon their burrows after an amount of time that is indistinguishable from their residence time under competition (see Chapter 2), and although male disputes over territory can be violent and prolonged, they are more frequently relatively passive and short with residents relinquishing territory ownership despite the lack of a fight and their well-documented ownership advantage (Fayed et al., 2008; Hyatt and Salmon, 1978) (see Chapter II). Therefore, the original resident may also act in his own self-interest while granting the new resident access to mating opportunities that would otherwise be beyond reach. It may then be possible that male *L. pugilator* participate in what is effectively cooperative breeding via by product mutualism to

overcome the harsh environmental condition that would, in all likelihood, drastically reduce the fitness of a male functioning in isolation.

The results of the current study lend credence to this hypothesis by demonstrating two things: (A) that males maintain mating burrows which contain cohabitating females frequently enough that encountering cohabitation in the wild is not rare (but see Christy, 1982 which suggests that males isolate females in distinct underground chambers that are separate from the mating burrows post-copulation), and (B) that males infrequently have paternity over the broods of cohabitating females and, when they do, paternity appears to be partial. There is no doubt that this behavior is utterly bereft of altruism, but the results of the current study demonstrate that, once in control of a territory, males promote the reproductive success of cohabitating females, and thus those females' mates, through continued burrow maintenance regardless of their own paternity status (Christy, 1982; Reaney et al., 2012).

The presence of partial paternity in *L. pugilator* is unexpected. Given the previous literature on mate-guarding and lack of surface mating in this species, it was expected that paternity would be nearly all-or-nothing, but the current results indicate that full-paternity may be exceedingly rare. The partial paternity reported here must then indicate one or more of three potentialities: (A) males do not mate guard as strenuously as suggested in previous literature, thus allowing new residents to acquire partial paternity when they obtain a burrow containing a female that has not yet oviposited, (B) that surface mating occurs leading to partial paternity for those males that actually attract a female to their burrow, or (C) that the paternity analysis conducted resulted in false positive paternity assignments. The expected incubation period for females in this species

137

is roughly 14 days, and given the fact that females were typically only held in captivity for 1-6 days following collection it seems likely that most of the collected females had already been incubating their eggs for some not-insubstantial amount of time before collection, though this does not account for the increase temperatures and subsequently shortened incubation periods that the female may have faced in captivity. If it is the case that the collected females had been incubating their larvae for a substantial period before collection, then when considered alongside the short residence times of male recorded in Chapter 2, it would seem that false positive paternity assignment is the likely culprit for the observed partial paternity. The likelihood of this being the case is further supported by the fact that the analysis used to properly account for pooled larval samples required the utilization of less strict paternity assignment thresholds, thus increasing the likelihood of false positive paternity. While an assessment of mate guarding and surface mating was never the intent of this study, neither of these possible reasons for the observed partial paternity can be absolutely ruled out. The previous observations regarding the absence of surface mating and strict mate gurading in *L. pugilator* are largely indirect, and future studies regarding these reproductive strategies are likely warranted regardless of the current results. However, in regard to the current hypothesis, the relaxed paternity thresholds used in the current analysis only serve to strengthen the 50% of observed burrows in which paternity was utterly lacking, and do not take away from the finding that males can be found maintaining the burrows of cohabitating female whose broods they lack paternity over.

This does not so much prove that males partake in by-product mutualism as much as it leaves the door open on the hypothesis by not refuting it. More work on this topic is

needed, particularly in regard to the role of the original resident, the exact frequency at which burrow abandonment and willing concession occurs, and the physiological state of residents at the time of burrow abandonment and concession. Perhaps even more important are investigations of female reproductive success when no male resident is present to maintain the burrow. Unfortunately, the reliance of females on male burrow maintenance remains, to my knowledge, a hypothesis based on indirect observations and assumptions (Christy, 1983). If it could be shown empirically that residents benefit in some way when leaving their burrows and that females require male burrow maintenance to reproduce successfully, then the hypothesize by-product mutualism might be demonstrated nearly beyond doubt.

Table 5.1 *Results of paternity analysis*

Burrow	Adult ID	Adult loci	Larvae Assn.	1	2	3	4	5	6	7	8	9	10
1	♂1	387	2/10	-32.92 (393)	-30.17 (393)	-18.77 (393)	**-7.71 (392)**	**-9.31 (384)**	-21.67 (393)	-22.54 (393)	-15.99 (393)	-30.96 (364)	-33.75 (389)
♂ x ♀	♀1	393	10/10	**19.01 (387)**	**25.44 (387)**	**31.01 (387)**	**18.13 (386)**	**24.39 (378)**	**19.86 (387)**	**19.73 (387)**	**28.89 (387)**	**25.06 (358)**	**12.97 (383)**
2	♂1	385	0/10	-128.02 (349)	-114.08 (335)	-128.87 (347)	-132.61 (347)	-127.57 (346)	-143.50 (346)	-125.55 (347)	-130.34 (348)	-120.47 (337)	-131.61 (343)
♂ x ♀	♀1	348	10/10	**216.56 (347)**	**200.07 (334)**	**215.66 (345)**	**212.60 (345)**	**215.44 (345)**	**196.26 (344)**	**222.12 (346)**	**215.05 (346)**	**191.70 (336)**	**201.01 (341)**
3	♂1	378	0/10	-27.66 (378)	-27.78 (378)	-26.42 (378)	-21.71 (378)	-20.19 (378)	-26.57 (378)	-21.94 (378)	-19.69 (378)	-16.22 (378)	-20.22 (378)
♂ x ♀	♀1	377	10/10	**28.08 (377)**	**29.04 (377)**	**17.87 (377)**	**33.36 (377)**	**16.50 (377)**	**27.72 (377)**	**28.20 (377)**	**28.84 (377)**	**16.06 (377)**	**25.41 (377)**
4	♂1	393	0/10	-42.06 (393)	-34.08 (393)	-55.28 (393)	-47.86 (393)	-37.48 (385)	-44.76 (393)	-45.90 (393)	-46.59 (393)	-38.08 (393)	-45.38 (385)
♂ x ♀	♀1	393	10/10	**67.67 (393)**	**60.45 (393)**	**61.52 (393)**	**65.48 (393)**	**51.24 (385)**	**55.05 (393)**	**61.26 (393)**	**58.28 (393)**	**54.58 (393)**	**57.17 (385)**
5	♂1	382	0/10	-35.13 (382)	-36.90 (382)	-43.52 (355)	-48.59 (380)	-38.87 (375)	-41.98 (382)	-45.39 (381)	-39.27 (382)	-33.54 (380)	-32.12 (343)
♂ x ♀	♀1	393	10/10	**15.47 (393)**	**17.92 (393)**	**11.76 (366)**	**-0.24 (391)**	**10.75 (386)**	**28.97 (393)**	**16.95 (391)**	**14.47 (393)**	**18.30 (391)**	**10.63 (354)**
6	♂1	393	6/10	-25.53 (378)	**-4.60 (388)**	**-9.82 (386)**	-18.67 (368)	**-10.13 (381)**	**-2.80 (392)**	-15.95 (367)	**-7.39 (393)**	-31.06 (352)	**-5.88 (393)**
♂ x ♀	♀1	391	10/10	**3.95 (376)**	**-11.32 (386)**	**5.92 (384)**	**-6.10 (368)**	**4.06 (379)**	**-6.90 (390)**	**-4.75 (365)**	**-4.01 (391)**	**3.38 (352)**	**2.14 (391)**
7	♂1	392	1/10	-17.74 (392)	-40.08 (391)	-24.69 (392)	**-13.70 (392)**	-20.13 (392)	-19.93 (392)	-17.16 (390)	-26.45 (392)	-20.04 (391)	-39.64 (343)
♂ x ♀	♀1	392	10/10	**33.93 (392)**	**21.01 (391)**	**31.69 (392)**	**37.18 (392)**	**29.27 (392)**	**28.60 (392)**	**33.48 (390)**	**32.56 (392)**	**30.99 (391)**	**25.40 (344)**
8	♂1	396	7/10	**-13.86 (369)**	**-8.62 (369)**	**-8.55 (369)**	**-5.46 (369)**	-15.22 (369)	**-6.20 (315)**	-17.43 (354)	**-11.91 (369)**	-18.04 (353)	**-9.96 (369)**
♂ x ♀	♀1	-		+	+	+	+	+	+	+	+	+	+
9	♂1	393	4/7	**-10.63 (390)**	-28.79 (370)	**-6.30 (335)**	**-1.39 (383)**	**-9.03 (355)**	-14.40 (385)	-16.12 (390)	-	-	-
♂ x ♀	♀1	-		+	+	+	+	+	+	+	-	-	-
10	♂1	393	0/10	-18.70 (351)	-34.71 (392)	-67.89 (349)	-25.63 (393)	-25.65 (393)	-29.90 (393)	-36.48 (331)	-32.61 (380)	-	-
♂ x 2♀	♀1	382	8/8	**15.97 (342)**	**9.73 (381)**	**17.58 (340)**	**25.85 (382)**	**33.77 (382)**	**28.83 (382)**	**14.92 (327)**	**42.99 (369)**	-	-
	♂1	393	0/10	-35.04 (381)	-28.46 (393)	-19.76 (393)	-27.56 (393)	-28.02 (393)	-20.72 (393)	-32.02 (393)	-25.89 (393)	-17.84 (392)	-
	♀2	-		+	+	+	+	+	+	+	+	+	-
11	♂1	393	0/10	-43.84 (393)	-49.42 (393)	-31.61 (393)	-39.69 (393)	-39.96 (393)	-32.49 (393)	-48.26 (393)	-34.86 (393)	-48.39 (393)	-43.59 (393)
♂ x 2♀	♀1	393	10/10	**46.78 (393)**	**36.49 (393)**	**47.08 (393)**	**45.30 (393)**	**45.70 (393)**	**39.66 (393)**	**36.98 (393)**	**39.52 (393)**	**42.04 (393)**	**30.64 (393)**
	♂1	393	0/7	-40.62 (390)	-38.51 (368)	-32.46 (393)	-96.93 (376)	-38.59 (393)	-43.95 (388)	-46.49 (366)	-	-	-
	♀2	-		+	+	+	+	+	+	+	-	-	-
12	♂1	349	4/8	**-13.83 (349)**	-27.55 (349)	**-11.24 (349)**	-16.71 (349)	**-8.32 (349)**	-26.76 (349)	-29.90 (349)	**-5.68 (347)**	-	-
2♂ x 3♀	♂2	393	0/8	-41.43 (393)	-38.99 (393)	-45.12 (393)	-37.90 (393)	-42.99 (393)	-50.15 (393)	-44.16 (393)	-53.84 (391)	-	-
	♀1	393	8/8	**56.00 (393)**	**45.57 (393)**	**59.45 (393)**	**56.25 (393)**	**55.75 (393)**	**50.25 (393)**	**51.94 (393)**	**38.10 (391)**	-	-
	♂1	349	2/9	-25.58 (312)	**-7.90 (291)**	-26.84 (349)	**-0.11 (346)**	-30.76 (393)	-36.83 (393)	-37.57 (393)	-16.33 (349)	-28.75 (349)	-
	♂2	393	0/9	-42.38 (343)	-49.62 (318)	-32.67 (393)	-42.97 (390)	-43.28 (349)	-40.19 (349)	-43.12 (349)	-40.85 (393)	-38.00 (393)	-
	♀2	385	9/9	**19.74 (338)**	**33.33 (312)**	**34.13 (385)**	**28.16 (382)**	**27.40 (385)**	**25.07 (385)**	**27.49 (385)**	**17.78 (385)**	**22.47 (385)**	-
	♂1	349	8/10	**11.38 (339)**	-25.23 (349)	**-2.42 (349)**	**-8.71 (349)**	-7.83 (348)	-25.83 (393)	**-6.25 (349)**	**-4.41 (349)**	-13.58 (344)	-12.88 (349)
	♂2	393	0/10	-40.56 (381)	-41.04 (393)	-27.65 (393)	-33.60 (393)	-29.23 (392)	-25.95 (349)	-29.86 (393)	-17.63 (393)	-34.14 (388)	-20.47 (393)
	♀3	368	8/10	**5.31 (359)**	-22.49 (368)	**-7.78 (368)**	**3.17 (368)**	**-1.39 (367)**	-26.58 (368)	**4.11 (368)**	-13.65 (368)	**-1.31 (363)**	**-3.91 (368)**

Results of paternity analysis. Burrow indicates the numerical ID and composition of excavated burrows. Adult ID indicates which individual is being tested on each row. Adult loci indicates the number of loci that were used for each testing parentage of each adult. Larvae assn. indicates the number of larval samples assigned to each potential parent. Laval subsample indicates the LOD score followed by the number of loci compared in parentheses. Minus signs indicate that an sample was of too low quality to include in the analysis, and plus signs indicate that a test was not possible due to lack of parental genome. Significantly positive parentage results (i.e., LOD score above threshold set by Cervus simulation) are in bold type.

CHAPTER VI – SYNTHESIS

The Atlantic sand fiddler crab *Leptuca pugilator* has a rich history of study. The species was originally catalogued and named by Louis A. G. Bosc (1802) who refers to the crab as "L'ocypode combatant" (English: the fighting ocypode). He describes the behavior of *L. pugilator* in terms of his own experience that "as soon as a man or an animal appeared in their midst they bent their great claw, holding it in front of them as though it were a challenge to combat." The species name chosen by Bosc is well suited to what he saw as their fighting nature: *pugilator* meaning boxer. However, as is often the case in original species' descriptions, Bosc's initial investigation of the newly catalogued species was superficial. His classification of *L. pugilator* as the "fighting ocypode" was not given based on the crab's true biology, but on his own intuition concerning its most readily observable and relatable behaviors.

This intuition concerning combat pervades the study of fiddler crab biology, and not without substantial evidence to support it. The single large claw of male fiddler crabs is an eye-catching and oft studied structure, and it seems to take the form of a weapon, as well as an ornament, which males very clearly use to communicate during territorial negotiations. The apparent conflict of these negotiations, their physicality, and the fact that one male always seems to emerge victorious over his opponent lends itself well to human intuitions concerning combat. However, if one traces the study of combat in fiddler crabs back as far as one can, it becomes apparent that the term "combat" was assigned from the onset to a set of signaling behaviors that weren't studied with real depth until well after they were designated as such. For better or worse, when the

141

territorial disputes of male fiddler crabs were originally reported, they were reported as combat, and it is from those initial reports that subsequent hypotheses were posed.

The traditional designation of male interactions as "combat" in fiddler crabs has not gone without question or caveat. The mother of fiddler crab biology herself, Joycelyn Crane, who likely logged more hours of direct observation of the genus than any other individual, dedicates an entire chapter of her cornerstone publication "Fiddler Crabs of the World" (1975; Chapter 6) to the discussion of how antagonistic combat between males can be reconciled with her own monumental experience with the genera. To summarize her work here is impossible, but notably she concludes her discussion of the function of combat in fiddler crabs with this quote by Fraser Darling which she believed to describe fiddler crabs as well as the birds which Darling studied:

> "The aggressive quality of bird song has, I think, been overemphasized. Proclamation, yes; *apparently* aggressive, yes; no more combative than a military tournament of befrogged dragoons, but probably even more stimulating. So-called fighting, and singing, are in my opinion often a form of social stimulation and have indirect survival value as aids to development of reproductive condition. I should think the term "aggressive behavior" could be dropped for a great deal of true display." (Darling, 1952)

Despite not viewing male interaction as aggressive, frequently noting the lack of true violence between males, Crane retained the view that combat in fiddler crabs is antagonistic and likely serves to ensure that only the most fit individuals are allowed to mate.

My own hypotheses concerning inter-male competition for territory and the experiments designed to test them presented in this dissertation were created based on the current state of our understanding of male-male interactions, which can be summarized as follows: **(A)** male fiddler crabs engage in antagonistic combat with one another over territory ownership (Crane, 1975); **(B)** combat in fiddler crabs is ritualized (Crane, 1975) and utilizes some form of assessment game to determine the winner (Jennions and Backwell, 1996; Pratt et al., 2003); **(C)** the resident male is far more likely to retain the territory than the intruding male is to take it due to a mechanical ownership advantage (Fayed et al., 2008; Hyatt and Salmon, 1978; Jennions and Backwell, 1996); **(D)** larger males have increased combative abilities and are therefore more likely to hold territory (Hyatt and Salmon, 1978; Jennions and Backwell, 1996; Morrell et al., 2005; Pratt and Mclain, 2002); and **(E)** males engage in endurance rivalry (Andersson, 1994) and are therefore likely to push themselves to retain territory for as long as physiologically possible in the face of the energetic and thermal cost of territory residency (Allen and Levinton, 2014, 2007; Darnell et al., 2013; Matsumasa and Murai, 2005; Munguia et al., 2017). These observations culminate in an image of territoriality in which males regularly engage in intense, combative interactions for territory that only the most physically dominant individuals are likely to win. That once a territory is secured, the resident male must continuously defend it from antagonistic intruders which aim forcefully evict the resident as soon as he becomes too weak to defend his territory. That the only advantage a resident has is in his ability to retreat to his burrow, and even retreat will not be enough to stave off his attackers once his physiological state has sufficiently declined. That

143

regardless of all else, every male who holds territory is eventually removed against his will due to either his own weakness or the strength of his opponent.

Ultimately, the findings of my dissertation call almost every aspect of this picture into question for *L. pugilator*. The finding that the presence or absence of intruding crabs has no impact on the residence time of territory-owning crabs indicates that it is the resident, and not a combative intruder, who determines when territory will be exchanged. The fact that territory-owning crabs are no more capable of obtaining or retaining territory than are any other males in the population indicates that males can own territory and mate without being particularly expert combatants or particularly capable defenders. The increased probability of territory exchange following low intensity interactions gives the impression that residents value territory ownership less than might be expected, and the relatively short residence times indicate a high rate of territory turnover. Finally, the correlation between courtship behavior and acute strength highlights the importance of physiological condition in territory ownership, while the decrease in residence times during peak mating periods, when costly courtship behaviors are expected to be most common, points to physiologically limited burrow tenure.

These findings change the perspective on male-male dynamics surrounding territory ownership from one of combat and ultimate endurance, in which the biggest and most powerful take and retain territory by force, to one of resident control, in which wanderers most frequently obtain territory when it is given to them by a resident who has decided it is time to leave the stressors of the high intertidal in order to recover in the low intertidal. For *L. pugilator,* who's tenure in the mating area is limited by environmental stressors, it may be that reproductive fitness is not determined by aggressive competition

144

between males, but by investment in one's own physiology, attractiveness to females, and ability to maximize reproductive output over relatively short periods of territory ownership. Simply put, the reproductive necessity of mating burrows constructed in the stressful environment of the high intertidal limits *L. pugilator*'s ability to mate effectively over long durations, and the subsequent increase in territory turnover between males seems to have reduced the need for antagonist combat. By and large, this conclusion is not at odds with previous work on the species, and similar phenomenon may be present in other species for whom truly violent interactions between males are rare.

If not antagonistic combat, then what exactly are male territorial interactions? What purpose do they serve if not to ensure that only the strongest can mate? In Chapter 5, I provide preliminary support of the hypothesis that males cooperate via by-product mutualism to raise their offspring in the face of harsh environmental conditions, and this frame of reference may help us to understand their interactions. When a resident engages with a wandering male in a territorial dispute, it may be that that the interaction is truly an assessment of the wander's strength as suggested previous studies (e.g., Jennions and Backwell, 1996; Pratt et al., 2003), but instead of assessing which the intruder's combat prowess, the resident is assessing his potential replacement's ability to maintain his offspring's burrow. This would ensure that the offspring are protected by a male in adequate physiological condition and could explain why it is the stronger males are more likely to hold territory than are weaker males (Chapter 2; McLain et al., 2015).

REFERENCES

Allen, B.J., Levinton, J.S., 2007. Costs of bearing a sexually selected ornamental weapon in a fiddler crab. Funct. Ecol. 21, 154-161.

Allen, B.J., Levinton, J.S., 2014. Sexual selection and the physiological consequences of habitat choice by a fiddler crab. Oecologia 176, 25–34.

Allen, B.J., Rodgers, B., Tuan, Y., Levinton, J.S., 2012. Size-dependent temperature and desiccation constraints on performance capacity: Implications for sexual selection in a fiddler crab. J. Exp. Mar. Bio. Ecol. 438, 93–99.

Andersson, M.B., 1994. Sexual Selection. Princeton University Press, Princeton, NJ.

Andrews, K.R., Hohenlohe, P.A., Miller, M.R., Hand, B.K., Seeb, J.E., Luikart, G., 2014. Trade-offs and utility of alternative RADseq methods: reply to Puritz et al. 2014. Mol. Ecol. 23, 5943–5946.

Axelrod, R., Hamilton, W.D., 1981. The evolution of cooperation. Science 211, 1390–1396.

Backwell, P.R., Jennions, M.D., 2004. Coalition among male fiddler crabs. Nature 430, 417.

Backwell, P.R.Y., Jennions, ., Christy, J.H., Passmore, N.I., 1999. Female Choice in the Synchronously Waving Fiddler Crab *Uca annulipes*. Ethology 105, 415–421.

Backwell, P.R.Y., Passmore, N.I., 1996. Time constraints and multiple choice criteria in the sampling behaviour and mate choice of the fiddler crab, *Uca annulipes*. Behav Ecol Sociobiol 38, 407–416.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., Johnson, Er.A., 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3, e3376.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.

Best, L.B., 1977. Territory quality and mating success in the field sparrow (*Spizella pusilla*). Condor 79, 192–204.

Bolton, J., Backwell, P.R.Y., Jennions, M.D., 2013. Density dependence and fighting in species with indeterminate growth: a test in a fiddler crab. Anim. Behav. 85, 1367–1376.

Booksmythe, I., Detto, T., Backwell, P.R.Y., 2008. Female fiddler crabs settle for less: the travel costs of mate choice. Anim. Behav. 76, 1775–1781.

Booksmythe, I., Jennions, M.D., Backwell, P.R.Y., 2010. Interspecific assistance: fiddler crabs help heterospecific neighbours in territory defence. Biol. Lett. 6, 748–750.

Bosc, L.A.G., 1802. Histoire naturelle des vers, contenant leur description et leurs mœurs; avec figures dessinées d'après nature. Tome premier. Deterville, Paris, France.

Brown, J.L., 1983. Cooperation - a biologists dilemma. Adv Study Behav 13, 1–37.

Callander, S., Jennions, M.D., Backwell, P.R.Y., 2012. The effect of claw size and wave rate on female choice in a fiddler crab. J. Ethol. 30, 151–155.

Campagna, C., Le Boeuf, B.J., 1988. Reproductive behaviour of southern sea lions. Behavior 104, 233–261.

Chong, Z., Ruan, J., Wu, C.-I., 2012. Rainbow: an integrated tool for efficient clustering and assembling RAD-seq reads. Bioinformatics 28, 2732–2737.

Christy, J.H., 1978. Adaptive significance of reproductive cycles in the fiddler crab *Uca pugilator*: a hypothesis. Science 199, 453–455.

Christy, J.H., 1982. Burrow structure and use in the sand fiddler crab, *Uca pugilator* (Bosc). Anim. Behav. 30, 687–694.

Christy, J.H., 1983. Female choice in the resource-defense mating system of the sand fiddler crab, *Uca pugilator*. Behav Ecol Sociobiol. 12, 169–180.

Christy, J.H., Backwell, P.R.Y., 1995. The sensory exploitation hypothesis. Trends Ecol. Evol. 10, 417–417.

Christy, J.H., Salmon, M., 1984. Ecology and evolution of mating systems of fiddler crabs (Genus Uca). Biol. Rev. Camb. Philos. Soc. 59, 483–509.

Clements, K.C., Stephens, D.W., 1995. Testing models of non-kin cooperation: mutualism and the Prisoner's Dilemma. Anim. Behav. 50, 527–535.

Crane, J., 1975. Fiddler crabs of the world: Ocypodidae: genus Uca. Princeton University Press, Princeton, NJ.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 1000 Genomes Project Analysis Group, 2011. The variant call format and VCFtools. Bioinformatics 27, 2156–2158.

Darling, F.F., 1952. Social behavior and survival. The Auk 69, 183-191.

Darnell, M.Z., Darnell, K.M., 2018. Geographic variation in thermal tolerance and morphology in a fiddler crab sister-species pair. Mar. Biol. 165, 26.

Darnell, M.Z., Fowler, K.K., Munguia, P., 2013. Sex-specific thermal constraints on fiddler crab behavior. Behav. Ecol. 24, 997–1003.

Darnell, M.Z., Munguia, P., 2011. Thermoregulation as an alternate function of the sexually dimorphic fiddler crab claw. Am. Nat. 178, 419–428.

Darnell, M.Z., Yeghissian, T.G., Lane, Z.M., 2020. Balancing risk and reward: mating opportunity influences thermal refuge use in fiddler crabs. Anim. Behav. 169, 51–56.

Davies, N.B., Houston, A.I., 1981. Owners and satellites: the economics of territory defence in the pied wagtail *Motacilla alba*. J. Anim. Ecol. 50, 157–180.

De Jaegher, K., Hoyer, B., 2016. By-product mutualism and the ambiguous effects of harsher environments - a game theoretical model. J. Theor. Biol. 393, 82–97.

deRivera, C.E., 2005. Long searches for male-defended breeding burrows allow female fiddler crabs, *Uca crenulata*, to release larvae on time. Anim. Behav. 70, 289–297.

deRivera, C.E., Vehrencamp, S.L., 2001. Male versus female mate searching in fiddler crabs: a comparative analysis. Behav. Ecol. 12, 182–191.

Dugatkin, L.A., Reeve, H.K., 1998. Game theory and communication, in: Game theory and animal behavior. Oxford University Press, New York, NY, pp. 38–63.

Emlen, S.T., Oring, L.W., 1977. Ecology, sexual selection, and the evolution of mating systems. Science 197, 215–223.

Fayed, S.A., Jennions, M.D., Backwell, P.R.Y., 2008. What factors contribute to an ownership advantage? Biol. Lett. 4, 143–145.

Fischer, E.A., 1984. Egg trading in the chalk bass, *Serranus tortugarum*, simultaneous hermaphrodite. Z. Tierpsychol. 64, 143–151.

Fu, L., Niu, B., Zhu, Z., Wu, S., Li, W., 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28, 3150–3152.

Garrison, E., Marth, G., 2012. Haplotype-based variant detection from short-read sequencing ArXiv, 1207.3907.

Gibson, R., Barker, P.L., 1979. The decapod hepatopancreas. Oceanography and Marine Biology 17, 285–346.

Goodall, J., 1986. The chimpanzee of Gombe: patterns of behavior. Belknap Press, Cambridge, MA.

Greenspan, B.N., 1980. Male size and reproductive success in the communal courtship system of the fiddler crab *Uca rapax*. Anim. Behav. 28, 387–392.

Hart, B.L., Hart, L.A., 1992. Reciprocal allogrooming in impala, *Aepyceros melampus*. Anim. Behav. 44, 1073–1083.

How, M.J., Zeil, J., Hemmi, J.M., 2009. Variability of a dynamic visual signal: the fiddler crab claw-waving display. J. Comp. Physiol. A, Neuroethol. Sens. Neural Behav. Physiol. 195, 55–67.

Hyatt, G.W., 1977. Field studies of size-dependent changes in waving display and other behavior in the fiddler crab,*Uca pugilator* (Brachyura, Ocypodidae). Mar Behav Physiol 4, 283–292.

Hyatt, G.W., Salmon, M., 1978. Combat in the Fiddler Crabs *Uca pugilator* and *U. pugnax*: A Quantitative Analysis. Behavior 65, 182–211.

Hyatt, G.W., Salmon, M., 1979. Combat in the fiddler crabs *Uca pugilator* and *U. pugnax*: a quantitative analysis. Behaviour 68, 1–23.

Jennions, M.D., Backwell, P.R.., Murai, M., Christy, J.H., 2003. Hiding behaviour in fiddler crabs: how long should prey hide in response to a potential predator? Anim. Behav. 66, 251–257.

Jennions, M.D., Backwell, P.R.Y., 1996. Residency and size affect fight duration and outcome in the fiddler crab *Uca annulipes*. Biological Journal of the Linnean Society 57, 293–306.

Jennions, M.D., Backwell, P.R.Y., 1998. Variation in courtship rate in the fiddler crab *Uca annulipes*: is it related to male attractiveness? Behav. Ecol. 9, 605–611.

Kahn, A.T., Holman, L., Backwell, P.R.Y., 2014. Female preferences for timing in a fiddler crab with synchronous courtship waving displays. Anim. Behav. 98, 35–39.

Kalinowski, S.T., Taper, M.L., Marshall, T.C., 2007. Revising how the computer program Cervus accomodates genotyping error increases success in paternity assignment. Mol Ecol 16, 1099–1106.

Kerr, K.A., Christy, J.H., Joly-Lopez, Z., Luque, J., Collin, R., Guichard, F., 2014. Reproducing on time when temperature varies: shifts in the timing of courtship by fiddler crabs. PLoS One 9, e97593.

Kim, T.W., Cho, J.C., 2003. The effect of food availability on the semilunar courtship rhythm in the fiddler crab *Uca lactea* (de Haan) (Brachyura: Ocypodidae). Behav Ecol Sociobiol 54, 210–217.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. Lmertest package: tests in linear mixed effects models. J. Stat. Softw. 82, 1–26.

Lailvaux, S.P., Reaney, L.T., Backwell, P.R.Y., 2009. Dishonest signalling of fighting ability and multiple performance traits in the fiddler crab *Uca mjoebergi*. Funct. Ecol. 23, 359–366.

Land, M., Layne, J., 1995. The visual control of behavior in fiddler crabs. Journal of Comparative Physiology 177, 81–90.

Layne, J., Land, M., Zeil, J., 1997. Fiddler crabs use the visual horizon to distinguish predators from conspecifics: a review of the evidence. J. Mar. Biol. Ass. 77, 43–54.

Levinton, J., Lord, S., Higeshide, Y., 2015. Are crabs stressed for water on a hot sand flat? Water loss and field water state of two species of intertidal fiddler crabs. J. Exp. Mar. Bio. Ecol. 469, 57–62.

Levinton, J.S., Allen, B.J., 2005. The paradox of the weakening combatant: trade-off between closing force and gripping speed in a sexually selected combat structure. Funct. Ecol. 19, 159–165.

Levinton, J.S., Judge, M.L., 1993. The relationship of closing force to body size for the major claw of *Uca pugnax* (Decapoda: Ocypodidae). Funct. Ecol. 7, 339–345.

Li, W., Godzik, A., 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22, 1658–1659.

Marler, C.A., Walsberg, G., White, M.L., Moore, M., 1995. Increased energy expenditure due to increased territorial defense in male lizards after phenotypic manipulation. Behav Ecol Sociobiol. 37, 225–231.

Marshall, T.C., Slate, J., Kruuk, L.E.B., Pemberton, J.M., 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7, 639–655.

Matsumasa, M., Murai, M., 2005. Changes in blood glucose and lactate levels of male fiddler crabs: effects of aggression and claw waving. Anim. Behav. 69, 569–577.

McLain, D.K., Logue, J., Pratt, A.E., McBrayer, L.D., 2015. Claw-pinching force of sand fiddler crabs in relation to activity and the lunar cycle. J. Exp. Mar. Bio. Ecol. 471, 190–197.

McLain, D.K., Pratt, A.E., 2007. Approach of females to magnified reflections indicates that claw size of waving fiddler crabs correlates with signaling effectiveness. J. Exp. Mar. Bio. Ecol. 343, 227–238.

Mesterton-Gibbons, M., Dugatkin, L.A., 1992. Cooperation among unrelated individuals: evolutionary factors. Q Rev Biol 67, 267–281.

Miller, C.C., 1961. The feeding mechanism of fiddler crabs, with ecological considerations of feeding adaptations. Zool. Sci. Contrib. N. Y. Zool. Soc. 46, 89–100.

Milner, R.N., Jennions, M.D., Backwell, P.R., 2010. Eavesdropping in crabs: an agency for lady detection. Biol. Lett. 6, 755–757.

Milner, R.N.C., Jennions, M.D., Backwell, P.R.Y., 2011. Know thine enemy's neighbor: neighbor size affects floaters' choice of whom to fight. Behav. Ecol. 22, 947–950.

Mooring, M.S., Hart, B.L., 1995. Costs of allogrooming in impala: distraction from vigilance. Anim. Behav. 49, 1414–1416.

Morgan, S.G., Christy, J.H., 1995. Adaptive significance of the timing of larval release by crabs. Am. Nat. 145, 457–479.

Morrell, L.J., Backwell, P.R., Metcalfe, N.B., 2005. Fighting in fiddler crabs *Uca mjoebergi*: what determines duration? Anim. Behav. 70, 653–662.

Munguia, P., Backwell, P.R.Y., Darnell, M.Z., 2017. Thermal constraints on microhabitat selection and mating opportunities. Anim. Behav. 123, 259–265.

Oliveira, R.F., Custódio, M.R., 1998. Claw size, waving display and female choice in the European fiddler crab, *Uca tangeri*. Ethology Ecology & Evolution 10, 241–251.

Ord, T.J., 2021. Cost of territoriality: a review of hypotheses, meta-analysis, and field study. Oecologia 197, 615–631.

Pagès, J., 2015. Factorial analysis of mixed data, in: Multiple factor analysis by example using R. CRC Press, Boca Raton, FL.

Peterson, B.K., Weber, J.N., Kay, E.H., Fidher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7, e37135.

Pope, D.S., 2000. Video Playback Experiments Testing the Function of Claw Waving in the Sand Fiddler Crab. Behavior 137, 1349–1360.

Pratt, A.E., Mclain, D.K., 2002. Antisymmetry in male fiddler crabs and the decision to feed or breed. Funct. Ecol. 16, 89–98.

Pratt, A.E., McLain, D.K., 2006. How dear is my enemy: intruder-resident and resident-resident encounters in male sand fiddler crabs (*Uca pugilator*). Behaviour 143, 597–617.

Pratt, A.E., McLain, D.K., Lathrop, G.R., 2003. The assessment game in sand fiddler crab contests for breeding burrows. Anim. Behav. 65, 945–955.

Puritz, J.B., Hollenbeck, C.M., Gold, J.R., 2014. dDocent: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. PeerJ 2, e431.

R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raubenheimer, B., Guza, R.T., 1999. Tidal water table fluctuations in a sandy ocean beach. Water Resour. Res. 35, 2313–2320.

Reaney, L.T., 2007. Foraging and mating opportunities influence refuge use in the fiddler crab, *Uca mjoebergi*. Anim. Behav. 73, 711–716.

Reaney, L.T., 2009. Female preference for male phenotypic traits in a fiddler crab: do females use absolute or comparative evaluation? Anim. Behav. 77, 139–143.

Reaney, L.T., Backwell, P.R.Y., 2007. Temporal constraints and female preference for burrow width in the fiddler crab, *Uca mjoebergi*. Behav Ecol Sociobiol (Print) 61, 1515–1521.

Reaney, L.T., Maurer, G., Backwell, P.R.Y., Linde, C.C., 2012. Paternity analysis of two male mating tactics in the fiddler crab, *Uca mjoebergi*. Behav Ecol Sociobiol (Print) 66, 1017–1024.

Reinsel, K.A., Rittschof, D., 1995. Environmental regulation of foraging in the sand fiddler crab *Uca pugilator* (Bosc 1802). J. Exp. Mar. Bio. Ecol. 187, 269–287.

Rissing, S.W., Pollock, G.B., Higgins, M.R., Hagen, R.H., Roan Smith, D., 1989. Foraging specialization without relatedness or dominace among co-founding ant queens. Nature 338, 420–422.

Robertson, R.J., Bancroft, K., Vermeer, G., Plaisier, K., 1980. Experimental studies on the foraging behavior of the sand fiddler crab *Uca pugilator* (Bosc, 1802). J. Exp. Mar. Bio. Ecol. 44, 67–83.

Salmon, M., Hyatt, G.W., 1983. Spatial and temporal aspects of reproduction in North Carolina fiddler crabs (*Uca pugilator* Bosc). J. Exp. Mar. Bio. Ecol. 70, 21–43.

Sanches, F.H.C., Costa, T.M., Barreto, R.E., Backwell, P.R.Y., 2017. Faster male displays and less complex choice are more attractive to female fiddler crabs as they reduce search costs. Anim. Behav. 124, 119–123.

Schweyen, H., Rozenberg, A., Leese, F., 2014. Detection and removal of PCR duplicates in population genomic ddRAD studies by addition of a degenerate base region (DBR) in sequencing adapters. Biol Bull 227, 146–160.

Silbiger, N., Munguia, P., 2008. Carapace color change in *Uca pugilator* as a response to temperature. J. Exp. Mar. Bio. Ecol. 355, 41–46.

Slate, J., Marshall, T., Pemberton, J., 2000. A retrospective assessment of the accuracy of the paternity inference program Cervus. Mol Ecol 9, 801–808.

Smith, W.K., Miller, P.C., 1973. The thermal ecology of two south Florida fiddler crabs: *Uca rapax* Smith and *U. pugilator* Bosc. Physiol. Zool. 46, 186–207.

Trivers, R.L., 1971. The evolution of reciprocal altruism. Q Rev Biol 46, 189–226.

Venables, W. N., Ripley, B. D., 2003. Modern applied statistics with S (statistics and computing), 4th ed. Springer, New York, NY.

Weisberg, F.J., 2019. An R companion to applied regression, 3rd ed. Sage, Thousand Oaks, CA.

Weissburg, M., 1992. Functional analysis of fiddler crab foraging: sex-specific mechanics and constraints in *Uca pugnax* (Smith). J. Exp. Mar. Bio. Ecol. 156, 105–124.

Weissburg, M., 1993. Sex and the single forager: gender-specific energy maximization strategies in fiddler crabs. Ecology 74, 279–291.

Wilkens, J.L., Fingerman, M., 1965. Heat tolerance and temperature relationships of the fiddler crab, *Uca pugilator*, with reference to body coloration. Biological Bulletin 128, 133–141.

Zar, J.H., 1999. Circular distributions: hypothesis testing, in: Biostatistical analysis. Prentice-Hall, Hoboken, NJ, pp. 616–663.

Zeil, J., 1998. Homing in fiddler crabs (*Uca lactea annulipes* and *Uca vomeris*: Ocypodidae). J Comp Physiol 183, 367–377.