ABSTRACT

Title of Document: A COMPARATIVE ANALYSIS OF SPERM
STORAGE IN SIX BRACHYURAN
SUPERFAMILIES: MATING BEHAVIOR,
ECOLOGICAL VARIATION AND PHYLOGENETIC
PATTERNS

Paula Jane Rodgers, Ph.D. 2010

Dissertation directed By: Professor Marjorie Reaka, Department of Biology
Dr. Anson Hines, Smithsonian Environmental Research
Center

While life history traits are shaped by allometric, phylogenetic, environmental and
behavioral factors, few comparative studies of brachyuran life history patterns have
considered sperm storage traits as important components of reproductive strategies. To
understand the evolutionary forces selecting for sperm storage and their interactions with
other life history traits, I (1) used controlled laboratory experiments and field mating
observations to examine variation in male sperm transfer patterns, (2) sampled variation
in female reproductive output and sperm storage for two species across a latitudinal
gradient, (3) conducted a survey of life history traits across a broad range of brachyuran
taxa, and (4) used phylogenetic analyses to identify patterns in the evolution of life
history traits in brachyurans.

From mating experiments and observations on five species, I found that males transfer
more sperm with longer than shorter copulation durations and that variation in copulation

duration was shaped by differences in the species' ecologies. Latitudinal surveys of two species with contrasting mating systems identified seasonal and geographical variation in female reproductive output. While the variation in most reproductive traits could best be explained at smaller spatial scales, a sperm storing species, *Callinectes sapidus* became sperm limited at low latitudes.

From a comparative survey of male and female life history traits across 61 species of brachyurans, I found that allometry, phylogeny and mating strategies explained much of the variation in life history traits. Using rigorous phylogenetic techniques, male life history traits showed more plasticity across the phylogeny than female traits suggesting male traits may be influenced more by behavioral and environmental factors. After correcting for phylogenetic signals, species with larger male sperm stores had larger amounts of sperm stored by the female. In summary this dissertation illustrates the importance of partitioning variation in mating behavior, phylogeny, environmental factors and allometry when examining the evolution of life history traits in brachyurans.

A COMPARATIVE ANALYSIS OF SPERM STORAGE IN SIX BRACHYURAN SUPERFAMILIES: MATING BEHAVIOR, ECOLOGICAL VARIATION AND PHYLOGENETIC PATTERNS

by

Paula Jane Rodgers

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2010

Advisory Committee:

Professor Marjorie Reaka, Co-chair
Dr. Anson Hines, Co-chair
Professor Alexa Bely
Professor Charlie Mitter
Professor Brad Stevens
Professor James Dietz

DEDICATION

I dedicate this dissertation to my loving husband, Josh Odell, and to our growing family. I

would also like to thank my parents, Chris and Frank Rodgers, and my sister, Vikki

Rodgers, for their continued love and support.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I

Chapter II

Chapter III

LIST OF APPENDICES

Chapter II

THEORETICAL BACKGROUND AND INTRODUCTION

Sexual selection in the form of both male competition and female choice are important forces shaping the evolution of life history traits such as sperm storage and reproductive output. This is brought about by a typically higher variation in reproductive success in males versus females (Bateman, 1948). It has been well documented that male-male competition, including sperm competition, can shape both male functional morphology (Diesel, 1991; Cordoba-Aguilar et al., 2003; Snow et al., 2006) and mating behavior (Diesel, 1991; Jivoff, 1997a; Wedell & Cook, 1999; Rondeau & Sainte-Marie, 2001; Wedell et al., 2002; Wada et al., 2005). Sexual selection likely played an important role in the evolution of female sperm storage. Similarly, female choice places selective pressure on male morphology and behavior, which can lead to the evolution of elaborate courtship behaviors and male morphologies. For example, male fiddler crabs have evolved intricate claw-waving patterns. In fact, each species displays a unique waving pattern which attracts conspecific females (Crane, 1975). Cryptic female choice, for example females differentially using sperm packets after copulation, also can lead to directional selection of male sperm traits (Birkhead, 1998). Although this mechanism has not received much attention, it is thought to play a role in the mating system of the cuttlefish *Sepia apama* (Naud et al., 2005).

By storing sperm, females guarantee they will have a readily available sperm source at the time of oviposition without having to engage in costly mate searching or courtship behaviors. Additionally, if females multiply mate, they can store sperm from more than one male and increase the genetic diversity of the brood (Johnston et al., 2006). However,

sperm storage is not only advantageous, but it can be costly to both males and females. Storage requires the evolution of male and female proteins and sperm metabolism that maintain the viability of sperm within the spermathecae (Diesel, 1991; Jeyalectumie & Subramoniam, 1991). There is a developmental cost (in both time and energy) to producing a spermatheca, and a metabolic cost associated with developing reproductive tissue (Stearns, 1992; Zera & Denno, 1997). Interestingly, recent experimental work has revealed additional costs associated with long-term sperm storage, such as a decreased immune response in attine ants (Baer et al., 2006), and decreased longevity in *Drosophila* (Lung et al., 2002). In molting arthropods, it is possible to lose sperm during ecdysis (Cheung, 1968; Morgan et al., 1983), and this can vary with phylogenetic molting patterns. In this dissertation, I examined variation in brachyuran life history traits, with particular emphasis on sperm storage patterns, in association with variation in mating behavior, environmental factors such as latitude and depth, and with phylogenetic patterns, in order to identify the selection pressures leading to the diverse array of life history traits found within this group.

Mating behavior shapes and is shaped by sexual selection and thus influences the evolution of reproductive traits. For example, male competition for access to limited females can lead to pre-copulatory guarding. Indeed, Jivoff (1997) found that, by increasing the sex ratio of *Callinectes sapidus,* the blue crab, so that males out-numbered females, males increased the amount of time they spent guarding females, and thus increased their energetic investment per female. In addition, sexual selection can shape functional morphology. For example, a high rate of sperm competition has led male

redback spiders to evolve the ability to break their sternite in the female reproductive tract. The broken sternite serves as a low cost plug to prevent another male from depositing a sperm packet, and assures the male's paternity (Snow et al., 2006). Intersexual selection can lead to the co-evolution of male and female traits, with the sexes acting in concurrence or in conflict with each other (Parker, 2006; Wedell et al., 2006). One example of intersexual conflict shaping morphology is the production of "toxic sperm" in *Drosophila*. Males have evolved proteins in their seminal fluid that can control female behavior by reducing a female's mating frequency and increasing ovulation but at the cost of decreasing female longevity (Lung et al., 2002; Harmer et al., 2006). While mating behavior and functional morphology can shape sperm storage patterns, these traits can in turn shape optimal mating behaviors and functional morphologies.

Environmental factors such as latitude, depth, season length, temperature, and food availability can alter optimal life history traits, and can shape mating behavior (Emlen & Oring, 1977). Variation in physiology caused by geographical and spatial factors has received considerable attention in the literature and has played an important part in shaping reproductive traits. Body size follows one of three possible trends with increasing latitude: 1) Bergman's rule (increasing body size), 2) converse Bergman's rule (decreasing body size), and 3) countergradient variation (varies based on the amount of genetic compensation) (Conover & Schultz, 1995; Atkinson & Sibly, 1997; Blanckenhorn & Demont, 2004). Recent work on marine arthropod species has shown that several follow Bergman's rule (Reaka, 1986; Blanckenhorn & Demont, 2004;

Lardies & Bozinovic, 2006), and thus reproductive output is expected to increase with latitude. This alters how sperm is used by the female and allocated by the male, and thus has the potential to lead to sperm limitation when females have a large clutch size (MacDiarmid & Butler, 1999; Hines et al., 2003). Since individual male sperm number can vary over relatively small spatial scales with males having fewer sperm in areas with low versus high sperm competition (Evans & Magurran, 1999) and in areas with high versus low fishing pressure (MacDiarmid & Butler, 1999; Gardner & Williams, 2002; Hines et al., 2003; Jivoff, 2003b; Alonzo & Mangel, 2004; Carver et al., 2005), then male sperm number can likely vary across a larger spatial scale, such as latitude. Similarly, female egg number varies with body size (Hines, 1982; McIntyre & Hutchings, 2003) and decreases with increasing egg size (Smith & Fretwell, 1974; Kolm et al., 2006b). Such traits have been shown to vary predictably across latitude (Cody, 1966) and with depth (Strathmann, 1977).

Lineage effects occur when a trait becomes fixed in an ancestor and constrains evolution in the descendants (Stearns, 1992). Such phylogenetic patterns are known to shape life history traits. For example, in stomatopods, body size is constrained by phylogeny; body size in turn alters life history traits and rates of speciation (Reaka, 1986). With the advancement of phylogenetic techniques, reproductive traits can be permuted across a phylogeny in order to test for a significant phylogenetic pattern in that trait. Once identified, phylogenetic correlations can be removed from the trait in order to test correlations between different life history traits. By identifying phylogenetic patterns in

life history traits, we can begin to understand how these traits have evolved across a phylogeny.

The Study System:

Brachyura, or true crabs, are a model group for comparative research on reproductive traits such as sperm storage and its correspondence with mating system dynamics and reproductive morphologies. Brachyurans are abundant, easily collected from the field and manipulated in the laboratory. They all have internal fertilization and possess a spermatheca, and thus can store sperm, at least for the short term (Diesel, 1991). While there are approximately 24 superfamilies and 71 families of brachyurans (Martin & Davis, 2001), this proposal will focus on species with diverse life history traits and mating strategies from six superfamilies: Cancroidea, Xanthoidea, Ocypodoidea, Portunoidea, Majoidea, and Grapsoidea.

Long-term sperm storage is thought to be ubiquitous and the basal condition within the brachyurans. It has been well documented in several commercially important brachyuran species, including the portunids *C. sapidus* (Jivoff, 2003b), and *Scylla serrata* (Jeyalectumie & Subramoniam, 1991), the majids *Chionoecetes opilio* (Sainte-Marie & Sainte-Marie, 1999), *Chionoecetes bairdi* (Paul, 1984)*,* and *Inachus phalangium* (Diesel, 1990), and the xanthids *Pseudocarcinas gigas* (Gardner & Williams, 2002) and *Menippe mercenaria* (Cheung, 1968). However, relatively little information is known about sperm storage in small bodied xanthids, ocypodids and grapsids in relation to their mating systems, environments and phylogenetic histories. Specifically, species with varying

mating behaviors have not been investigated in a comparative framework. Although mapping sperm storage patterns in brachyurans on a phylogenetic tree is beyond the scope of this dissertation (but is a long term research goal), this dissertation research provides a first step in examining the phylogenetic patterns of sperm storage and reproductive output in this group.

<u>Objectives for each chapter:</u>

In order to understand the relationship between sperm storage and mating systems, in chapter one, I examined male sperm transfer and female sperm storage in five species of brachyuran crabs from three superfamilies (Ocypodoidea, Grapsoidea, and Xanthoidea). Using controlled laboratory mating experiments and field mating observations, I was able to collect mated pairs for each of the five species, calculate the amount of sperm transferred to the female at mating for each species, and determine if (1) copulation duration and the time until mating are longer for larger males and larger females, and for males with more sperm, (2) more sperm and a heavier ejaculate are transferred with long copulation durations, by large males and to large females, and (3) large, fecund females accumulate more sperm after mating. While copulation duration and time until mating were not longer for large versus small individuals, more sperm was transferred with longer copulation durations, to larger females and by larger males. More fecund species did not accumulate more sperm after mating than less fecund species; in fact, the reverse trend emerged. I suggest that these fecund females might be mating more frequently in order to meet the sperm storage demands for their large, numerous broods.

The differences in sperm transfer and copulation duration in these five species matched with differences in their mating ecologies. The two xanthids species that occurred lower in the subtidal and that lived a more cryptic existence had long copulation durations and transferred large ejaculates to females at mating. These males did not react quickly to the presence of a mate and I suggest that the time between mating might be longer for these species. In contrast, the three intertidal and semi-terrestrial species had short copulation durations, small ejaculate transfer and males reacted quickly to the presence of the mate. While there is a large degree of variation in mating systems within these habitats, I suggest that, for these small bodied species, selection for short copulation durations leads to small sperm transfer and has likely decreased the selective pressures on long term sperm storage in those species.

For chapter 2, I conducted an intraspecific study of the variation in reproductive output and sperm storage traits for two species of crabs, *Eurypanopeus depressus* and *Callinectes sapidus.* I tested the hypothesis that reproductive output is high early in the season and at high latitudes (Lardies & Bozinovic, 2006) and that this has important consequences for female sperm storage in *C. sapidus*, a known sperm-storing species. While we did not find consistent patterns in body size with latitude, we did find that egg size generally decreased late in the season, likely due to energetic constraints on yolk accumulation. Moreover, species produce smaller, yet more numerous brood masses late in the season. While there were latitudinal differences in female sperm stores, they did not vary consistently across a latitudinal gradient suggesting that local environmental variation may be a better predictor of variation in sperm storage traits. Since more broods

are produced during the longer reproductive seasons at low versus high latitudes, populations of *C. sapidus* were sperm limited at low latitudes consistent with previous assessments (Hines et al., 2003). Thus, it would be beneficial to include spatial patterns in reproductive output and sperm storage into fisheries models in order to better manage declining stocks.

I conducted a comparative analysis of life history traits across a broad array of species of brachyurans in chapter 3 in order to partition the variation in life history traits among allometric, phylogenetic, environmental, and behavioral factors. I predicted that: (1) life history traits would scale by positive allometry, (2) superfamilies would differ in both reproductive output and sperm storage traits, (3) species at high latitudes and in deep water would have the largest investment in reproductive output and sperm storage traits, and (4) that species with a 'female defense' mating strategy (males directly defend females) would have larger male and female sperm stores than species with 'resource defense' (males defend resources valuable to females) and 'encounter/scramble' (males defend neither females nor resources) strategies (Christy, 1987a).

Most life history traits scaled by positive allometry. Several traits (egg number, female sperm number, male gonopod length and male gonopod volume) displayed consistent allometries, and thus body size explains most of variation in these traits. Phylogeny played an important role in shaping variation in life history traits. Egg size, spermathecal % fullness and female sperm/egg ratio displayed significant phylogenetic patterns, and most of the other life history traits varied allometrically across superfamilies. In

particular, species within the Grapsoidea had negative allometries for most sperm storage traits, suggesting an important role for phylogeny in shaping life history traits.

While latitude and depth explained less of the variation in life history traits, egg size varied significantly by depth. Deep water species had larger eggs than shallow water species, likely due to greater nutrient provisioning as a result of long development times in deep waters. Since egg number decreases with increasing egg size, ecological selection on egg size can have important implications for sperm storage and use. Species with a 'female defense' mating strategy typically had larger male and female sperm stores than species with a 'resource defense' strategy. However, species with 'encounter/scramble' strategies did not significantly differ from those with 'female defense' strategies. I suggest that increased sperm competition in the 'resource defense' strategy has likely led to a small male investment in reproduction in sperm storage in the Ocypodoidea and Grapsoidea.

In chapter 4 I conducted a comparative analysis of the correlated evolution of male and female traits and predicted that (1) life history traits display a significant phylogenetic signal, and that (2) male and female sperm storage traits are correlated in brachyurans after correcting for phylogenetic relatedness. Using permutation tests I found that, while all of the female traits displayed a significant phylogenetic signal, few of the male sperm storage traits varied across the phylogeny. I suggest that male sperm storage traits might be responding more to variation in sperm competition and are thus more variable across the phylogeny. On the other hand, female sperm storage is likely restricted by

reproductive output, a trait that is constrained by superfamilial patterns (Hines, 1982; Hines, 1986). While nearly all male and female traits were correlated prior to correcting for phylogeny, only four of the seven initially tested correlations remained after incorporating phylogenetic relatedness into the model. These results emphasize the importance of incorporating phylogeny into comparative analyses of life history traits in brachyurans.

CHAPTER I

A comparative analysis of sperm storage and mating strategies in five species of brachyuran crabs

ABSTRACT

Variation in female sperm storage across taxa is explained, in part, by the amount of sperm transferred at mating and thus by variation in mating behavior. Several studies that focused on a single species have shown that male sperm transfer can vary with body size, sex-ratio, copulation duration, and season. In this study, I compared the amount of sperm transferred, and thus female sperm storage, across five brachyuran species (*Rhithropanopeus harrisii, Eurypanopeus depressus, Pachygrapsus transversus, Uca beebei,* and *U. terpsichores*) with varying mating strategies. Using mating experiments and field mating observations, I found that species with longer copulation durations transferred more sperm at mating, and this was directly related to variation in their mating systems. The semi-terrestrial species, which rely heavily on vision and for which predation pressure is high during copulation, had shorter copulation durations, transferred less sperm at mating, but were more fecund per brood and per season and had lower sperm/egg ratios. Thus variation in mating behavior shapes, and is shaped by, the evolution of sperm transfer, sperm competition and sperm storage. Comparisons of patterns of sperm transfer across different mating strategies provide a better understanding of the evolution of female sperm storage.

INTRODUCTION

Female sperm storage has evolved multiple times and occurs in a diverse assemblage of taxa from acoel flatworms (Grae & Kozloff, 1999), insects (Eberhard, 1997), decapods (Salmon, 1983), salamanders (Sever et al., 2001), birds (Malecki et al., 2004), to mammals (Taggart & Templesmith, 1991). There is a large degree of variation in how and for how long females store sperm, whether this is externally (MacDiarmid & Butler, 1999), internally in their reproductive tract (Bucklandnicks & Darling, 1993), or in specialized internal sperm storage organs called spermathecae (Sainte-Marie & Sainte-Marie, 1998). It is unclear why only certain species have evolved the ability to store sperm and what factors account for the diverse array of sperm storage tactics. While females obviously benefit from storing sperm, storage also can be costly. For example, attine queen ants suffer reduced immunity with increased sperm storage (Baer et al., 2006). Similarly, sperm storage can decrease female life span (Lung et al., 2002), reduce re-mating frequency, and thus allow males to manipulate female mating behavior (Harmer et al., 2006).

The amount and composition of male ejaculate that is transferred at mating can determine the amount and viability of the sperm stored by the female. In fact, since sperm production can be costly to males (Dewsbury, 1982; Olsson et al., 1997; Wedell et al., 2002), it has led to sperm limitation in some females (Levitan & Peterson, 1995; Shapiro & Giraldeau, 1996), including sperm-storing species (Rondeau & Sainte-Marie, 2001; Gardner & Williams, 2002; Wedell et al., 2002; Hines et al., 2003). When there is consistently high variation in female reproductive success, as with sperm limited species,

species can evolve a polyandrous mating strategy (Bateman, 1948). When females multiply mate, sperm competition within the storage organ can be high, leading to unique reproductive strategies such as cryptic sperm choice by the females (Eberhard, 1997), removal of competitors' sperm by the male (Cordoba-Aguilar et al., 2003), and different sperm allocation tactics (delBarco-Trillo & Ferkin, 2004). Therefore sperm storage, sperm allocation and mating systems are all tightly coupled.

To understand the evolution of sperm allocation and sperm storage and their correspondence with mating behavior, it is important to understand how these traits vary within a species. Males likely alter the amount of sperm transferred to females by varying the duration of mating; however, this is not the case for web-building spiders (Ramos et al., 2005). Since males can transfer both seminal fluid and sperm, a long mating can result from large seminal fluid transfer or the transfer of a sperm plug, rather than from transfer of larger quantities of sperm. Studies have shown that sperm allocation often is correlated with body size. For example, in *Homarus americanus* (American lobster), larger males deliver more sperm to females (Gosselin et al., 2003) and, in *Acheta domesticus* (house cricket), males transfer more sperm to larger, more fecund females (Gage & Barnard, 1996). In addition to small body size, less sperm typically is transferred when mating encounters are frequent. For example, in *Callinectes sapidus* (blue crab), males transfer less sperm during subsequent matings (Jivoff, 1997a; Kendall et al., 2002) and the amount they transfer depends on the time required for males to replenish their sperm stores.

Mating encounters also vary with the synchronicity of female reproductive cycles, which can alter the operational sex ratio (Emlen & Oring, 1977). For example, in some species of fiddler crab, female receptivity is morphologically restricted to a lunar cycle (Christy, 2003), while, in lobsters and snow crabs, mating is typically induced by the ripening of female ovaries (Waddy & Aiken, 1991; Bublitz et al., 2008), which can occur synchronously for some species (Jensen et al., 1996). When females have synchronous cycles, the operational sex ratio approaches equality, or even is skewed toward females, and rate of encounters increases. By manipulating the sex ratio of snow crabs, Rondeau and Sainte-Marie (2001) found that males invested less per mating event when encounter rates were high. The probability of future mating encounters also can shape male sperm transfer. In a simulation model of sperm allocation, males transferred more sperm later in the season when the chances for future reproduction were low (Galvani & Johnstone, 1998). Thus, morphology, mating behavior and ecological factors all influence male sperm transfer within a species. However, it is not known how sperm transfer varies across species and how this potential variation corresponds with differences in their mating systems.

Brachyura, or true crabs, are a model group for comparative research on sperm allocation and storage and its correspondence with mating system dynamics. They all have internal fertilization and possess a spermatheca, and thus can store sperm, at least for the short term (Hartnoll, 1969). Male and female reproductive tracts are paired: females have two spermathecae with separate opercula for each organ, and males have two separate vas deferens tracts, each leading into identical intromittent organs. Males transfer their non-

motile sperm at mating in hundreds to thousands of simple, small spermatophores (Subramoniam, 1991). The amount and composition of transferred seminal fluid likely varies across species, as it forms a hard, transient sperm plug in some species but not in others (Sainte-Marie & Sainte-Marie, 1999). Sperm and seminal fluid are transferred during copulation, which can vary from short encounters in some species of fiddler crab to long copulation durations of up to twelve hours in *C. sapidus*, the blue crab (Jivoff, 1997b). After sperm is transferred to the spermathecae, it can be stored for days, or even up to years (Morgan et al., 1983; Paul, 1984; Yamaguchi, 1998; Wolcott et al., 2005; Penha-Lopes et al., 2006).

Brachyuran mating systems are divided into three broad categories: 'female defense,' 'resource defense' and 'encounter/scramble' (Emlen & Oring, 1977; Christy, 1987a; Orensanz et al., 1995). These strategies were defined by Christy (1987a) due to male mate acquisition strategies and vary primarily in the object of competition by the males, the costs associated with mate searching, and the frequency of mating (Christy, 1987a). In a 'female defense' strategy, males search for and defend females (resulting in prolonged pre- and post copulatory guarding (Jivoff, 1997a; Jivoff & Hines, 1998)) and thus invest large amounts of energy in each mating encounter. Conversely, in a 'resource defense' strategy, males focus their energy on defending resources that are valuable to females. Females can either stay on the male resource until production of the brood or can move among male resources; the latter greatly decreases a male's confidence of paternity (Christy, 1987a). The third mating strategy, 'encounter/scramble,' has been less studied. It differs from the two previous strategies because males neither set up territories

nor directly compete for females, and it can be optimal at either low or very high population densities (Christy, 1987a). In contrast, Orensanz (1995) concluded from his work on cancroid crabs that mating strategies are best defined by the structure of the habitat and by female receptivity patterns rather than by male acquisition strategies. Regardless, all three of these mating strategies have been identified in brachyuran crabs. Patterns in male sperm transfer and in female sperm storage across these mating strategies have not been investigated.

My goal in this study was to determine if there was variation in copulation duration, sperm transfer and sperm storage across five similarly sized brachyurans with different mating behaviors. By determining how variation in these traits varies across species, we can better identify the selective forces shaping the evolution of sperm storage across brachyurans. In some species, males are able to assess female receptivity and thus mate only with females close to spawning, thus increasing their assurance of paternity. Similarly, females can release pheromones (Gleeson, 1991; Bublitz et al., 2008) or elicit behavioral cues to advertise their receptivity to mating (deRivera et al., 2003). If males can assess female receptivity to mating, then I predict that mating is more likely to occur with females of late ovary stages *(mating behavior)*. Given that large male blue crabs copulated for longer than small males, and that males guarded large females for longer periods than smaller females (Jivoff, 2003a), I predict that copulation duration and time until mating are longer for larger males and larger females, and for males with more sperm *(copulation)*.

While longer copulation durations result in larger ejaculate transfer in blue crabs (Jivoff, 1997b), this is not the case for the white butterfly since stimulatory behaviors, sperm plug transfer and sperm removal can all increase copulation duration without increasing the number of sperm transferred (Wedell & Cook, 1999; Cordoba-Aguilar et al., 2003). Given that stimulatory behaviors and sperm removal are unknown in brachyurans, if copulation duration varies across brachyuran species, then I predict that more sperm and a heavier ejaculate are transferred with longer copulation durations, by larger males and to larger females *(sperm transfer)*. Similarly, if large, more fecund females receive more sperm at mating, then I predict that large, fecund females accumulate more sperm after mating *(egg production)*. I predict there are differences between species in each of the four traits (mating behavior, copulation, sperm transfer and egg production) which allows us to identify ecological and behavioral traits that might be driving this variation.

METHODS

Study Species

Five small bodied species of brachyurans, including two shallow subtidal species *R. harrisii*, *E. depressus* (Superfamily: Xanthoidea), and three semi-terrestrial and intertidal species *P. transversus* (Superfamily: Grapsoidea), *U. beebei,* and *U. terpsichores* (Superfamily: Ocypodoidea) were used in this study. Average carapace width of the species ranged from 8 mm for *U. beebei* to 13 mm for *E. depressus,* with average widths of 11 mm for both *P. transversus* and *R. harrisii* and 10 mm for *U. terpsichores*. *Rhithropanopeus harrisii* and *E. depressus* are both cryptic, slow moving crabs that live on oyster reefs. Males likely defend crevices in the reef, but their mating strategies are

unknown. The semi-terrestrial and intertidal species are all conspicuous, fast moving, and susceptible to both aquatic and terrestrial predators. *Pachygrapsus transversus* inhabits rocky intertidal areas and worm reefs, where males defend crevices in the reef. Gravid and non-gravid females seek refuge in the crevices but move among these male-defended resources (resource defense) (Christy, 1987a). *Uca terpsichores* and *U. beebei* live in burrows that are exposed at low tide along sand and mud banks. Females of *U. terpsichores* mate and remain in male burrows until spawning (female defense) where as *U. beebei* has a variable mating strategy. Mating can occur on the sediment surface or in male defended burrows; however, the burrow is not an essential resource for brooding females (Christy, 1987b).

Laboratory Mating Experiments

Rhithropanopeus harrisii, *E. depressus* and *P. transversus* were collected from the field and brought into the lab during the summer of 2006 and 2007. I collected a total of 150 males, 150 females and 10 brooding females of *R. harrisii* from oyster bars along the Chesapeake Bay in 2006 and 2007. For *E. depressus,* I collected 110 males, 110 females, and 10 brooding females along artificial oyster banks at the Virginia Institute of Marine Science, VA, in 2007. I collected 111 male, 111 female, and 18 brooding female *P. transversus* in 2007 along a rocky jetty in the Indian River Lagoon, FL. All crabs were brought back to the laboratory and used for mating experiments.

To prevent sperm transfer from both sides of the paired male reproductive tract during mating, one of the two male gonopods was clipped. To serve as controls for clipping the

male gonopod, 25% of the males used in the experiment were left intact. Each male was held in a separate mating tank two days prior to running the experiment. This allowed for sperm replenishment and acclimation. Mating containers consisted of a 750 ml container with a screen mesh lid. Each container held one 1x1 inch piece of PVC pipe, which mimicked crevices males defend in the field. Three to four females were held in each tank for 2-3 days before the experiment and a white stripe was painted (using nontoxic artist paint) on the dorsal surface of the carapace of each female to distinguish females from males during mating observations. Painting a thin stripe of nontoxic paint has been used routinely in the lab for visualizing behaviors in blue crabs and is not predicted to alter behavior. Only mature, non-gravid females were used in the mating experiments.

Mating experiments were initiated by adding a female to a male mating container. For *E. depressus* and *P. transversus*, approximately 30 mating pairs were run simultaneously, of which 25% [of the 30] were controls. Mating behavior was observed continuously in the 30 mating containers for a total of 8 hours for *E. depressus* and 2 hours for *P. transversus* (preliminary experiments on *P. transversus* indicated that no mating events occurred between 2 and 8 hours in the mating containers). Experiments on *R. harrisii* were run in 2006 and 2007. In 2006, all 50 male gonopods were left intact and in 2007 one side of all of the 100 male gonopods were clipped. Since all pairs were observed simultaneously for *R. harrisii* each year, observations were surveyed in approximately 10 minute increments. Mating status (mated or unmated), copulation duration, and time until mating was initiated, were recorded. Mated and unmated pairs were dissected and the reproductive tissue of the mated pairs was stored for sperm counts.

Field Mating Observations

All observations were made along a mud and sand flat exposed at low tide at the Rodman Naval Base in Panama City, Panama. I observed two species, *U. terpsichores* and *U. beebei*, for roughly 4-5 hours a day at low tide for a total of 10 days. All observations were made during the mating peaks, which occur approximately 12-14 days before the highest spring tides when the high tide corresponded with either dusk or dawn. Areas of high densities of the species were chosen; observation sites and binoculars were used to view details of courtship while minimizing disturbances due to the observer.

Uca beebei was observed mating on the surface of the mud near the entrance to the female burrow. While all 17 of the females that were observed mating were collected, it was possible to collect only 10 of the 17 males (after mating the males scurry to their burrows and are easily lost to the observer). An additional twenty brooding females were collected and saved for sperm and egg counts. Mate searching behaviors by *U. terpsichores* females and the initiation of mate choice, which is signaled by a female entering a male burrow and the male closing the entrance to the burrow, were recorded. To mark the burrows of mating pairs, males were startled just prior to closing the burrow entrance, a few brightly colored beads were dropped into the terminal end of the burrow, and a piece of nylon line with a flexible anchor was threaded into the burrow. The thread was tied to a stake with a brightly colored, numbered flag. Approximately 24 hours later, I carefully dug the pair from the burrow and brought them into the lab for dissections. I collected 20 brooding females by first digging a trench in the sand and then by carefully scraping away layers of sand from the sediment surface to the bottom of the trench until a

burrow with a brooder was identified. All mated pairs and brooders were brought into the lab for dissections.

Dissections and sperm counts

Carapace width, body weight (wet weight) and molt stage were measured for all collected individuals. Males and females were dissected; the stage of females' ovaries ('0'= not visible and highly reduced, '1'= ¼ full, not full of yolk, '2'= developed, mature oocytes present) and egg stage ('1'= yolk, no organ development, '2'= 50% yolk, some organ development, '3'= little to no yolk, eyespots present) were recorded. The percent fullness of the spermatheca was determined visually as the percent of the spermatheca that was occupied by ejaculate contents. For the mated individuals, the reproductive tissue -- female spermathecae and male vas deferens -- were weighed (wet weights) and fixed in 3% glutaraldehyde in a 0.1M sodium phosphate buffer. The fixative was removed after 24 hours and the tissue was rinsed and stored in a 0.1M sodium phosphate buffer. Sperm were counted by first grinding the tissue in a known volume of 0.1M sodium phosphate buffer using a handheld glass Dounce homogenizer. Three subsample counts were conducted on a Petroff-Hausser Counting Chamber. The average sperm number was calculated for each sample. The total weight, percent fullness of the spermatheca, and sperm number after mating were calculated for all species using the spermatheca that received sperm at mating. In the laboratory experiments, ejaculate weight, percent fullness of the spermatheca and sperm number transferred were calculated as the difference between the spermathecae that did and did not receive sperm at mating.

Broods from the egg-bearing females were fixed in 3% glutaraldehyde, and stored in 70% ethanol. Egg number was estimated volumetrically. The egg mass was placed in a known volume of 0.1M phosphate buffer and the volume displaced was recorded +/- 1µl. The eggs were then removed from the pleopods and the volume of water displaced was subtracted from the total egg mass. The average ovum volume was calculated for each brooding female by measuring the diameter of twenty eggs per egg mass at 100x magnification, and the spherical ovum volume was calculated using the formula: *ovum vol. $(mm^3) = (4/3)*\pi*(diameter/2)^3$*. The average egg number for each female was calculated using the equation: *no. of eggs = vol. displaced by the egg mass/ave ovum vol.* The total number of broods per year was obtained from the literature for *R. harrisii* (4 broods) and *E. depressus* (2 broods) (Hines, 1982). Brood production in *U. terpsichores* and *U. beebei* follows a lunar cycle with approximately two brood cycles per month (Christy, 2003). Given that approximately one third of the population reproduces on any given cycle and that the reproductive season spans approximately 7-8 months (Christy, pers. comm.), it was estimated that *U. terpsichores* and *U. beebei* produce approximately 4-6 broods per year. While brood production in *P. transversus* likely follows a lunar cycle, reproductive frequency has not been well described for this species.

Statistical Analysis

All statistical analyses were conducted in SAS v.9. A likelihood ratio G-test was conducted in order to test for a significant association between mating status and ovary stage. Paired t-tests were run using proc t-test in order to examine the differences in sperm number, ejaculate weight, and % fullness of the spermathecae before versus after

mating (α=0.05). ANCOVA models were conducted using the proc glimmix procedure for both data sets from the mating experiments and brooding female collections. Response and explanatory variables are listed in Table 1 and were transformed to meet assumptions of normality and heterogeneity of variance (the best transformation was chosen for each variable). Any negative numbers in the laboratory mating data for sperm transfer were set to zeros. To identify collinearity among explanatory variables, a regression analysis with all continuous variables of interest was examined and the variance inflation estimated. If the variance inflation estimate was >2 for two or more of the variables in the model statement (Graham, 2003), then the primary variable of interest (typically body size) was used in the analysis and the collinear variable removed (the collinear variables are not shown in Table 1).

Differences between species were tested using the least squares means procedure (lsmeans) with a tukey's adjustment. A step-wise model reduction was used to remove insignificant interaction and covariate terms from the model, while non-significant class variables were retained in the model statement. Because separate analyses were run for each response variable, the alpha level was divided by the total number of tests conducted for each data set (0.00625 for mating data and 0.01 for brooding female data). This Bonferroni adjustment prevents inflation of the overall experimental error as a result of conducting multiple tests (Quinn & Keogh, 2002).

RESULTS

(1) Mating behavior: mating is more likely to occur with females of late ovary stages.

Eurypanopeus depressus, *R. harrisii*, and *P. transversus* varied in the percent of pairs that mated in the experiments with 12, 18 and 24 % mating, respectively. A larger proportion of the mated females were characterized by late vs. early stages of the ovary (G_2^2=12.59, p=0.0018) and this pattern was similar for each species (Fig. 1). Therefore, mating was more likely to occur for females with late stage ovaries as was predicted.

While pre-copulatory behaviors were not obvious for *E. depressus* and *R. harrisii*, one distinct behavior, initially described from field observations (Abele et al., 1986), was observed for *P. transversus*. Males tapped and vibrated their legs in a repeated fashion first on the female's legs and then on the dorsal portion of her carapace. This interaction lasted approximately 5 seconds and occurred in 67% of the pairs that mated but in only 23% of the pairs that remained unmated (G_1^2=10.76, p=0.001). However, the male's behavior was not associated with female ovary development (G_2^2=2.56, p=0.28).

(2) Copulation: copulation duration and time until mating are longer for larger males and females.

Copulation duration varied significantly among the four species ($F_{3, 55}$=327.48, p<0.0001) with average durations of 79, 72, 0.59, and 1.3 min for *E. depressus, R. harrisii, P. transversus* and *U. beebei*, respectively. Copulation duration was longer for the shallow subtidal *E. depressus* and *R. harrisii* compared to the semi-terrestrial and intertidal *U. beebei* and *P. transversus* (Fig. 2). Copulation duration did not vary with size of males or females as was predicted, and did not differ with ovary stage, time until mating or with the type of experiment (control vs. experimental).

Time until mating differed across species ($F_{2, 43}=24.95$, $p<0.0001$), with *P. transversus* requiring an average of only 18 minutes before mating, compared to 114 and 154 minutes for *E. depressus* and *R. harrisii,* respectively (Fig. 2). Time until mating was not recorded for *U. beebei* or *U. terpsichores,* since mating was opportunistically observed in the field. Larger males and females did not have longer times until mating as was predicted, and time until mating did not differ with ovary stage, copulation duration or with the type of experiment (control vs. experimental). However, species with long copulation durations typically had a long time until mating (Fig. 2).

*(3) **Sperm transfer:** more sperm and a heavier ejaculate are transferred with longer copulation durations, by larger males and to larger females.*

There was an increase in sperm number and in ejaculate weight in the female spermatheca after mating for each of the three species, respectively (*R. harrisii*: $t_{17}=4.85$, $p=0.0002$, $t_{16}=4.47$, $p=0.0004$; *E. depressus*: $t_8=3.95$, $p=0.0043$, $t_9=3.75$, $p=0.0045$; *P. transversus*: $t_{25}=3.46$, $p=0.0019$, $t_{21}=4.16$, $p=0.0004$) (Figs. 3, 4). The percent fullness of the spermatheca increased after mating for *R. harrisii* ($t_{18}=3.62$, $p=0.0019$) and for *E. depressus* ($t_{12}=2.31$, $p=0.0396$), but not for *P. transversus* ($t_{25}=1.44$, $p=0.1618$) (Fig. 5).

The number of sperm transferred ($F_{2, 39}=11.52$, $p=0.0001$), the weight of the ejaculate transferred ($F_{2, 43}=6.02$, $p=0.005$), and the change in the percent fullness of the spermathecae after mating ($F_{36}=8.76$, $p=0.0008$) differed significantly among species. Male *R. harrisii* transferred the largest number of sperm at mating (1.41×10^7; Fig. 6), produced an ejaculate that weighed 1.45g (Fig. 7) and increased the percent fullness of the spermatheca by 30% after mating (Fig. 8). While male *E. depressus* transferred fewer

sperm than *R. harrisii* (4.86×10^6, Fig. 6), it produced the heaviest spermathecal load (1.75g, Fig. 7) and the greatest increase in spermathecal fullness (45%, Fig. 8). In contrast, *P. transversus* transferred 9.45×10^5 sperm (the least of the three species, Fig. 6), transferred the lightest ejaculate (0.18g, Fig. 7), failed to produce a significant increase in the percent fullness of the spermatheca (Fig. 8), and had the largest percent fullness of the spermatheca prior to the observed mating (Fig. 5).

The number of sperm transferred increased with female body size ($F_{1, 39} = 12.93$, $p = 0.0009$), and the relationship between percent fullness of the spermatheca and female body size varied by species ($F_{1, 36} = 11.71$, $p = 0.0016$): the increase in percent fullness of the spermatheca after mating increased with female body size for *E. depressus* and *R. harrisii* but not for *P. transversus*. The change in percent fullness of the spermatheca after mating increased with male body size ($F_{1, 36} = 10.03$, $p = 0.0031$), and the number of sperm transferred and the change in percent fullness of the spermatheca increased with copulation duration ($F_{1, 39} = 29.90$, $p < 0.0001$; $F_{1, 36} = 17.49$, $p = 0.0002$), respectively. Therefore, as predicted, more sperm and a larger ejaculate were transferred with longer copulation durations, by large males and to large females.

Spermathecal load after mating

Spermathecae of recently mated females were approximately 60% full while those of females that did not mate during the experiment were on average 10% full. Thus, recently mated females had significantly more full spermathecae after mating ($F_{1, 323} = 160.03$, $p < 0.0001$). Weight of the spermathecal load and sperm number were not calculated for

crabs that did not mate during the experiment and thus not tested in this analysis. The percent fullness of the spermathecae after mating increased with female size ($F_{1, 323}$=24.94, p<0.0001) and with ovary stage ($F_{1, 323}$=10.12, p=0.0016), and this varied by species for ovary stage ($F_{4, 323}$=4.31, p=0.0021). *Rhithropanopeus harrisii* exhibited the greatest increase in percent fullness of the spermatheca after mating with increasing ovary stages (from 7.5% full at stage 0 to 62.3% full at 'stage 2.') while the spermatheca of *U. terpsichores* was less full at later ovary stages; however, only stages '0' and '1 were recorded for this species. Overall the percent fullness of the spermatheca after mating did not differ significantly by species. However, *R. harrisii* had the largest percent fullness of the spermatheca after mating and the greatest increase in percent fullness after mating, and *P. transversus* had the least change in percent fullness of the spermatheca after mating (Fig. 3).

The weight of the spermathecal contents ($F_{4, 75}$=36.32, p<0.0001) and the number of sperm in the female spermatheca ($F_{4, 76}$=35.79, p<0.0001) after mating varied significantly across the five study species. *Rhithropanopeus harrisii* had the most sperm after mating (average 1.54×10^7), and then *E. depressus* (average, 8.27×10^6) (Fig. 3). Their post-mating spermathecal contents weighed more than twice as much as those of *P. transversus*, *U. beebei*, or *U. terpsichores* (Fig. 4). *Rhithropanopeus harrisii* had a significantly heavier spermatheca weight than all other species (Fig. 4), and *P. transversus, U. terpsichores* and *U. beebei* had less sperm than *R. harrisii* while *E. depressus* had more sperm than *P. transversus* (Fig. 3). Large females had heavier spermathecal loads ($F_{1, 75}$=58.69, p<0.0001) and more numerous sperm ($F_{1, 76}$=9.67, p=0.0026) after mating than smaller females.

27

Therefore, the amount of sperm and ejaculate accumulated in the spermatheca after mating increased with female size and varied across species.

(4) Egg production: larger, more fecund females accumulate more sperm after mating.

Average egg number per brood for each species ($F_{4, 65}=17.31$, $p<0.0001$), as well as the average egg number per year ($F_{3, 49}=41.48$, $p<0.0001$), varied significantly for the five species (Table 2). Egg number per brood and egg number per year increased with increasing female body weight ($F_{1, 65}=155.95$, $p<0.0001$; $F_{1, 49}=1, 110.4$, $p<0.0001$) and decreased with increasing egg size ($F_{1, 65}=17.62$, $p<0.0001$; $F_{1, 49}=14.48$, $p=0.0004$). The number of sperm in brooding females increased with carapace width and the equation of the line varied by species ($F_{3, 60}=576.15$, $p<0.0001$). Percent fullness of the spermatheca ($F_{1, 69}=14.07$, $p=0.0004$) and spermatheca weight ($F_{1, 67}=35.84$, $p<0.0001$) increased with female carapace width. Species differed in the average percent fullness of the spermatheca of the brooding females ($F_{4, 69}=11.70$, $p<0.0001$) (Fig. 5). *Eurypanopeus depressus* had the least full spermatheca but the largest sperm number remaining after brood production (Figs. 3, 5). Species also differed in sperm to egg ratios in brooding females ($F_{4, 68}=8.14$, $p<0.0001$), with *R. harrisii* (8,100) and *E. depressus* (3,700) having larger ratios than *P. transversus* (230, Fig. 9). Sperm used (or wasted) in fertilizing an egg can vary from 1 to as many as 170 sperm (Parker, 1970; Hines et al., 2003). Using only the high sperm to egg ratio estimate (170), *E. depressus* and *R. harrisii* can fertilize multiple additional broods, while *U. beebei* and *U. terpsichores* can fertilize up to two additional broods; *P. transversus* can fertilize only one additional brood (Table 2).

Species with high numbers of eggs per brood did not have larger amounts of sperm after mating $(F_{1, 3}=8.37, p=0.63)$ nor did species that produce larger number of eggs per year $(F_{1, 2}=4.18, 0.18)$ as was predicted. In fact, the reverse pattern appeared to emerge: more fecund females generally had less sperm in the female spermatheca after mating than less fecund females for these five species (Fig. 10). However, this was not statistically significant, likely due to the small sample size, and more studies are needed to verify or refute this potentially interesting finding.

DISCUSSION

Variation in male sperm transfer associated with varying mating systems can have strong implications for the evolution of female sperm storage. Sperm transfer and storage patterns have been well documented within certain species. In taking a comparative approach, I was able to document variation in patterns of male sperm transfer across five species of brachyurans with different body sizes, female receptivities and mating patterns.

(1) Mating behavior

Even though *E. depressus*, *R. harrisii* and *P. transversus* are morphologically receptive to continuous mating, males were more likely to mate with females with late ovary stages regardless of female size and fecundity. In other sperm-storing animals such as reptiles (Mendonca & Crews, 2001), insects (Okutani-Akamatsu et al., 2007), and lobsters (Waddy & Aiken, 1991), males also mate with females in later reproductive stages. This assures that females will have enough viable sperm to fertilize their eggs, and increases

the males' confidence of paternity for that clutch (Urbani et al., 1998). The visual and physiological cues that females can use to advertise their receptivity are quite diverse, ranging from a nonaggressive stance in hyenas (Szykman et al., 2007) and distinct rejection behavior by unreceptive sepsid flies (Teuschl & Blackenhorn, 2006; Baena & Eberhard, 2007) to long distance pheromones in the red-back spider (Perampaladas et al., 2008) and in the blue crab (Gleeson, 1991). Uridine DiPhosphate (UDP), a byproduct released from ripening ovaries, recently was identified as a pheromone responsible for eliciting mate searching behavior in several species of brachyurans (Bublitz et al., 2008).

While it is unclear from this study which of these mechanisms is driving mating behavior for each of the five species of crabs studied, visual cues are known to play an integral role in communication and mate attraction in the semi-terrestrial and intertidal species *P. transversus* (Abele et al., 1986), *U. beebei* and *U. terpsichores* (Crane, 1975; Salmon, 1983). Semi-terrestrial and intertidal species rely heavily on their well developed visual system for mate detection and predator avoidance (Christy, 1987a). The rapid initiation of mating and short copulation time of *P. transversus* males, and their pre-copulatory leg tapping behavior, indicates that males of this species respond quickly to the presence of females. Since this unique leg tapping behavior of *P. transversus* was not directed more towards females with late stage ovaries, males are likely responding to the visual presence of females rather than to pheromones such as UDP. Semi-terrestrial and intertidal crabs also use their visual systems for predator avoidance. They must avoid both terrestrial and aquatic predators and thus typically respond quickly to any visual

stimulation (Christy, 1987a), which likely explains the necessity for shorter copulation durations in order to reduce the risk of predation while in copula.

Visual communication between male-female pairs was observed for neither *E. depressus* nor *R. harrisii.* The turbidity of the shallow subtidal habitat has lead to their cryptic life-styles and suggests that chemical cues probably play an important role in their mating, foraging and predator avoidance strategies (Bublitz et al., 2008). Mating pheromones have been identified in several subtidal decapods such as the blue crab, spiny lobster, and American lobster (Raethke et al., 2004). In fact, one study discovered that, in blue crabs, visual cues alone would not elicit courtship behaviors in males without the presence of female molting hormone (Gleeson, 1991). Pheromones are most effective across large distances and in turbid waters. With decreased risk of predation by visual predators in these two cryptic subtidal species, the risk of being preyed upon while mating is likely lower. Thus, they can have longer copulation durations and can spend longer searching for and attracting mates, and males can invest more in each mating event. *Eurypanopeus depressus* and *R. harrisii* also had generally larger sperm to egg ratios than the semi-terrestrial and intertidal species suggesting a possible role for sperm storage for these species. In fact, sperm storage has been documented in the laboratory for *R. harrisii* (Morgan et al., 1983). Therefore, variation in mating behavior of these three species likely shapes mate attraction and copulation duration patterns, and has the potential to influence female sperm storage.

(2 & 3) Copulation & Sperm transfer

Longer copulation durations resulted in a larger number of sperm transferred and in fuller spermathecae, but not necessarily in the transfer of heavier ejaculates, since males can transfer differing amounts of both sperm and seminal fluid at mating. For example, *E. depressus* transferred the largest ejaculate weight at mating but not the most sperm. Therefore, weight of the ejaculate is not synonymous with sperm number. Males of *E. depressus* likely transferred more seminal fluid than sperm, which accounted for the heavier weight. Seminal fluid can act to increase sperm viability, reduce microbial activity (Hinsch, 1991), or function as a sperm plug which reduces sperm competition within the female storage organ (Wedell & Cook, 1999). Species differences in the composition of their ejaculates could be related to different degrees of sperm competition or due to differences in the length of time sperm are stored in the female tract. This would be an interesting direction for further investigation.

Male and female size influenced the amount of sperm transferred at mating. Thus, males alter their optimal mating strategy with a female based on her body size. Since larger females within a species are more fecund (Hines, 1982), males allocate more of their sperm reserves to larger females. This pattern has been well described in the American and spiny lobsters, and in the brachyuran crab *Hemigrapsus sexdentatus* (Sainte-Marie, 2007). By processing both visual and physiological information on female size and receptivity, males may be able to maximize their reproductive success. In fact, in some spider crabs, males approach and visually assess the brood stage of females on their territory in order to determine the optimal time for mating (Christy, 1987a). Thus male

mating decisions in many brachyuran species may be based on multiple decision factors and may be much more complex than initially thought.

Like blue crabs (Jivoff, 1997b), in these five species of crabs, males with large sperm reserves transferred large numbers of sperm at mating. This relationship held across species: species with large male sperm reserves typically mated for longer durations. *Eurypanopeus depressus* and *R. harrisii* both had large male sperm reserves, long copulations, and longer times until mating. They both inhabit oyster reefs and likely mate within the crevices of the reef, and are thus less susceptible to predation while mating, allowing longer mating durations. Consequently, they are able to invest more in each female by transferring more sperm and a heavier ejaculate (Wolcott et al., 2005).

Variance in mating patterns of the semi-terrestrial and intertidal species in this study is well documented. *Uca beebei* and *P. transversus* occur in dense clusters, have intricate visual and acoustic displays (Crane, 1975), and are highly susceptible to both aquatic and terrestrial predators while courting. Not surprisingly, they have short copula durations. In fact, I observed two pairs of mating *U. beebei* eaten by avian predators while in copula on the sediment surface, hence the need for short mating interactions. While *P. transversus* mates in or near male defended crevices, females move continuously from crevice to crevice (Christy, 1987a; Brockerhoff & McLay, 2005). Sperm competition is likely high in this system, as evidenced by the large percent fullness of the female spermatheca prior to mating. Thus, males had short copulation durations, and invested less sperm per female.

While pre-copulatory displays and searching are costly for *U. terpsichores,* they have reduced predation pressure during copula by mating inside the male burrow. Pairs stay in the burrow until the female produces an egg mass, at which point the male leaves to build a new burrow (Christy et al., 2002). This behavior not only protects them from predation, but reduces sperm competition. While it is unknown how long the pairs mate or if they mate repeatedly while inside the burrow, copulation duration is no longer restricted by predation pressure and this species theoretically could have longer copulations inside burrows. It would be particularly useful to compare male sperm transfer in surface mating versus burrow mating *U. beebei,* since local variations in ecological traits have been known to shape mating systems (Brockerhoff & McLay, 2005). In this study, *U. beebei* only were observed to mate on the surface of the sediment, and no females were observed entering a male's burrow to mate.

(4) Egg Production

The duration of copulation explained the amount of sperm transferred at mating but not the total sperm accumulation after mating. Instead, females with larger body sizes tended to accumulate more ejaculate. These large females may be mating more frequently (as occurs in the American lobster (MacDiarmid & Butler, 1999)), have longer copulation durations, or both. Surprisingly, when fecundity (egg number) was measured, not only were more fecund species not accumulating more sperm after mating (before oviposition), but the relationship appeared to be inversely related: more fecund species may accumulate less sperm after mating than less fecund species. The species with higher fecundity, *U. beebei* and *P. transversus* mated for shorter amounts of time. These more

fecund semi-terrestrial and intertidal species might be mating more frequently in order to accumulate enough sperm for their egg production. In fact, they may need to re-mate each time they produce a brood. In contrast, *E. depressus* and *R. harrisii* receive more sperm at each mating and have higher sperm to egg ratios (higher than ratios that have been calculated for known sperm-storing brachyurans (Hines et al., 2003)) than the semi-terrestrial and intertidal species studied. These results suggests that they have the potential to fertilize multiple broods after mating, and that they may rely on stored sperm to fertilize multiple broods (Morgan et al., 1983). Therefore, species that mate frequently, and for shorter durations, are more likely to mate prior to producing a brood. Variation in mating strategies has potentially important implications for the evolution of sperm storage patterns.

When sperm competition is high, as was likely for the semi-terrestrial and intertidal species in this study, males have been known to remove rival's sperm (Cordoba-Aguilar et al., 2003; Wada et al., 2005), guard the female after mating (Jivoff & Hines, 1998) and produce sperm plugs (Snow et al., 2006). None of these strategies were exhibited by any of the species in the present study. Instead, they seemed to transfer small ejaculates per mate. While male snow crabs increase the amount of sperm they transfer when sperm competition increases (Rondeau & Sainte-Marie, 2001), selection for reduced copulation duration for *U. beebei* and *P. transversus* may limit the ability for males to transfer larger amounts of sperm. Therefore, it might be optimal for these species to transfer smaller ejaculates to multiple mates; however we did not directly measure mating frequency in this study. This strategy is likely optimal in *P. transversus,* given the large percent

fullness of the spermatheca prior to additional matings; this would leave little room for males to transfer large amounts of ejaculate at mating and increase the potential for sperm competition. Therefore, species differences in fecundity combined with variation in mating behaviors may best explain female sperm storage patterns.

CONCLUSIONS

A possible ecological trend in mating systems and sperm storage patterns is identified for five study species of intertidal, semi-terrestrial and subtidal brachyurans. More sperm and larger ejaculate volume was correlated with longer copulation durations. The semi-terrestrial and intertidal species, *U. beebei* and *P. transversus,* had shorter copulation durations and thus transferred less sperm than the subtidal species *R. harrisii* and *E. depressus*. As predicted, larger bodied males transferred more sperm at mating than smaller species, and this relationship held across species. Males transferred more sperm, but not ejaculate weight, to larger than smaller females and were more likely to mate with females with later than earlier ovary stages, but did not provide the females with late ovary stages with more sperm. While larger bodied females accumulated more sperm after mating (likely securing more mating events) than smaller bodied females, the more fecund species tended to accumulate a smaller total amount of sperm after mating and could fertilize a smaller number of broods after mating than the more fecund species; sperm storage might be less prevalent in the former species.

Species that live in 'risky' habitats for mating, such as *U. beebei* and *P. transversus*, have short copulation durations and males transferred small amounts of sperm at mating. On

the other hand, *R. harrisii* and *E. depressus* live cryptically in crevices within the reef, had longer copulation durations, and transferred more sperm at mating than *U. beebei* and *P. transversus*. This suggests that selection on copulation duration can have strong implications for the amount of sperm transferred at mating and stored in the female spermatheca. By examining sperm allocation in a comparative framework, we have identified ecological factors that might be driving differences in the mating systems and thus sperm storage patterns of these five small-bodied brachyuran species.

Table 1: The explanatory and response variables used in mixed models for the mating experiments (top box) and for surveyed brooding females (bottom box). Variable transformations are displayed in parentheses (the best transformation was chosen for each variable). All interactions between fixed factors (c) and covariates, and between the fixed factors were tested (not shown). Explanatory variables with the largest correlation with the response variables are indicated by *.

Response Variable	Explanatory Variables
(H2) Copulation	
Copulation duration ($\log_{10}$) width	species(c), type(c), ovary stage, ♀ carapace width ($\log_{10}$), ♂ carapace ($\log_{10}$),
Reaction time ($\log_{10}$)	species(c), type(c), ovary stage, ♀ carapace width ($\log_{10}$), copulation duration($\log_{10}$)*, ♂ carapace width ($\log_{10}$)
(H3) Sperm transfer	
Weight transferred (geometric)	species(c), ovary stage, ♀ carapace width ($\log_{10}$), copulation duration ($\log_{10}$)*, ♂ carapace width ($\log_{10}$)
Sperm no. transferred (geometric)	species(c), ovary stage, ♀ carapace width ($\log_{10}$), copulation duration ($\log_{10}$)*, ♂ carapace width ($\log_{10}$)
% Full spermatheca transferred (geometric)	species(c), ovary stage, ♀ carapace width ($\log_{10}$), copulation duration ($\log_{10}$)*, ♂ carapace width ($\log_{10}$)
Weight spermatheca ($\log_{10}$) _(after mating)_	species(c), type(c), ovary stage, ♀ carapace width ($\log_{10}$), copulation duration ($\log_{10}$)*
Sperm number ($\log_{10}$) _(after mating)_	species(c), type(c), ovary stage, ♀ carapace width ($\log_{10}$), copulation duration ($\log_{10}$)*
% Full spermatheca [+0.0001 (arc sin)] _(after mating)_	species(c), type(c), ovary stage, ♀ carapace width ($\log_{10}$)*, mated(c),
(H4) Egg production	
Egg no. per brood ($\sqrt{}$)	species(c), ovary stage, egg size, ♀ body weight ($\log_{10}$)*
Egg no. per year ($\sqrt{}$)	species(c), ovary stage, egg size, ♀ body weight ($\log_{10}$)*
Sperm no.	species(c), ♀ carapace width ($\log_{10}$)
% Full spermatheca ($\log_{10}$)	species(c), ♀ carapace width ($\log_{10}$)
Weight spermatheca ($\sqrt{}$)	species(c), ♀ carapace width ($\log_{10}$)

Table 2: Average egg number per brood and estimated average egg number per year for brooding female *R. harrisii, E. depressus, P. transversus, U. beebei*, and *U. terpsichores.* Average female sperm number after mating and the number of additional broods that can be produced based on the number of sperm remaining in brooding females (using sperm egg ratios of 1 and *170).

Species	Egg diameter (um)	Egg no./brood	# Broods/year	Egg no./Year	Female sperm number	Number of additional fertilizable broods
R. harrisii	328 +/- 9	1901 +/- *532*	4	7,603 +/- *4,251*	1.54×10^7 +/- *1.22×10^6*	multiple, *multiple
E. depressus	307 +/- 8	2263 +/- *1021*	2	4,526 +/- *1021*	8.27×10^6 +/- *1.00×10^6*	multiple, *multiple
P. transversus	287 +/- 5	4935 +/- *908*	?	?	1.11×10^6 +/- *1.81×10^5*	multiple, *1
U. beebei	229 +/- 4	4028 +/- *1192*	5	15,263 +/- *2,467*	1.08×10^6 +/- *1.24×10^5*	multiple, *2
U. terpsichores	262 +/- 7	6016 +/- *966*	5	24,066 +/- *1,432*	1.97×10^6 +/- *2.29×10^5*	multiple, *2
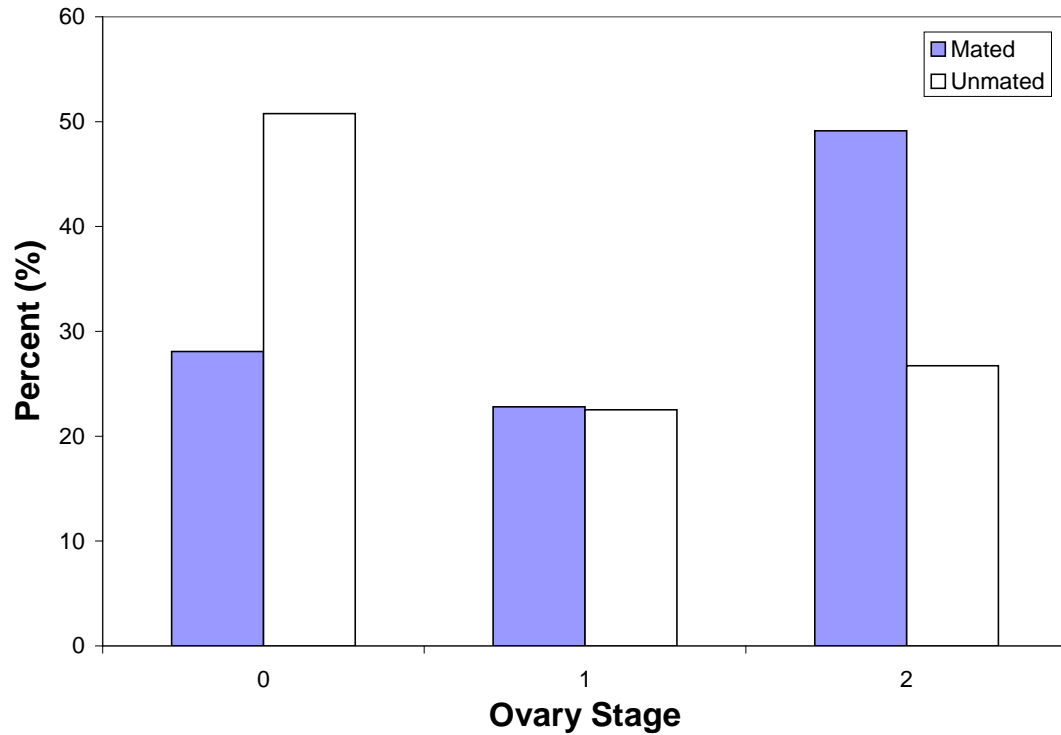
Figure 1: The percent of females of a given ovary stage (0=immature, 1= 50% developed, 2=mature) for both mated and unmated crabs of *R. harrisii*, *E. depressus*, *P. transversus*, *U. terpsichores* and *U. beebei* (all species showed similar patterns) Crabs that mated had later ovary stages than unmated crabs (G_2^2=12.59, p=0.0018).
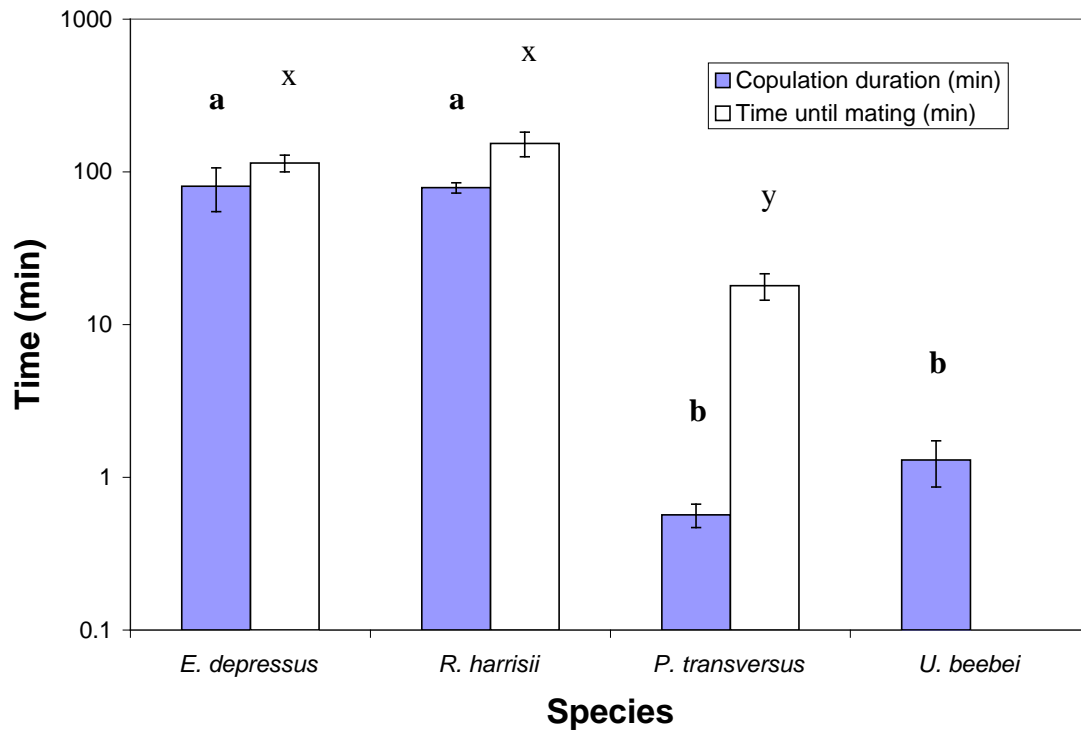
Figure 2: Copulation duration in minutes (back transformed) +/- 1 SE of mated pairs of *E. depressus, R. harrisii* and *P. transversus* from laboratory experiments and *U. beebei* from field mated pairs (grey), and time until mating in minutes (back transformed) +/- 1SE of mated pairs *E. depressus*, *R. harrisii* and *P. transversus* (white). Different letters indicate significant differences (p<0.00625) between species from separate ANOVAs with species and type as fixed effects. *E. depressus* and *R. harrisii* had longer copulation times than both *P. transversus* and *U. beebei* and had a longer time until mating than *P. transversus*.
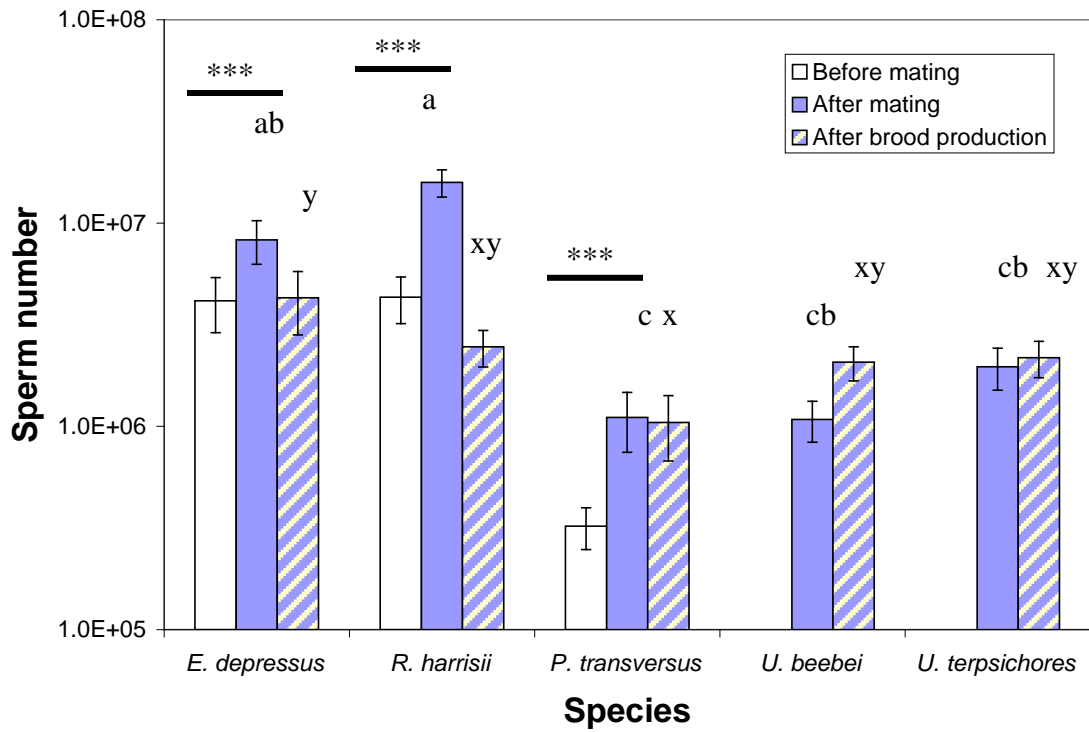
Figure 3: The average sperm number in females +/- 1SE before mating (open bar), after mating (solid bar) and in brooding females (diagonal lines) for females of five different species of brachyuran crabs. Different letters represent significant differences after mating (p<0.00625) from an ANCOVA with species and type as fixed effects and female body size as the covariate, and significant differences in sperm number of brooding females (p<0.01) from an ANCOVA with species as the fixed effect. Horizontal bars represent paired t-tests conducted on the before and after values for each species (***=significant).
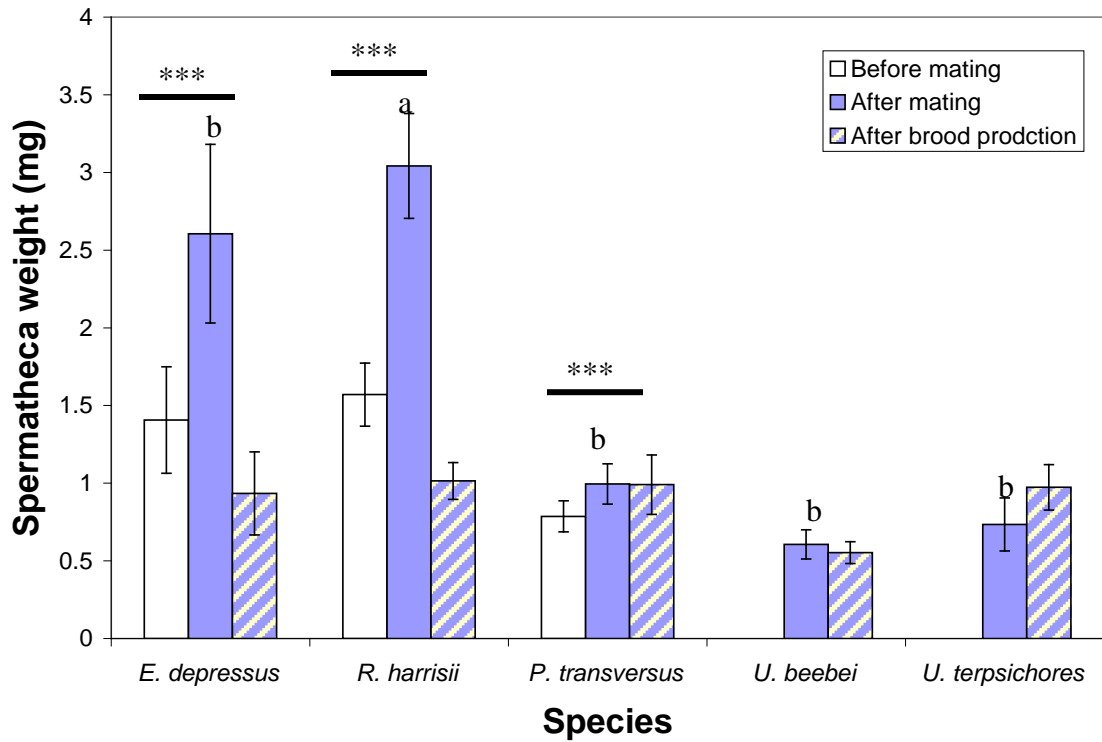
Figure 4: The average weight of the female spermatheca (mg) +/- 1SE (back transformed) before mating (open bar), after mating (solid bar) and in brooding females (diagonal lines) for females of five different species of brachyuran crabs. Different letters represent significant differences in female spermathecae weight after mating from an ANCOVA with significant species and female carapace width effects. Horizontal bars represent paired t-tests conducted on the before and after values for each species (***=significant).
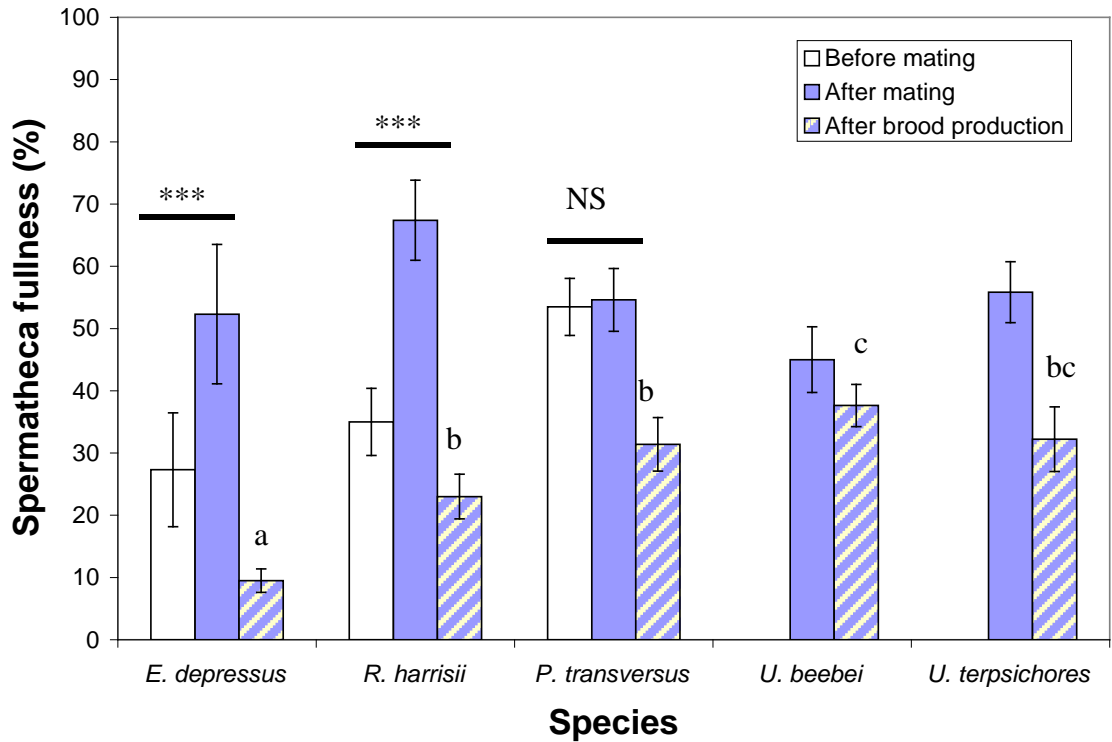
Figure 5: The average percent fullness of the female spermatheca +/- 1SE before mating (open bar), after mating (solid bar) and in brooding females (diagonal lines) for five different species of brachyuran crabs. Different letters represent significant differences between brooding females (back transformed) based on an ANCOVA model with species as the fixed effect and female carapace width as the covariate. Horizontal bars represent paired t-tests conducted on the before and after values for each species (NS=not significant, ***=significant).
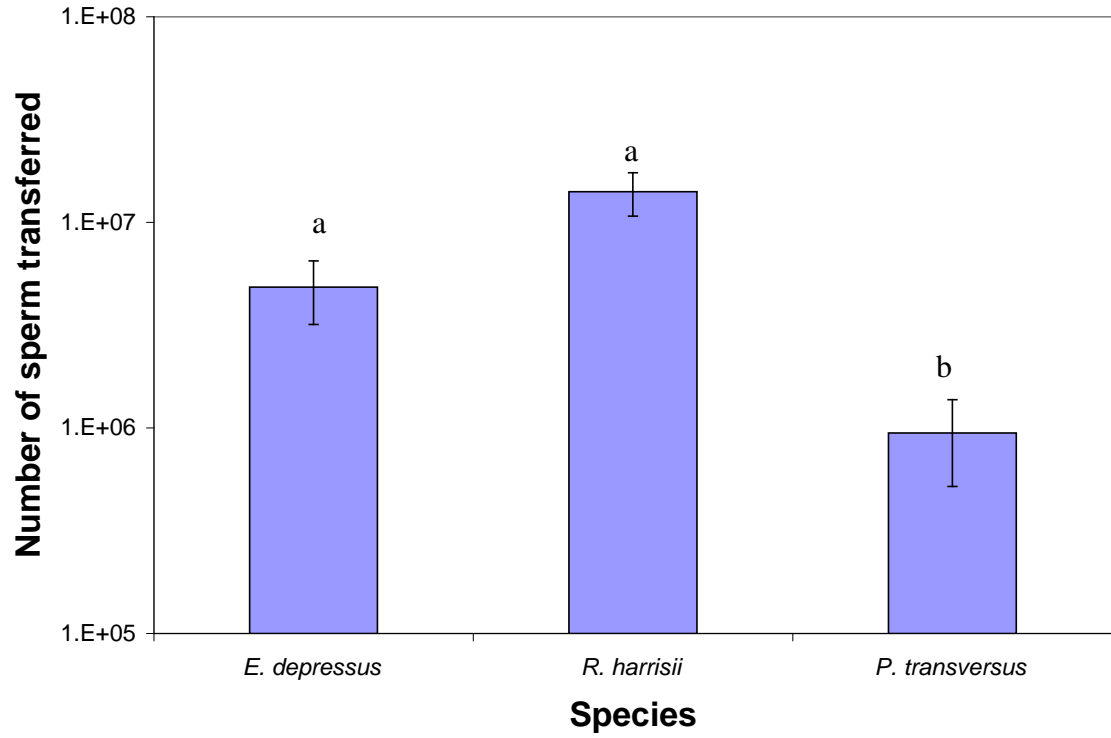
Fig. 6: The number of sperm transferred at mating +/- 1SE for three species of brachyuran crabs. Different letters represent significant differences ($p < 0.00625$) in number of sperm transferred across species from an ANOVA with species as the fixed effect.
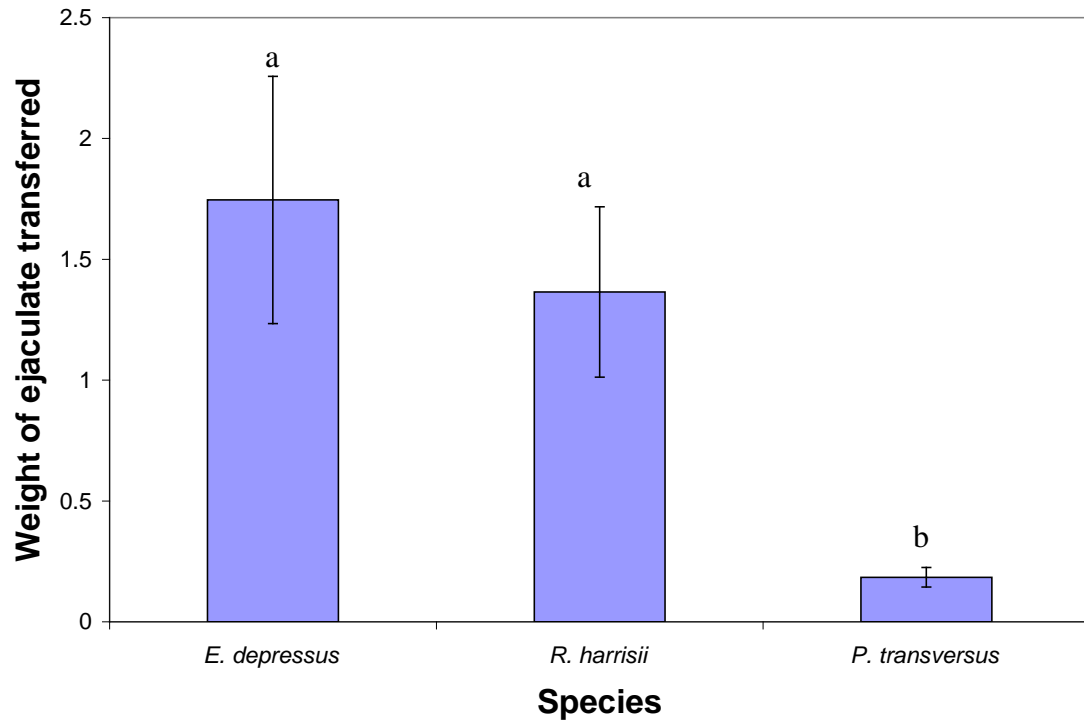
Fig. 7: The weight of the ejaculate transferred at mating +/- 1SE for three species of brachyuran crabs. Different letters represent significant differences (p<0.00625) in the weight of the ejaculate transferred across species from an ANOVA with species as the fixed effect.
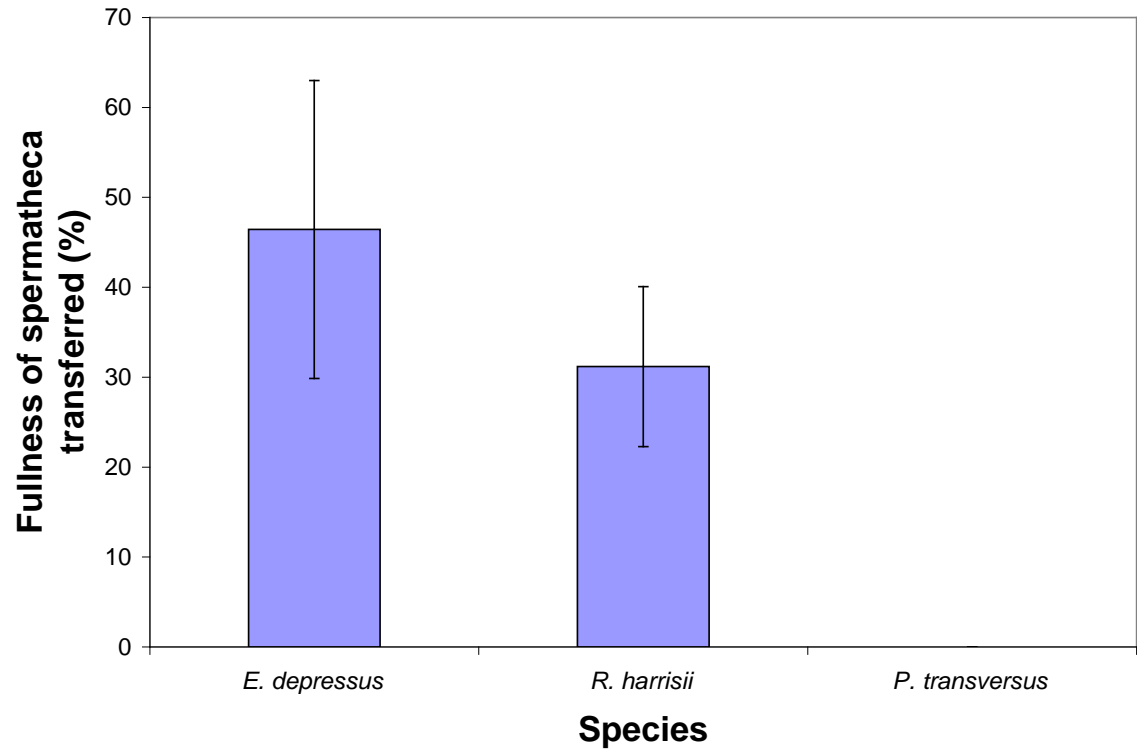
Fig. 8: The increase in the percent fullness of the female spermatheca at mating +/- 1SE for three species of brachyuran crabs.
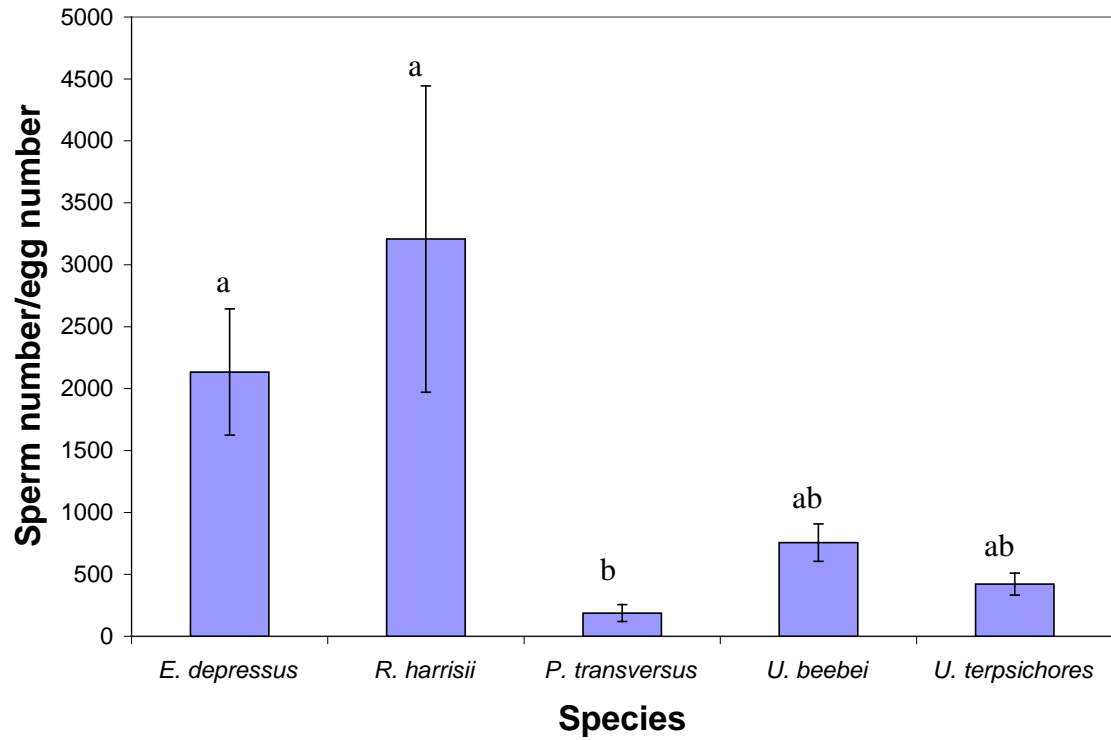
Figure 9: Sperm to egg ratio in brooding females +/- 1 SE for the five study species. Different letters represent significant differences (p<0.00625) from an ANCOVA with species as the fixed effect. Species differed significantly in the sperm to egg ratios of brooding females with *P. transversus* having the lowest ratio.
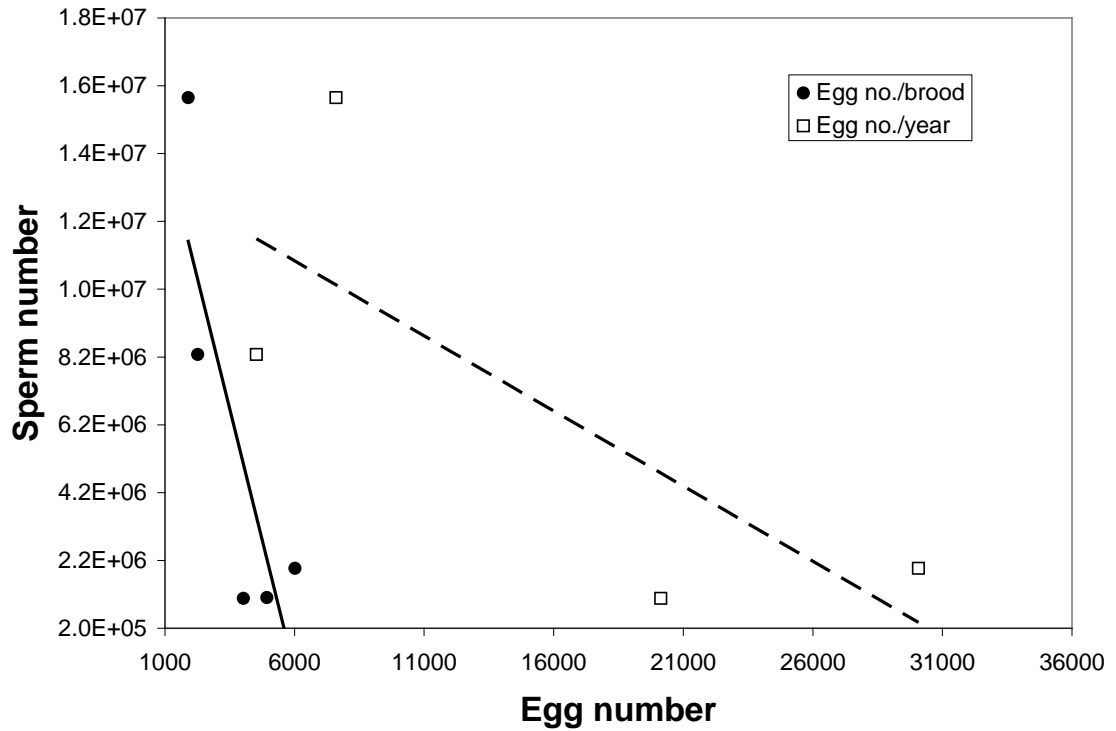
Figure 10: The relationship the number of sperm in female spermatheca after mating (back transformed) versus number of eggs per brood (black circles), and number of eggs per year (white squares). Equations: *egg no. per brood = -3.09x10³*(sperm no. in mated females) + 2x10⁷, r²=0.70, p=0.63*; egg *no. per year = -4.43x10²*(sperm no. in mated females) + 1x10⁷, r²=0.58, p=0.18.*

CHAPTER II

Geographic and seasonal variation in reproductive output and sperm storage of fished, *Callinectes sapidus,* and un-fished, *Eurypanopeus depressus,* (Brachyura) species

ABSTRACT

Plasticity in life history traits in response to ecological variation alters reproductive output and sperm storage within a species. Previous research has demonstrated that body size varies by latitude, following Bergmann's (larger at higher latitudes), converse Bergmann's (smaller at higher latitudes) or countergradient variation (varies depending on genetic compensation) trends, and also varies across the season. To better define the influence of ecological variation on reproductive output and sperm storage, I measured body size, egg traits and sperm storage across a latitudinal gradient (from New Jersey to Florida) and across the season for two species, *Eurypanopeus depressus* and *Callinectes sapidus*, with different life history strategies. *Eurypanopeus depressus* mates repeatedly throughout the breeding season, whereas *C. sapidus* mates once and stores sperm for its lifetime production of broods. *Callinectes sapidus* is also a fished species and fishing pressure varies by location, thus shaping body size and population dynamics. Both species typically produced smaller yet more numerous eggs later in the season when chances for future reproduction were low. Sperm/egg ratios decreased across the season for *C. sapidus* but remained constant for *E. depressus*. In fact, *C. sapidus* became sperm limited at lower latitudes and was predicted to become depleted of stored sperm by the

50

end of the sampling year. While broad latitudinal trends can explain some of the variation in reproductive output, variation at smaller scales is likely responsible for a large portion of the differences in reproduction. Particularly, variation in fishing pressure can alter life history traits when fishing pressure changes by location or by season. Future studies should focus on smaller scale patterns in order to define which ecological factors are driving these differences.

INTRODUCTION

Life history theory predicts that species will maximize fitness by optimally allocating resources between reproduction and survival (Cody, 1966; Stearns, 1992). Differences in how species, and individuals, allocate these resources are shaped by ecological factors which can vary both spatially and across the season (Lack, 1948; Pianka, 1970). Cody demonstrated that clutch size in birds increased with increasing latitude, and thus broad ecological trends could explain variation in reproductive output (Cody, 1966). Ecological trends in body size, which can have a profound impact on life history traits (Reaka, 1986; Hines, 1992; Kolm et al., 2006a), are well known and three opposing trends have emerged; with increasing latitude, body size (1) increases - Bergman's Rule (Bergmann, 1847; McNab, 1971), (2) decreases - converse Bergmann's Rule (Mousseau, 1997), and (3) variable – countergradient variation (Conover & Schultz, 1995).

While Bergmann's Rule was initially described for homeotherms (Bergmann, 1847; McNab, 1971) it has been shown to generally hold true for ectotherms (Reaka, 1986; Partridge & Coyne, 1997). The underlying mechanisms that have been proposed to explain this rule vary from surface to volume ratios (McNab, 1971), the influence of

temperature on cell size (Van Voorhies, 1996), physiological constraints (Atkinson & Sibly, 1997), high predation pressure at low latitudes (Hines, 1989), and developmental trade-offs (Blanckenhorn & Demont, 2004). However, it has been argued that shorter season length at high latitudes leads to converse Bergmann's Rule in some ectotherms (Mousseau, 1997), and that selection can act in opposition to ecological factors, leading to a countergradient pattern (Conover & Schultz, 1995). These forces are not mutually exclusive, since principles of both Bergmann's and converse Bergmann's Rule have been found in the same species, leading to sex specific differences (Defeo & Cardoso, 2002) and to intermediate patterns (Blanckenhorn & Demont, 2004). While all of these latitudinal trends have been identified in the literature, Bergmann's Rule has generally received the most support for arthropods (Blanckenhorn & Demont, 2004).

Not only does body size shape reproductive output (Hines, 1982; Charnov & Ernest, 2006), but reproductive investment can vary with latitude (Heibo et al., 2005; Traynor & Mayhew, 2005) and across the season. Individuals that mature late in the season can become energy depleted leading to smaller body size and a smaller reproductive investment (Magrath et al., 2009). Generally, a large reproductive investment is described at high latitudes since less energy is allocated to metabolism and more energy is reserved for reproduction at these cooler temperatures (Brante et al., 2004; Lardies & Bozinovic, 2006). In contrast, at low latitudes, the reproductive season is long and there is the potential for iteroparous species to produce many more broods per season, leading to a large investment in reproduction by the end of the reproductive season.

While different combinations of these trends in life history traits have been identified for various taxa, less is known about how these ecological patterns influence female sperm storage, and depletion, in sperm-storing species. Patterns of sperm storage rely heavily on mating strategies and on the reproductive output of the female. If reproductive output varies with latitude or season, then sperm-storing species can become sperm limited, depending on their geographical location and on seasonal patterns (Hines et al., 2003). For example, long breeding seasons at low latitudes can lead to sperm limitation towards the end of the reproductive season for sperm-storing species; this is not likely for species that do not store sperm. These patterns can be confounded by anthropogenic stressors, such as overfishing. Fisheries often target large males disproportionately and create a skewed sex ratio, which in turn alters mating system dynamics (Kendall et al., 2001; Gardner & Williams, 2002; Alonzo & Mangel, 2004). When large males are removed, females are left to mate with smaller, less fecund males. Since small males often transfer less sperm at mating (Gosselin et al., 2003; Sato et al., 2006) and require longer amounts of time to replenish their sperm stores after mating than large males (Kendall et al., 2002), females that mate with these small males can be sperm limited (Hines et al., 2003). These factors can interact synergistically or antagonistically with latitudinal trends in reproductive output and impact sperm storage patterns of females.

Since the amounts of sperm that are stored across successive broods is determined by the reproductive output of the female and frequency of brood production, this study investigated reproductive output and sperm depletion in two species of brachyurans (*Callinectes sapidus* and *Eurypanopeus depressus*) with different life history strategies

across a latitudinal gradient. Brachyuran crabs are ideal study organisms since reproductive output varies with body size (Hines, 1982), and females possess a sperm storage organ (Hartnoll, 1969) but vary in the amount and length of time they store sperm depending on species and circumstances.

Callinectes sapidus is a large-bodied species that inhabits soft sediment habitats in estuaries and bays and sustains valuable fisheries in the US from New Jersey to the Gulf Coast (Uphoff, 1998). Females mate only once during their terminal molt to maturity, at which point they receive their lifetime supply of sperm. Females store this sperm across the season and even across years to fertilize their life time production of broods, which can be greater than 18 clutches (Hines et al., 2003). Due both to intense fishing pressure, in which large males are removed from the population, and to the large number of broods produced, females are at risk of becoming sperm limited (Hines et al., 2003). Conversely, *E. depressus* is a small bodied species that is commercially unexploited yet plays an important ecological role on oyster reefs. While females possess a sperm storage organ, they mate during the intermolt phase, are continuously receptive to mating and thus capable of mating after each spawning event. Therefore, sperm limitation is less likely for this species.

The objective of my research was two-fold: to identify latitudinal and seasonal patterns in female reproductive output; and to examine the potential for sperm limitation seasonally and spatially across species with different life history strategies. Given that crustaceans primarily follow Bergmann's Rule, with larger body size, more eggs, and larger egg size

at higher latitudes (Lardies & Castilla, 2001) and early in the season (Morgan et al., 1983), and that sperm storage patterns vary between the two species, I tested several predictions: (1) body size, body weight, egg number and egg size decrease with decreasing latitude (Bergmann's Rule) for both species, (2) due to the longer reproductive season at lower latitudes, spermathecal load and sperm number are predicted to decrease with decreasing latitude for both species and sperm-egg ratios decrease with decreasing latitude for the sperm storing species *C. sapidus*, yet remain constant for the continuously receptive species *E. depressus*, (3) egg number and egg size are smaller later versus early in the season due to seasonal depletion of nutrients, and (4) spermatheca weight, sperm number, and sperm-egg ratio decrease late in the season for *C. sapidus* yet remain constant across the season for *E. depressus* since they can re-mate after producing a brood.

METHODS

Field Collections

I collected brooding female blue crabs, *C. sapidus,* and mud crabs, *E. depressus*, from the field during the reproductive season in 2008. When possible, collections were made both early and late in the season for both species. Collection dates varied by location to reflect the differences in timing and length of the season at different latitudes. *Callinectes sapidus* were collected from local fishermen and researchers using trot lines and pots in four different geographic locations: 25 *C. sapidus* from Tuckerton, New Jersey (16 in June and 9 in August 2008), 25 from Gloucester, Virginia (all in May 2008), 39 from Beaufort, North Carolina (24 in May and 15 in September 2008), and 16 from Sebastian

Inlet, Florida (all in March 2008) (Fig. 1). *Eurypanopeus depressus* were collected by hand along oyster and rock banks from three different geographic locations: 50 *E. depressus* from Virginia (25 in May and 25 in September 2008), 26 in North Carolina (all in May 2008), and 47 from Florida, (25 in May, 23 in September 2008) (Fig. 1). All specimens were returned to the lab for further analyses.

Dissections

Individuals were sealed in plastic bags, frozen and stored for no longer than three months in a -20°C freezer. Each crab was weighed, carapace width measured, and the egg mass removed. *E*gg masses of *E. depressus* were fixed in 3% glutaraldehyde overnight and then rinsed in 0.1M phosphate buffer and stored in 70% Ethanol. *Callinectes sapidus* broods were stored frozen; however, a sub-sample of 20-30 eggs was fixed in glutaraldehyde, rinsed in buffer, and then stored in 70% ethanol. Each crab was dissected and the ovary stage recorded ('0'= not visible or highly reduced, '1'= ¼ full, not full of yolk, '2'= developed, mature oocytes present). One of the two spermathecae was removed from each crab, weighed, and fixed overnight in 3% glutaraldehyde solution and stored in 0.1M phosphate buffer.

Egg traits

A random sub-sample of twenty-eggs from each brood, previously fixed in 3% glutaraldehyde, was used for measurements of egg size. The average ovum volume was calculated for each brooding female by measuring the diameter of twenty eggs per egg mass at 100x magnification, and the spherical ovum volume was calculated using the

formula: *ovum vol. (mm^3) = (4/3)*π*(diameter/2)^3*. The average egg stage was recorded ('1'= mostly yolk with no visible organ development, '2'= 50% yolk with some organ development, '3'= developed eyespots, little to no yolk).

Egg counts were conducted using a volumetric method of displacement of the egg mass in a 0.1M phosphate buffer for all samples, with the exception of the *C. sapidus* egg masses from Florida, which were thawed and measured in 70% ethanol (these samples were shared with another researcher). For each egg mass, the pleopods with attached eggs were separated from the abdomen, blotted with paper to remove excess liquid, placed in a known volume of liquid (either phosphate buffer or ethanol) and the volume displaced recorded to the nearest +/- 1 µl. To subtract the volume of the pleopods from the egg volume, the eggs were stripped from the pleopods using tweezers and the pleopods again placed in a known volume of 0.1M phosphate buffer.

Egg number was calculated from the known volume displaced by the egg mass and the average volume of an egg from that brood:

Egg no. = ave. egg vol. x (vol. displaced by egg mass and pleopods – vol. displaced by pleopods).

The average volume of individuals eggs for *C. sapidus* were measured a second time in the same solution used for the volumetric method (either ethanol or 0.1M phosphate buffer). Therefore, the measures for average egg volume used for the egg number calculations were treated identically to those in which the egg mass was sampled volumetrically.

Sperm traits

Each spermatheca was ground using a glass, hand-held Dounce® homogenizer in a known volume of 0.1M phosphate buffer. A subsample of the homogenate was placed on a Petroff-Hausser Counting Chamber® and allowed to settle for 1-2 minutes before adding a coverslip. Three replicate sperm counts per individual were conducted at 100x using phase contrast microscopy. The number of sperm was calculated from the average of the replicate counts and the known volume of the counting chamber:

(no. of sperm = no. of sperm in subsample x ml)/ (vol. /square x no. of squares counted).

Sperm-egg ratio was calculated for each individual and the number of additional broods that could be fertilized from the remaining stored sperm was calculated using known sperm-egg ratios of 20:1 for *C. sapidus* (calculated from Hines et al. 2003) and 60:1 for *E. depressus* (calculated from Rodgers, chapter 1 of this dissertation).

Statistical Analyses

<u>Univariate Models</u>

ANCOVAs models were conducted in SAS v.9.1 using the proc mixed procedure for each species (analyzing species together caused severe deviations from normality). Response and explanatory variables are listed in Table 1 and were transformed to meet assumptions of normality and heterogeneity of variance. Season was modeled as a class variable (early, late) and geographic location as a continuous variable, latitude (1=Florida, 3=North Carolina, 4=Virginia, and 5=New Jersey; numbers represent relative distances between locations) (Underwood, 1997). To avoid problems of collinearity with numerous correlated response variables, we first chose the explanatory variable with the

highest Pearson's correlation coefficient and then included other explanatory variables that had a variance inflation estimate (vif) <2 (Mansfield & Helms, 1982; Graham, 2003).

A step-wise reduction model was used to remove insignificant interactions and covariate terms from the model; however, non-significant factors of interest (latitude and season) were kept in the model statement. Because separate analyses were run for each response variable, we adjusted the alpha level by dividing it by the number of tests conducted for each data set, α=0.0071. This Bonferroni adjustment prevents inflation of the overall experimental error as a result of conducting multiple tests (Quinn & Keogh, 2002). Since not all locations were sampled both early and late in the season, this created an unbalanced design. To alleviate problems associated with unbalanced designs, two reduced data sets, (1) balanced location – collections made only early in the season, and (2) balanced season - only locations with collections made both early and late in the season, were analyzed in addition to the full data set. Significant latitude and season effects were only reported if they were also significant in the reduced data sets.

Multivariate Models

Multivariate models were conducted for each species in Primer v.6. Individuals with missing variables were excluded from the analysis. Each response variable (egg number, egg size, spermatheca weight, sperm number, sperm egg ratio, ovary stage and egg stage) was transformed to meet assumptions of multivariate normality and then regressed against either female carapace width or body weight (Table 1). If the regression was significant, the residuals were used as the response variables. Since sperm/egg ratios were

significantly collinear with egg number and with sperm number (vif >2), it was removed as a response variable. Response variables were normalized in Primer ($\mu = 0$, $\sigma = 1$) and the resemblance matrix constructed using Euclidean distances (Clarke et al., 2006). I tested for significant clustering due to latitude and season using an analysis of similarity (ANOSIM), a nonparametric method which uses permutations to test for significant clustering due to a priori groupings (Clarke et al., 2006). The relative contribution of each variable to the differences between groups was examined using a similarity percentage routine (SIMPER). The % contribution of each variable to the average dissimilarities (squared distances) between groupings was calculated as well as the contribution corrected for the standard deviation (sq. dist/sd).

RESULTS

Body Size/Bergman's Trend

Callinectes sapidus weighed more and had larger carapace widths than *E. depressus* (Table 2). Species weighed more (*C. sapidus*: $F_{1, 102}$=22.50, p<0.0001; *E. depressus*: $F_{1, 119}$=37.98, p<0.0001) and had larger carapace widths (*C. sapidus*: $F_{1, 102}$=31.06, p<0.0001; *E. depressus:* $F_{1, 119}$=26.43, p<0.0001) early than late in the season. However, carapace widths of *E. depressus* varied across the season differently in Florida vs. Virginia ($F_{1, 119}$=8.59, p=0.004). Few heavy and large *E. depressus* and *C. sapidus* individuals were collected late versus early in the season and a large number of small and light size class individuals were collected late in the season in Florida for *E. depressus* (Fig. 2) and in North Carolina and New Jersey for *C. sapidus* (Fig. 3). At lower latitudes, *C. sapidus* had larger carapace widths ($F_{1, 102}$=8.01, p=0.0056) indicative of converse Bergmann's Rule (Fig. 4). *Eurypanopeus depressus,* on

the other hand, displayed a polynomial trend with increasing latitude for both body weight ($F_{1,\ 119}=15.60$, $p=0.0001$) and carapace with ($F_{119}=12.65$, $p=0.0005$), where individuals weighed more and were larger at higher latitudes (Fig. 4).

Egg Traits

Egg size increased with egg development in both species (*C. sapidus*: $F_{1,\ 101}=34.30$, $p<0.0001$; *E. depressus*: $F_{1,\ 116}=63.95$, $p<0.0001$). Egg size was larger early in the season for both species (*C. sapidus*: $F_{1,\ 100}=34.20$, $p<0.0001$; *E. depressus*: $F_{1,\ 119}=19.32$, $p<0.0001$). While there was no significant latitudinal trend for either species, *C. sapidus* collected in Virginia had larger eggs ($p<0.0005$) than at any of the other locations, even after correcting for variation in egg stages (Fig. 5). After using a conservative approach of first removing the effect of egg stage and then testing for differences between locations using a sequential ANCOVA, egg size was still largest in Virginia ($p<0.0005$).

Egg number per brood increased with increasing body weight in both species (*C. sapidus*: $F_{1,\ 97}=73.00$, $p<0.0001$; *E. depressus*: $F_{1,\ 116}=79.48$, $p<0.0001$), decreased with egg development for *C. sapidus* (*C. sapidus*: $F_{1,\ 97}=20.18$, $p<0.0001$), and decreased with increasing egg size *(E. depressus*: $F_{1,\ 116}=25.99$, <0.0001). There were no significant seasonal or latitudinal effects on egg number for *E. depressus*. However, in *C. sapidus,* egg number increased late in the season ($F_{1,\ 97}=20.52$, $p<0.0001$, $\beta=0.96$) and increased with decreasing latitude ($F_{1,\ 97}=34.92$, $p<0.0001$); egg number was lowest in Virginia (Fig. 5).

Sperm Traits

Spermatheca weight in the female increased with carapace width (*C. sapidus*: $F_{1, 98}=56.99$, p<0.0001; *E. depressus*: $F_{1, 120}=26.88$, p<0.0001). It varied by latitude only for *C. sapidus* where spermatheca weight displayed a third order polynomial trend with latitude ($F_{1, 98}=20.69$, p<0.0001), with the heaviest spermatheca for females in North Carolina and the lightest for females in Virginia (Fig. 6).

Sperm number in female spermathecae increased with carapace width (*C. sapidus*: $F_{1, 101}=22.45$, p<.0001; *E. depressus*: $F_{1, 118}=8.57$, p=0.0041) and increased at a greater rate late in the season for *E. depressus* ($F1, _{118}=14.88$, p=0.0002). Sperm number followed a concave polynomial trend with latitude for *E. depressus* with the lowest numbers at mid latitudes ($F_{118}=11.59$, p=0.0009) (Fig. 6), but did not vary with latitude in *C. sapidus*.

Sperm-egg ratios were lower later in the season for *C. sapidus* ($F_{1, 99}=30.57$, p<0.0001). Sperm-egg ratios were greatest for *C. sapidus* at mid-latitudes, following a polynomial trend ($F_{1, 99}=8.92$, p=0.0035) (Fig. 6). However, for *E. depressus* they showed a reverse pattern, lowest in North Carolina and highest in Florida and Virginia ($F_{1, 115}=10.86$, p=0.0013) (Fig. 6). Given the relatively small sample sizes for *C. sapidus*, results from power analyses for all of the statistical analyses presented above can be found in Appendix I.

The total number of additional broods that could be fertilized from the sperm remaining in the spermatheca for individuals collected at each location and across the season was

calculated for *C. sapidus* (Fig. 7). Crabs collected from New Jersey and Virginia had enough sperm to fertilize more than six broods. Assuming they produced three broods per year (Hines et al. 2003, in review), this would support at least two additional years of brooding. However, blue crabs in North Carolina retained enough sperm to fertilize 5-11 additional broods. Assuming they produced 5-8 broods per season (Dickinson et al. 2006; Hines et al., in review), their remaining sperm stores would be enough for 1-2 additional brooding seasons. Remaining sperm stored in the Florida population were enough to fertilize 6 additional broods, which would be enough only for one additional season of 6-8 broods (Hines et al., in review).

Multivariate Analysis

There were significant effects of season (R=0.13, p=0.005) and latitude (R=0.309, p=0.001) for *C. sapidus.* The largest differences between latitudes occurred between Florida and Virginia (R=0.537, p=0.001), Virginia and North Carolina (R=0.41, p=0.001), and Virginia and New Jersey (R=0.322, p=0.001) (Fig. 8). From the SIMPER analysis, the contribution of spermatheca weight and ovary stage explained the largest amount of the dissimilarity between the early and late collections (Table 3). In general, egg size explained a large amount of the dissimilarity of the crabs collected from Virginia compared to the other three sites (Table 3). While latitude (R=0.075, p=0.001) and season (R=0.05, p=0.009) were significant for *E. depressus*, the low R values indicate little separation between the groupings, are considered to be of little importance, and thus not analyzed further.

DISCUSSION

Variation in ecological factors across a latitudinal gradient and across the season can drastically alter life history traits. This has important implications for sperm storage and use. If species rely on stored sperm to fertilize their lifetime supply of broods, they can become sperm limited towards the end of the season and in locations where total reproductive output is large. By examining variation in reproduction across a latitudinal gradient, we can identify patterns of potential sperm limitation and thus devise effective management policies, particularly for exploited species.

Body Size/Bergman's Trend

Species exhibited opposite latitudinal trends in body size and weight; *E. depressus* had smaller body size at intermediate to low latitudes (similar to Bergmann's Rule) and *C. sapidus* were smallest at high latitudes (converse Bergmann's Rule). It is not unusual for related species, such as these two brachyuran species, to display different patterns in body size with latitude. For example, in a comprehensive survey of the Hymenoptera, both latitudinal trends were found within this group (Traynor & Mayhew, 2005). There may not be one simple explanation for body size patterns in ectotherms, and species patterns may vary according to their behavior and physiology (Angilletta & Dunham, 2003). One striking difference between the two species in the present study is in their growth patterns – *C. sapidus* females have determinate growth, *E. depressus* has indeterminant growth; therefore, foraging efficiency and food availability during *C. sapidus's* limited time until maturity may be more important in determining final body size than physiological processes. *E. depressus,* on the other hand, continues to grow

across multiple years and may be more directly influenced by the physiological constraints described by Bergmann's Rule (Lonsdale & Levinton, 1985).

Another conspicuous difference that can affect body size of the two species is fishing pressure. High levels of fishing pressure were responsible for an 8% reduction in female body size of the *C. sapidus* population in the lower Chesapeake Bay (Lipcius & Stockhausen, 2002). State regulations differ in both the intensity of fishing permitted and in the sex-specific removal of *C. sapidus*. For example, egg-bearing females are fished at certain times of the year in Virginia and in North Carolina (Henry, 1998; Sharov et al., 2003), whereas their removal is prohibited in Florida and New Jersey (Steele & Bert, 1998; Stehlik et al., 1998). Thus fishing pressure would reduce female body size in Virginia and North Carolina by selective removal of large females. In fact, fishing pressure could be altering the ecological trend in this species of large body size at mid latitudes. Since life history traits of the Virginia population differed significantly from the other populations, with the majority of the variation explained by egg number and egg size, local differences in fisheries practices may be responsible for placing selection pressure on life history traits. Regardless of selection due to the fisheries, it is clear that the life histories of the two species are shaped differently by ecological factors across the latitudinal gradient.

While both species were smaller and weighed less late compared to early in the season, differences in population abundance and differential mortality likely explains these patterns. Although *E. depressus* continues to molt past maturity, the larger individuals

may die off towards the end of the season, leaving behind a large number of small, new recruits into the population. *Callinectes sapidus* does not molt after maturity and thus the larger sizes early in the season could be due to both differential mortality of larger individuals late in the season and due to differences in the size at maturity for crabs that mature early versus late in the season. Regardless of the source of this seasonal difference in body size, the smaller body size late in the season has potentially important consequences for mating interactions and for fecundity.

Egg Traits

Consistent with life history theory, there was a trade-off between egg number and egg size for both species and this varied across the season (Smith & Fretwell, 1974; Bass et al., 2007). *Callinectes sapidus* produced more numerous, smaller eggs later in the season and thus could be 'hedging their bets' by investing less in each offspring late in the season when chances for future reproduction are low (Morgan et al., 1983; Lips, 2001; Simons, 2007). Conversely, individuals could simply be depleted of energy stores and ovarian yolk proteins by the end of the season, leading to smaller and possibly inferior eggs (Brante et al., 2004). While *E. depressus* produced smaller eggs late in the season, they did not produce more eggs, giving further support to the idea that seasonal depletion of yolk leads to smaller eggs late in the season. Thus, investment in reproduction, and reproductive output, can vary within a species across the season.

Egg number varied by latitude only for *C. sapidus*. The larger, less numerous eggs produced at higher latitudes could be the result of physiological processes described by

Bergmann's Rule. Low temperatures result in lower metabolic costs and thus larger cell size (Lardies & Castilla, 2001). Egg number typically declined with larger egg size; however, the most northern population, New Jersey, had a low number of eggs and also had one of the smallest egg sizes. Females from this population are investing less in reproduction than populations at other locations. Rather than a consistent trend in egg number and size across both species, reproductive variation can best be explained on smaller spatial and temporal scales.

Recent studies have shown a large degree of variation in reproductive output at small spatial scales (Lester et al., 2007). These patterns can be driven by predation pressure (Polovina, 1989), density (Lestang et al., 2003), food supply, and/or productivity (Dugan et al., 1991; Ruttenberg et al., 2005; Castilho et al., 2007). A combination of the above factors can lead to a patchwork of variation in reproductive output (Bass et al., 2007). For example, factors such as food availability and population density have been used to explain small scale geographic variation in life history traits of several brachyuran species (Hines, 1989).

Sperm Traits

There was significant spatial variation in the number of sperm stored in the spermathecae for *E. depressus* and in the weight of the spermathecal load for *C. sapidus.* The two species displayed opposite trends in sperm storage. Males pass both seminal fluid and sperm to females (Jivoff, 2003b), and the weight of the female storage organ depends on the relative composition of each of these two male components. Since sperm storage

varied by latitude even after correcting for body size and reproductive output, it is likely that ecological differences played an important role in determining the amount of ejaculate females received at mating (Sato et al., 2006). Males can vary spatially in sperm production (Evans & Magurran, 1999; Carver et al., 2005) and could be allocating different starting amounts of ejaculate due to differences in mating history (Wedell & Cook, 1999; Sato et al., 2006), female size (Jivoff, 1997b) or by population structure (Gage & Barnard, 1996; Rondeau & Sainte-Marie, 2001). Similarly, males may allocate sperm differently within a location due to seasonal variation (Galvani & Johnstone, 1998), thereby drastically altering the amount of sperm received by females and thus the sperm-egg ratio.

Not surprisingly, *C. sapidus* has lower sperm to egg ratios late in the season, as they used up their sperm reserves to fertilize broods. The sperm-egg ratio decreased further at lower latitudes late in the season, as more broods were produced at low latitudes, causing females to deplete their sperm reserves. *Eurypanopeus depressus*, on the other hand, displayed no seasonal change in sperm-egg ratios, since they re-mated throughout the breeding season and thus did not become depleted of sperm stores.

It cannot be determined from examining a female brachyuran how many broods she has produced that season or in her lifetime, but general patterns for brood production exist along a latitudinal gradient. Females in the northern regions, with shorter brooding seasons, produce fewer broods than those in the south, which have longer reproductive seasons. For *C. sapidus*, overall lifetime reproductive output is much smaller in northern

latitudes than in the North Carolina and Florida populations, where up to eight broods can be produced within a single reproductive season (in contrast to the 2-3 broods for New Jersey populations; (Hines et al., 2003; Dickinson et al., 2006)).

Geographic variation in brood size and number will alter the potential for sperm limitation in a species that stores sperm. There was no decrease in sperm-egg ratios across the season for *E. depressus,* while there was for *C. sapidus* than in *E. depressus*. This is a further indication that *E. depressus* re-mate after spawning. While there is still the potential for sperm limitation in *E. depressus* due to alterations in male sperm allocation, the potential for sperm limitation is much greater in *C. sapidus*. *C. sapidus* females from NC and FL had lower sperm-egg ratios than females from NJ and VA, and they are likely to become sperm limited within a single season, whereas NJ and VA females are not likely to become sperm limited due to the fewer number of broods per season at higher latitudes. However, Hines et al. (2003) showed that sperm stores in Virginia were significantly lower than those in Florida and thus sperm limitation is possible due to fishing pressure. A combination of latitudinal variation in the number of broods per season, geographic variation in egg number and sperm allocation, and seasonal variation in egg number all shape the total lifetime reproductive output and the potential for sperm limitation in these crabs (Hines et al., 2003).

CONCLUSIONS

A single latitudinal trend across all life history traits could not be detected in either of the two study species. Complex interactions between life history traits and species-specific

physiology and behavior have brought about geographic variation in reproductive output. This has important implications for sperm limitation in brachyuran species. If females become sperm depleted by the end of the season in lower latitudes, fisheries might be permitted to collect brooding females only towards the end of the reproductive season, while it might be advantageous to protect the largest males from removal (having both a minimum and a maximum capture size). Although latitudinal trends are often related to temperature, recent work has shown that moisture, environmental stress, and seasonality all correlate with latitudinal trends and explain significant variation in reproductive output (Quin et al., 1996; Stillwell et al., 2007). Studies that examine variation in reproductive output and sperm storage at smaller spatial scales could identify which ecological variables are responsible for creating these patterns. Regardless, fisheries management and conservation strategies of the blue crab could be enhanced by incorporating the spatial variation in sperm stores and reproductive output defined in this study.

Table 1: Explanatory and response variables tested in the ANCOVA proc mixed procedures. Transformations for each variable are displayed in parentheses. All interactions between class variables (c) and covariates and class variables were tested (not shown).

Response Variable	Explanatory Variables
Body weight ($\log_{10}$)	latitude, laitude^2, latitude^3, time(c)
Carapace width	latitude, latitude^2, latitude^3, time(c)
Egg number ($\log_{10}$)	egg stage, ovary stage, egg size, time(c), body weight ($\log_{10}$), latitude, latitude^2, latitude^3
Egg size	egg stage, egg number ($\log_{10}$), ovary stage, time(c), body weight ($\log_{10}$), latitude, latitude^2
Spermatheca weight ($\surd$)	carapace width*, latitude, latitude^2, latitude^3, time(c), ovary stage
Sperm number ($\surd$)	carapace width*, ovary stage, time(c), latitude, latitude^2, latitude^3
Sperm egg ratio ($\surd$)	body weight ($\log_{10}$), egg stage, ovary stage, time(c), latitude, latitude^2, latitude^3

Table 2: The reproductive output of brooding female *E. depressus* and *C. sapidus* from the four study sites collected early and late in the season are listed below. Numbers in parentheses are +/- 1 standard error from the mean. The predicted number of broods that could be fertilized for each species was calculated from the residual sperm number in females, the average egg number, and from known values of sperm used/wasted for each egg (predicted # broods).

Species	Location	Season	Sample size	Body weight (g)	Spermatheca (mg)	Egg #	Egg diameter (μm)	Sperm no.	Sperm-egg ratio	Predicted # broods
C. sapidus	Tuckerman, NJ	Early	15	158 (+/-7)	194 (+/-18)	$1.63E^6$ (+/-$1.76E^5$)	261 (+/-5)	$3.33E^8$ (+/-$9.9E^7$)	209 (+/-60)	9
		Late	9	144 (+/-13)	198 (+/-24)	$2.72E^6$ (+/-$3.73E^5$)	241 (+/-4)	$1.88E^8$ (+/-$4.55E^7$)	113 (+/-53)	3
	Gloucester, VA	Early	25	186 (+/-8)	246 (+/-14)	$1.39E^6$ (+/-$1.55E^5$)	288 (+/-2)	$5.49E^8$ (+/-$5.48E^7$)	466 (+/-52)	18
	Beaufort, NC	Early	24	219 (+/-12)	377 (+/-21)	$2.26E^6$ (+/-$1.24E^5$)	267 (+/-4)	$5.22E^8$ (+/-$5.04E^7$)	252 (+/-33)	10
		Late	15	141 (+/-10)	175 (+/-12)	$1.98E^6$ (+/-$1.76E^5$)	246 (+/-3)	$1.67E^8$ (+/-$3.14E^7$)	87 (+/-14)	4
	Sebastian Inlet, FL	Early	16	199 (+/-13)	309 (+/-30)	$4.01E^6$ (+/-$5.05E^5$)	261 (+/-3)	$5.62E^8$ (+/-$8.23E^7$)	161 (+/-37)	6
E. depressus	Gloucester, VA	Early	25	0.88 (+/-0.07)	1.38 (+/-0.10)	$6.03E^3$ (+/-$7.65E^2$)	314 (+/-5)	$8.63E^5$ (+/-$1.81E^5$)	204 (+/-49)	2
		Late	25	0.61 (+/-0.04)	1.38 (+/-0.12)	$4.66E^3$ (+/-$5.19E^2$)	295 (+/-4)	$1.92E^6$ (+/-$4.26E^5$)	448 (+/-101)	7
	Beaufort, NC	Early	26	0.54 (+/-0.04)	1.06 (+/-0.11)	$4.096E^3$ (+/-$3.92E^2$)	314 (+/-5)	$3.87E^5$ (+/-$7.16E^4$)	113 (+/-22)	2
	Fort Pierce, FL	Early	25	0.65 (+/-0.06)	1.21 (+/-0.15)	$3.62E^3$ (+/-$3.45E^2$)	318 (+/-4)	$1.58E^6$ (+/-$3.03E^5$)	502 (+/-84)	7
		Late	23	0.34 (+/-0.04)	0.71 (+/-0.09)	$2.59E^3$ (+/-$2.89E^2$)	309 (+/-3)	$7.71E^5$ (+/-$1.50E^5$)	303 (+/-48)	5

Table 3: Relative influence of response variables to the pairwise differences between the groupings 'season' and 'latitude.' for *C. sapidus*. The average squared distance corrected for the standard deviation (Sq. Dist/SD) and the percent contribution of the variable to the average group squared distances (% Contribution) are represented in the table below.

Factor	Variables	Sq. Dist/SD	% Contribution
Season -early vs. late	Spermatheca weight	0.86	21.24
	Ovary stage	0.86	20.58
	Egg stage	0.74	15.09
	Sperm number	0.73	17.72
	Egg size	0.58	15.56
Latitude: -North Carolina vs. Virginia)	Egg size	1.14	18.99
	Spermatheca weight	0.97	25.22
	Egg stage	0.89	16.62
	Egg number	0.80	13.45
	Ovary stage	0.79	15.20
	Sperm number	0.72	10.52
Latitude: -New Jersey vs. Virginia	Egg stage	0.98	24.37
	Egg size	0.86	20.66
	Sperm number	0.84	22.74
	Egg number	0.76	14.92
	Spermatheca weight	0.68	12.36
Latitude: -Florida vs. Virginia	Egg number	1.16	36.81
	Egg size	1.16	18.00
	Egg stage	0.80	13.75
	Sperm number	0.77	11.19
	Spermatheca weight	0.58	11.66
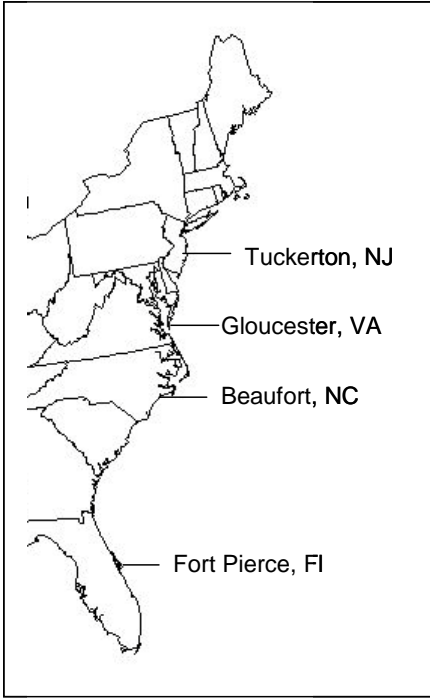
Figure 1: Collecting sites for *E. depressus* and *C. sapidus* during the spring and fall of 2008.

Virginia
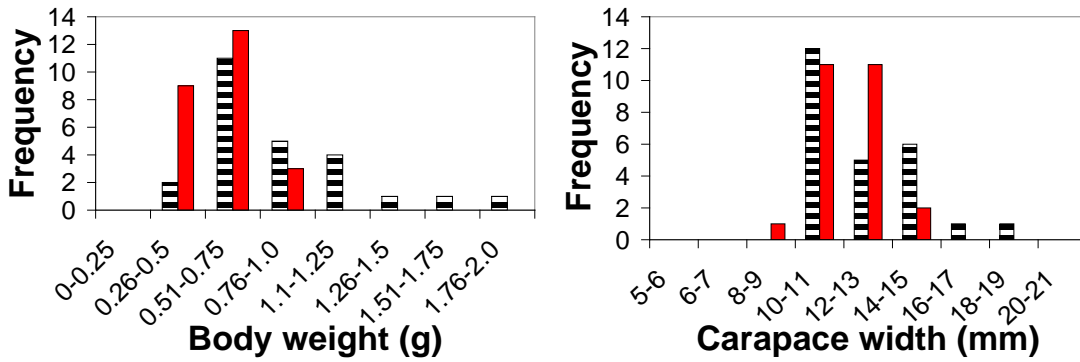
Florida
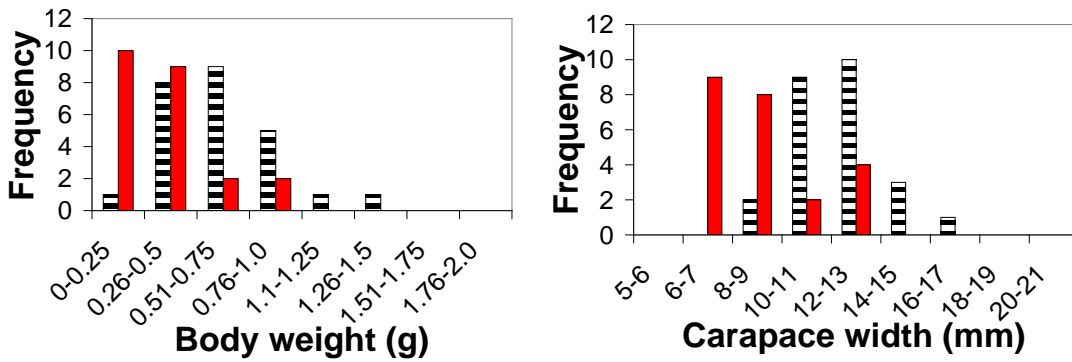
Figure 2: Frequency histograms for body weight (left) and carapace width (right) of *E. depressus* collected from Virginia (top) and Florida (bottom) early (black striped) and late (solid red) in the season.

North Carolina
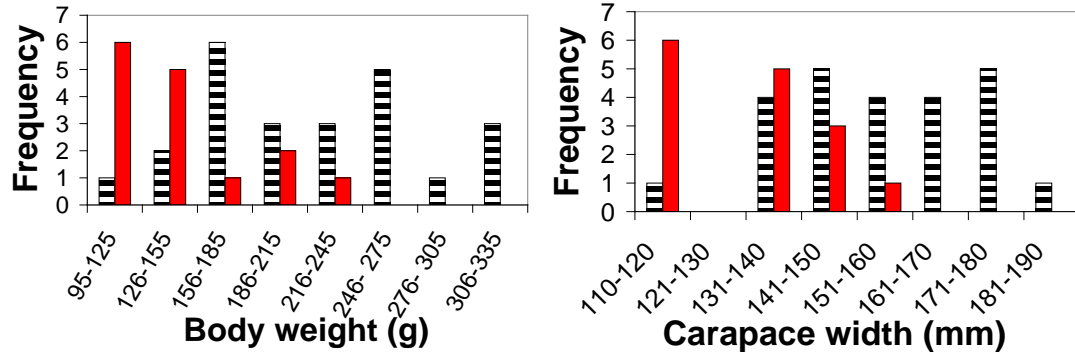
New Jersey
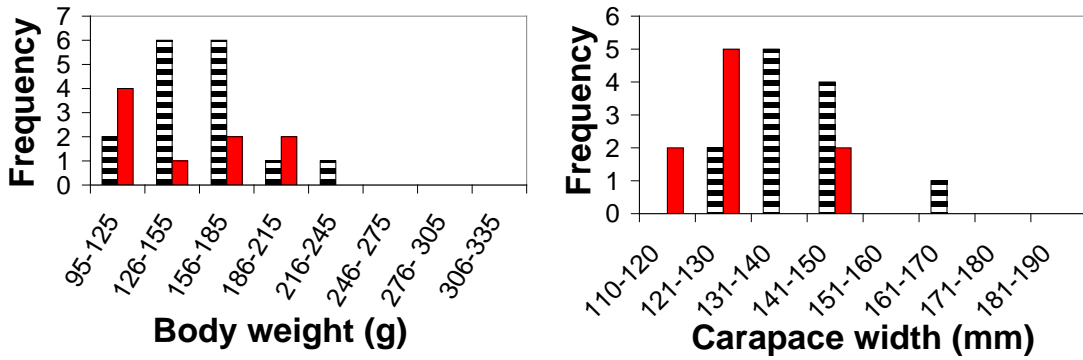
Figure 3: Frequency histograms for body weight (left) and carapace width (right) of *C. sapidus* collected from North Carolina (top) and New Jersey (bottom) collected early (black striped) and late (solid red) in the season.
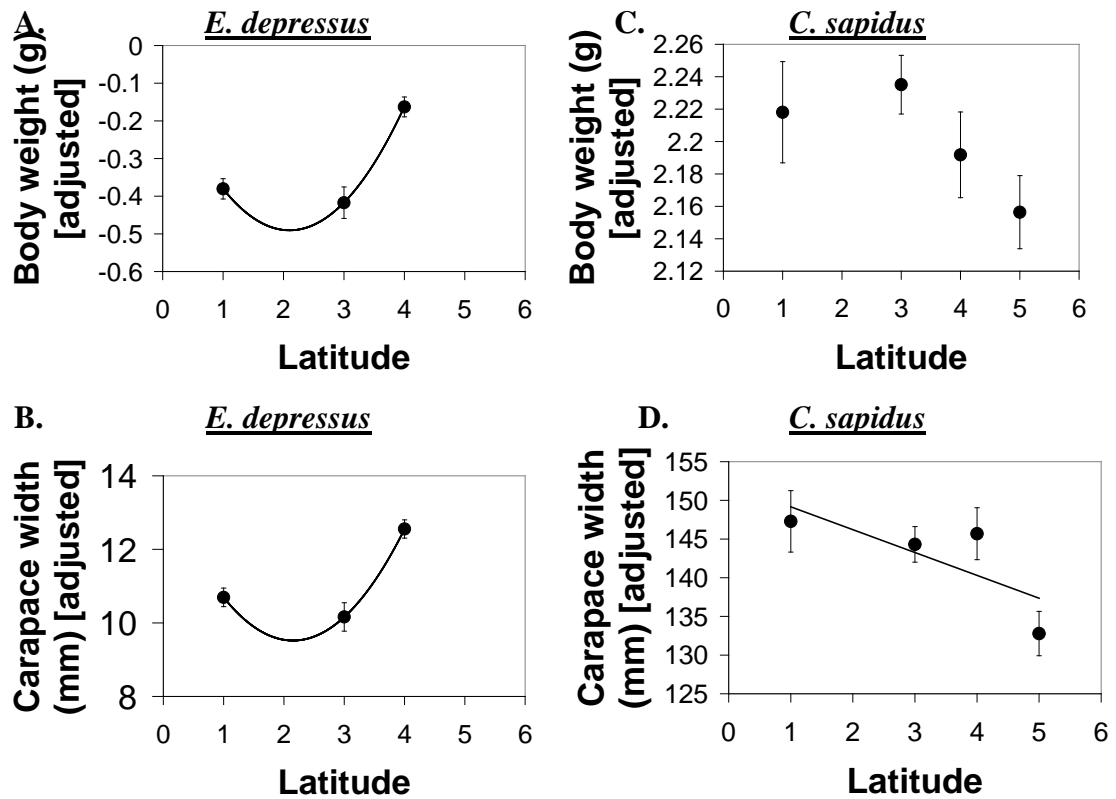
Figure 4: Body weight (mg) (top) and carapace width (mm) (bottom) for *E. depressus* (left) and *C. sapidus* (right) collected at one of four locations; 5=New Jersey, 4 = Virginia, 3 = North Carolina, 1 = Florida. Bars represent +/- 1 standard error from the mean and the trendlines and equations are displayed after adjusting for significant effects of time from ANCOVA models: (A) *body weight = 0.09*latitude^2 –0.38*latitude -0.089,* (B) *carapace width = 1.46*latitude^2 -4.92*latitude + 14.15,* (D) *carapace width = -2.96*latitude + 152.12.*
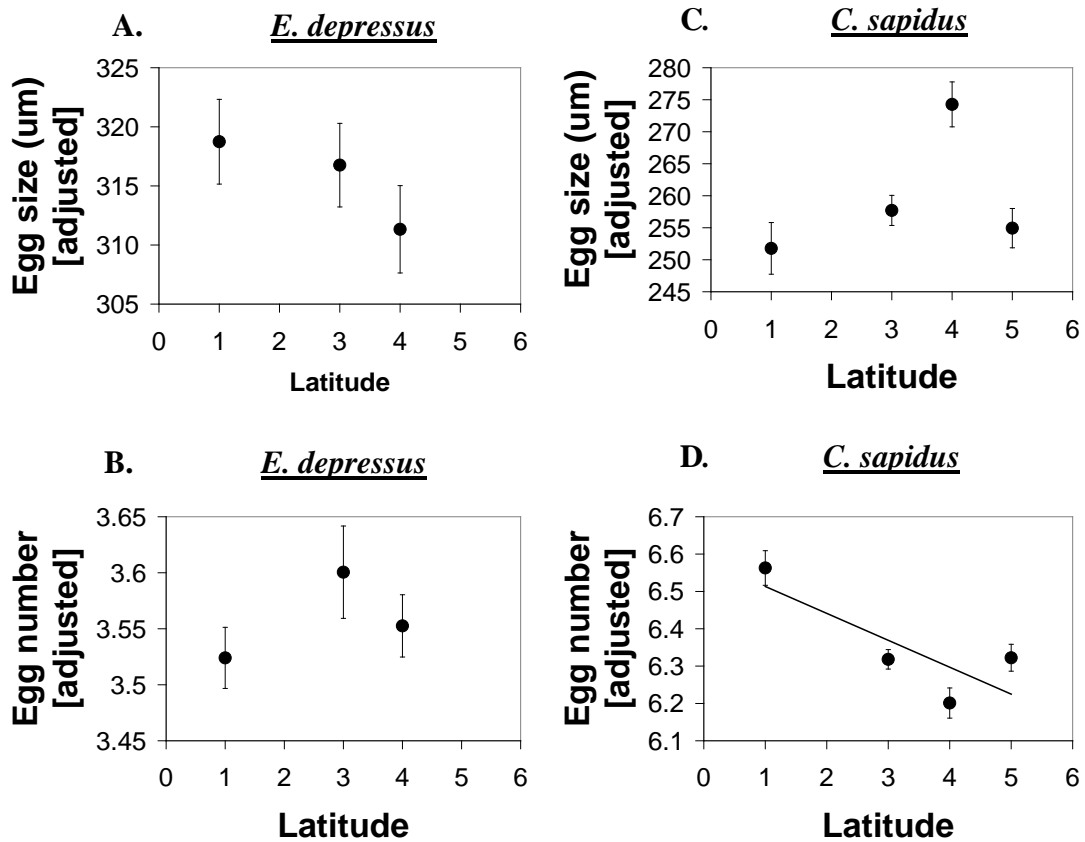
Figure 5: Egg size (top) and egg number ($\log_{10}$) (bottom) for *E. depressus* (left) and *C. sapidus* (right) collected from four locations (5=New Jersey, 4=Virginia, 3= North Carolina, 1=Florida). Error bars represent +/- 1 standard error from the mean and the trendlines and equations are displayed after adjusting for covariates and for significant time effects using an ANCOVA: (D) *Egg number = -0.07*latitude-6.59.*
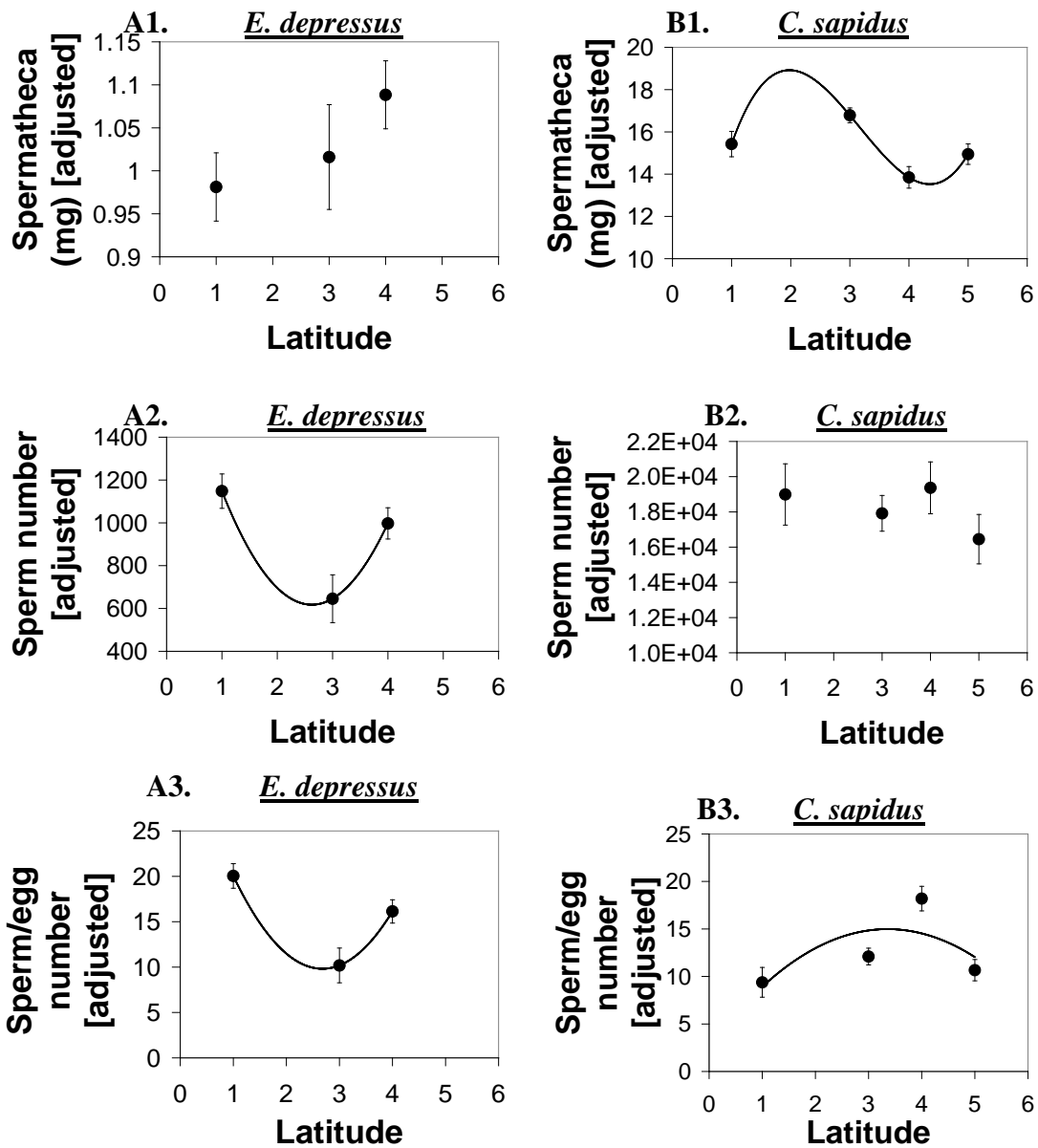
Figure 6: Spermatheca weight ($\sqrt{}$) (top), sperm number ($\sqrt{}$) (middle) and sperm/egg ratio ($\sqrt{}$) (bottom) for *E. depressus* (left) and *C. sapidus* (right) collected from four locations (5=New Jersey, 4=Virginia, 3= North Carolina, 1=Florida). Error bars represent +/- 1 standard error from the mean and the trendlines and equations are displayed after adjusting for covariates and for significant time effects using an ANCOVA: (A2) *Sperm no. = 201.02*latitude^2 -1055.4*latitude + 2002.5,* (A3) *Sperm/egg no.=3.63*latitude^2 - 19.44*latitude + 35.86,* (B1) *Spermatheca weight = 0.805*latitude^3 – 7.65*latitude^2 + 20.80,* (B3) *Sperm/egg no.=-1.09*latitude^2 + 7.32*latitude +2.70.*
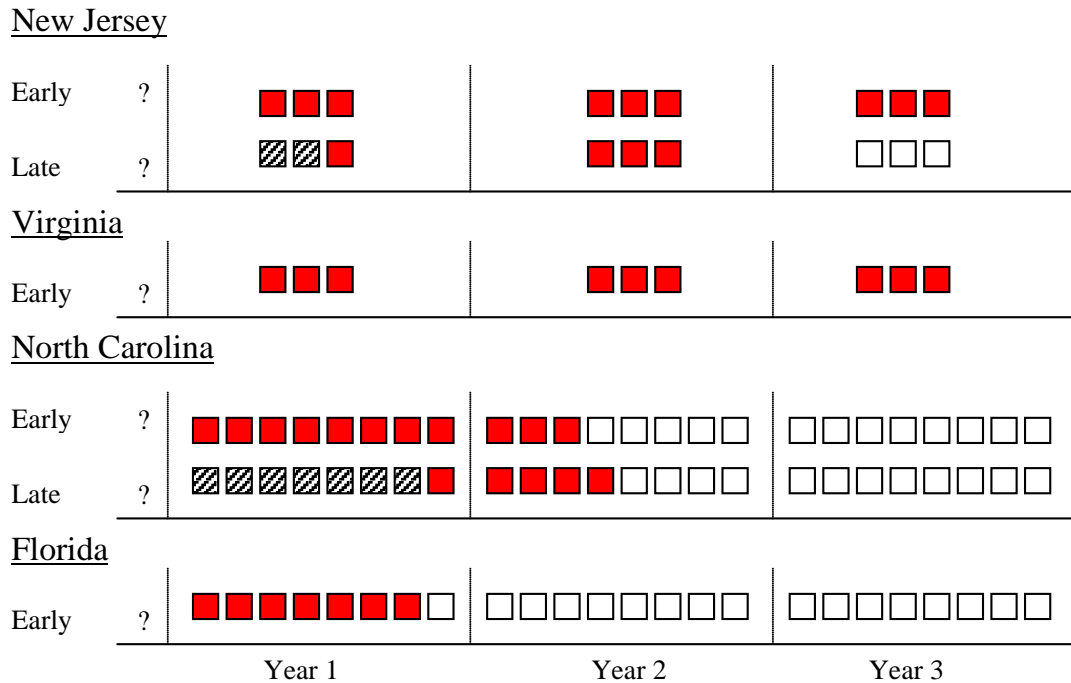
Figure 7: Predicted future brood production for *C. sapidus* at the four collection locations. Estimates of number of broods produced at each location were determined from the literature as NJ = 3, VA = 3, NC = 8, Fl = 8 (Dickinson et al. 2006, Hines et al. 2003). The number of broods was calculated using the amount of residual sperm in the females collected both early and late in the season, and from the known amount of sperm used to fertilize a brood (Hines et al., 2003); (? = unknown if females were collected during their first brooding season, filled red boxes= fertilized broods, open boxes = unfertilized broods, striped boxes = unknown brooding history prior to collection).
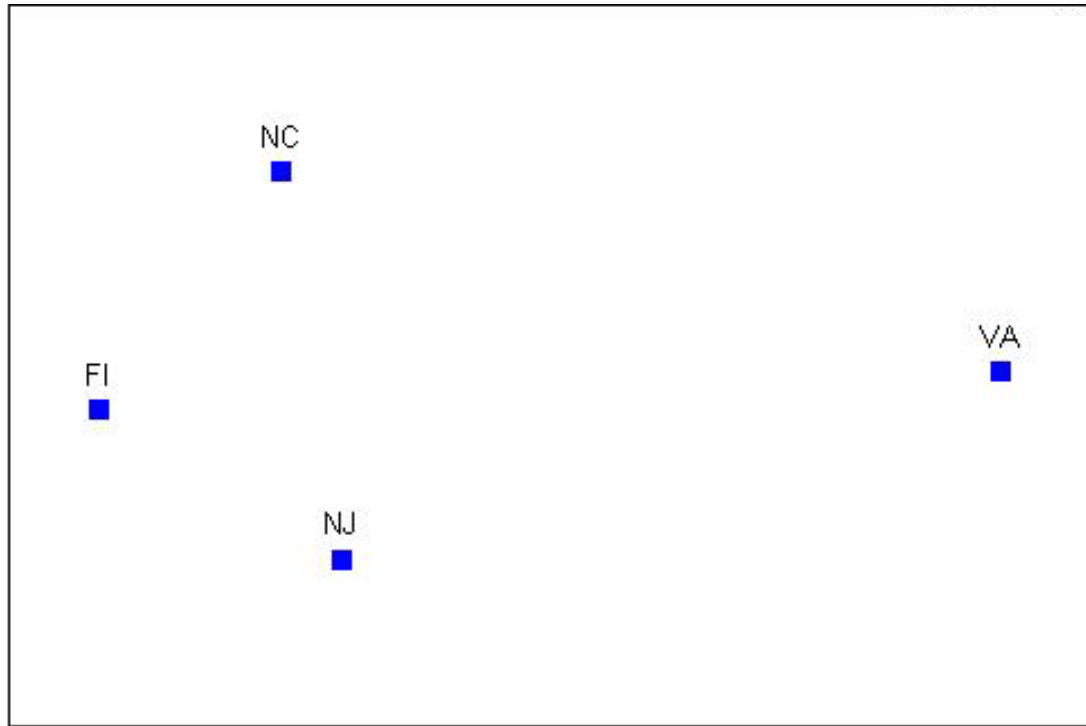
Figure 8: MSD nonparametric ordination graphs for clustering of life history traits for *C. sapidus* across the four collection locations (Florida=FL, North Carolina=NC, Virginia=VA, New Jersey=NJ) using the resemblance matrix from the ANOSIM analysis.

CHAPTER III

Comparative analysis of life history traits in brachyuran crabs

ABSTRACT

Species' life history traits are shaped by allometric, phylogenetic, ecological and behavioral factors. Although allometric and phylogenetic patterns in female reproductive output have been identified across brachyuran crabs, less is known about how life history traits vary with mating strategies and ecological factors. Despite the universal prevalence of internal fertilization and complex mating behavior in the Brachyura, few comparative studies of brachyuran life history patterns have considered sperm storage traits as important components of reproductive strategies. By conducting a comparative survey of life history traits in six superfamilies of brachyuran crabs, I was able to partition variation in life history traits by body size, phylogeny, habitat, and mating strategies using nested and sequential ANCOVAs. Variables of female reproductive output and of male and female sperm storage traits generally displayed positive allometries; thus, larger bodied species invested proportionally more in these traits than smaller bodied species. Phylogeny played a large role in shaping life history patterns in brachyurans. Not only did female reproductive output vary, but the allometric patterns of male sperm storage, sperm number and vas deferens weight also varied by lineage at the superfamily level. Differences in larval development, female molting patterns, male sperm plug production, and habitat preferences likely best explain these differences in superfamilies. Some lineages showed contrasts to the general patterns across the Brachyura. Species within the Grapsoidea typically displayed negative allometries for these traits, suggesting that a unique allometric pattern has emerged within this lineage. While egg size increased for

deep water species, none of the other life history traits varied by depth or consistently across latitude. The contrasting superfamily patterns suggest a strong impact of phylogeny on the evolution of life history traits in this group. Male and female sperm storage patterns varied predictably with mating strategies. Species with a 'female defense' strategy, in which males invest heavily in each mating encounter, had larger female sperm stores and male reproductive output than those with a 'resource defense' strategy. Allometry and phylogeny had the greatest influence on female reproductive output and sperm storage traits. However, mating strategies also influenced the amount of sperm stored by males and females.

INTRODUCTION

Although species have been categorized by one of two primary life history strategies based on the stability of their habitat (r-strategies in unpredictable environments versus K-strategies in more stable environments) (MacArthur & Wilson, 1967; Pianka, 1970), species often do not fit nicely into the r vs. K paradigm (Reaka, 1979; Reaka, 1980; Stearns, 1992). In addition to environmental predictability, at least four other groups of variables are important in shaping life history traits: allometric scaling with body size (Hines, 1982; Berrigan, 1991; Stearns, 1992); phylogeny (Stearns, 1992; Blomberg et al., 2003), habitat (Reaka, 1986; Morrison & Hero, 2003); and mating behavior (Moller, 1991). While the objective of this research was to determine how life history traits vary across these four primary factors, many of these factors are interrelated and interpretation of the effects of one factor can be confounded by the effects of the other factors. To explain the evolution and plasticity of life history traits in brachyuran crabs, we applied a

variety of statistical techniques in an attempt to partition the variation among these factors.

One focus of this research was to identify evolutionary scaling of life history traits to body size across species (Stern & Emlen, 1999) as described by allometric relationships of the traits to variation in body size (Stearns, 1992). Positive allometries have been well described for morphological traits that are used in visual courtship displays (Emlen & Nijhout, 2000; Kodric-Brown et al., 2006); and negative allometries have been shown to occur in genital traits in which the sizes of the average male and female genitalia must match in order for successful intromission (Eberhard et al., 1998). Scaling of life history traits can vary within the same group. For example, brachyuran crabs exhibit isometric scaling of brood weight to body weight, yet negative allometry in egg size to body weight (Hines, 1982). The differential investment in life history traits across body size has important implications for understanding the constraints on, and plasticity of, the evolution of life history traits.

Life history traits can be constrained by phylogeny (Stearns, 1983). For example, phylogenetic history accounted for up to 64% of the variance in life history traits for iguanid lizards (Miles & Dunham, 2009) and accounted for a large proportion of the variation in life history traits in birds (Bohning-Gaese & Oberrath, 1999). While overall phylogenetic patterns in life history traits have been identified, lineages also can vary in the allometric scaling of these traits to body size (Gould, 1971). For example, while brood weight scales isometrically with body weight for brachyurans generally (Hines,

1982), pinnotherid crabs invest proportionately more overall in brood weight (Hines, 1992). In fact, these species have extended their ovaries into their abdomen allowing for larger brood weights relative to their body size. By examining only average differences in traits between lineages, important phylogenetic patterns in allometries may be obscured. Differences in allometries can explain much of the variation in life history traits among lineages and must be analyzed to fully identify phylogenetic patterns.

Variation due to environmental factors such as temperature, seasonality and food availability have been shown to shape life history patterns and can vary predictably across latitude and depth. High metabolic costs associated with warm temperatures at low latitudes has lead to the development of smaller eggs in isopods (Lardies & Bozinovic, 2006) and crabs (Lardies & Castilla, 2001), and smaller clutch sizes in birds (Cooper et al., 2005) and amphibians (Morrison & Hero, 2003). Cold temperatures also can lead to large egg sizes as a result of greater nutrient provisioning due to long incubation times (Lardies & Castilla, 2001). High productivity and thus food availability, which is typically greater during a short growing season at high latitudes, led to greater fecundity in mole crabs (Defeo & Cardoso, 2002). Since egg number trades off with egg size (Smith & Fretwell, 1974), variations in temperature and food availability can have important implications for fecundity across latitudes and depth. Also, longer reproductive seasons at lower latitudes allows species to produce more broods in a single reproductive season (Hines et al., 2003), leading to a larger total reproductive output per year in blue crabs at lower latitudes. However, reproductive output may simply be a function of degree days, such that similar total life time outputs may be accomplished faster at lower

latitude than higher latitudes (Darnell et al., 2009). Many of these habitat traits are not independent of phylogeny or body size. Species of large body size are generally found at higher latitudes (Bergmann, 1847). Similarly, a single or several lineages may radiate into a new habitat, thus confounding differences due to habitat and phylogeny. By recognizing and addressing such confounded patterns, we can begin to tease apart these factors and attribute variation in traits due to ecology versus phylogeny.

Life history traits, particularly male sperm traits, also can be shaped by mating behaviors. With the evolution of polyandrous mating systems and sperm storing females, post copulatory selection has lead to sperm competition within the female reproductive tract (Parker, 1970). In turn, males have evolved sperm plugs (Hartnoll, 1969; Jensen et al., 1996) and sperm removal behaviors (Cordoba-Aguilar et al., 2003; Wada et al., 2005). Males may also optimally allocate sperm to females (Gage & Barnard, 1996; Wedell & Cook, 1999), and have evolved mate guarding behaviors (Jivoff, 1997a; Rondeau & Sainte-Marie, 2001) to reduce the impact of sperm competition and assure their paternity. In bird mating systems that have a high degree of sperm competition, males have evolved larger testes, and thus produce greater sperm number, for their body size (Moller, 1991). Thus mating behavior can shape the evolution of life history traits, particularly sperm storage traits. But, ecological factors also shape species' mating strategies. In fact, many of the primary divisions in mating system strategies are defined by differences in ecological variables (Emlen & Oring, 1977). In stomatopod crustaceans mate guarding occurs more frequently in species which inhabit hard cavities versus those that burrow in soft sediments (Caldwell, 1991). Similarly, mating strategies can be constrained within a

lineage. Thus, the influence of mating strategies can best be teased apart from phylogeny and habitat in groups with plasticity in mating strategies both within lineages and across habitats.

The objective of this research was to identify patterns in life history traits with body size, across the phylogeny, within various habitats and across different mating strategies. Given the complexities and potential overlap in these factors, our goal was to partition the variation in these traits while accounting for the allometric relationships to body size. Brachyurans can serve as a model group because they inhabit a diverse range of habitats from terrestrial to the deep water and many species are broadly distributed across latitude. They all have internal fertilization with females possessing a paired sperm storage organ (spermatheca) that stores sperm for varying durations, depending on species and circumstances (Hartnoll, 1969). Brachyuran mating strategies have been divided into one of three broad categories based on how males compete for mates (Christy, 1987a) and on the environmental potential for polygyny (EPP) (Emlen & Oring, 1977; Orensanz et al., 1995): these generally fall into categories called 'female defense,' 'resource defense' and 'scramble/encounter' competition. There are approximately 21 superfamilies of brachyurans, and this study focused on six: Majoidea, Portunoidea, Cancroidea, Xanthoidea, Grapsoidea, and Ocypodoidea. These six superfamilies have been well investigated for patterns of many life history and reproductive traits, such as larval development, sperm plug production, mating receptivity and female molting (Table 1); and these characteristics likely shape the evolution of sperm transfer and thus sperm storage.

The goal of this study was to partition the variation in life history traits in brachyuran crabs by phylogeny, ecological factors, and mating strategy and to test for allometric relationships:

(1) *Allometry:* Given the large fitness pay-off for investment in life history traits (Stearns, 1992), female reproductive output and sperm storage traits are predicted to scale by positive allometry. However, I predicted a negative allometry for the male intromittent organ, since its size must match the reproductive structures of a wide range of female sizes.

(2) *Phylogeny:* Since brachyuran morphology, larval development, mating receptivity and ejaculate traits vary by superfamily and there are previously established superfamily patterns in female reproductive output (Hines, 1982), I predicted that sperm storage traits, in addition to female reproductive output, would vary significantly among superfamilies.

(3) *Ecological factors:* Since metabolic investment is expected to be lower at higher than lower latitudes and at deeper that shallower depths (Lardies & Bozinovic, 2006), and longer development times in deep waters select for larger egg sizes (Strathmann, 1977), I predicted that species would produce more eggs, have larger eggs, and larger male and female sperm stores at higher than lower latitudes and at deeper than shallower depths.

(4) *Mating strategy:* In species with a 'female defense' mating strategy, males directly compete for limited females and typically invest more in each mating event, as opposed to a 'resource defense' strategy in which males monopolize

resources utilized by females, or in a 'scramble/encounter' strategy in which males are unable to monopolize resources and/or mates (Christy, 1987a). Therefore, I predicted that species with a 'female defense' strategy would have larger male sperm reserves and more stored sperm in the female storage organ than species with 'resource defense' and 'scramble/encounter' strategies.

METHODS

Study Species

A total of 61 species were collected in the field from 17 families and six superfamilies of brachyuran crabs (Majoidea, Xanthoidea, Cancroidea, Portunoidea, Grapsoidea, Ocypodoidea) and information for females of 13 of these species was compiled from previous studies (Hines, 1982; Hines, 1988; Hines, 1991) (Table 2). For data collected in the field, approximately 10 males and 10 females were collected per species. However, several species were rare or difficult to find, and fewer specimens were collected for each of those species. The range of sampling locations included the eastern seaboard of the United States from Nova Scotia to Florida, the Caribbean coast of Panama, and the Pacific coastline from Panama to Coquimbo, Chile (Table 1). Species were defined by the general habitat categories (depth and latitude/region) in which they were collected and generalized by references from the literature. Latitude categories were defined by one of four geographic regions (cold temperate, warm temperate, semi-tropical, or tropical). Depth was defined as semi-terrestrial (species active primarily above the tide line and in the upper intertidal), intertidal/shallow subtidal (intertidal species and species whose range extends into the lower intertidal and shallow subtidal – this category includes

species which can be found in abundance in both the intertidal and subtidal), subtidal (species which can inhabit depths below 10m and whose range does not extend into the lower intertidal), and deep water (species found in deep submarine canyons whose ranges do not typically extend shallower than 50m). While most specimens were collected opportunistically by hand, some of the deep water specimens were collected by trawl. The mating strategy (if known) was identified from the literature and from field mating observations (Table 2).

Dissections and sperm counts

The following variables were recorded for all individuals collected: carapace width (maximum, mm), wet body weight (g), and molt stage (premolt, intermolt, postmolt). Males and females were dissected; female ovary stage ('0'= not visible and highly reduced, '1'= ¼ full, not full of yolk, '2'= developed, mature oocytes present) and egg stage ('1'= yolk, no organ development, '2'= 50% yolk, some organ development, '3'= little to no yolk, eyespots present) were recorded. The percent fullness of the spermatheca was estimated visually as the percent of the spermathecae that was occupied by ejaculate contents. One side of the male vas deferens was removed and carefully separated into anterior, median and posterior vas deferens sections (Cronin, 1947), and each section weighed. One side of the paired reproductive organ -- female spermathecae or male vas deferens -- was removed, weighed and fixed in 3% glutaraldehyde in a 0.1M sodium phosphate buffer. The fixative was removed after 24 hours and the tissue was rinsed and stored in 0.1M sodium phosphate buffer. Sperm were counted by first grinding the tissue in a known volume of 0.1M sodium phosphate buffer using a handheld glass Dounce®

homogenizer. Three subsample counts were conducted on a Petroff-Hausser Counting Chamber® using 400x magnification on a phase contrast microscope. The average sperm number was calculated for each sample.

Egg Traits

Broods from egg-bearing females were fixed in 3% glutaraldehyde and stored in 70% ethanol. A volumetric method was used to estimate the number of eggs. The egg mass was placed in a known volume of 0.1M sodium phosphate buffer and the volume displaced was recorded +/- 1µl. The eggs then were removed from the pleopods and the volume of water displaced by the pleopods was subtracted from the volume displaced for the total egg mass. The average ovum volume was calculated in 0.1M phosphate buffer for each brooding female by measuring the diameter of twenty eggs per egg mass at 100x or 400x magnification, and the spherical ovum volume was calculated using the formula: *ovum vol. $(mm^3) = (4/3)*\pi*(diameter/2)^3$*. The average egg number for each female was calculated using the equation: *no. of eggs = vol. displaced by the egg mass/ave ovum vol.*

Gonopod Traits

One gonopod was removed from each male and stored in 70% ethanol. Measurements of length (base to tip excluding any setae) and average width (measured at the base, midline and near the tip on both sides of the gonopod) were conducted under a dissection microscope or using a vernier caliper depending on the size of the gonopod. Volume of the gonopod was calculated by multiplying the length by the average width.

Statistical Analyses

Univariate Hypothesis Testing

I conducted nested ANCOVA models in SAS v.9.1 using the proc mixed procedure for each sex. Response and explanatory variables are listed in Table 3 and each was transformed to meet assumptions of normality and heterogeneity of variance. Variance inflation estimates above 2 were used as indicators of collinear variables (Graham, 2003). Two statistical models were used to examine the data. First, nested ANCOVA models were used to test for significant body size, superfamily, depth and latitude/region effects. All interactions between factors and between the covariates and superfamily were tested. Species nested within each superfamily, as well as the interactions with the covariates, were written into a random statement allowing for a more conservative test of superfamily effects (McKone & Lively, 1993). If there were significant superfamily, depth or latitude/region effects, a least squares mean statement with a Tukey's adjustment was used to test for pairwise differences. A step-wise model reduction approach was used to remove insignificant covariates and interaction terms from the model. Because separate analyses were run for each response variable, the alpha level was adjusted by dividing it by the number of tests conducted for each data set (α=0.0083). This Bonferroni adjustment prevents inflation of the overall experimental error as a result of conducting multiple tests (Quinn & Keogh, 2002).

In the second model, a sequential ANCOVA was used to remove the significant factors and covariates from the first model (not including species nested within superfamily), and the residuals from the analysis were used to test for species differences and a priori

contrast statements were used to test for differences between mating strategies ('female defense' vs. encounter/scramble,' 'female defense' vs. 'resource defense', and 'encounter/scramble' vs. 'resource defense'). This model was run a second time comparing mating strategies for species within the superfamilies Grapsoidea and Ocypodoidea only.

Multivariate Analyses

A dataset of the species averages for the reproductive traits combined for both sexes was used to conduct nonparametric multivariate analyses in Primer v.6. Species with missing variables were excluded from the analysis, leaving 31 of the original 61 species. Each response variable was transformed to reduce skew and then regressed against either male or female carapace width (Table 4). If variables were significantly collinear (variance inflation estimate >2), one variable was dropped from the analysis, leaving a combination of noncollinear response variables. The residuals from the regressions were then normalized in Primer ($\mu = 0$, $\sigma = 1$) and the resemblance matrix constructed using Euclidean distances (Clarke et al., 2006). Significant clustering due to the factor variables listed in Table 3 was tested using an analysis of similarity (ANOSIM), which uses permutations to test for significant clustering due to a priori groupings (Clarke & Gorley, 2006). The relative contribution of each variable to the significant group effects was examined using a similarity percentage routine (SIMPER). The % contribution of each variable to the average dissimilarities between groupings was calculated as well as the contribution corrected for the standard deviation (sim/sd).

RESULTS

<u>Univariate Analyses</u>: Statistical results are presented in Table 5 and species averages for each of the life history traits are presented in Appendices II and III.

(1) Allometry

As predicted, most of the life history traits displayed positive allometries with body size, meaning that larger bodied species invested proportionately more in these reproductive traits than smaller bodied species. Species' egg number displayed a positive allometry with carapace width (Fig. 1) and decreased with increasing egg size (Fig. 2). While species' egg size did not vary with carapace width, it did increase with developmental stage (Table 5). Female spermatheca weight (Fig. 3) and female sperm number (Fig. 4) displayed positive allometries with carapace width across species, but the slope of the allometry for spermatheca weight varied by superfamily (see phylogeny below). As predicted, male gonopod length displayed a negative allometry with carapace width - larger bodied species had less of an increase in gonopod length with increasing body size (Fig. 5). However, gonopod volume displayed a positive allometry with body size (Fig. 6). Male sperm number (Fig. 7), vas deferens weight (Fig. 8), anterior & median vas deferens weight (Fig. 9) and posterior vas deferens weight (Fig. 10) generally displayed positive allometries across species but the slope of the allometries varied by superfamily (for details, see phylogeny below).

Phylogeny

Egg size varied across superfamilies; the Majoidea had the largest eggs (598 µm), while the Ocypodoidea had the smallest eggs (266 µm; Table 6, Fig. 11). The Majoidea produced significantly fewer eggs per brood than species within the other five superfamilies ($p<0.0083$) when egg number was not adjusted for egg size (Table 6). Percent fullness of the spermatheca also varied by superfamily; with the Grapsoidea and Majoidea having spermathecae that were fuller than the other four superfamilies (Table 6, Fig. 12). Sperm/egg ratio varied by superfamily, with the Majoidea having the highest ratio and the Ocypodoidea and Portunoidea with the lowest ratios (however, least squares means were not significant) (Fig. 13). Since the remaining life history traits had varying allometries across superfamilies, phylogenetic variation in these life history traits are best explained by superfamily relationships with body size. The Portunoidea and Grapsoidea had less of an increase in spermatheca weight with increasing carapace width (slope) than was found in the other three superfamilies, and the Xanthoidea and Ocypodoidea had lower spermathecal weights for their body size (lower intercepts), averaging 83 mg and 6 mg, respectively, than the Majoidea, with an average weight of 226 mg (Table 6, Fig. 3).

Male reproductive traits generally displayed positive allometries, with the Grapsoidea as the main exception; they had negative allometries for male sperm number (Fig. 7), vas deferens weight (Fig. 8), anterior and median vas deferens weights (Fig. 9), and posterior vas deferens weights (Fig. 10). While all of the other superfamilies had positive allometries, the relative slopes of these relationships varied across superfamilies. The

Portunoidea generally had less of an increase in vas deferens weight with body size. However, only two relatively large bodied species were collected within this superfamily requiring caution when interpreting a superfamily pattern for this group. The Majoidea generally had the greatest increase in male reproductive traits with body size. The Xanthoidea and Ocypodoidea had slightly lower vas deferens weights for their body size (intercepts), averaging 288 mg and 40 mg, respectively (Fig. 8), but they had larger sperm numbers than the Majoidea for their body size (Table 7, Fig. 7). Thus, the Majoidea had considerably heavier vas deferens (weighing 1,539 mg; Table 6) and invest more in vas deferens products and sperm number with increasing body size, but on average had lower sperm numbers (after correcting for body size) than other lineages.

Ecological factors

The relative fullness of the female spermatheca seemed to vary by region, with species in the semi-tropical region appearing to have the least full spermatheca; however, least squares means did not differ significantly among regions (Fig. 14). Interestingly, gonopod volume varied significantly across the regions, with species in the tropical region having smaller gonopod volumes than those in the warm temperate region (Fig. 15). As predicted, species in the deep water habitat had the largest egg volume, but intertidal species had the smallest egg volume; and there was no difference between semi-terrestrial, subtidal or intertidal species (Fig. 16).

Mating Strategy

Differences in life history traits across mating strategies were tested using contrast statements across all superfamilies after removing the significant effects for each response variable (see Table 5) using a sequential ANCOVA. As a more conservative test of mating strategy, any significant differences in mating strategies that were identified across all superfamilies then were compared within only the Ocypodoidea and Grapsoidea; because these superfamilies included representative species of all three mating strategies. Results of significant mating strategy differences are only presented if they were significant in the full (all superfamilies) and reduced (only Grapsoidea and Ocypodoidea) data set. Species with a 'resource defense' strategy had less sperm stored in the female (Fig. 17), smaller sperm/egg ratios (Fig. 18), and lighter posterior vas deferens (Fig. 19) than species with either 'female defense' or 'encounter/scramble' strategies. Similarly, species with a 'resource defense' strategy had lighter vas deferens (Fig. 20) than species with a 'female defense' strategy.

Multivariate Analyses

Species differed significantly by superfamily (r=0.291, p=0.001), with the greatest differences between the Ocypodoidea and Portunoidea (R=0.81, p=0.036), and between the Majoidea and Ocypodoidea (r=0.519, p=0.004) (Fig. 21). The differences between these superfamilies were primarily determined by variation in female egg traits and in female spermatheca weight (Appendix IV). The Ocypodoidea differed from the Grapsoidea (R=0.34, p=0.012) primarily due to differences in female sperm storage traits, and the Xanthoidea differed from the Ocypodoidea (R=0.35, p=0.004) by male sperm traits (Fig. 21,

Appendix IV). Species clustered significantly by female molting pattern (R=0.27, p=0.008), and by the presence versus absence of a male sperm plug in the female storage organ (R=0.525, p=0.022); this is likely explained by superfamily patterns (Table 1). Female sperm storage traits had the greatest influence on the grouping 'sperm plug', whereas female fecundity had the greatest influence on the grouping 'female molt pattern' (Appendix IV).

DISCUSSION

By conducting a comparative survey of life history traits in six superfamilies of brachyuran crabs, we were able to document the variation in these traits and to partition the variation by body size, phylogeny, ecological factors and mating behavior. Several of the life history traits displayed consistent allometries, whereas other traits varied allometrically across lineages. Sperm storage patterns generally varied as predicted by mating strategy. This comparative approach allowed us to examine the relative importance of several factors on the evolution of brachyuran life history traits.

(1) Allometries

As predicted, most life history traits displayed a positive allometry with body size. Species of larger body size invested proportionately more in these life history traits relative to their body size. Therefore, any factor leading to a change in body size has a disproportionate affect on reproduction. An increase in fecundity can be brought about by selection for larger body size. This was exemplified by new world monkeys in which two species displayed the same allometric slopes for life history traits but one species delayed

maturity and thus attained a larger body size (Marroig, 2007). While many of the life history traits studied in our research varied across lineages (particularly the sperm storage traits), they are discussed further as lineage-specific effects (see phylogeny). Several traits remained invariant across lineages and were tightly coupled to body size. Life history traits such as clutch size and adult mortality, and clutch size and age at first reproduction are known to be invariant across certain lineages (Charnov, 1993). Not surprisingly, the allometry of female clutch size and the trade-off between egg number and egg size were consistent across brachyuran lineages. In the insect orders Diptera and Hymenoptera, ovary volume scaled isometrically with body size across lineages and was likely constrained by the energetic costs associated with developing larger flight muscles (Berrigan, 1991). While brachyurans do not seem to be energetically constrained, they do have a fixed amount of body cavity space, which scales with body size, for ovary production (Hines, 1982). Females can then partition their yolk reserves to produce many small eggs or few larger eggs. Since egg number scaled with body size, these larger bodied species likely require an increasing number of sperm in order to fertilize their eggs. While we did not directly measure sperm transfer in these species, we did measure the amount of sperm remaining in the female after producing a brood. This residual sperm number displayed a similar positive allometry, meaning that larger bodied females either receive more sperm at mating or mate more frequently. Thus, female brood production, which is constrained by body size, has important consequences for sperm storage and use.

The length of the gonopod was the only trait that was measured which showed negative allometry across lineages. This supports our prediction that sexual selection on the male intromittent organ has been relaxed in order to "fit" the morphology of a range of female sizes. This result is consistent with past studies on insect genitalia (Eberhard et al., 1998; Emlen & Nijhout, 2000; Funke & Huber, 2005). However, measures of gonopod volume displayed positive allometries suggesting that the relationship between body size and genitalia shape might be complex within this group and there may be selection to transmit a larger volume of sperm even if the organ does not increase proportionately in length. It would be valuable to measure female reproductive morphology in these crabs to see if there are negative or positive allometries in female structures which could explain the patterns found in the males.

(2) Phylogeny

There is no single explanation for the phylogenetic patterns in life history traits for brachyurans. Variation in larval development, molting patterns, sperm plug production and habitat preferences likely impact life history strategies differently within each superfamily. The larger than average egg size in the Majoidea result from the reduced and relatively fixed number of larval instars in this group (Hines, 1986). Since egg size trades-off with egg number, investing in egg size has important consequences for female fecundity. Thus, the Majoidea produced fewer eggs per brood (which also varies based on parity (Somerton & Meyers, 1983)) but many species produce multiple broods per year, yielding approximately equal yearly fecundity to other superfamilies (Hines, 1982).

Variation in female reproductive output can have important consequences for how sperm is stored in the female spermatheca. For example, the Portunoidea, Ocypodoidea and Cancroidea produced larger numbers of eggs per brood, had lower sperm-egg ratios and less full spermatheca than the Majoidea and Grapsoidea. Consequently, the number of sperm stored and the fullness of the spermatheca in brooding females may be inversely related to the number of eggs produced per brood. Some of these superfamily patterns in spermathecae traits could be explained by a trade-off between ovarian development and spermatheca volume, since both must occupy the same space in the body cavity (Sainte-Marie, 2007). Some Portunoidea, Majoidea and Cancroidea males transfer large sperm plugs at mating which may or may not be broken down prior to ovarian development. In the Majoidea, male sperm plugs are displaced posteriorly in the spermatheca at mating and up to eight sperm plugs have been identified in one female (Diesel, 1990). The sperm plug typically deteriorates prior to ovarian development in the Portunoidea and in some species of Cancroidea which may account for the lighter and less full spermatheca found in brooding females within these superfamilies than in the Majoidea (Hartnoll, 1969; Wolcott et al., 2005).

Variation in female molting patterns can also influence the length and amount of sperm stored in the spermatheca. Species within the superfamilies Ocypodoidea, Grapsoidea and Xanthoidea (and some Portunoidea) continue to mate after the female maturity molt (Hartnoll, 1969) which can pose challenges for sperm storage. While some brachyuran species can retain sperm across molts (Cheung, 1968), others from the same superfamily cannot (Morgan et al., 1983). Thus, trans-molt retention of sperm in the storage organ

may be a special adaption of species within some of the superfamilies with indeterminate growth. On the other hand, if reproduction can occur only when the female is in a soft molting stage, species with a terminal molt can not mate again; sperm storage is mandatory and sperm stores may become depleted. These superfamilial patterns in sperm storage may arise from complex interactions among molting, female receptivity, sperm plug production, and female reproductive output.

The allometric patterns in female spermatheca weight were similar to those for the total vas deferens weight in males. The Majoidea had heavier vas deferens and spermatheca, whereas the Xanthoidea and Ocypodoidea generally had lighter vas deferens and spermatheca weights. The Portunoidea and Grapsoidea had lower vas deferens and spermatheca weights relative to their body size than the other three superfamilies. These patterns suggest a possible correlated evolution of male vas deferens weight and female spermatheca weight (Rodgers, chapter 4 of this dissertation); species with heavier vas deferens either transfer heavier ejaculates at mating or females mate more frequently.

Male sperm storage traits typically displayed positive allometries in all superfamilies except for the Grapsoidea. Since the Grapsoidea had negative allometries for sperm number, vas deferens weights and gonopod volume, larger species invested proportionally less in these traits for their body size compared to other superfamilies. It is not clear why the Grapsoidea, which form a monophyletic group with the Ocypodoidea and often share similar habitats, differ so drastically from the other superfamilies. It is possible that these species have some change in body design, or have evolved to invest

more in other behaviors or traits at the cost of investment in these reproductive traits. For example, male fish that exert large amounts of energy on territorial behaviors have less sperm than non-territorial fish (Yamauchi, 2000). Large bodied Grapsoidea might have additional energy expenditures that prevent them from investing more in reproduction as body size increases. Phylogenetic history may play a significant role in male reproductive output in this lineage.

(3) Ecological factors

While egg number, female sperm storage traits, and male sperm number did not vary with depth, egg size was larger for deep than shallow water species, suggesting that deep water species may be allocating more to reproduction for each brood than species at shallower depths. At least one deep water brachyuran species has evolved a larger space in its cephalothorax for ovary development, allowing for increased investment in each brood at the expense of producing fewer broods per year (Hines, 1988). The deep water habitat poses unique challenges for larval dispersal, development, and survival. Since productivity is lower in deep water and low temperatures may lengthen the duration of larval development, larvae likely require greater nutrient provisioning and require a larger size at metamorphosis (Strathmann, 1977) in order to increase larval survival. In fact, Hines (1986) discovered that the size of the last zoeal instar correlated with adult body size in brachyurans. Therefore, selection for larger egg size may have led to the evolution of increased space for ovary production seen in some deep water brachyurans. Egg size in semi-terrestrial species, on the other hand, was slightly larger than predicted. It is possible that semi-terrestrial species also may require larger egg sizes in order to

overcome the greater fluctuations in temperature, salinity and desiccation in this habitat than in deeper habitats, as was documented for the estuarine crab, *Neohelice granulata* (Silva et al., 2009). Therefore, the influence of ecological factors on egg size may drive variation in patterns of reproductive investment across these habitats and in turn could have consequences for how sperm are allocated and utilized to fertilize a brood.

Although latitudinal trends in female fecundity have been identified within species for turtles (Litzgus & Mousseau, 2006), terrestrial isopods (Lardies & Bozinovic, 2006), certain brachyurans (Henmi, 1993; Lardies & Castilla, 2001) and several other taxa, we did not detect clear latitudinal patterns in reproductive traits among species in this study (for intraspecific trends see Rodgers, chapter 2 of this dissertation). While species in the warm temperate region generally had larger gonopod volumes than species in tropical regions, there is no clear adaptive explanation for the variation in morphology between these two specific regions. Since superfamilies are often distributed differently across latitudes, it is likely that phylogenetic history of different superfamilies may explain more of the variation in these traits than environmental factors. For example, the Cancroidea are distributed in cold waters while the Ocypodoidea and Grapsoidea are more common in semi-tropical and tropical regions. It can be challenging to completely separate variation due to phylogeny and habitat. However, when we tested for regional variation in female reproductive traits within the Xanthoidea and Ocypodoidea (traits and superfamilies with adequate sample sizes to run these analyses), there were no regional differences within either of these superfamilies. It is clear that superfamily patterns

explained more of the variation in these reproductive traits than did variation by latitude/region.

(4) Mating Strategy

As predicted, species with a 'female defense' mating strategy generally had larger sperm stores, male sperm reserves, and sperm-egg ratios in females than species with a 'resource defense' mating pattern. Since the operational sex ratio in this strategy is typically skewed towards males (Orensanz & Gallucci, 1988), males typically invest a large amount of energy in each mating encounter, copulation durations are typically long and a large ejaculate is transferred at mating. Since males with a 'female defense' strategy typically had heavier vas deferens weights than those with a 'resource defense' strategy, they are allocating more energy to this reproductive trait than species with a 'resource defense' strategy.

It is interesting to note that species with an 'encounter/scramble' strategy tended to have similar sperm storage patterns to species with a 'female defense' strategy, contrary to our predictions. Female sperm stores were lower for species with a 'resource defense' strategy than for either the 'encounter/scramble' or 'female defense' strategies. In a 'resource defense' strategy, females require access to male resources as oviposition sites and the identity of individual females on these sites varies (Christy, 1987a). Therefore, males likely only gain paternity for one breeding cycle and thus it would be optimal for males to invest less ejaculate in each female. Also, defense of the resource may require energy that then is unavailable to the male for mating. In 'encounter/scramble' and

'female defense' strategies, females do not require male resources for oviposition and thus could produce multiple broods from stored sperm reserves. Males could gain greater paternity by investing more in these mating events.

These analyses were restricted mainly to species within the Ocypodoidea and Grapsoidea lineages for which mating strategies have best been described. Orensanz et al. 1995 noted that the previously described brachyuran mating strategies (Christy, 1987a) were defined primarily by male mate acquisition strategies. He suggested that male acquisition strategies can vary within mating strategies for the Cancroidea and this led to misclassification of mating strategies within this group (Orensanz et al., 1995). For example, long copulations with pre- and post guarding have been described for one subtidal species of the Cancroidea with a 'resource defense' strategy and for another subtidal species with a 'scramble/encounter' strategy, suggesting that long copulations and mate guarding are not exclusive to 'female defense' strategies in this group (Orensanz et al., 1995). Variation in mating strategies with similar male acquisition strategies could lead to differences in sperm storage tactics among superfamilies or across habitats. Regardless, sperm storage patterns vary predictably with mating strategies in several of these species for which there is a direct correspondence between mating strategy and male acquisition strategy, suggesting an important role for mating behavior in the evolution of male and female sperm storage traits.

Conclusions

Brachyuran life history traits are shaped by a combination of allometric, phylogenetic, ecological and behavioral patterns, many of which interact with one another. Several of these life history traits were constrained by allometry across lineages indicating the importance of identifying patterns in allometry first in comparative studies. Most traits displayed a phylogenetic pattern, suggesting that phylogenetic history has an important impact on the evolution of life history traits. While few ecological patterns emerged, sperm storage traits did vary with mating strategies. In the future, more comparisons should be made between habitats and mating strategies within a single superfamily. Given the small number of species of Cancroidea and knowledge of their reproductive output and mating strategies (Diesel, 1991; Hines, 1991; Orensanz et al., 1995), this group could serve as a model to further test for variation in life history traits across mating strategies in the aquatic habitat. Similarly, the broad depth and latitudinal ranges that both the Majoidea and Xanthoidea inhabit might make this group ideal for testing the impact of ecological factors on life history patterns. Regardless of ecological factors, it is clear that phylogenetic patterns and allometric constraints have played an integral role in the evolution of life history traits of brachyuran crabs and have shaped the possible trajectories of evolution of these traits within this group.

Table 1: A summary of the superfamilial patterns in habitat, male and female molting, timing of mating, female gonopore morphology, and ejaculate traits.

Superfamily	Habitat	Molting patterns (Hinsch 1972)	Larval development (Hines 1986)	Time of mating (Hartnoll 1969)	Female gonopore morphology (Hartnoll 1969; Brockerhoff and McLay 2005)	Ejaculate traits (Diesel 1991)
Cancroidea	Intertidal to subtidal	No terminal molt to maturity for either sex	5 zoea stages 65 larval days	Mate at molt, some species can mate on the intermolt	Continuous or limited	Some species are known to produce a sperm plug
Portunoidea	Intertidal to subtidal	Terminal molt at maturity (for most species) for females only	5.5 zoea stages 40 larval days	Mate at molt	Limited, decalcifies at molt	Some species are known to produce a sperm plug
Majoidea	Intertidal to subtidal	Terminal molt at maturity for males and females	2 zoea stages 30 larval days	Mate at maturity molt (primiparous) and continue to mate on the intermolt (multiparous)	Most species likely continuously receptive	Some species are known to produce a sperm plug
Ocypodoidea	Semi-terrestrial to intertidal	No terminal molt, molting decreases with age	5 zoea stages 29 larval days	Mate on the intermolt	Varies. Some species are continuously receptive, others the opercula decalcifies on a lunar cycle.	Presence of sperm plug unknown
Xanthoidea	Intertidal to subtidal	No terminal molt, molting decreases with age	4.2 zoea stages 31 larval days	Mate on the intermolt	Most species have continuous receptivity but some likely have limited receptivity	Presence of sperm plug unknown
Grapsoidea	Semi-terrestrial to intertidal	No terminal molt, molting decreases with age	4.5 zoea stages 39 larval days	Mate on the intermolt	Varies. Some species are continuously receptive, others the opercula decalcifies on a lunar cycle.	Presence of sperm plug unknown

Table 2: Species of brachyuran crabs collected from 2005 to 2009 from the field and from the literature. Superfamily, collection habitat, mating strategy (if known), collection location are listed for each species. Asterisks represent species for which egg number and egg size were compiled from the literature (sperm storage traits were not available for these species).

Taxon	Superfamily	Family	Habitat	Mating Strategy	Collection location
Cancer irroratus	Cancroidea	Cancridae	Subtidal	Female	Nova Scotia, Canada
*Cancer antennarius**	Cancroidea	Cancridae	Intertidal/shallows		Hines 1991
*Cancer anthonyi**	Cancroidea	Cancridae	Subtidal		Hines 1991
*Cancer borealis**	Cancroidea	Cancridae	Subtidal	Female	Hines 1991
*Cancer gracilis**	Cancroidea	Cancridae	Intertidal/shallows		Hines 1991
*Cancer magister**	Cancroidea	Cancridae	Subtidal	Encounter	Hines 1991
*Cancer oregonensis**	Cancroidea	Cancridae	Intertidal/shallows	Resource	Hines 1991
*Cancer pagurus**	Cancroidea	Cancridae	Subtidal	Female	Hines 1991
*Cancer productus**	Cancroidea	Cancridae	Intertidal/shallows	Female	Hines 1991
*Cardisoma guanhumi**	Grapsoidea	Gecarcinidae	Semi-terrestrial	Resource	Hines 1982
Aratus pisonii	Grapsoidea	Grapsidae	Semi-terrestrial	Encounter	Fort Pierce, FL, USA
Pachygrapsus transversus	Grapsoidea	Grapsidae	Intertidal/shallows	Resource	Fort Pierce, FL, USA
Sesarma cinereum	Grapsoidea	Sesarmidae	Semi-terrestrial	Encounter	Point Lookout, MD, USA
Sesarma ricordii	Grapsoidea	Sesarmidae	Semi-terrestrial		Fort Pierce, FL, USA
Sesarma occidentale	Grapsoidea	Sesarmidae	Semi-terrestrial		NAOS, Panama
Sesarma reticulatum	Grapsoidea	Sesarmidae	Semi-terrestrial	Resource	Point Lookout, MD, USA
Hemigrapsus sanguiensis	Grapsoidea	Varunidae	Intertidal/shallows		Tuckerton, NJ, USA
Pachygrapsus gracilis	Grapsoidea	Grapsidae	Intertidal/shallows		Fort Pierce, FL, USA
Cyclograpsus cinereus	Grapsoidea	Varunidae	Intertidal/shallows		Coquimbo, Chile
Acanthonyx petiverii	Majoidea	Epialtidae	Intertidal/shallows		Fort Pierce, FL, USA
Stenorhynchus seticornis	Majoidea	Inacidae	Subtidal		Caribou Caye, Belize, USA
Mithrax forceps	Majoidea	Mithracidae	Intertidal/shallows		Peanut Island, Fl, USA
Mithrax sculptus	Majoidea	Mithracidae	Intertidal/shallows		Caribou Caye, Belize, USA
Mithrax spinosissimus	Majoidea	Mithracidae	Subtidal		Portobello, Panama
Macrocoeloma trispinosum	Majoidea	Mithracidae	Sudtidal		Portobello, Panama
Mithrax coryphe	Majoidea	Mithracidae	Subtidal		Portobello, Panama
Chionoectes opilio	Majoidea	Oregoniidae	Subtidal		Nova Scotia, Canada
Hyas araneus	Majoidea	Oregoniidae	Subtidal		Nova Scotia, Canada
Hyas coarctatus	Majoidea	Oregoniidae	Subtidal		Nova Scotia, Canada
Libinia dubia	Majoidea	Pisidae	Subtidal		Gloucester, VA, USA

Species	Superfamily	Family	Habitat		Location
Pisoides edwardsi	Majoidea	Pisidae	Subtidal		Coquimbo, Chile
Pitho lherminieri	Majoidea	Tychidae	Intertidal/shallows		Peanut Island, FL, USA
Uca thayeri	Ocypodoidea	Ocypodidae	Semi-terrestrial	Encounter	Fort Pierce, FL, USA
Uca pugilator	Ocypodoidea	Ocypodidae	Semi-terrestrial	Resource	Fort Pierce, FL, USA
Uca minax	Ocypodoidea	Ocypodidae	Semi-terrestrial		Point Lookout, MD, USA
Uca pugnax	Ocypodoidea	Ocypodidae	Semi-terrestrial		Tuckerton, NJ, USA
Uca beebei	Ocypodoidea	Ocypodidae	Semi-terrestrial	Encounter	Rodman, Panama City, Panama
Uca rapax	Ocypodoidea	Ocypodidae	Semi-terrestrial	Resource	Fort Pierce, FL, USA
Ocypode quadrata	Ocypodoidea	Ocypodidae	Semi-terrestrial		Fort Pierce, FL, USA
Uca stylifera	Ocypodoidea	Ocypodidae	Semi-terrestrial	Female	Rodman, Panama City, Panama
Uca terpsichores	Ocypodoidea	Ocypodidae	Semi-terrestrial	Female	Rodman, Panama City, Panama
Uca stenodactylus	Ocypodoidea	Ocypodidae	Semi-terrestrial	Female	Rodman, Panama City, Panama
Carcinus maenas	Portunoidea	Portunidae	Intertidal/shallows	Female	Avery Point, CT, USA
Ovalipes ocellatus	Portunoidea	Portunidae	Subtidal		Tuckerton, NJ, USA
Callinectes sapidus	Portunoidea	Portunidae	Subtidal	Female	Gloucester, VA, USA
*Geryon fenneri**	Portunoidea	Geryonidae	Deep		Hines 1991
*Geryon quinquidens**	Portunoidea	Geryonidae	Deep		Hines 1991
*Portunus gibbesi**	Portunoidea	Portunidae	Subtidal		Hines 1991
*Portunus spinicarpus**	Portunoidea	Portunidae	Subtidal		Hines 1991
*Menippe nodifrons**	Xanthoidea	Menippidae	Subtidal		Hines 1991
Panopeus lacustris	Xanthoidea	Panopeidae	Intertidal/shallows		Fort Pierce, FL, USA
Panopeus herbstii	Xanthoidea	Panopeidae	Intertidal/shallows		Fort Pierce, FL, USA
Eurypanopeus depressus	Xanthoidea	Panopeidae	Intertidal/shallows		Fort Pierce, FL, USA
Euypanopeus dissimilis	Xanthoidea	Panopeidae	Intertidal/shallows		Fort Pierce, FL, USA
Rhithropanopeus harrisii	Xanthoidea	Panopeidae	Intertidal/shallows		Edgewater, MD, USA
Panopeus americanus	Xanthoidea	Panopeidae	Intertidal/shallows		Caribou Caye, Belize, USA
Xanthodius sternberghii	Xanthoidea	Xanthidae	Intertidal/shallows		Caribou Caye, Belize, USA
Cataleptodius floridanus	Xanthoidea	Xanthidae	Intertidal/shallows		Fort Pierce, FL, USA
Paraxanthus barbiger	Xanthoidea	Xanthidae	Subtidal		Coquimbo, Chile
Eriphia gonagra	Xanthoidea	Xanthidae	Intertidal/shallows		Fort Pierce, FL, USA
Homolaspis plana	Xanthoidea	Xanthidae	Subtidal		Coquimbo, Chile
Raninoides louisianensis	Raninoidea (section Raninoida)	Raninidae			

Table 3: Explanatory and response variables tested in the ANCOVA proc mixed procedures. Transformations for each variable are displayed in parentheses and class variables marked by [c]. All interactions between covariates and superfamily, as well as the interaction between factors, were tested.

Females

Response Variable	Explanatory Variables
Egg number ($\log_{10}$)	superfamily [c], habitat [c], carapace width ($\log_{10}$), egg diameter ($\log_{10}$), egg stage, region [c]
Egg diameter (um) ($\log_{10}$)	superfamily [c], habitat [c], carapace width ($\log_{10}$), egg stage, region [c]
% full spermathecae ($\log_{10}$)	superfamily [c], habitat [c], ovary stage, carapace width ($\log_{10}$), region [c]
Spermatheca weight (mg) ($\log_{10}$)	superfamily [c], habitat [c], carapace width ($\log_{10}$), ovary stage, region [c]
Sperm number ($\log_{10}$)	superfamily [c], habitat [c], carapace width ($\log_{10}$), ovary stage region [c]
Sperm egg ratio ($\log_{10}$)	superfamily [c], habitat [c], carapace width ($\log_{10}$), egg stage, ovary stage, region [c]

Males

Response Variable	Explanatory Variables
Sperm number ($\log_{10}$)	superfamily [c], depth [c], carapace width ($\log_{10}$), latitude [c]
Vas deferens weight (mg) ($\log_{10}$)	superfamily [c], depth [c], carapace width ($\log_{10}$), latitude [c]
AVD&MVD weight (mg) ($\log_{10}$)	superfamily [c], depth [c], PVD ($\log_{10}$), latitude [c], carapace width ($\log_{10}$)
PVD weight (mg) ($\log_{10}$)	superfamily [c], depth [c], AVD&MVD ($\log_{10}$), latitude [c], carapace width ($\log_{10}$)
Gonopod length (mm) ($\log_{10}$)	superfamily [c], carapace width ($\log_{10}$). depth [c]
Gonopod volume (mm^3) ($\log_{10}$)	superfamily [c], carapace width ($\log_{10}$), depth [c]

Table 4: Response and explanatory variables tested in Primer using nonparametric multivariate clustering and MSD analyses. Transformations for each variable are displayed in parentheses. Two data sets were run since both egg number and egg size were collinear yet both of direct interest.

Factors	Superfamily, family(superfamily), mating strategy, depth, female molt, female receptivity, sperm plug, latitude [c]
Variables	
Set 1	Gonopod length ($\log_{10}$), vas deferens weight ($\log_{10}$), male sperm no. ($\log_{10}$), ovary stage, egg stage, egg number ($\log_{10}$), spermatheca weight ($\log_{10}$), % fullness of spermatheca, female sperm number ($\log_{10}$)
Set 2	Gonopod length ($\log_{10}$), vas deferens weight ($\log_{10}$), male sperm no. ($\log_{10}$), ovary stage, egg stage, egg size ($\log_{10}$), spermatheca weight ($\log_{10}$), % fullness of spermatheca, female sperm number

Table 5: Statistical results for male and female variables each tested in an ANCOVA model. Primary factors of interest, carapace width, phylogeny, depth and their interactions were tested in a single ANCOVA model. F values, numerator and denominator degrees of freedom, and p values are listed for each variable.

	Body size	Phylogeny	Depth	Region	Covariates
Egg number	$F_{1, 42}$=442.51, p<0.0001	$F_{5, 44}$=1.83, p=0.1271	$F_{3, 228}$=0.63, p=0.5945	$F_{3, 228}$=0.60, p=0.6175	Egg size: $F_{1, 37}$=65.27, p<0.0001
Egg size	NS	$F_{5, 44}$=15.63, p<0.0001	$F_{3, 267}$= 10.92, p<0.0001	$F_{3, 267}$=2.41, p=0.067	Egg number : $F_{1, 42}$=19.75, p<0.0001 Egg stage: $F_{1, 33}$=28.33, p<0.0001
Spermatheca % full	NS	$F_{5, 32}$=8.32	$F_{2, 190}$=2.53, p=0.0820	$F_{3, 190}$=4.71, p=0.0034	
Spermatheca (mg)	$F_{1, 24}$=184.72, p<0.0001 [CW*Superfamily]: $F_{2,24}$=9.43, p<0.0001	$F_{4, 33}$=6.14, p<0.0008	$F_{2, 190}$=1.33, p=0.2680	$F_{3, 190}$=0.97, p=0.4075	
Female sperm number	$F_{1, 26}$=30.97, p<0.0001	$F_{5, 31}$=2.40, p=0.0595	$F_{2, 176}$=0.28, p=0.7540	$F_{3, 176}$=1.85, p=0.1399	
Female sperm-egg ratio	NS	$F_{4,30}$=5.35, p=0.0023	$F_{2,142}$=0.22, p=0.8073	$F_{3,142}$=3.05, p=0.0308	
Male sperm number	$F_{1, 33}$=213.94, p<0.0001 [CW*Superfamily]: $F_{5, 33}$=8.14, p<0.0001	$F_{5, 32}$=10.50, p<0.0001	$F_{2, 284}$=0.63, p=0.5322	$F_{4, 284}$=2.38, p=0.0523	
Vas deferens (mg)	F1,35=332.24, p<0.0001 [CW*Superfamily]: $F_{5, 35}$=9.17, p<0.0001	$F_{5, 32}$=11.63, p<0.0001	$F_{2, 324}$=0.83, p=0.5083	$F_{5, 35}$=9.17, p<0.5083	
Anterior & median vas deferens (mg)	$F_{1, 31}$=247.14, p<0.0001 [CW*Superfamily]: $F_{5, 31}$=5.79, p=0.0007	$F_{5, 28}$=6.76, p=0.0003	$F_{2, 269}$=0.30, p=0.7404	$F_{4, 269}$=01.83, p=0.1235	
Posterior vas deferense (mg)	$F_{1, 31}$=224.38, p<0.0001 [CW*Superfamily]: $F_{5, 31}$=11.60, p<0.0001	$F_{5, 28}$=13.69, p<0.0001	$F_{2, 267}$=0.18, p=0.8387	$F_{4, 267}$=0.46, p=0.7645	
Gonopod length (mm)	$F_{1, 37}$=355.58, p<0.0001	$F_{5, 30}$=3.88, p=0.0078	$F_{2, 308}$=0.21, p=0.8071	$F_{3, 308}$=3.41, p=0.0180	
Gonopod volume (mm^3)	$F_{1, 28}$=142.71, p<0.0001	$F_{5, 21}$=1.83, p=0.1505	$F_{2, 181}$=0.98, p=0.3760	$F_{3, 181}$=6.20, p=0.0005	

Table 6: Female life history traits averaged across species for six different superfamilies of brachyuran crabs. Standard error of the mean +/- 1 is presented in parentheses for each average.

Superfamily	Carapace width (mm)	Egg number	Egg size (µm)	Spermath-eca (mg)	Spermath-eca (% full)	Sperm number	Sperm number/ egg number
Cancroidea	107.4 (14.2)	1.06×10^6 (2.61×10^5)	376.9 (16.8)	90	5	8.25×10^7	-
Portunoidea	82.8 (15.9)	3.99×10^5 (2.62×10^5)	408.8 (65.8)	143 (67)	20 (6)	1.36×10^8 (1.13×10^8)	2.51×10^2 (30)
Majoidea	27.0 (5.2)	1.17×10^4 (6.14×10^3)	598.4 (30.6)	226 (105)	49 (6)	1.91×10^8 (9.40×10^7)	3.25×10^4 (1.63×10^4)
Ocypodoidea	14.4 (2.1)	1.50×10^4 (4.65×10^3)	266.4 (10.6)	6 (2)	27 (6)	4.16×10^6 (9.90×10^5)	4.13×10^2 (84)
Xanthoidea	32.8 (7.9)	7.44×10^4 (3.83×10^4)	365.5 (21.9)	83 (72)	24 (5)	5.74×10^7 (2.39×10^7)	3.25×10^3 (1.02×10^3)
Grapsoidea	21.9 (6.3)	3.92×10^4 (2.91×10^4)	401.8 (74.5)	18 (5)	58 (7)	5.86×10^7 (3.42×10^6)	2.81×10^4 (2.45×10^4)

Table 7: Male life history traits averaged across species for six different superfamilies of brachyuran crabs. Standard error of the mean +/- 1 is presented in parentheses for each average.

Superfamily	Carapace width (mm)	Sperm number	Vas deferens (mg)	Anterior & median vas deferens	Posterior vas deferens	Gonopod length (mm)	Gonopod volume (mm^3)
Cancroidea	85.7	8.23×10^8	702	253	615	17	62
Portunoidea	111.3 (55.8)	2.28×10^9 (1.77×10^9)	1285 (800)	849 (533)	436 (268)	28 (15.8)	9.6
Majoidea	25.7 (7.7)	1.96×10^9 (9.74×10^8)	1539 (713)	808 (381)	921 (433)	10.4 (2.0)	11.2 (4.9)
Ocypodoidea	19.5 (2.7)	2.06×10^8 (1.43×10^8)	33.9 (20.3)	20 (13)	17 (12)	7.6 (1.2)	2.1 (1.1)
Xanthoidea	30.9 (8.4)	9.33×10^8 (4.82×10^8)	288 (203)	236 (166)	80 (56)	8.0 (1.9)	11.8 (7.7)
Grapsoidea	16.0 (1.5)	6.13×10^7 (1.84×10^7)	25.0 (6.4)	17 (4)	8 (2)	4.2 (0.5)	2.3 (0.7)

Figure 1: The linear relationship between egg number (log$_{10}$) and carapace width (log$_{10}$) (CW) for 54 species of brachyurans from six superfamilies; Cancroidea (purple +), Grapsoidea (green, square), Majoidea (red, x), Ocypodoidea (yellow, triangle), Portunoidea (blue, diamond), Xanthoidea (black, circle). Equation of the line: *Log$_{10}$(egg number)= 2.39*log$_{10}$(carapace width) + 0.78, r^2=0.82, p=<0.0001*.
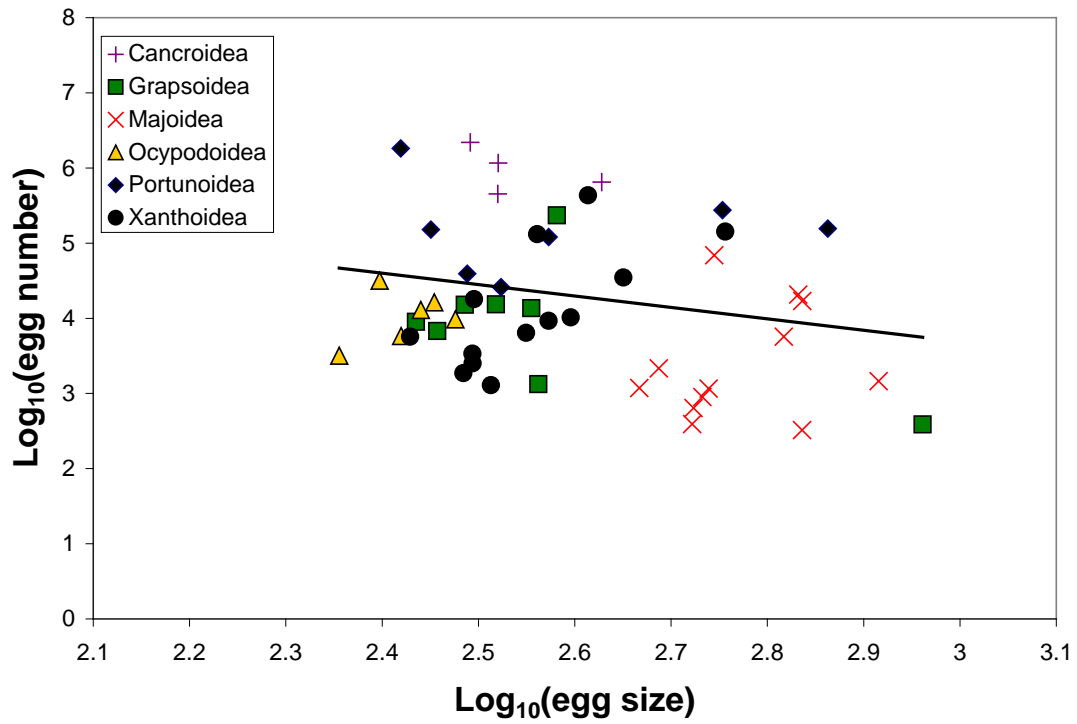
Figure 2: The linear relationship between egg number ($\log_{10}$) and egg size ($\log_{10}$) for 54 brachyuran species from six superfamilies; Cancroidea (purple +), Grapsoidea (green, square), Majoidea (red, x), Ocypodoidea (yellow, triangle), Portunoidea (blue, diamond), Xanthoidea (black, circle). Equation of the line: *Log_{10}(egg number)= -1.52*log_{10}(egg size) + 8.25, r^2=0.04, p=<0.0001.*

Figure 3: The relationship between spermatheca weight (log_{10}) and carapace width (log_{10}) (CW) for 41 species of brachyurans from six superfamilies; Cancroidea (purple), Grapsoidea (green, square): $Log_{10}(spermatheca) = 1.606*log_{10}(CW) - 0.8062, r^2=0.12$, Majoidea (red, x): $2.77* log_{10}(CW) -2.37, r^2=0.68$, Ocypodoidea (yellow, triangle): $2.22* log_{10}(CW) - 1.96, r^2=0.83$, Portunoidea (blue, diamond): $1.20* log_{10}(CW) - 0.25, r^2=0.55$, Xanthoidea (black, circle): $2.84* log_{10}(CW) -2.80, r^2=0.95$.

Figure 4: The linear relationship between female sperm number ($\log_{10}$) and carapace width ($\log_{10}$) (CW) for 39 species of brachyurans from six superfamilies; Cancroidea (purple +), Grapsoidea (green, square), Majoidea (red, x), Ocypodoidea (yellow, triangle), Portunoidea (blue, diamond), Xanthoidea (black, circle). Equation of the line: *$\log_{10}(sperm\ number) = 2.22 * \log_{10}(CW) + 4.01$, $r^2 = 0.54$, $p = <0.0001$.*
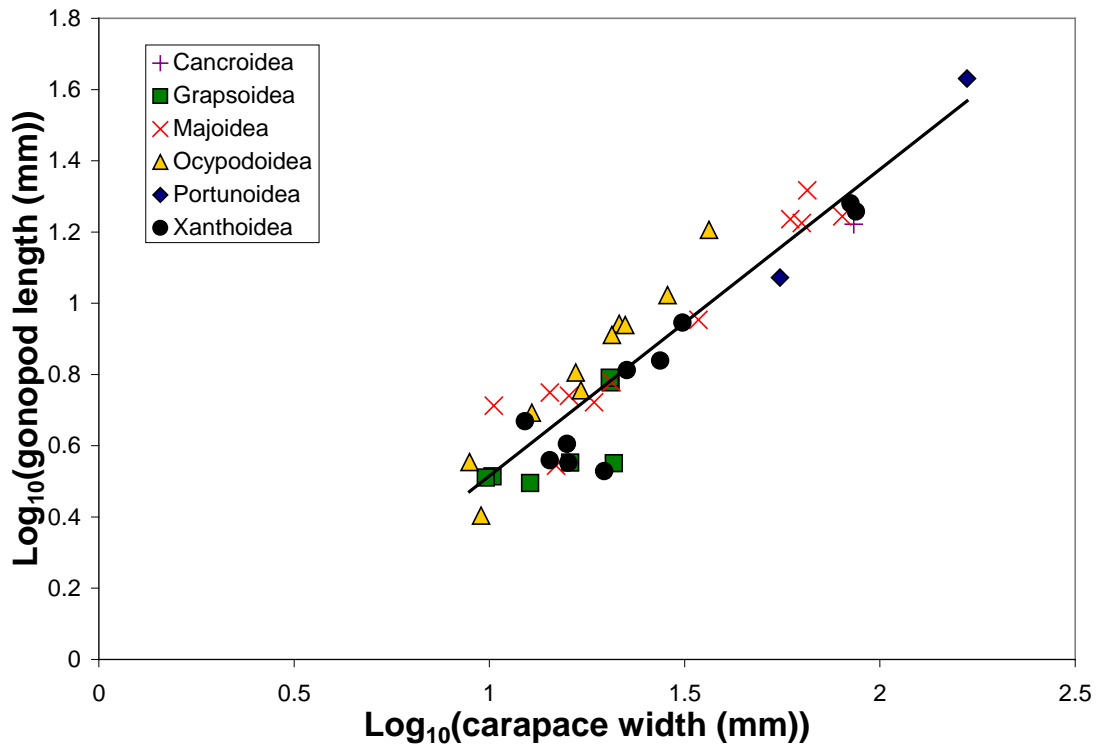
Figure 5: The relationship between the species' average of gonopod length ($\log_{10}$) and male carapace width ($\log_{10}$) (CW) for 41 species of brachyurans from six superfamilies: Cancroidea (purple +), Grapsoidea (green, square), Majoidea (red, x), Ocypodoidea (yellow, triangle), Portunoidea (blue, diamond), Xanthoidea (black, circle). Equation of the line: *Log_{10} (gonopod length) = 0.79*log_{10} (CW) - 0.25, r^2=0.73.*
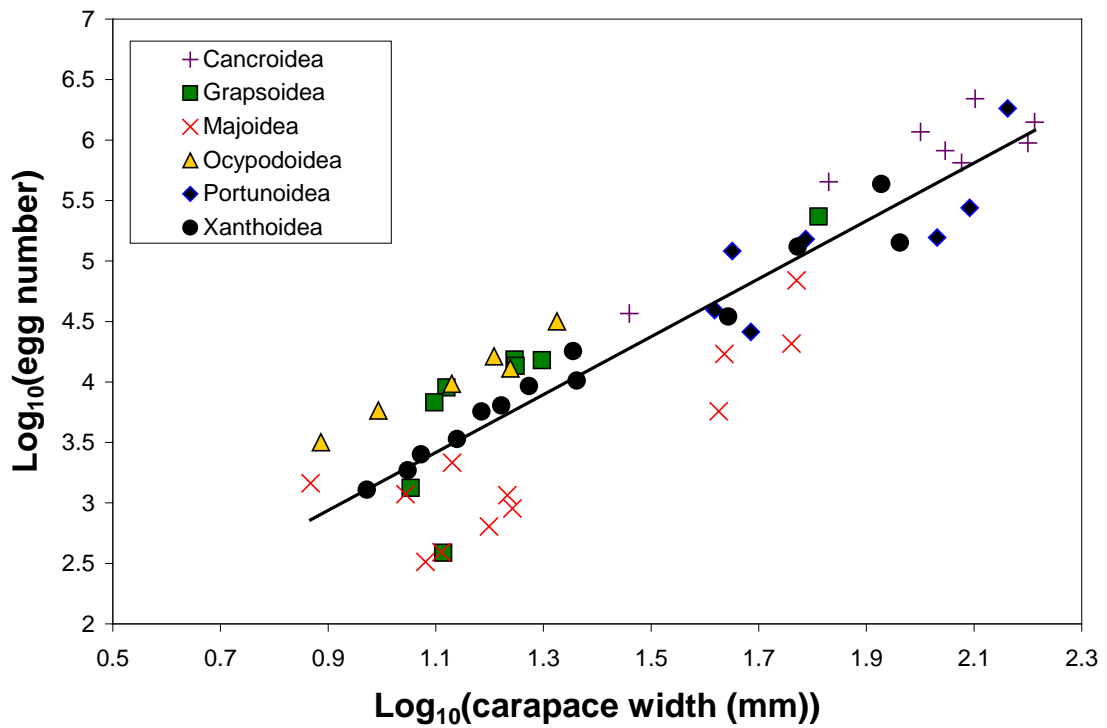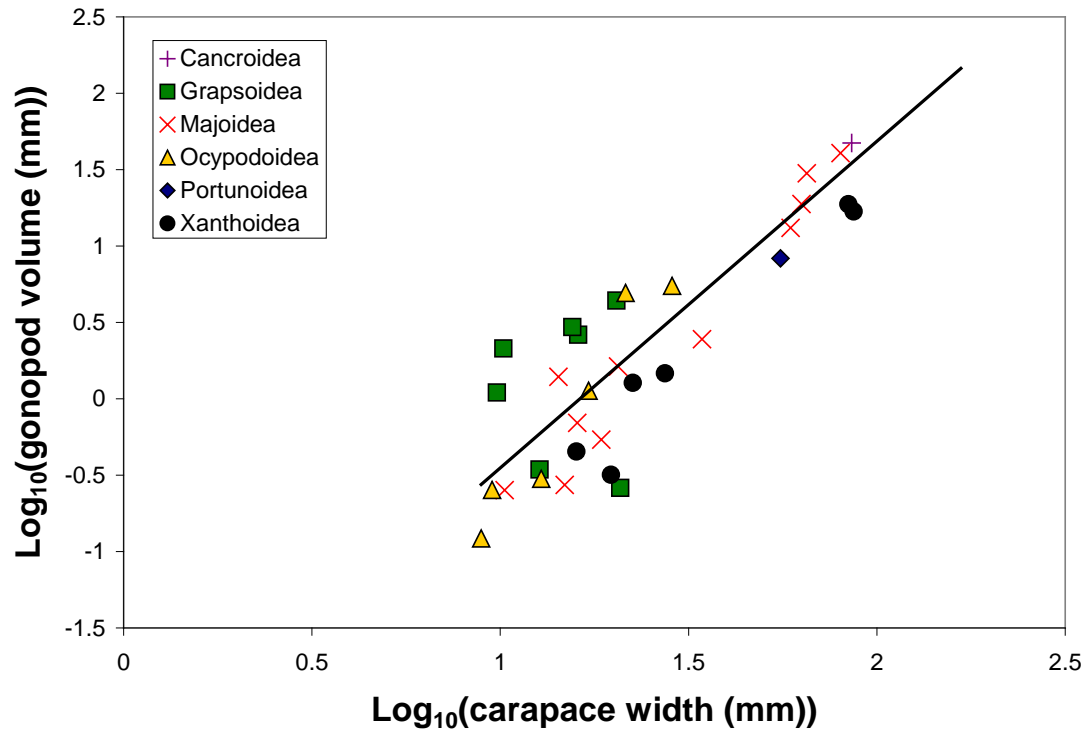
Figure 6: The relationship between gonopod volume ($\log_{10}$) and male carapace width ($\log_{10}$) (CW) for 32 species of brachyurans from six superfamilies: Cancroidea (purple +), Grapsoidea (green, square), Majoidea (red, x), Ocypodoidea (yellow, triangle), Portunoidea (blue, diamond), Xanthoidea (black, circle). Equation of the line: *$\log_{10}$(gonopod volume): 2.1*$\log_{10}$(CW) -2.59.*
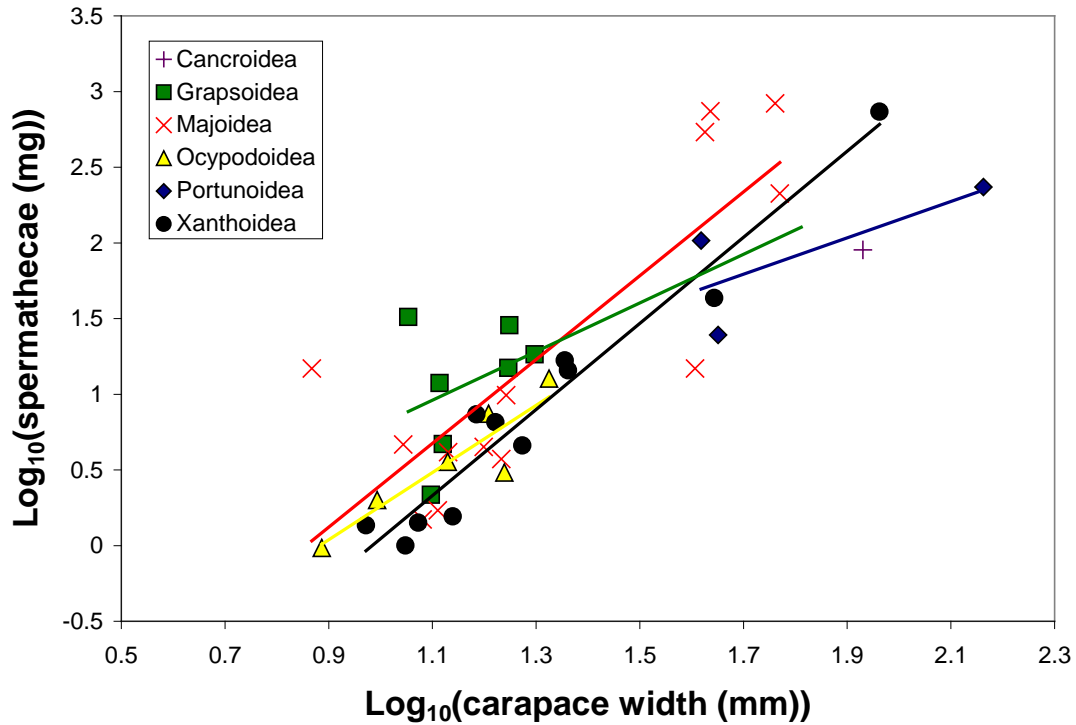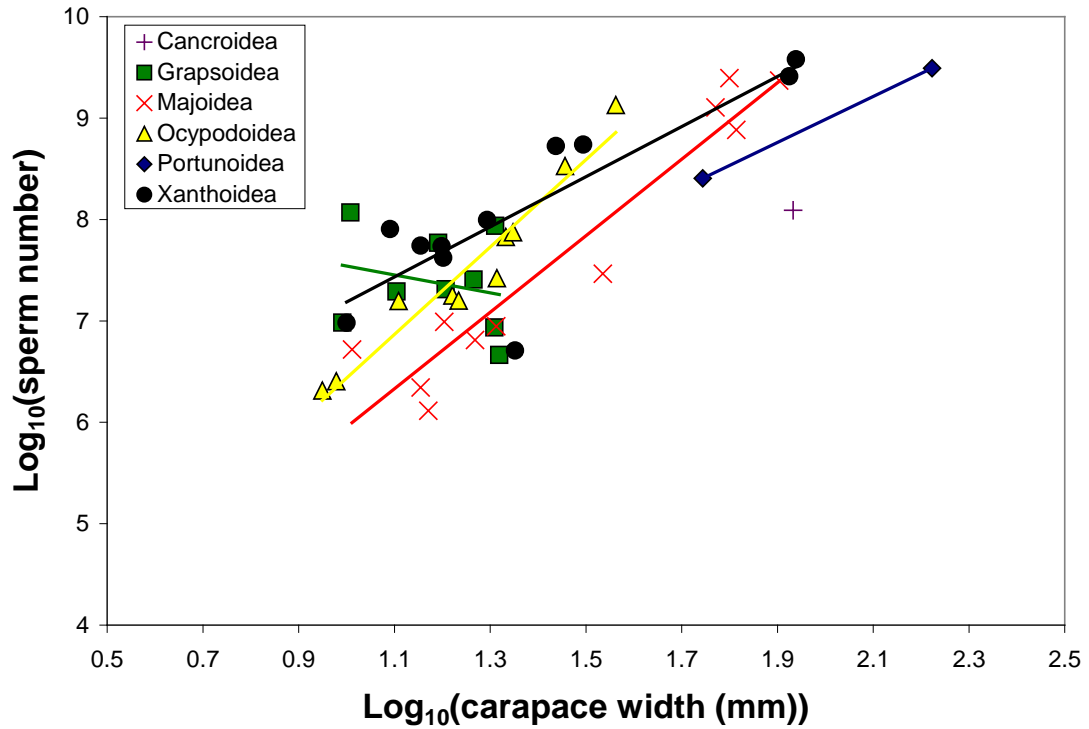
Figure 7: The relationship between male sperm number ($\log_{10}$) and carapace width ($\log_{10}$) (CW) for 44 species of brachyurans from six superfamilies; Cancroidea (purple, plus), Grapsoidea (green, square): *Log_{10}(sperm number) = -0.88*log_{10}(CW) + 8.42, r^2=0.06*, Majoidea (red, x): *3.94* log_{10}(CW) + 2.24, r^2=0.83*, Ocypodoidea (yellow, triangle): *4.31* log_{10}(CW) + 2.1, r^2=0.94*, Portunoidea (blue, diamond): *2.26* log_{10}(CW) + 4.45, r^2=1.0*, Xanthoidea (black, circle): *2.47* log_{10}(CW) + 4.71, r^2=0.71*.
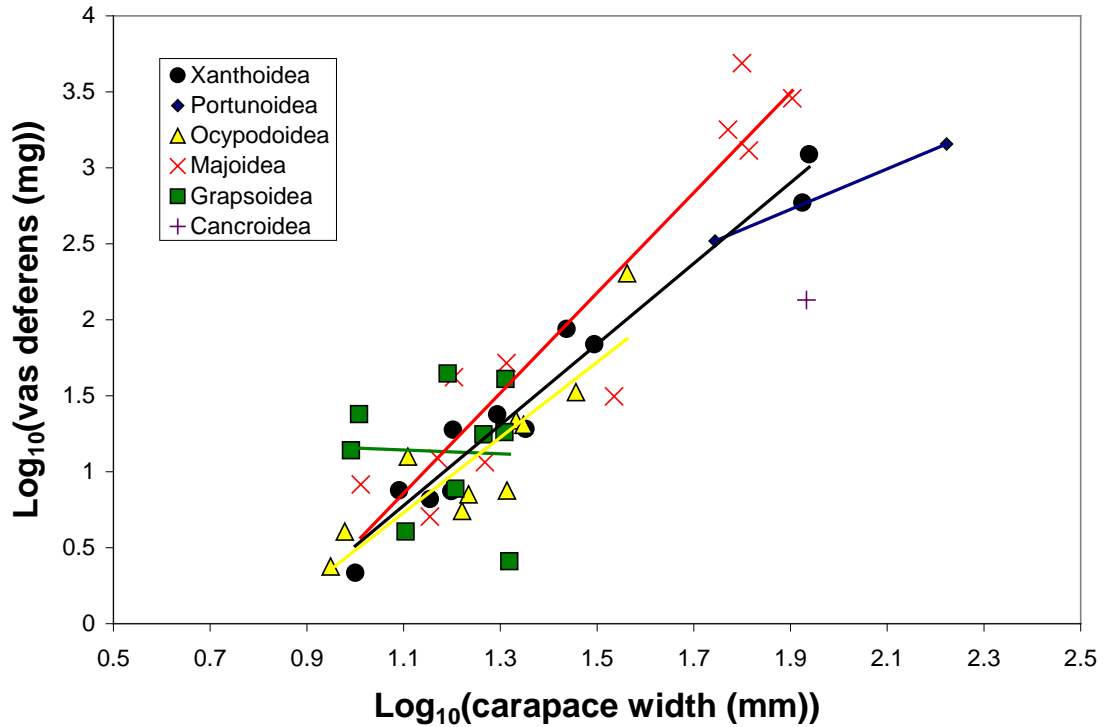
Figure 8: The relationship between vas deferens weight (mg) ($\log_{10}$) (VD) and male carapace width ($\log_{10}$) (CW) for 44 species of brachyurans from six superfamilies; Cancroidea (purple, plus), Grapsoidea (green, square): $Log_{10}(VD) = 1\text{-}0.13* log_{10}(CW) + 1.28, \ r^2=0.0001$, Majoidea (red, x): $3.30* log_{10}(CW) \text{ - } 2.8, \ r^2=0.88$, Ocypodoidea (yellow, triangle): $2.48* log_{10}(CW) \text{ - } 4.2.0, \ r^2=0.77$, Portunoidea (blue, diamond): $1.33* log_{10}(CW) + 0.19, \ r^2=1$, Xanthoidea (black, circle): $2.66* log_{10}(CW) \text{ - } 2.14, \ r^2=0.96$.
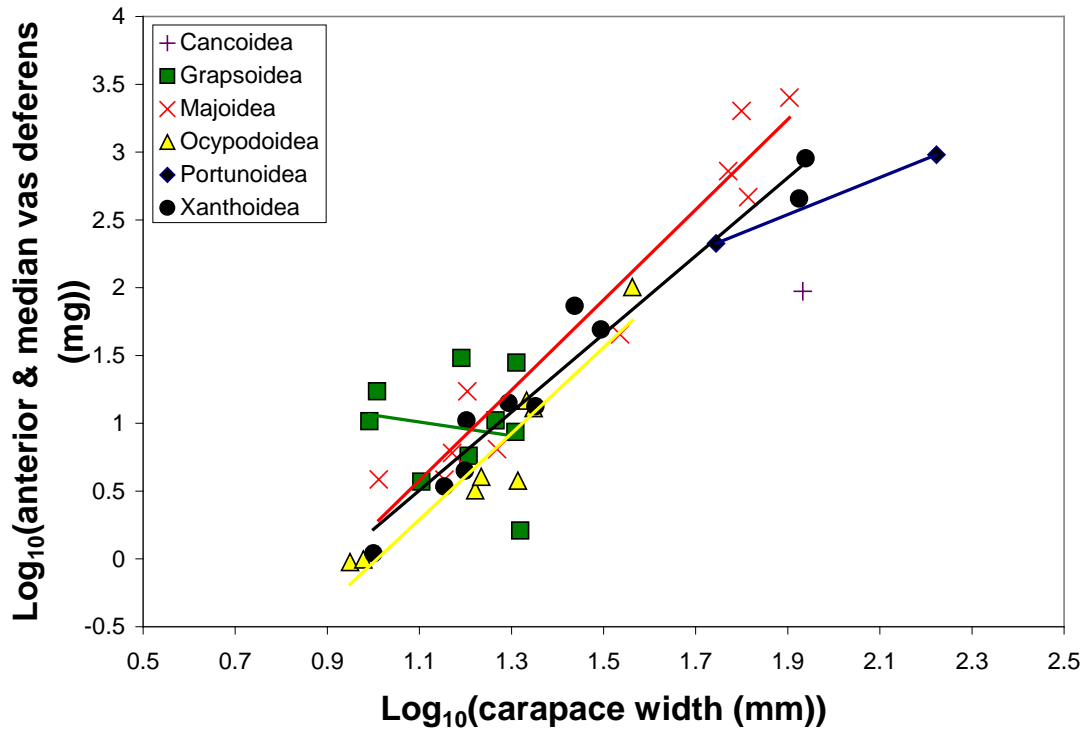
Figure 9: The relationship between the anterior & median vas deferens weight (mg) ($\log_{10}$) (AVDMVD) and male carapace width ($\log_{10}$) (CW) for 40 species of brachyurans from six superfamilies; Cancroidea (purple, plus), Grapsoidea (green, square): $Log_{10}(AVDMVD) = -0.51* \log_{10}(CW) + 1.57, r^2=0.025$, Majoidea (red, x): $3.33* \log_{10}(CW) - 3.088, r^2=0.94$, Ocypodoidea (yellow, triangle): $3.17* \log_{10}(CW) - 3.20, r^2=0.90$, Portunoidea (blue, diamond): $1.37* \log_{10}(CW) - 0.056, r^2=1$, Xanthoidea (black, circle): $2.88* \log_{10}(CW) - 2.67, r^2=0.96$.
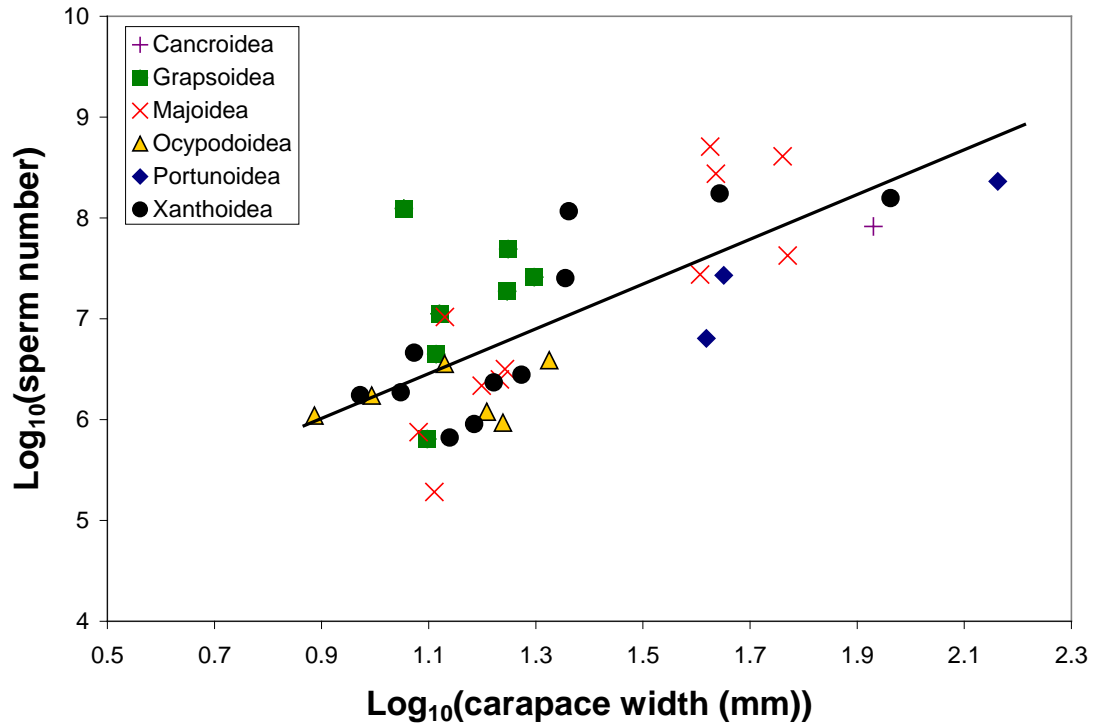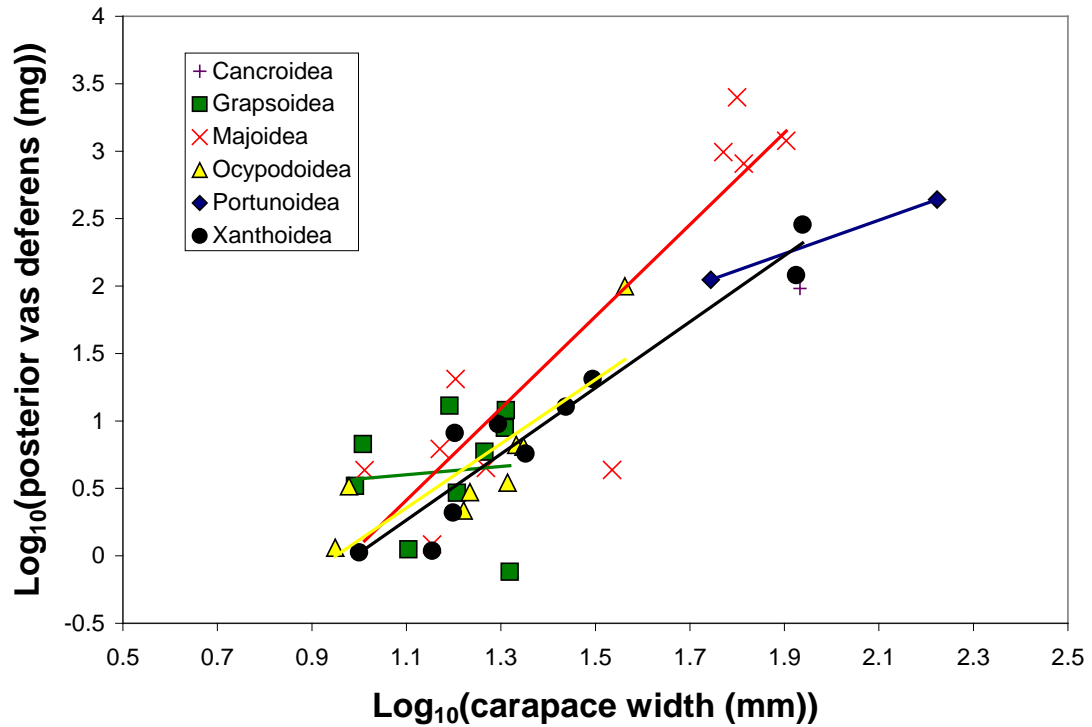
Figure 10: The relationship between posterior vas deferens weight (mg) ($\log_{10}$) (PVD) and male carapace width ($\log_{10}$) (CW) for 40 species of brachyurans from six superfamilies; Cancroidea (purple, plus), Grapsoidea (green, square): $Log_{10}(PVD) = 0.31* \log_{10}(CW) + 0.26$, $r^2=0.008$, Majoidea (red, x): $3.40* \log_{10}(CW) - 3.33$, $r^2=0.80$, Ocypodoidea (yellow, triangle): $2.38* \log_{10}(CW) - 2.26$, $r^2=0.68$, Portunoidea (blue, diamond): $1.24* \log_{10}(CW) - 0.12$, $r^2=1$, Xanthoidea (black, circle): $2.45* \log_{10}(CW) - 2.43$, $r^2=0.92$.
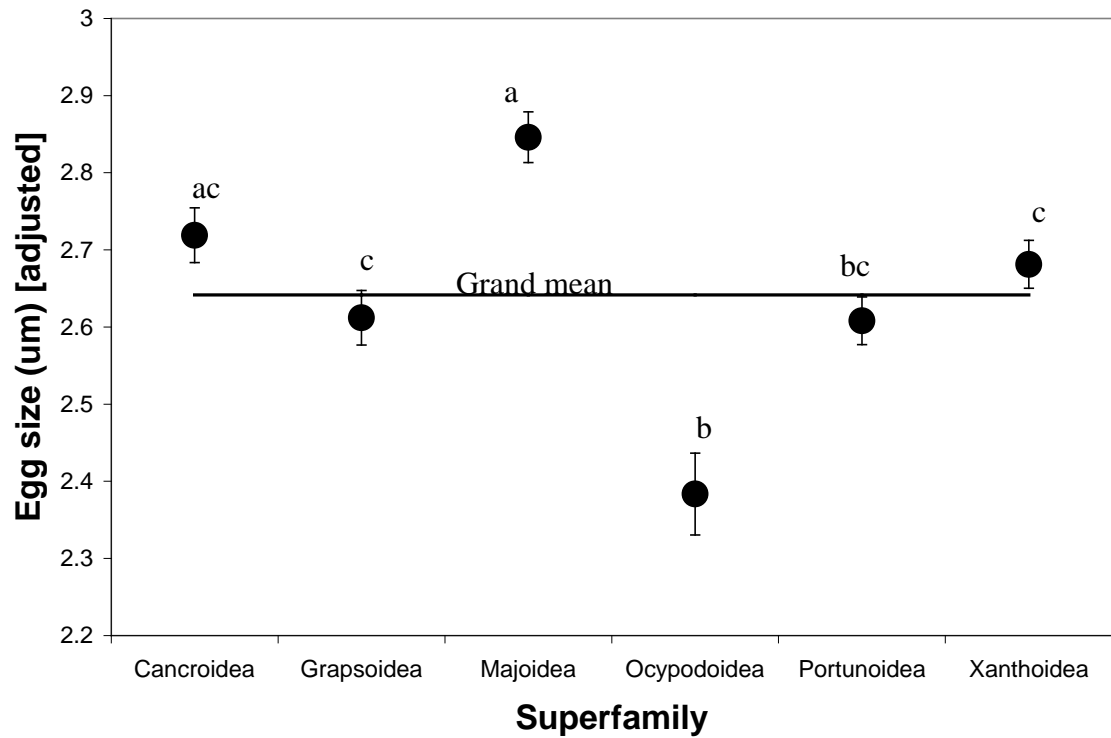
Figure 11: The average egg size +/- 1SE for 54 species of brachyurans from each of the six superfamilies: Cancroidea, Grapsoidea, Majoidea, Ocypodoidea, Portunoidea, and Xanthoidea. Superfamily means were adjusted for egg number, egg stage, and habitat. Different letters represent significant differences between superfamilies and the horizontal bar represents the grand adjusted mean.
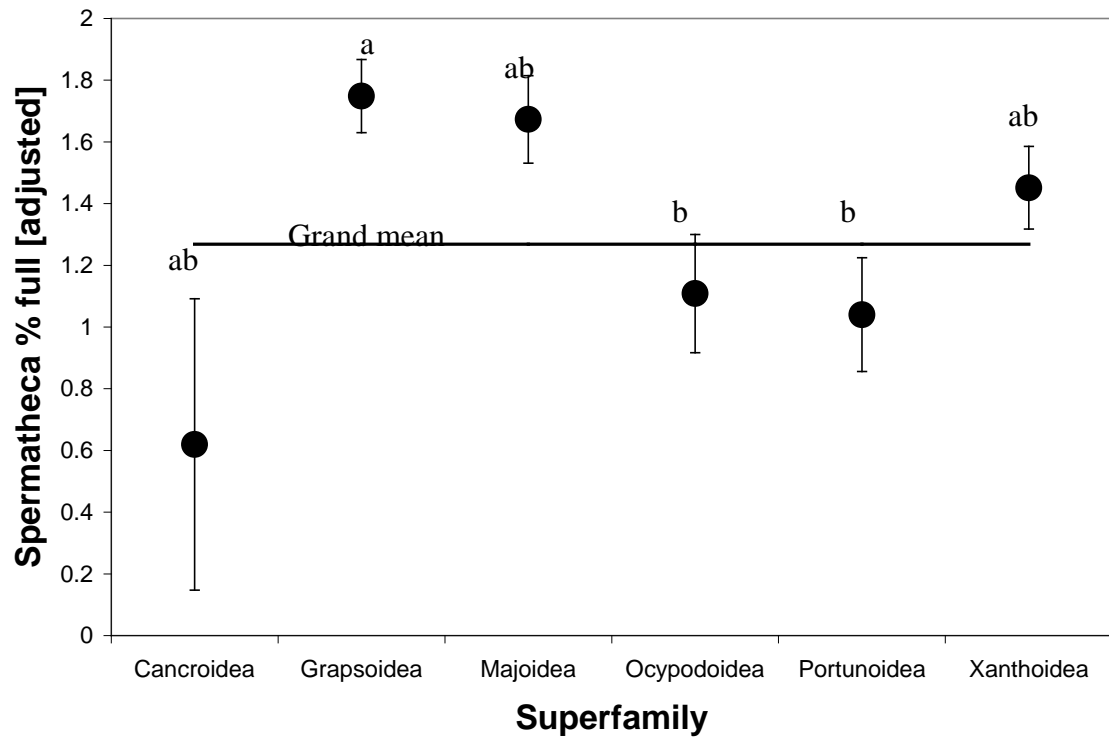
Figure 12: The average spermatheca % fullness +/- 1SE for 40 species of brachyurans from each of the six superfamilies: Cancroidea, Grapsoidea, Majoidea, Ocypodoidea, Portunoidea, and Xanthoidea. Superfamily means were adjusted for habitat and region. The horizontal bar represents the grand adjusted mean.
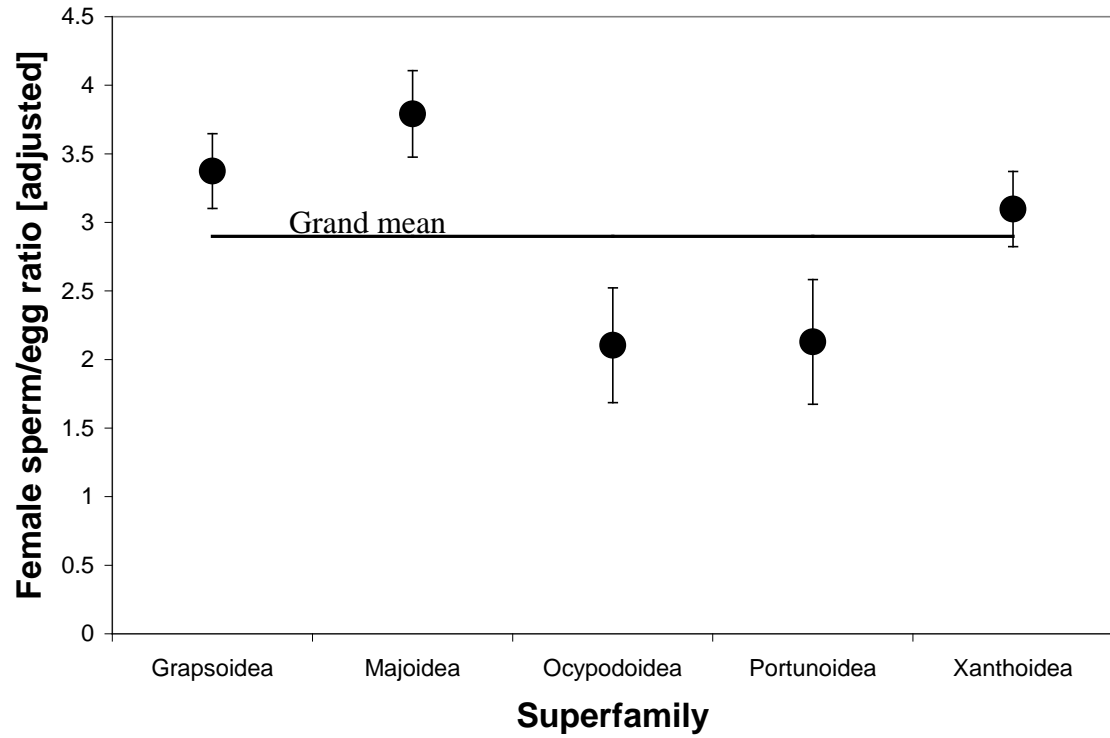
Fig. 13: The average female sperm/egg ratio +/- 1SE for 54 species of brachyurans from five different superfamilies: Grapsoidea, Majoidea, Ocypodoidea, Portunoidea, and Xanthoidea. Superfamily means are adjusted for habitat. The horizontal bar represents the grand adjusted mean.
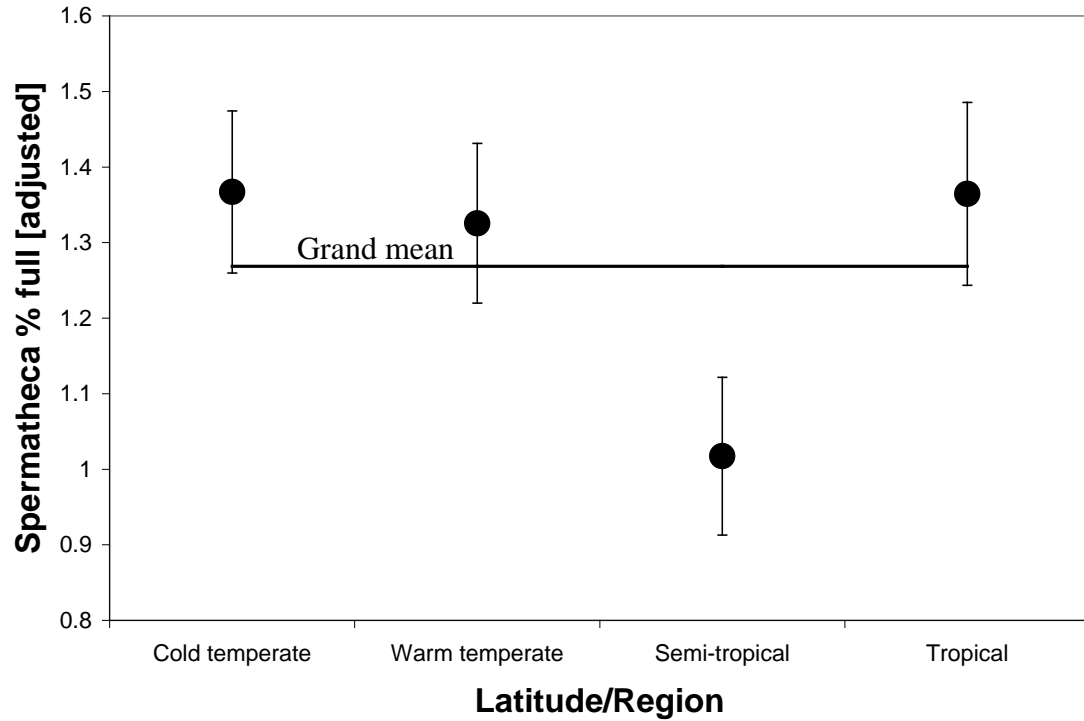
Fig. 14: The average spermatheca % fullness +/- 1SE for 54 species of brachyurans from four different geographical regions: cold temperate, warm temperate, semi-tropical, tropical. Regional means are adjusted for depth and superfamily. The horizontal bar represents the grand adjusted mean.
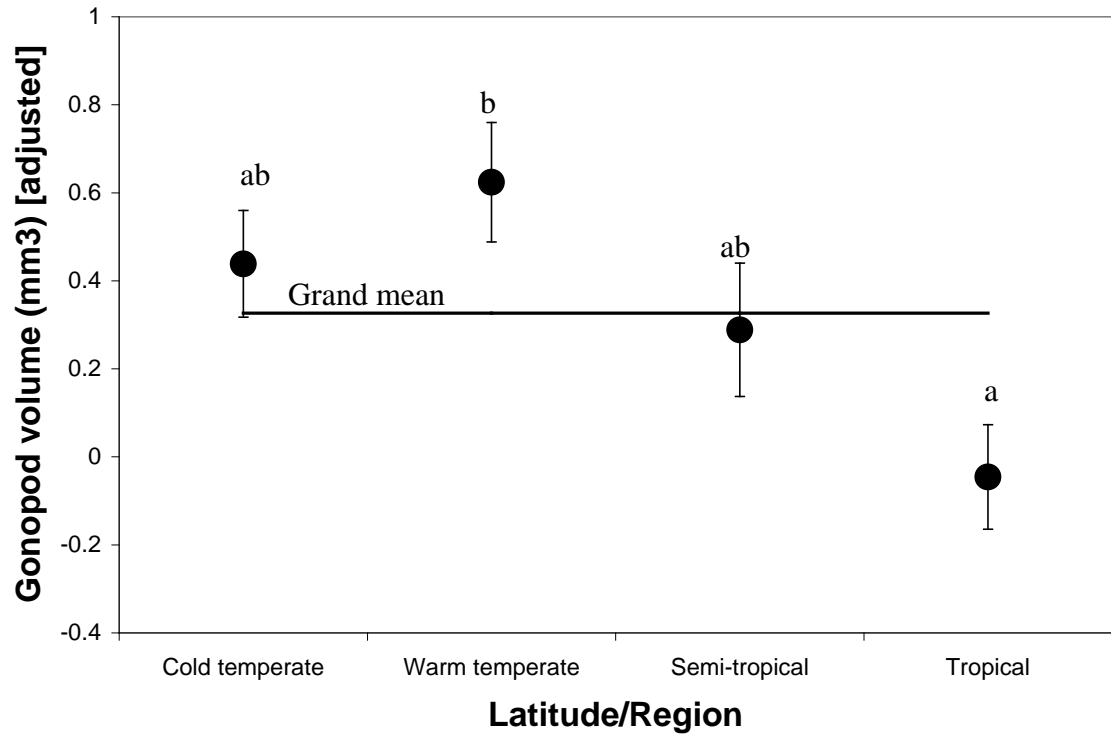
Fig. 15: The average gonopod volume (mm^3) +/- 1SE for 54 species of brachyurans from four different geographical regions: cold temperate, warm temperate, semi-tropical, tropical. Regional means are adjusted for depth and superfamily. Letters represent significant differences between region and the horizontal bar represents the grand adjusted mean.
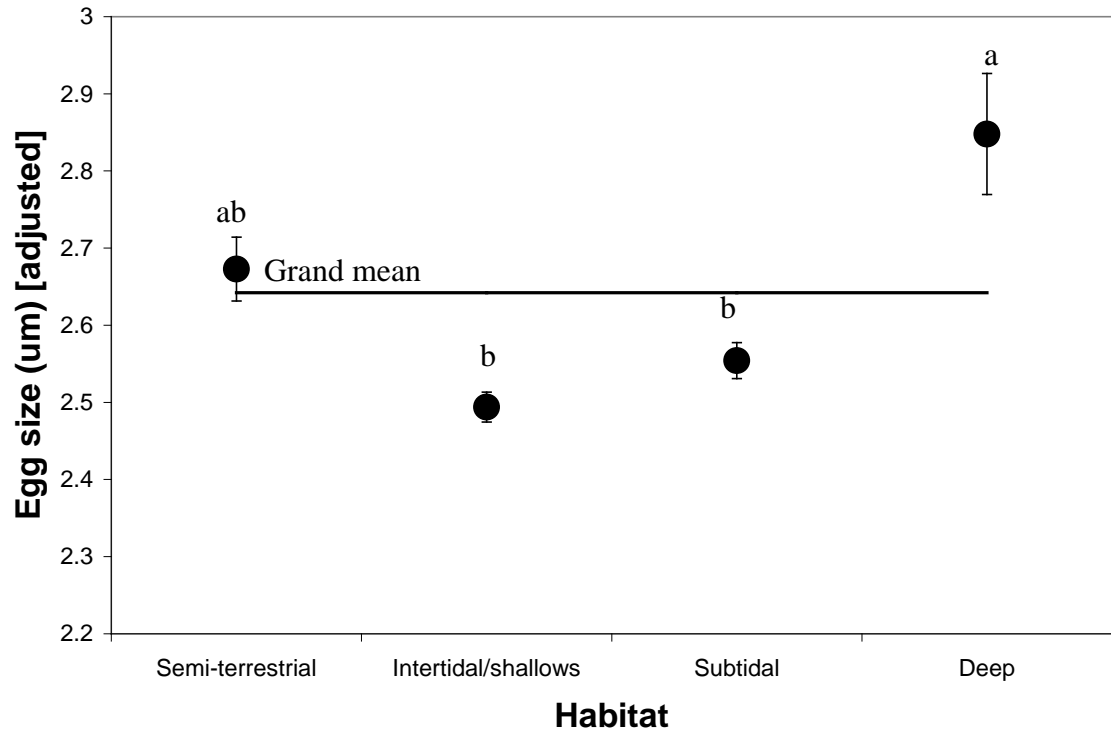
Figure 16: The average egg size +/- 1SE for 54 species of brachyurans from five different depths: semi-terrestrial, intertidal-shallows, subtidal, deep. Habitat means are adjusted for egg number, egg stage, and superfamily. Different letters represent significant differences between habitats and the horizontal bar represents the grand adjusted mean.
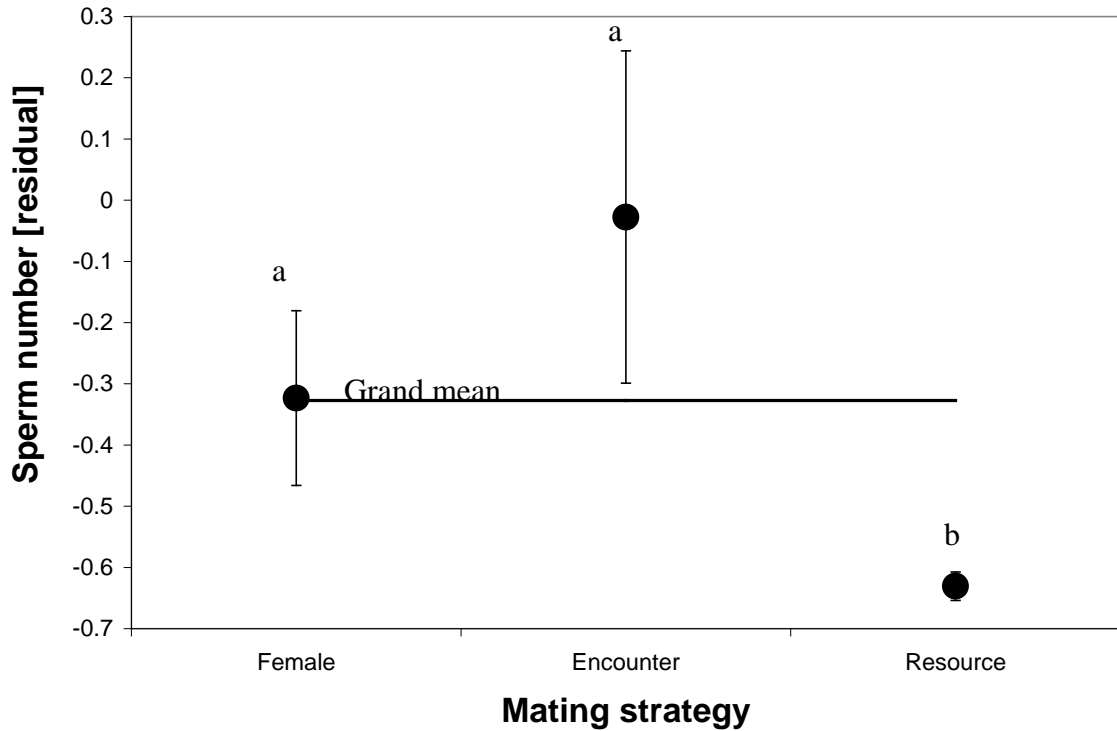
Figure 17: The average female sperm number +/- 1SE for 10 species of brachyurans with one of three mating strategies: 'female defense,' 'encounter/scramble,' and 'resource defense.' Mating strategy means were calculated after removing the significant effect of superfamily from a sequential ANCOVA. Letters represent significant differences between mating strategies.
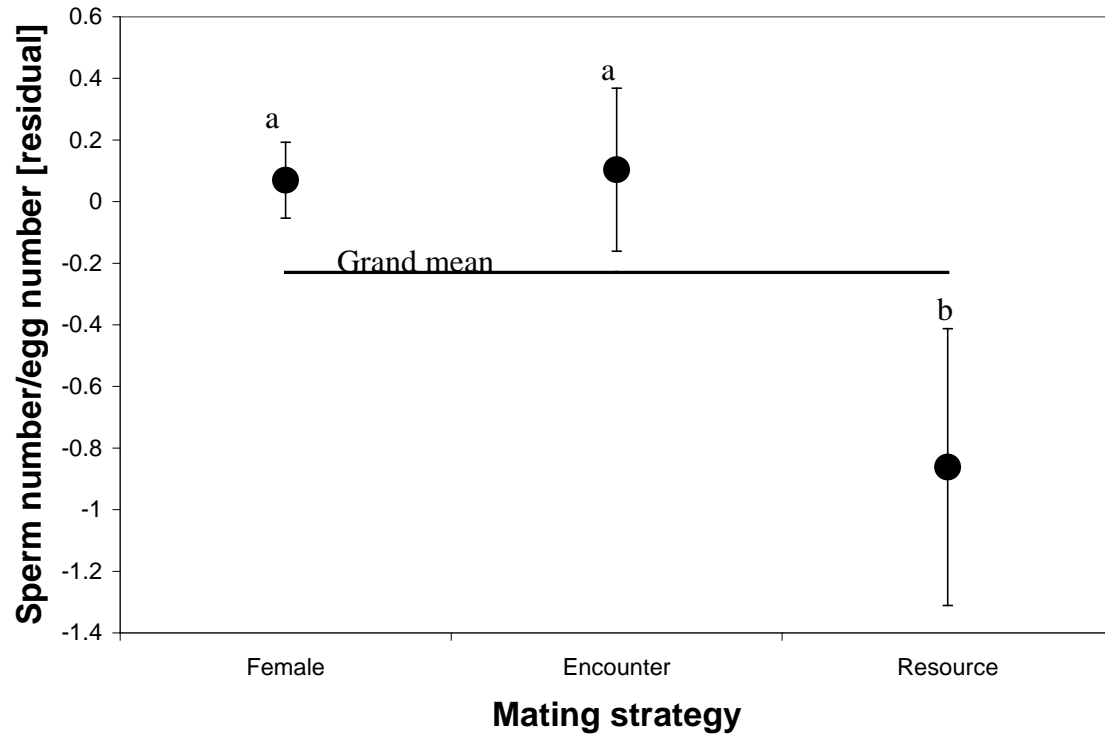
Figure 18: The average sperm/egg ratio +/- 1SE for 10 species of brachyurans with one of three mating strategies: 'female defense', 'encounter/scramble,' and 'resource defense.' Mating strategy means were calculated after removing the significant effect of superfamily from a sequential ANCOVA. Letters represent significant differences between mating strategies.
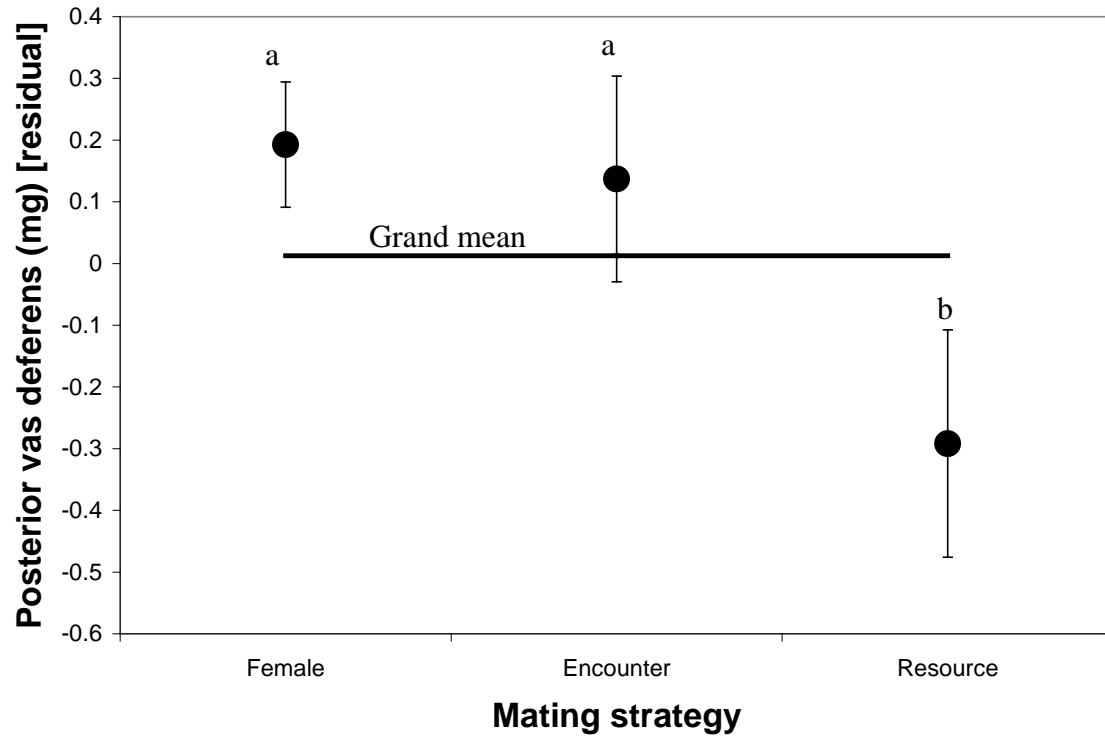
Figure 19: The average posterior vas deferens weight (mg) +/- 1SE for 12 species of brachyurans with one of three mating strategies: 'female defense,' 'encounter/scramble,' and 'resource defense.' The residuals of mating strategy means were calculated after removing the significant effects of carapace width, superfamily and the interaction of carapace width and superfamily from a sequential ANCOVA. Letters represent significant differences between mating strategies.
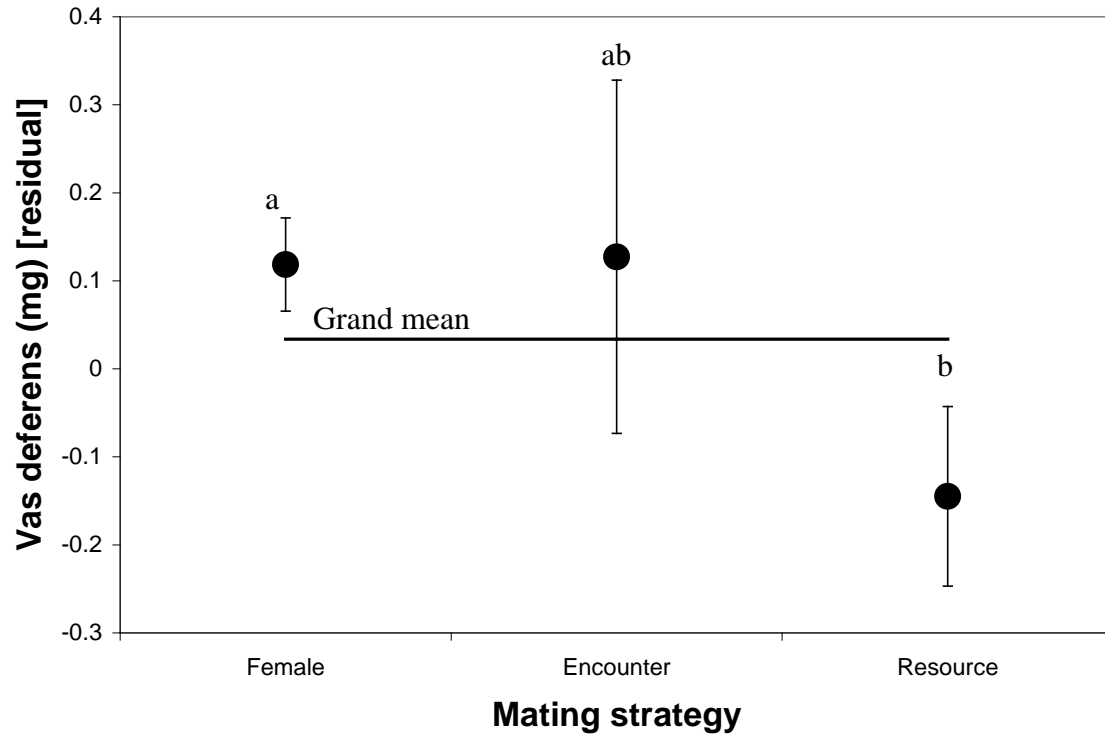
Figure 20: The average vas deferens weight (mg) +/- 1SE for 14 species of brachyurans with one of three mating strategies: 'female defense,' 'encounter/scramble,' and 'resource defense.' The residuals of mating strategy means were calculated after removing the significant effects of carapace width, superfamily and the interaction of carapace width and superfamily from a sequential ANCOVA. Letters represent significant differences between mating strategies.
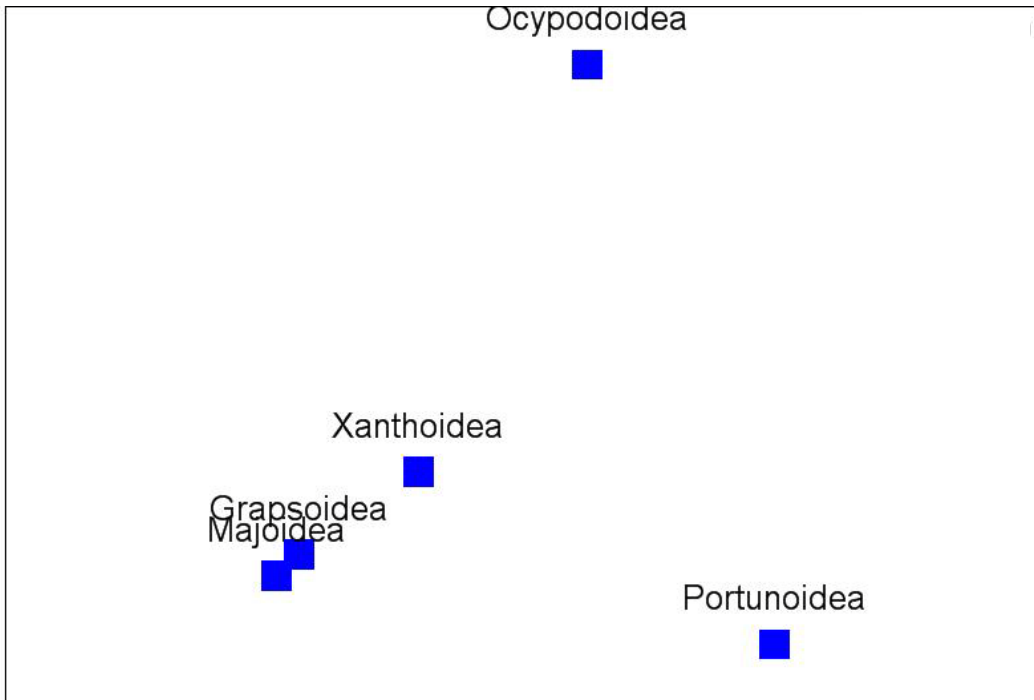
Figure 21: MSD nonparametric ordination graphs for clustering of life history traits by superfamily (Cancroidea, Majoidea, Portunoidea, Xanthoidea, Grapsoidea, Ocypodoidea) for 31 species of brachyurans using the resemblance matrix from the ANOSIM analysis.

CHAPTER IV

Testing for phylogenetic patterns in life history traits for six superfamilies of brachyuran crabs

ABSTRACT

Phylogeny has profound implications on the evolution of life history traits. With recent advances in phylogenetic methods, previously described life history trade-offs and correlations among male and female life history traits can now be evaluated within a phylogenetic context. To better define phylogenetic patterns in brachyurans, I first reconstructed a Bayesian phylogeny using 16S rDNA and measured variation in six male and four female reproductive traits for 36 species of brachyurans from 6 superfamilies (Majoidea, Xanthoidea, Cancroidea, Portunoidea, Grapsoidea, Ocypodoidea). I tested for a significant phylogenetic signal in each of several key life history traits by conducting permutation tests across the phylogeny. Most life history traits displayed a significant phylogenetic signal; however, male traits were less constrained by phylogeny than female traits. After accounting for phylogeny, I corroborated previous studies which showed that egg number and egg size are negatively correlated. I also show that species whose males possess heavier vas deferens weights have females with heavier spermatheca and fuller sperm storage organs (after correcting for body size) than species with lighter vas deferens. This study suggests phylogeny has played an important role in the evolution of life history traits, and that it is imperative to account for phylogeny in tests of correlation between life history traits in brachyurans.

INTRODUCTION

Male and female life history traits display significant phylogenetic patterns across a diverse array of taxa (Stearns, 1992; Charnov, 1993). Trade-offs between these traits have been well described (Lack, 1948; Cody, 1966; Williams, 1966), and male and female life history traits also have been shown to co-evolve. Co-evolution of male and female traits has been known for more than a century and explains many of the unique and diverse secondary sexual traits seen in various plants and animals (Darwin, 1874). More recently, this Darwinian view has been expanded to include post copulatory traits such as male sperm traits, and the co-evolution of male and female traits has received strong support (Dybas & Dybas, 1981; Eberhard, 1997; Minder et al., 2005; Anthes et al., 2008).

Studies on the evolution of life history traits have begun to incorporate rigorous phylogenetic analyses to test for independence of these traits from lineage effects (Arnqvist et al., 2000; Blomberg et al., 2003; Anthes et al., 2008). Since species share a common phylogenetic history, they cannot be considered independent units, and by ignoring phylogeny, the assumption of independence of units underlying statistical analyses is violated (Felsenstein, 1985). Various statistical techniques have been employed to account for phylogeny in comparisons among species: nested analyses (Clutton-Brock & Harvey, 1977); independent contrasts (Felsenstein, 1985); concentrated changes (Lorch & Eadie, 1999); and phylogenetic regressions (Grafen, 1989; Martins & Hansen, 1997). In addition, increased software capabilities in computer modeling and analyses (e.g., Bayesian analysis and maximum likelihood) have allowed the inclusion of

more complex models of evolution and are becoming widely used techniques in comparative studies.

While many of these analyses can be employed to identify a phylogenetic signal in a trait, identification of a signal does not indicate the underlying cause of the pattern. For example in stomatopods, shifts in habitat can be constrained across the phylogeny and these in turn shape body size and thus life history traits (Reaka, 1986; Caldwell, 1991). A similar link between habitat and phylogeny is also observed in brachyurans. Brachyurans are thought to have evolved in the littoral zone from a shared common ancestor with the dromiid crabs and subsequently radiated into the deep-sea and semi-terrestrial zones by the late Cretaceous (Stevcic, 1971). With a shift to semi-terrestrial habitats, their bodies became more stream-lined and adapted for speed. There were multiple independent invasions from terrestrial to freshwater habitats (Diesel et al., 2000), which lead to the production of lecithotrophic larvae and species thus produced fewer, larger eggs (Diesel et al., 2000). Similar changes in habitat likely shaped patterns in sperm storage. Habitat correlates with morphology of the female spermatheca in stylommatophoran snails and slugs; those inhabiting rocky coasts have simple fertilization pouches, whereas those in woodland habitats tend to have complex organs with multiple pouches for sperm storage (Beese et al., 2008). Thus, broad shifts in habitat may shape many of the phylogenetic patterns observed for life history traits.

Brachyurans provide a good model group for the study of phylogenetic patterns in life history traits and for the study of correlations in male and female life history traits,

138

particularly sperm storage traits. All brachyurans have internal fertilization and females possess a paired spermatheca for sperm storage (Hartnoll, 1969). There is a large degree of variation across species in the duration of female sperm storage, in the amount of sperm that females store in their spermathecae, and in the amount of sperm and seminal fluid produced by the male. While there is a well established trade-off between egg number and egg size (Hines, 1982), and there are significant superfamily patterns for many brachyuran life history traits (Hines, 1982; Hines, 1986), these patterns are highly interactive with and confounded by, effects of habitat and mating strategies (Rodgers, chapter 3 of this dissertation). Despite the fact that these superfamily patterns have been well studied in brachyuran crabs, these traits have not been tested rigorously across the phylogeny. By first identifying the presence of a phylogenetic signal in these life history traits, I then used a phylogenetic regression which accounts for phylogenetic relatedness to test for correlations between male and female life history traits independent of phylogenetic relatedness.

While the phylogeny of brachyuran crabs continues to be revised, several molecular and morphological phylogenies have been established within the main superfamilies. The first published molecular phylogeny across the main brachyuran superfamilies (Ahyong et al., 2007) has a similar topology to previous phylogenies based on adult and larval morphology for the major superfamilies of brachyurans (Rice, 1983; Martin & Davis, 2001). However, patterns in life history traits have yet to be tested across the brachyuran phylogeny.

The goal for this study was to first construct a phylogenetic tree for six superfamilies of brachyuran crabs (Grapsoidea, Majoidea, Portunoidea, Ocypodoidea, Xanthoidea, Cancroidea), and to use this phylogeny to test for phylogenetic patterns in several key life history traits. After accounting for significant phylogenetic effects, I then test for correlations between male and female life history traits to better understand the evolution of these traits. Given that many life history traits vary significantly across superfamilies (Rodgers, chapter 3 of this dissertation), I predicted that each of several key life history traits (female reproductive output, male sperm traits, female sperm storage traits) would show significant phylogenetic patterns. I also predicted that egg number is inversely related to egg size, and that species with more eggs, higher sperm-egg ratios and larger stored female sperm correlate with large male sperm reserves (sperm number and seminal fluid weight). By using a phylogenetic approach, we were able to take a first step at incorporating rigorous phylogenetic analyses in studies of life history traits for brachyurans.

METHODS

I collected a total of 36 species from 14 different families and six superfamilies of brachyuran crabs (Majoidea, Xanthoidea, Cancroidea, Portunoidea, Grapsoidea, Ocypodoidea) (Table 1). Approximately 10 males and 10 females were collected for most species; however, several species were rare or difficult to find and fewer specimens were collected for these species. The range of sampling locations spanned a broad range of latitudes, including the eastern seaboard of the United States from Nova Scotia to Florida, the Caribbean coast of Panama, and the Pacific coastline from Panama to Coquimbo,

Chile (Table 1). While most specimens were collected opportunistically by hand, some of the deeper specimens were collected by trawl.

Construction of Phylogeny

A 520 base pair portion of the 16S rDNA nucleotide sequence was acquired from Genbank for each of the 36 study species and for *Raninoides louisianensis,* the outgroup (Table 1). Given the difficulty of aligning sequences using 16S rDNA (Schubart et al., 2000a), the data were separated by superfamily and analyzed individually: Majoidea, Xanthoidea, Portunoidea and Cancroidea (Brosing et al., 2006), Grapsoidea, and Ocypodoidea. Multiple alignments were conducted in ClustalX v.2. The models of nucleotide substitution were determined in JModelTest v.3 (Posada, 2008), and the best model of nucleotide evolution was chosen using the Akaike Information Criteria (AIC) model selection criteria (Steel, 2005) for each superfamily: Majoidea (TVM+G), Xanthoidea (TIM3+G), Portunoidea & Cancroidea (GTR+G), Grapsoidea (GTR+I+G), Ocypodoidea (TPM1uf+G) (for a description of the parameters for each model of nucleotide evolution listed above, see Posada 2008).

Maximum likelihood (ML) and Bayesian analysis (BI) were used to construct phylogenies for each data set using the models of evolution selected by JModelTest. Garli v.0951 (Zwickl, 2006) was used to construct trees by maximum likelihood and 1000 bootstrap replicates were conducted on the data. Bayesian analyses were run in Mr. Bayes v.3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) and each dataset was run with two MCMC (Markov Chain Monte Carlo) chains; the analyses were

run until the two chains converged on a stable distribution (standard deviation <0.08) or when the log likelihood converged on a stable value (Grapsoidea 20,000; Majoidea 20,000; Ocypodoidea 10,000; Portunoidea and Cancroidea 10,000; Xanthoidea 30,000) (Schubart et al., 2000c). Consensus trees were constructed using the 50% majority rule and the posterior probabilities reported. Phylograms were drawn and rooted in TreeView (Page, 1996).

While the topologies of the Bayesian consensus trees and maximum likelihood trees were quite similar, the Bayesian consensus trees resulted in topologies that best matched those of published brachyuran phylogenies that used molecular (Schubart et al., 2000b; Rosenberg, 2001; Schubart et al., 2006; Robles et al., 2007; Hultgren & Stachowicz, 2008) and morphological (Rosenberg, 2001) traits. Since the relationships across the six superfamilies have previously been established (Brosing et al., 2006; Ahyong et al., 2007), the complete phylogeny was compiled in TreeView using the topologies and branch lengths from our Bayesian analysis to identify within-superfamily patterns and the topology from the literature to construct the relationship across superfamilies. Branch lengths of 1 were assigned only to the branches separating superfamilies and the phylogeny was labeled 'estimated branch length' (Fig. 1). A second tree was constructed using the topology of the 'estimated branch length' phylogeny but with all branch lengths set as 1. This second tree, 'equal branch length,' makes fewer model assumptions about branch lengths (Blomberg et al., 2003). Phylogenetic signal and co-evolution of traits were tested against each phylogeny.

Measurements of life history traits

For all collected individuals, I measured carapace width (mm) and removed one side of the paired sperm storage organs: spermatheca (females), vas deferens (males). Percent fullness of the spermatheca was determined visually as the percent of the spermatheca that was occupied by ejaculate contents. The male vas deferens was carefully separated into anterior, median and posterior vas deferens sections (Cronin, 1947), and each section was weighed (wet weight). One side of the reproductive tissue -- female spermatheca or male vas deferens -- was weighed (wet weight) and fixed in 3% glutaraldehyde in a 0.1M sodium phosphate buffer. The fixative was removed after 24 hours and the tissue was rinsed and stored in 0.1M sodium phosphate buffer. Sperm were counted by first grinding the tissue in a known volume of 0.1M sodium phosphate buffer using a handheld glass Dounce homogenizer®. Three subsample counts were conducted on a Petroff-Hausser Counting Chamber® using 400x magnification on a phase contrast microscope. The average number of sperm was calculated for each sample.

Broods from egg-bearing females were fixed in 3% glutaraldehyde, and stored in 70% ethanol. A volumetric method was used to estimate the number of eggs. The egg mass was rinsed three times in 0.1M phosphate buffer and placed in a known volume of 0.1M phosphate buffer and the volume displaced was recorded +/- 1µl. The eggs were then removed from the pleopods and the volume of buffer displaced by the pleopods was subtracted from the volume displaced for the total egg mass. The average ovum volume was calculated for each brooding female by measuring the diameter of twenty eggs per egg mass in 0.1M phosphate buffer at 100x or 400x magnification, and the spherical

ovum volume was calculated using the formula: *ovum vol. (mm^3) = (4/3)*π*(diameter/2)^3*. The average egg number for each female was calculated using the equation: *no. of eggs = vol. displaced by the egg mass/ave ovum vol.*

One gonopod was removed from each male and stored in 70% ethanol. Measurements of length (base to tip excluding any setae) and average width (measured at the base, midline and near the tip on both sides of the gonopod) were conducted under a dissection microscope or using a vernier caliper in accordance with the size of the gonopod. Volume of the gonopod was calculated by multiplying the length by the average width.

Phylogenetic Signal

To test for significant phylogenetic signals for each of the life history traits, a modified generalized least squares model (GLS) was conducted in the analysis of phylogenetics and evolution (ape) package of R (Paradis et al., 2004). Each trait was first transformed for normality and regressed against male or female body size. A GLS model was used to calculate the variance in the trait using a Brownian motion model of evolution (assumes the trait evolves randomly along branches) and phylogenetic relatedness was incorporated into the error term. The log Likelihood score was computed which measured the phylogenetic component of the trait variance (Blomberg et al., 2003). Permutation tests were then used to randomly sample the trait across the phylogeny 1000 different times without replacement (this removes any phylogenetic signal). The range of log Likelihood scores were calculated for each permuted data set and frequency histograms of the log Likelihood scores were constructed. A log Likelihood score of the observed data that was greater than 95% of the log Likelihood scores for the permuted data indicated a

significant phylogenetic signal (related species had more similar trait values). Conversely, a log Likelihood score of the observed data that was lower than 95% of the permuted data sets indicated an anti-signal (closely related species had less similar trait values than expected by chance alone) (Blomberg et al., 2003). Permutations were run on both the 'equal branch length' and 'estimated branch length' phylogenies.

Correlated Evolution of Traits

Phylogenetic regressions were run in R using the ape package to test for regressions between male and female life history traits independent of phylogenetic relatedness. Regressions were conducted on both the 'estimated branch length' and 'equal branch length' phylogenies. All life history variables were first transformed to meet normality and regressed against female or male body size and the residuals used as the response variables in the phylogenetic regression (Table 5). Using a modified generalized least squares model that incorporates phylogenetic distance in the error term of the model and a Brownian motion of trait evolution (assumes the trait evolves randomly along branches), I tested for significant regressions between male and female life history traits (Grafen, 1989; Martins & Hansen, 1997).

RESULTS

Phylogeny construction

The Bayesian superfamily analyses returned phylogenies with high posterior probability support that ranged from 0.54-1.00 (Fig. 1). The phylogeny returned one polytomy

among *E. depressus, E. dissimilis,* and *R. harrisii,* but all other relationships between species were resolved (Fig. 1).

Phylogenetic Signal

When using the 'estimated branch length' phylogeny, four variables, in addition to male and female carapace widths, displayed significant phylogenetic signals (log-likelihood scores above the 95% confidence interval for the permuted data): spermathecae % fullness, spermatheca weight, female sperm number, and male gonopod volume (Table 2). Compared to the 'estimated branch length phylogeny,' more of the variables displayed a significant phylogenetic signal on the 'equal branch length' phylogeny: male carapace width, female carapace width, egg number, egg size, spermathecae % fullness, spermathecae weight, female sperm-egg ratio, female sperm number, male gonopod volume, and posterior vas deferens weight (Table 2). Since the data best fit the 'equal branch length' phylogeny, the results of the phylogenetic signal permutation histograms are displayed only for this phylogeny (Figs. 2, 3). Most of the variables with a significant phylogenetic signal displayed a higher log Likelihood than the permuted data, and thus a greater signal between closely related species than predicted by chance alone. However, the log Likelihood value for the observed gonopod volume was much lower than the range of log Likelihood values expected from the permuted data, an anti-signal, meaning that closely related species had more dissimilar gonopod volumes than predicted by chance alone (Fig. 2, 3). The log Likelihood value for the posterior vas deferens was also lower than predicted by chance, however it was the only sperm storage trait that

displayed an anti-signal, and the log Likelihood value was fairly close to the range computed for the permuted data (Fig. 3).

Correlated Evolution of Traits

Prior to correcting for phylogenetic effects, seven of the measured variables displayed significant regression equations (Table 3). After correcting for phylogeny using the 'estimated branch length' phylogeny, only three regressions were significant: egg number and egg size, female spermatheca weight and male vas deferens weight, and female sperm number and male sperm number (Table 3). However, the 'equal branch length' phylogeny produced the best model fit (lowest AICc values) (Table 3), and these results are displayed graphically. Using the 'equal branch length' phylogeny, there were four significant regressions: egg number decreased with increasing egg size (Fig. 4); spermathecae % fullness increased with increasing total male vas deferens weight (Fig. 5); and spermatheca weight increased with increasing total male vas deferens weight (Fig. 6), and with increasing anterior + median vas deferens weight (Fig. 7).

DISCUSSION

Phylogenetic Signals

Most brachyuran life history traits displayed a significant phylogenetic signal, meaning that closely related species had similar life history traits. Therefore, these life history traits are at least partially constrained to phylogenetic patterns and not completely free to vary with nonphylogenetic factors. From a metanalysis of 35 phylogenies, life history, body size and morphological traits were typically more constrained by phylogeny than

147

ecological, behavioral and physiological traits (Blomberg et al., 2003). In fact all of the female reproductive and sperm storage traits in this study displayed a significant phylogenetic signal. In brachyurans, female reproductive output, egg number and egg size are constrained by the volume available in the body cavity for ovary development (Hines, 1982). Even after removing the effects of body size, significant phylogenetic patterns in reproductive output emerged. Thus, using a more rigorous phylogenetic approach, this study confirms earlier findings in which reproductive output varied across superfamilies for brachyurans (Hines, 1982).

Not only did female reproductive output display phylogenetic patterns, but sperm storage patterns also showed significant phylogenetic signals consistent with superfamily trends previously described for these traits (Rodgers, chapter 3 of this dissertation). Since morphological features of the brachyuran spermatheca are known to vary across superfamilies (Jensen et al., 1996; Sainte-Marie & Sainte-Marie, 1998; Lopez-Greco et al., 2009), it is not surprising that spermathecal capacity displayed a phylogenetic pattern. Spermathecal capacity may in turn be constrained by reproductive output. Since female spermathecae and ovaries occupy the same space in the body cavity, a lineage that has evolved large ovaries has less room available for the development of large spermathecae and for the storage of large ejaculate volumes (Sainte-Marie, 2007). Thus the evolution of large sperm storage organs constrains, and is constrained by, the size of the ovaries.

Since only a few of the male reproductive traits displayed significant phylogenetic signals, male traits appear to be less constrained by phylogeny. While detection of a

phylogenetic signal is sensitive to sample size up to approximately 20 species (Blomberg et al., 2003), our data set contained 36 species, suggesting that there was adequate power to detect phylogenetic signals if they existed. According to our results, male reproductive traits are more labile and may be under greater selection due to ecological and mating system effects. It is known that as sperm competition increases, size of the testes increases in many bird species (Moller, 1991), and number of sperm increases in both voles (delBarco-Trillo & Ferkin, 2004) and guppies (Evans & Magurran, 1999). Thus, allocation to larger sperm stores in males may be the result of increased sperm competition and depends greatly on the mating system strategy. The relative importance of sperm competition is not known for most brachyurans due to the challenges associated with observing mating events in the field and identifying multiple sperm packets within the female spermatheca. However, it is known that males alter mating tactics -- guarding behavior (Jivoff, 1997a), copulation duration (Wedell & Cook, 1999), seminal fluid allocation (Kendall et al., 2001; Kendall et al., 2002), and sperm allocation (Gage & Barnard, 1996) -- with the presence of competitors and with increased sperm competition. Since males can invest in large copulations or frequent mating, male reproductive traits might respond to selection from ecological and behavioral factors more than female reproductive traits.

Surprisingly, gonopod volume displayed a significant anti-phylogenetic signal. Closely related species may have dissimilar gonopod morphologies which act as physical barriers to matings between species. Some species of male fiddler crabs have been observed to court and even attempt to mate with females of a different but closely related species

(Christy, pers. comm.); and male *Callinectes sapidus* have been observed to exhibit mating displays and copulated with female *C. similis* (Hines, pers. comm.). A morphological barrier to cross-species mating could prevent the occurrence of nonfunctional copulations. However, conclusions await further investigation.

Co-evolution/Correlated Evolution

If phylogenetic relatedness were not accounted for, I would have erroneously concluded that most of the male and female life history traits were significantly correlated. Instead, phylogenetic relatedness explained the correlation between many of these life history traits. Since the data best fit the 'equal branch length' phylogeny (determined by smaller AICc values), it was best to make fewer assumptions about the branch lengths when there was less certainty about the actual lengths (Blomberg et al., 2003). By assuming potentially erroneous branch lengths, the error in the model can increase, thus decreasing statistical power to detect significant correlations when they exist.

As the number of eggs per brood increased, the relative egg size decreased. Given that females have a limited amount of energy that can be allocated to reproduction, they can either produce many small eggs or few large eggs. While this trade-off is well described in the literature for a broad array of taxa (Smith & Fretwell, 1974; Hines, 1982; Charnov & Ernest, 2006) and across brachyuran superfamilies (Hines, 1982), we were able to test this trade-off after correcting for phylogenetic relatedness. Interestingly, the decline in number of eggs with increasing egg size was greater after correcting for phylogeny, confirming that females must allocate their energy to producing many small eggs or few

large eggs in this group. This has important implications not only for female reproductive output, but also for sperm allocation and storage.

Several of the male and female life traits remained correlated even after accounting for phylogeny. The weights of the male vas deferens and of the anterior + median vas deferens correlated with spermatheca weight as well as with the percent fullness of the spermatheca. In species for which males produced heavier vas deferens, females had larger sperm storage capacities. Females can accumulate large ejaculate stores either by receiving large amounts of ejaculate at mating or by mating frequently. While polyandrous mating has been documented for most brachyuran species, the size of male sperm stores explains a significant amount of variation in the amount of ejaculate stored by females across species. Interestingly, the weight of the posterior vas deferens did not correlate with capacity of the female spermatheca. The posterior vas deferens contains mostly seminal fluid but no spermatophores (Cronin, 1947), and is thought to contain seminal products important in anaerobic sperm metabolism (Jeyalectumie & Subramoniam, 1991) that might be used up at different rates in species, depending on the length of sperm storage in the female organ. Nevertheless, the amount of sperm in the anterior vas deferens and seminal fluid in the median vas deferens best explains larger ejaculate stores in brooding females.

CONCLUSIONS

The results from this study reveals the vital important of incorporating phylogeny into the study of life history traits. Not only did many of the life history traits display a significant

phylogenetic signal, but the correlation between male and female traits differed with the incorporation of phylogenetic analyses. The male life history traits tested in this study displayed a greater degree of phylogenetic plasticity and likely respond more strongly to selection from ecological and mating system effects than female traits. Species with large male vas deferens weights had females with large sperm stores, suggesting that these traits are correlated in brachyurans. By correcting for phylogeny, we were able to tease apart correlations in male and female traits versus phylogenetic trends in life history traits. With the impending publication of the brachyuran tree of life, these methods can be used in the future to rigorously test hypothesis across the complete brachyuran phylogeny.

Table 1: Species of brachyuran crabs collected from 2005 to 2009 for this study. Superfamily, collection location and Genbank accession numbers for 16S rDNA are listed for each species.

Taxon	Superfamily	Family	Location	Accession No
Cancer irroratus	Cancroidea	Cancridae	Nova Scotia, CANADA	AJ130812
Aratus pisonii	Grapsoidea	Grapsidae	Fort Pierce, FL, USA	AJ784012
Pachygrapsus transversus	Grapsoidea	Grapsidae	Fort Pierce, FL, USA	AM180259
Sesarma cinereum	Grapsoidea	Sesarmidae	Point Lookout, MD, USA	AJ784010
Sesarma ricordii	Grapsoidea	Sesarmidae	Fort Pierce, FL, USA	AJ225876
Sesarma occidentale	Grapsoidea	Sesarmidae	NAOS, Panama City, PANAMA	AJ225856
Sesarma reticulatum	Grapsoidea	Sesarmidae	Point Lookout, MD, USA	AJ130799
Hemigrapsus sanguiensis	Grapsoidea	Varunidae	Tuckerton, NJ, USA	AJ493053
Acanthonyx petiverii	Majoidea	Epialtidae	Fort Pierce, FL, USA	EU682803
Mithrax forceps	Majoidea	Mithracidae	Peanut Island, FL, USA	EU682782
Mithrax sculptus	Majoidea	Mithracidae	Caribow Caye, BELIZE	EU682785
Chionoectes opilio	Majoidea	Oregoniidae	Nova Scotia, CANADA	AB188684
Hyas araneus	Majoidea	Oregoniidae	Nova Scotia, CANADA	EU682773
Hyas coarctatus	Majoidea	Oregoniidae	Nova Scotia, CANADA	EU682774
Libinia dubia	Majoidea	Pisidae	Gloucester, VA, USA	EU682794
Pitho lherminieri	Majoidea	Tychidae	Peanut Island, FL, USA	EU682789
Uca thayeri	Ocypodoidea	Ocypodidae	Fort Pierce, FL, USA	Z79647
Uca pugilator	Ocypodoidea	Ocypodidae	Fort Pierce, FL, USA	Z79662
Uca minax	Ocypodoidea	Ocypodidae	Point Lookout, MD, USA	Z79670
Uca pugnax	Ocypodoidea	Ocypodidae	Tuckerton, NJ, USA	Z79672
Uca beebei	Ocypodoidea	Ocypodidae	Rodman, Panama City, PANAMA	Z79646
Uca rapax	Ocypodoidea	Ocypodidae	Fort Pierce, FL, USA	Z79676
Ocypode quadrata	Ocypodoidea	Ocypodidae	Fort Pierce, FL, USA	Z79681
Uca stylifera	Ocypodoidea	Ocypodidae	Rodman, Panama City, PANAMA	Z79688
Carcinus maenas	Portunoidea	Portunidae	Avery Point, CT, USA	AJ130811
Ovalipes ocellatus	Portunoidea	Portunidae	Tuckerton, NJ, USA	FJ716615
Callinectes sapidus	Portunoidea	Portunidae	Gloucester, VA, USA	AJ130813
Panopeus lacustris	Xanthoidea	Panopeidae	Fort Pierce, FL, USA	AJ274681
Panopeus herbstii	Xanthoidea	Panopeidae	Fort Pierce, FL, USA	AJ130815
Eurypanopeus depressus	Xanthoidea	Panopeidae	Fort Pierce, FL, USA	AJ274688
Euypanopeus dissimilis	Xanthoidea	Panopeidae	Fort Pierce, FL, USA	AJ274689
Rhithropanopeus harrisii	Xanthoidea	Panopeidae	Edgewater, MD, USA	AJ274697
Panopeus americanus	Xanthoidea	Panopeidae	Caribow Caye, BELIZE	AJ274683
Xanthodius sternberghii	Xanthoidea	Xanthidae	Caribow Caye, BELIZE, USA	AM076785
Cataleptodius floridanus	Xanthoidea	Xanthidae	Fort Pierce, FL, USA	AJ274698
Paraxanthus barbiger	Xanthoidea	Xanthidae	Coquimbo, CHILE	FJ031221
Raninoides louisianensis	Raninoidea	Raninidae	Outgroup	AF436044

Table 2: Log Likelihood scores measuring the phylogenetic component of variance for each life history trait ($\log_{10}$ transformed and corrected for body size) are displayed for both the 'estimated branch length' and 'equal branch length' phylogenies (AVD= anterior vas deferens, MVD= median vas deferens, PVD= posterior vas deferens). Asterisks signify significant phylogenetic signals ($\alpha=0.05$) compared to a permutated data set created by randomly assigning traits across the phylogeny 1000 different times.

	Estimated branch length	Equal branch length
Variable	Log Likelihood	Log Likelihood
Male carapace width [$\log_{10}$]	-2.7*	-5.4*
Female carapace width [$\log_{10}$]	-2.7*	-0.3*
Egg number	-35.1	-14.1*
Egg size	79.5	100.5*
Spermathecae % fullness	-21.6*	-14.0*
Spermatheca weight	-17.1*	-12.6*
Female sperm/egg ratio	-292.3	-288.5*
Female sperm number	-31.3*	-24.1*
Male sperm number	-45.3	-32.5
Male gonopod length	35.0	47.9
Male gonopod volume	-36.4*	-20.6*
Male VD weight	-48.4	-31.4
Male AVD&MVD weight	-45.7	-30.4
Male PVD weight	-23.4	-8.9*

Table 3: P values for the phylogenetic regressions of male and female life history traits (log$_{10}$ transformed and corrected for body size) corrected for either the 'estimated branch length' or 'equal branch length' phylogenies (AVD= anterior vas deferens, MVD= median vas deferens, PVD= posterior vas deferens). Asterisks signify significant differences (α=0.05) and values in parenthesis represent the AICc model fit.

Response	Explanatory	No phylogeny	Estimated branch length	Equal branch length
Egg number	Male sperm number	0.95	0.43 (73.1)	0.79 (30.1)
	Egg size	<0.0001*	<0.0001* (19.5)	<0.0001* (3.7)
Spermatheca weight	Male VD weight	0.0004*	0.015* (30.6)	0.01* (18.4)
	Male AVD&MVD weight	0.0013*	0.052 (22.3)	0.04* (9.4)
	Male PVD weight	0.09	0.29 (25.4)	0.21 (12.4)
Female sperm/egg ratio	Male sperm number	0.06	0.83 (569.2)	0.20 (560.2)
% fullness of spermatheca	Male VD weight	0.003*	0.05 (43.3)	0.003* (28.0)
	Male AVD&MVD weight	0.0028*	0.11 (39.4)	0.09 (24.5)
	Male PVD weight	0.0091*	0.16 (40.2)	0.10 (24.9)
Female sperm number	Male sperm number	0.0076*	0.01* (58.8)	0.84 (50.2)

R. louisianensis

L. dubia (P)
0.73
P. lherminieri (T)
0.71
C. opilio (O)
1.00
0.54
H. araneus (O)
H. coarctatus (O)

Majoidea

0.73
A. petiverii (E)
0.97
M. forceps (M)
M. sculptus (M)

C. irroratus (C)

Cancroidea

0.60
C. sapidus (Po)
0.70
C. maenus (Po)
O. ocellatus (Po)

Portunoidea

1.00
P. americanus (Pa)
1.00
P. herbstii (Pa)
0.76
P. lacustris (Pa)
0.94
E. depressus (Pa)
E. dissimilis (Pa)
R. harrisii (Pa)
0.68
P. barbiger (X)
1.00
C. floridanus (X)
X. sternberghii (X)

Xanthoidea

O. quadrata (Oc)
1.00
U. stylifera (Oc)
1.00
U. beebei (Oc)
U. thayeri (Oc)
0.73
0.99
U. pugilator (Oc)
1.0
U. rapax (Oc)
0.98
U. pugnax (Oc)
U. minax (Oc)

Ocypodoidea

H. sanguiensis (V)
0.73
P. transversus (G)
1.00
S. reticulatum (S)
0.97
A. pisonii (S)
S. ricordii (S)
0.98
0.89
S. occidentale (S)
S. cinereum (S)

Grapsoidea

1.0 (across superfamily branches)
- - - - - - -
0.1 (for branches within superfamilies)
─────

156

Fig 1: Phylogenetic tree based on 16S rDNA using Bayesian Inference (BI) for brachyuran crabs from six different superfamilies: Majoidea (open), Xanthoidea (horizontal stripes), Grapsoidea (vertical stripes), Ocypodoidea (solid), Cancroidea (dotted), and Portunoidea (diagonal stripes). Numbers represent posterior probabilities for each node. Branches between superfamilies (dashed lines) were determined from published molecular (Ahyong et al., 2007) and morphological (Rice, 1983; Martin and Davis 2001) phylogenies and all of these were set at branch lengths of 1.0. Branch lengths within superfamilies (solid lines) were estimated as indicated above: 'estimated branch length phylogeny.' Letters in parentheses represent families: Sesarmidae (S), Varunidae (V), Grapsidae (G), Ocypodidae (Oc), Xanthidae (X), Panopeidae (P), Portunidae (Po), Cancridae (C), Mithracidae (M), Epialtidae (E), Oregoniidae (O), Pisidae (P), Tychidae (T).

(a) Egg number
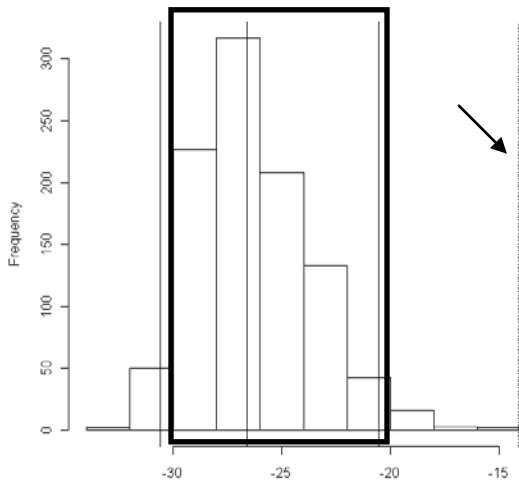
(b) Egg size
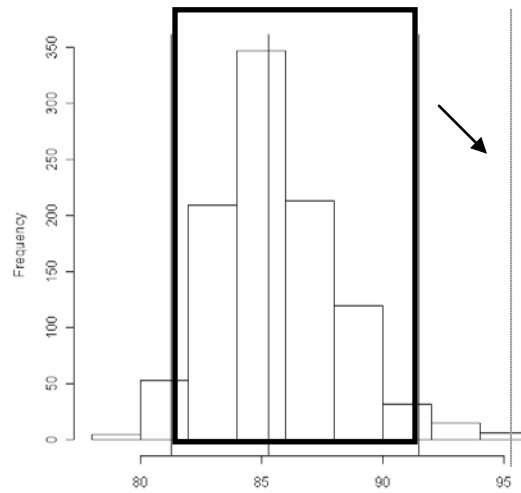
(c) % Fullness of spermatheca
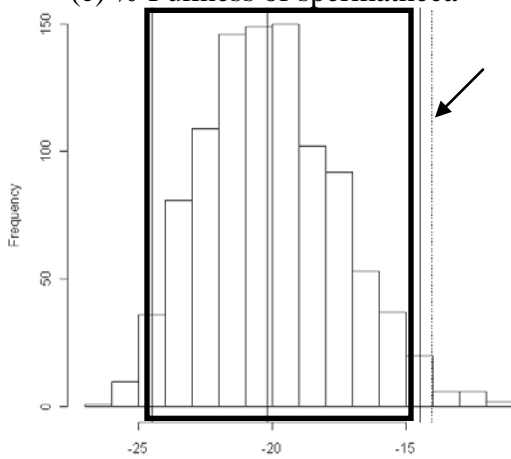
(d) Spermatheca weight
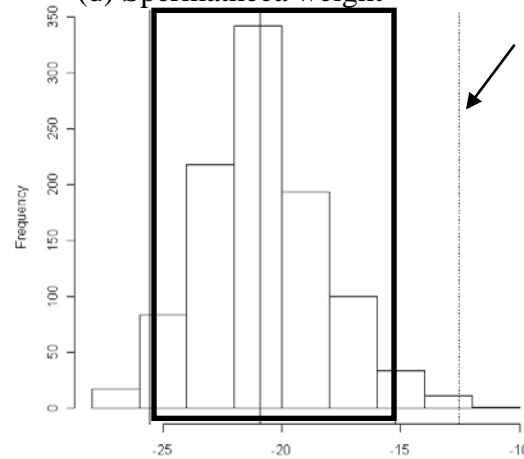
(e) Female sperm number/ egg number
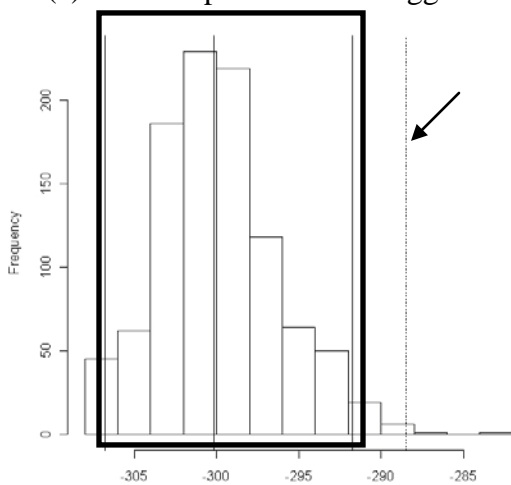
(f) Female sperm number

158
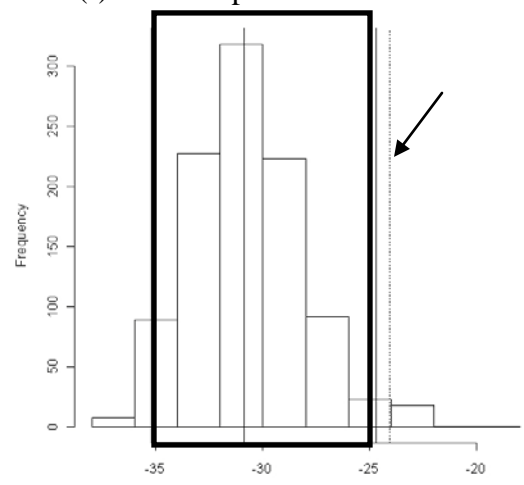
Fig. 2: Permutation test results across the 'equal branch length' phylogeny for the female traits. The boxed region represents the 95% confidence region of the log Likelihood scores for the permuted data and the arrow points to the dashed line at the actual log Likelihood score for the observed data.
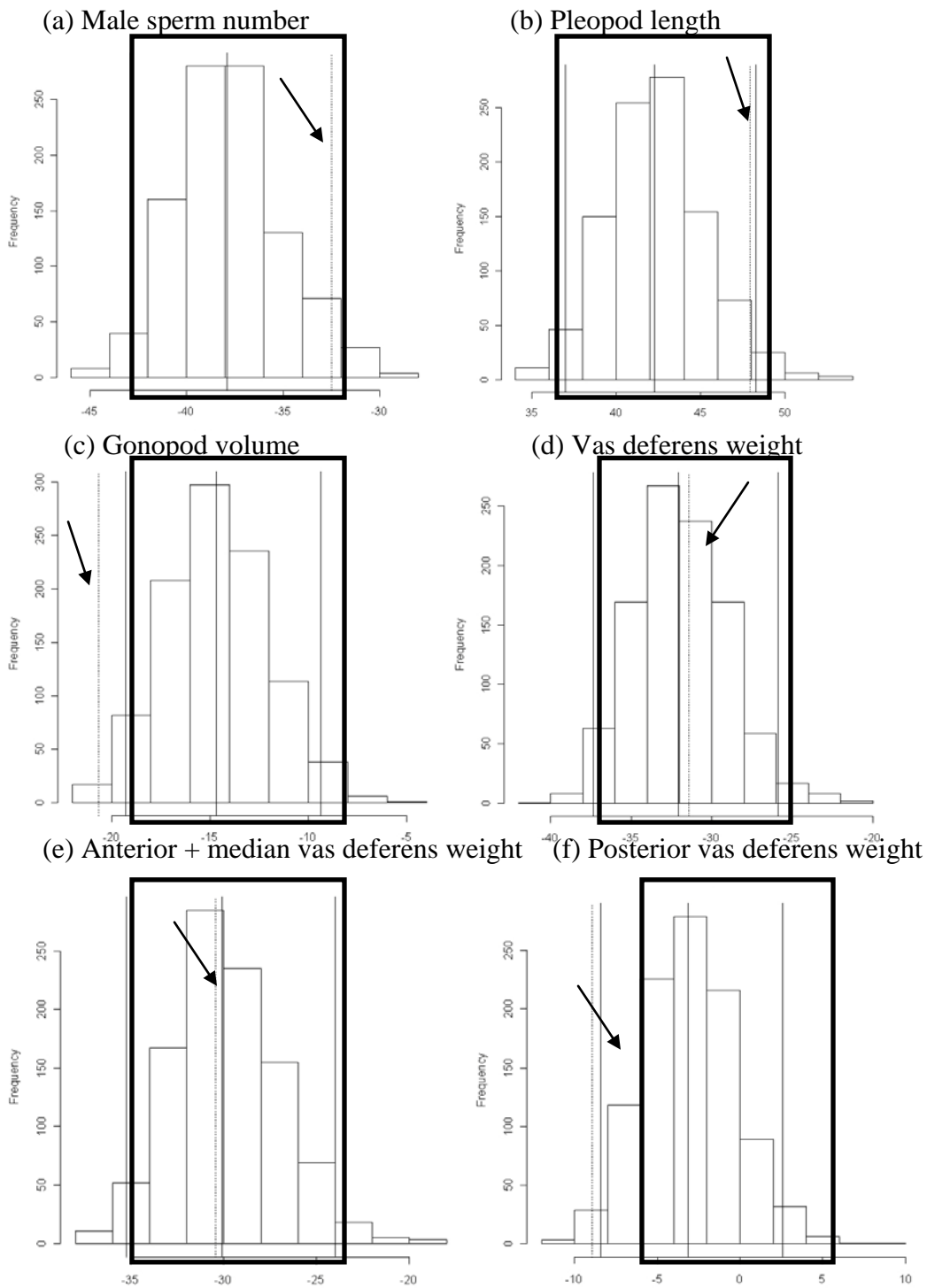
Fig. 3: Permutation test results across the 'equal branch length' phylogeny for the male traits. The boxed region represents the 95% confidence region of the log Likelihood scores for the permuted data and the arrow points to the dashed line at the actual log Likelihood score for the observed data.
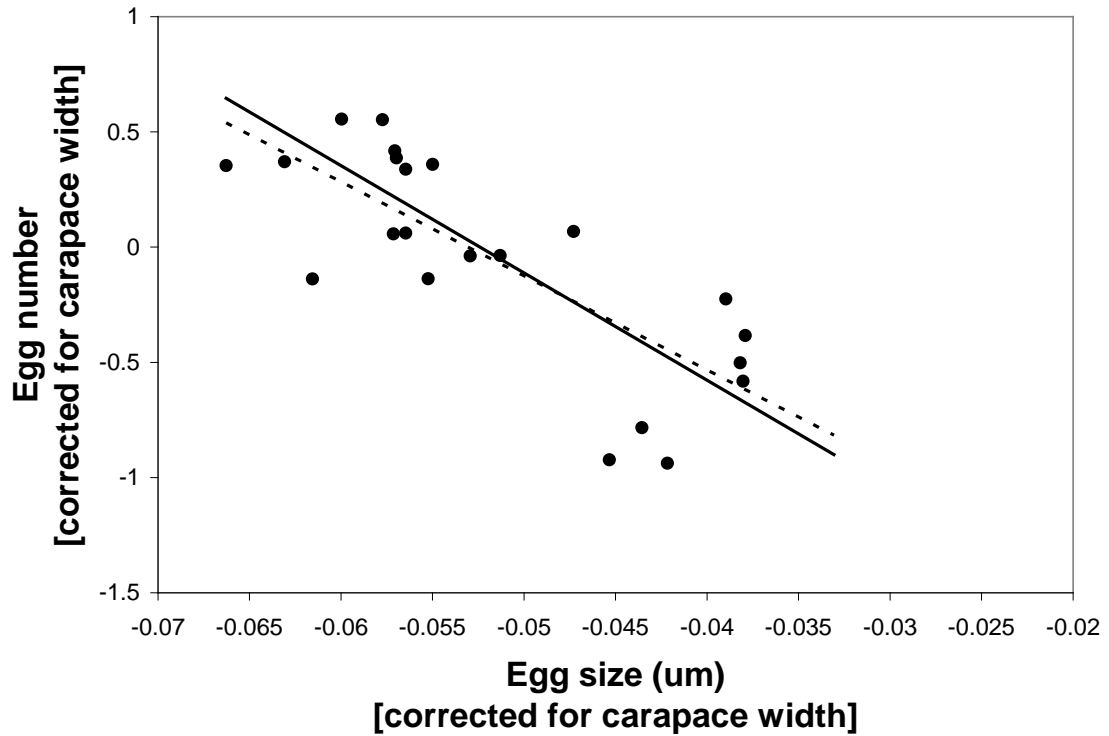
Fig 4: The relationship between egg number and egg size (both corrected for female carapace width) for 29 species of brachyuran crabs. Dashed line equation: *Egg No. = -40.84*Egg size - 2.2*. The solid line represents the relationship of egg number and egg size after correcting for phylogenetic relatedness using the 'equal branch length' phylogeny. Solid line equation: *Egg No. =-46.5512*Egg size - 2.44, F=40.66, p<0.0001*. R^2 values are not calculated for generalized least squares covariance models.
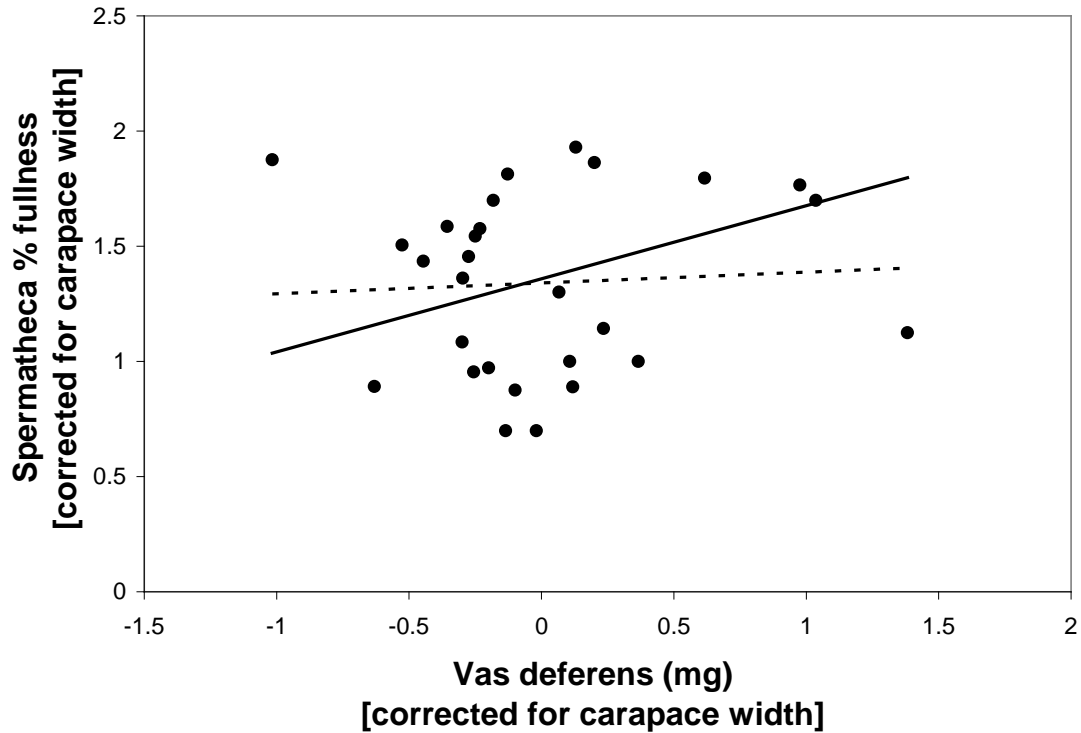
Fig 5: The relationship between spermatheca % fullness and vas deferens weight (VD) (both corrected for female carapace width) for 27 species of brachyuran crabs. Dashed line equation: *Spermatheca % full= 0.05*VD +1.34*. The solid line represents the relationship of spermatheca % fullness and vas deferens weight after correcting for phylogenetic relatedness using the 'equal branch length' phylogeny. Solid line equation: *Spermatheca % full= 0.3171*VD+1.3587726, F = 4.854, p=0.037.* R^2 values are not calculated for generalized least squares covariance models.
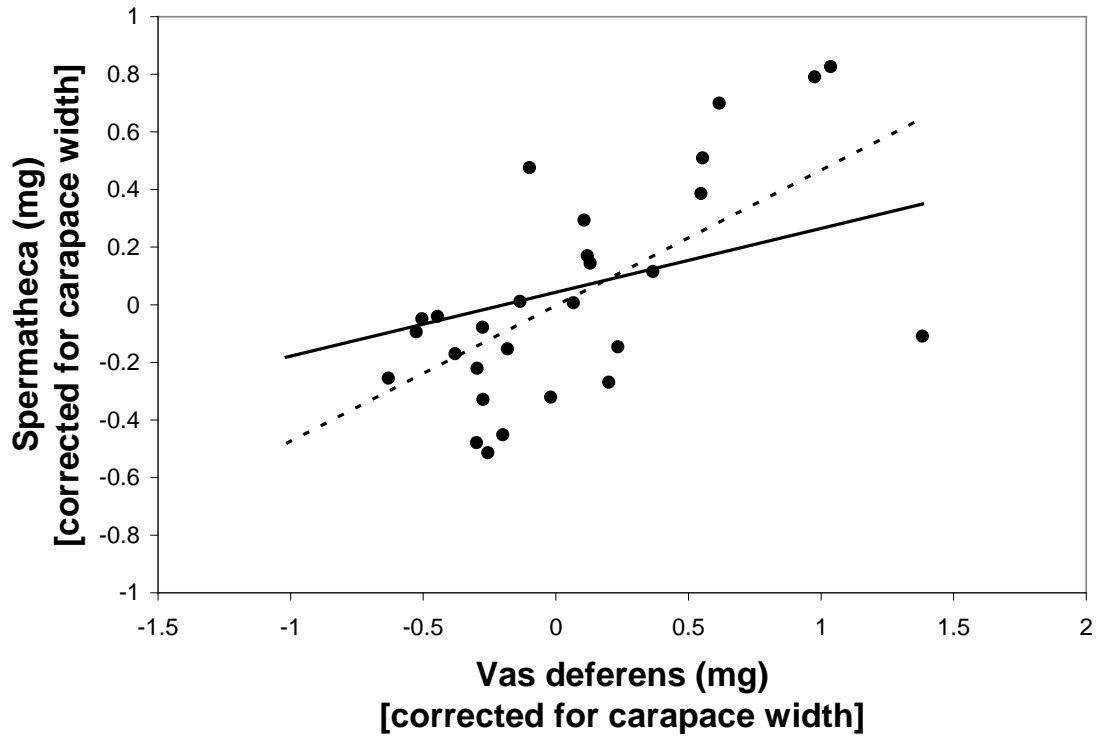
Fig 6: The relationship between spermatheca weight and vas deferens weight (VD) (both corrected for female carapace width) for 28 species of brachyuran crabs. Dashed line equation: spermatheca weight = *0.39*VD + 0.022*. The solid line represents the relationship of spermatheca weight and vas deferens weight after correcting for phylogenetic relatedness using the 'equal branch length' phylogeny. Solid line equation: spermatheca weight = *0.3229*VD-0.0097, F=7.35, p= 0.0117*. R^2 values are not calculated for generalized least squares covariance models.

163
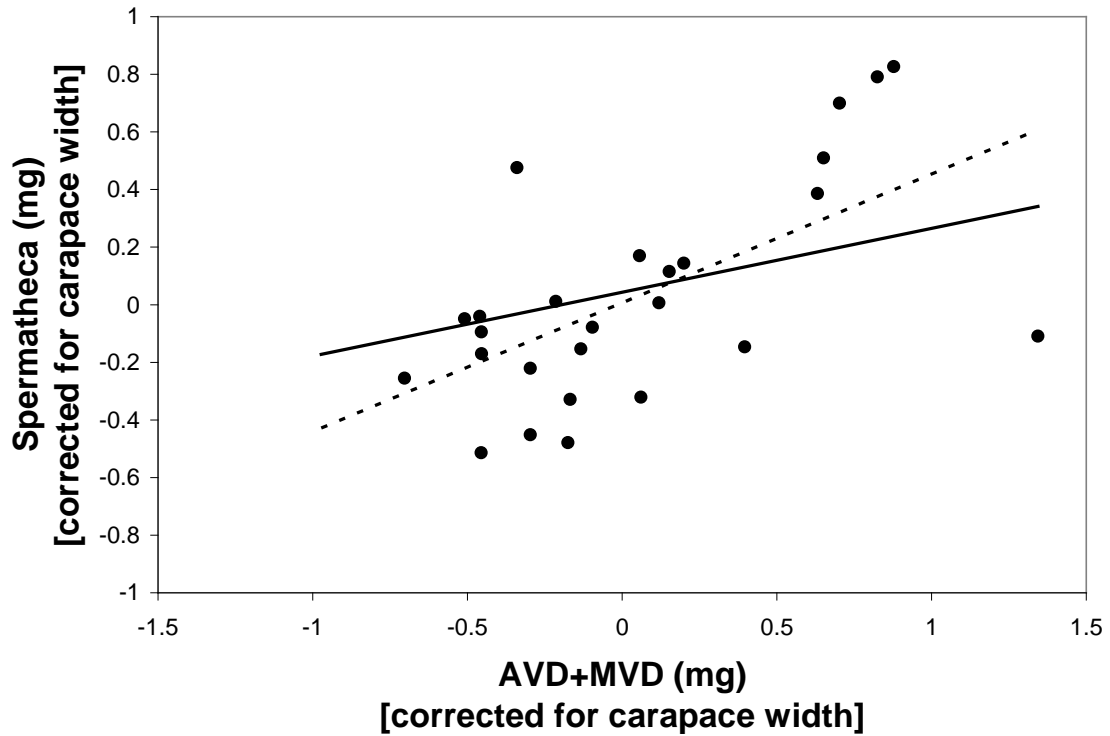
Fig 7: The relationship between spermatheca weight and AVD+MVD weight (both corrected for female carapace width) for 26 species of brachyuran crabs (AVD= anterior vas deferens, MVD= median vas deferens). Dashed line equation: *Spermatheca weight = 0.4468*(AVD+MVD) + 0.0064*. The solid line represents the relationship of spermatheca weight and VD weight after correcting for phylogenetic relatedness using the 'equal branch length' phylogeny. Solid line equation: *Spermatheca weight = 0.2214*(AVD+MVD) + 0.0434, F=4.57, p= 0.0429*. R^2 values are not calculated for generalized least squares covariance models.

Appendices

Appendix I: Power analysis results for all ANCOVAs run on *C. sapidus* and *E. depressus* listed by response variable. Power of the test is listed in parentheses next to each explanatory variable.

Response Variable	Explanatory Variables	
Body weight ($\log_{10}$)	*C. sapidus:*	latitude (0.286), time (0.975)
	E. depressus:	latitude (0.728), latitude^2 (0.884), time (0.999)
Carapace width	*C. sapidus:*	latitude (0.535), time (0.998)
	E. depressus:	latitude (0.701) latitude^2 (0.862), time (0.996), latitude*time (0.577)
Egg number ($\log_{10}$)	*C. sapidus:*	weight (1.0), egg stage (0.957), latitude (0.984), time (0.999), latitude^2 (0.873)
	E. depressus:	weight (1.0), egg size (0.998), latitude (0.050), time (0.033)
Egg size	*C. sapidus:*	latitude (0.250), egg stage (0.999), time (0.999)
	E. depressus:	latitude (0.715), egg stage (1.0), time (0.967)
Spermatheca weight (sq. rt.)	*C. sapidus:*	CW (1.0), latitude (0.993), time (0.976), latitude^2 (0.981), latitude^3 (0.962), latitude*time (0.915)
	E. depressus:	CW (0.992), latitude (0.166), time (0.012)
Sperm number (sq. rt.)	*C. sapidus:*	CW (0.975), latitude (0.027), time (0.472)
	E. depressus:	CW (0.576), latitude (0.730), time (0.751), latitude^2 (0.656), CW*time (0.866)
Sperm egg ratio (sq. rt.)	*C. sapidus:*	latitude (0.726), time (0.997), latitude^2 (0.595)
	E. depressus:	weight (0.020), latitude (0.804), time (0.607), latitude^2 (0.709), weight*time (0.571)

Appendix II: The average of the female life history traits for each species. Standard error of the mean +/-1 is presented in parentheses for each species average (CW= carapace width).

Superfamily	Taxon	CW (mm)	Egg number	Egg size	Spermatheca (mg)	Spermatheca (%full)	Sperm number
Cancroidea	*Cancer irroratus*	85.2	-	-	90	5	8.25×10^7
	Cancer antennarius	102 (6.9)	1562314 (354326)	333 (9.0)	-	-	-
	Cancer anthonyi	127.7 (5.2)	2377643 (246421)	311 (4.3)	-	-	-
	Cancer borealis	120 (2.6)	676712 (58345)	428 (11.4)	-	-	-
	Cancer gracilis	68.1 (2.3)	484761 (51397)	333 (6.5)	-	-	-
	Cancer magister	159 (2.8)	973369 (79526)	442 (5.1)	-	-	-
	Cancer oregonensis	29.3 (1.6)	41449 (5984)	339 (14.1)	-	-	-
	Cancer pagurus	163 (7.9)	1556266 (316655)	396 (6.0)	-	-	-
	Cancer productus	112 (5.8)	838853 (88143)	400 (39.8)	-	-	-
Grapsoidea	*Cardisoma guanhumi*	65.2 (4.0)	242052 (34163)	382 (6.1)	-	-	-
	Aratus pisonii	18.0 (1.2)	16950 (2490)	331 (5.5)	17 (3.6)	65 (3.9)	2.57×10^7 (7.97×10^6)
	Pachygrapsus transversus	12.7 (0.7)	8250 (2297)	288 (6.6)	2.8 (6.6)	24.5 (5.9)	1.74×10^6 (6.16×10^5)
	Sesarma cinereum	13.0	387	914	11.8	75.0	4.47×10^6
	Sesarma ricordii	17.8 (1.2)	15043 (6409)	359 (6.6)	28.6 (1.0)	85.0 (5.0)	4.93×10^7 (4.67×10^6)
	Hemigrapsus sanguiensis	20.0 (1.0)	20080 (5683)	307 (6.4)	26 (7.8)	50.0 (8.0)	4.99×10^7 (1.35×10^7)
	Pachygrapsus gracilis	13.3 (0.5)	9589 (1118)	274 (6.3)	5.5 (0.9)	50 (5.5)	1.96×10^7 (5.28×10^6)
	Cyclograpsus cinereus	11.5 (0.6)	1528 (212)	369 (12.6)	37.4 (7.1)	54.5 (6.5)	2.59×10^8 (1.25×10^8)
Majoidea	*Acanthonyx petiverii*	13.5	2155	487	4.1	50.0	1.04×10^7
	Stenorhynchus seticornis	11.1 (0.3)	1194 (67.9)	466 (8.9)	6.0 (1.8)	29.3 (9.3)	-
	Mithrax forceps	12.9	391.0	527.2	1.7		1.92×10^5
	Mithrax sculptus	17.7 (0.8)	1073 (184)	541.7 (9.6)	11.9 (2.5)	28.5 (8.1)	3.17×10^6
	Mithrax spinosissimus	40.4	-	-	14.7	75.0	2.75×10^7
	Macrocoeloma trispinosum	7.4	1455	823.2	14.7	75.0	-
	Chionoectes opilio	58.0 (1.9)	27481 (5560)	696 (38)	1006 (156)	73 (5)	8.14×10^8 (2.06×10^8)
	Hyas araneus	43.6 (2.1)	19387 (3641)	691 (23.6)	789 (98)	62.5 (8.9)	4.89×10^8 (2.31×10^8)
	Hyas coarctatus	42.5 (3.2)	11595 (5696)	658 (28.1)	816 (505)	58.3 (10.1)	6.83×10^8 (1.35×10^7)
	Libinia dubia	59.3 (2.7)	73071 (8232)	563 (24.1)	270.0 (69.2)	36.3 (7.5)	6.68×10^7 (2.24×10^7)
	Pisoides edwardsi	16.0 (1.1)	1295 (643)	530.8 (16.1)	5.0 (1.3)	45.0 (5.0)	2.98×10^6 (9.76×10^5)
	Pitho lherminieri	12.1	326	686	1.4	10.0	7.5×10^5
Ocypodoidea	*Uca thayeri*	17.8 (1.2)	14967 (2234)	278 (8)	6.6 (2.4)	7.5 (1.5)	2.75×10^6 (1.20×10^6)

	Uca pugilator	16.2 (0.6)	19496 (4793)	285 (4.8)	7.7 (0.7)	7.8 (2.2)	3.12x10^6 (1.63x10^6)
	Uca minax	21.4 (1.1)	35227 (6565)	251.3 (7.6)	15.2 (2.9)	27.2 (6.2)	7.60x10^6 (2.86x10^6)
	Uca pugnax	13.6 (0.5)	10281 (1151)	300.1 (4.8)	3.9 (0.5)	37.7 (3.6)	6.87x10^6 (2.24x10^6)
	Uca beebei	7.8 (0.3)	4077 (649)	227.6 (3.7)	1.1 (0.1)	38.5 (3.3)	1.94x10^6 (3.35x10^5)
	Uca terpsichores	9.9 (0.3)	6189 (633)	265.4 (7.9)	2.2 (0.4)	41.7 (5.9)	2.68x10^6 (5.97x10^5)
Portunoidea	*Carcinus maenas*	46.9 (5.78)	40060 (7561)	308 (8.4)	148 (48)	32 (9)	1.94x10^7 (7.62x10^6)
	Ovalipes ocellatus	44.8	120926	374.1	24.6	20.0	2.70x10^7
	Callinectes sapidus	146.1 (3.3)	1957236 (154797)	263.9 (4.0)	256.6 (26.6)	9.0 (1.0)	3.61x10^8 (6.67x10^7)
	Geryon fenneri	124 (2.7)	282807 (19945)	567 (4.4)	-	-	-
	Geryon quinquidens	108 (1.7)	158709 (9337)	731 (11.6)	-	-	-
	Portunus gibbesi	61.5 (1.7)	204467 (55175)	283.2 (4.9)	-	-	-
	Portunus spinicarpus	48.7 (1.6)	30265 (5562)	335.6 (8.2)	-	-	-
Xanthoidea	*Menippe nodifrons*	59.6 (1.9)	140014 (15162)	364.9 (6.1)	-	-	-
	Panopeus lacustris	20.4 (2.9)	19419 (9947)	380 (20)	8.9 (3.1)	13.9 (3.8)	2.63x10^7 (1.83x10^7)
	Panopeus herbstii	22.9 (1.0)	23362 (5931)	314 (5.3)	19.0 (3.1)	35.0 (3.3)	7.39x10^7 (3.81x10^7)
	Eurypanopeus depressus	11.5 (0.7)	2917 (829)	306 (5.0)	1.3 (0.4)	9.4 (1.2)	3780990 (1039653)
	Euypanopeus dissimilis	14.0 (0.9)	3617 (429)	314 (8.3)	1.7 (0.3)	5 (0)	1.64x10^6 (6.32x10^5)
	Rhithropanopeus harrisii	9.5 (0.4)	1901 (531)	327.8 (8.6)	1.8 (0.3)	23.0 (3.6)	2.46x10^6 (5.03x10^5)
	Xanthodius sternberghii	11.9 (1.0)	2518	312	1.7 (0.6)	13.3 (6.0)	4.88x10^6 (1.22x10^6)
	Cataleptodius floridanus	15.4 (0.6)	6083 (645)	268.9 (3.5)	8.8 (1.9)	29.0 (5.4)	2.65x10^6 (1.86x10^6)
	Paraxanthus barbiger	44.0	3.4811	447.2	43.2	10.0	1.75x10^8
	Eriphia gonagra	23.0	10275	394.4	14.3	60.0	1.16x10^8
	Homolaspis plana	92.3 (7.0)	247955 (114100)	590.9 (81.5)	801.6 (241.6)	50.0 (14.4)	2.20x10^8 (1.17x10^8)

Appendix III: The average of the male life history traits for each species. Standard error of the mean +/-1 is presented in parentheses for each species average. (CW= carapace width, AVD&MVD = anterior and median vas deferens, PVD= posterior vas deferens).

Superfamily	Taxon	CW (mm)	Sperm number	Vas deferens (mg)	AVD & MVD (mg)	PVD (mg)	Gonopod length (mm)
Cancroidea	*Cancer irroratus*	10.2 (0.5)	1.84×10^8 (4.60×10^7)	29.2 (5.1)	20.9 (3.6)	9.3 (2.1)	3.4 (0.2)
Grapsoidea	*Aratus pisonii*	20.5 (0.8)	1.10×10^8 (2.88×10^7)	43.6 (4.9)	30.4 (3.4)	13.2 (2.1)	6.1 (0.3)
	Pachygrapsus transversus	12.7 (0.5)	4.72×10^7 (1.16×10^7)	5.1 (0.6)	4.6 (0.8)	1.6 (0.3)	3.2 (0.1)
	Sesarma cinereum	15.5 (0.9)	7.89×10^7 (3.02×10^7)	57.2 (21.8)	42.8 (19.1)	14.4 (3.0)	-
	Sesarma ricordii	9.8 (0.7)	1.57×10^7 (8.67×10^6)	17.4 (7.2)	12.7 (5.2)	4.6 (2.1)	3.3 (0.1)
	Sesarma occidentale	20.8 (1.1)	1.2×10^7 (8.19×10^6)	3.3 (1.2)	2.1 (0.8)	1.1 (0.4)	3.6 (0.3)
	Sesarma reticulatum	18.4 (1.0)	3.36×10^7 (2.18×10^7)	18.5 (5.6)	12.4 (6.7)	6.0 (1.1)	-
	Hemigrapsus sanguiensis	20.4 (1.4)	3.72×10^7 (1.55×10^7)	42.0 (13.9)	21.6 (7.0)	20.4 (7.8)	6.4 (0.5)
	Pachygrapsus gracilis	16.1 (0.5)	2.90×10^7 (8.04×10^6)	8.8 (1.2)	6.2 (0.9)	3.4 (0.7)	3.6 (0.2)
Majoidea	*Stenorhynchus seticornis*	10.3 (0.8)	1.02×10^7 (8.78×10^5)	11.8 (5.0)	6.0 (2.8)	5.8 (2.3)	5.2 (0.2)
	Mithrax forceps	14.3 (0.7)	6.55×10^6 (6.16×10^6)	6.84 (4.6)	5.2 (3.6)	1.6 (1.0)	5.8 (1.3)
	Mithrax sculptus	18.6 (0.9)	1.28×10^7 (1.11×10^7)	18.5 (7.0)	9.1 (2.9)	9.3 (4.1)	5.3 (0.2)
	Mithrax spinosissimus	34.4 (2.7)	2.92×10^7	34.7 (14.9)	45.4	4.3	9.0 (0.5)
	Mithrax coryphe	14.8	1.30×10^6	12.2	6.0	6.2	3.5
	Chionoectes opilio	80.1 (7.3)	5.73×10^9 (1.36×10^9)	4402.6 (737.9)	3084 (464.9)	1711.0 (330.0)	18.3 (1.8)
	Hyas araneus	59.0 (4.4)	8.68×10^9 (2.77×10^9)	4600.0 (1138.4)	1878.1 (475.6)	2721.8 (693.0)	17.7 (1.2)
	Hyas coarctatus	63.0 (2.7)	6.13×10^9 (1.79×10^9)	6274.6 (1043.2)	2511.8 (424.1)	3762.8 (723.3)	17.8 (2.61)
	Libinia dubia	65.2 (2.2)	9.26×10^8 (1.84×10^8)	1457.5 (239.2)	513.9 (78.5)	943.6 (177.9)	20.9 (0.8)
	Pisoides edwardsi	16.0 (1.6)	3.0×10^7 (2.31×10^7)	60.6 (37.2)	17.9 (3.5)	42.7 (33.9)	5.5
	Pitho lherminieri	20.6	8.87×10^6	51.9	-	-	6
Ocypodoidea	*Uca thayeri*	20.6 (0.5)	2.74×10^7 (2.48×10^6)	7.7 (0.6)	3.9 (0.3)	3.8 (0.6)	8.2 (0.3)
	Uca pugilator	16.6 (0.3)	1.94×10^7 (2.37×10^6)	6.0 (0.7)	3.5 (0.5)	2.4 (0.4)	6.4 (0.2)
	Uca minax	34.3 (2.7)	2.92×10^7	34.7 (14.9)	45.4	4.3	9.0 (0.5)
	Uca pugnax	17.2 (0.6)	1.91×10^7 (4.09×10^6)	7.4 (0.7)	4.3 (0.5)	3.1 (0.3)	5.7 (0.2)
	Uca beebei	8.9 (0.3)	4.57×10^6 (1.85×10^6)	3.1 (0.5)	1.1 (0.2)	1.4 (0.2)	3.7 (0.2)
	Uca rapax	22.3 (0.6)	8.89×10^7 (1.84×10^7)	23.6 (3.9)	16.2 (3.6)	7.4 (1.3)	8.7 (0.3)
	Ocypode quadrata	36.5 (2.0)	1.46×10^9 (2.98×10^8)	214.5 (39.2)	112.2 (28.7)	102.3 (11.6)	16.1 (0.5)
	Uca stylifera	26.6 (0.8)	3.40×10^8 (2.82×10^7)	33.6 (3.4)	-	-	10.5 (0.3)
	Uca terpsichores	9.5 (0.2)	3.66×10^6 (8.02×10^5)	4.4 (0.4)	1.1 (0.2)	3.6 (0.5)	2.7 (0.2)

	Uca stenodactylus	12.8 (0.1)	1.75×10^7 (3.25×10^6)	14.8 (2.8)	-	-	5.0 (0.2)
Portunoidea	*Carcinus maenas*	55.5 (4.1)	5.02×10^8 (8.82×10^7)	484.7 (81.7)	316.3 (57.2)	168.3 (28.9)	12.2 (0.9)
	Callinectes sapidus	167.1 (2.8)	4.05×10^9 (8.32×10^8)	2084.7 (321.7)	1381.4 (206.5)	703.3 (121.1)	43.8 (2.2)
Xanthoidea	*Panopeus lacustris*	31.2 (2.2)	9.65×10^9 (3.19×10^8)	91.3 (21.5)	64.4 (21.2)	36.0 (13.5)	9.5 (1.3)
	Panopeus herbstii	27.4 (2.0)	8.39×10^8 (2.37×10^8)	116.9 (28.4)	100.0 (24.5)	17.0 (4.2)	7.1 (0.5)
	Eurypanopeus depressus	15.8 (0.5)	6.79×10^7 (9.70×10^6)	8.4 (0.9)	5.0 (0.7)	2.6 (0.3)	4.1 (0.2)
	Euypanopeus dissimilis	14.3 (1.1)	2.79×10^8 (2.35×10^8)	8.1 (2.0)	3.9 (0.9)	1.2 (0.3)	3.7 (0.2)
	Rhithropanopeus harrisii	12.3 (0.9)	1.44×10^8 (4.48×10^7)	11.3 (2.8)	-	-	4.7 (0.2)
	Panopeus americanus	22.5 (0.6)	5.10×10^6	23.2 (8.3)	16.4 (6.0)	6.8 (2.3)	6.5 (0.2)
	Xanthodius sternberghii	10	9.58×10^6	2.2	1.1	1.1	-
	Cataleptodius floridanus	15.9 (0.7)	5.12×10^7 (9.54×10^6)	21.6 (3.6)	12.2 (2.1)	9.4 (1.8)	3.7 (0.3)
	Paraxanthus barbiger	84.1 (1.3)	2.6×10^9 (1.96×10^9)	605.4 (66.6)	461.0 (37.6)	144.4 (42.1)	19.1 (0.6)
	Homolaspis plana	86.8 (4.3)	5.14×10^9 (1.53×10^9)	2248.6 (805.0)	1676.0 (587.9)	572.6 (245.1)	18.3 (0.9)

Appendix IV: Relative influence of response variables for the groupings 'superfamily' 'sperm plug' and 'female molt.' The average squared distance corrected for the standard deviation and the percent contribution of the variable to the average group similarities are represented in the table below.

Factor	Groups	Variables	Sim/SD	% Contribution
Superfamily	Majoidea vs. Portunoidea (r=0.237, p=0.156)	Egg stage	0.90	21.80
		Spermatheca weight	0.80	17.57
		Egg number	1.16	14.54
		Vas deferens weight	0.90	12.71
		Spermatheca % full	0.94	9.76
		Female sperm number	0.76	9.46
		Male sperm number	1.06	7.57
	Ocypodoidea vs. Portunoidea (r=0.813, p=0.036*)	Ovary stage	1.17	36.80
		Egg stage	1.01	31.59
		Spermatheca weight	1.74	7.67
		Egg number	0.94	6.6
		Spermatheca % full	0.94	5.4
		Spermatheca weight	0.85	4.67
	Grapsoidea vs. Xanthoidea (r=0.158, p=0.04)	Spermatheca % full	1.12	20.59
		Gonopod length	0.48	17.74
		Female sperm number	0.69	14.61
		Spermatheca weight	0.74	11.05
		Vas deferens weight	0.66	9.12
		Ovary stage	0.67	7.67
		Male sperm number	0.91	7.25
		Egg number	0.65	6.34
	Majoidea vs. Xanthoidea (r=0.281, p=0.003*)	Male sperm number	0.82	16.02
		Gonopod length	0.45	15.73
		Spermatheca weight	0.83	13.44
		Egg number	1.45	11.18
		Spermatheca % full	0.96	9.92
		Spermatheca weight	0.87	9.67
		Egg stage	0.94	9.12
		Female sperm number	0.93	8.18
	Ocypodoidea vs. Xanthoidea (r=0.35, p=0.004*)	Gonopod length	0.45	25.01
		Male sperm number	1.47	19.34
		Ovary stage	1.11	16
		Vas deferens weight	0.57	14.66
		Egg stage	0.83	8.58
		Spermatheca % full	0.92	5.38
		Egg number	1.65	4.88
	Portunoidea vs. Xanthoidea (r=0.241, p=0.244)	Egg stage	1.08	23.24
		Gonopod length	0.44	20.03
		Male sperm number	1.42	16.07
		Ovary stage	0.68	10.72
		Vas deferens weight	0.52	10.49
		Spermatheca weight	1.02	7.63
		Female sperm number	0.71	5
	Grapsoidea vs. Majoidea (r=0.106,	Egg number	1.4	17.79
		Female sperm number	0.77	34.46
		Spermatheca weight	0.93	47.11

170

	p=0.128)	Male sperm number	0.77	59.29
		Spermatheca % fullness	0.78	69.35
		Ovary stage	0.60	79.15
		Egg stage	0.91	88.89
		Vas deferens weight	0.80	97.70
	Grapsoidea vs. Ocypodoidea (r=0.34, p=0.012*)	Spermatheca % fullness	0.99	18.57
		Female sperm number	0.70	16.67
		Ovary stage	1.07	12.54
		Vas deferens weight	0.96	12.31
		Egg number	0.53	10.44
		Spermatheca weight	0.74	9.24
		Male sperm number	0.72	8.16
		Egg stage	0.72	7.33
	Majoidea vs. Ocypodoidea (r=0.519, p=0.004*)	Egg number	2.27	22.65
		Ovary stage	1.14	18.40
		Vas deferens weight	1.05	15.37
		Spermatheca weight	0.88	10.60
		Egg stage	0.90	9.28
		Spermatheca % full	0.84	7.52
		Male sperm number	1.05	7.37
	Grapsoidea vs. Portunoidea (r=0.299, p=0.25)	Egg stage	1.07	20.94
		Female sperm number	0.82	17.71
		Spermatheca % full	1.12	16.46
		Spermatheca weight	0.79	13.24
		Ovary stage	0.70	10.38
		Vas deferens	0.85	6.91
		Egg number	0.62	6.66
Sperm plug	Plug present vs. plug absent (r=0.525, p=0.022*)	Spermatheca weight	1.52	20.73
		Ovary stage	1.23	16.62
		Female Sperm number	0.95	13.10
		Vas deferens weight	1.22	12.49
		Egg stage	1.00	11.12
		% fullness of spermatheca	1.02	9.54
Female molt	Terminal molt to maturity vs. continuous molting after maturity (r=0.27, p=0.008*)	Egg number	1.21	14.79
		Ovary stage	0.74	12.18
		Egg stage	0.88	11.90
		Spermatheca weight	0.84	11.90
		Male sperm number	0.76	11.49
		Vas deferens weight	0.80	10.62
		Female sperm number	0.71	10.24

Literature Cited

ABELE, L. G., CAMPANELLA, P. J. & SALMON, M. (1986). Natural history and social organization of the semiterrestrial grapsid crab *Pachygrapsus transversus* (Gibbes). — Journal of Experimental Marine Biology and Ecology 104, 153-170.

AHYONG, S. T., LAI, J. C. Y., SHARKEY, D., COLGAN, D. J. & NG, P. K. L. (2007). Phylogenetics of the brachyuran crabs (Crustacean: Decapoda): The status of Podotremata based on small subunit nuclear ribosomal RNA. — Molecular Phylogenetics and Evolution 45, 576-586.

ALONZO, S. H. & MANGEL, M. (2004). The effects of size-selective fisheries on the stock dynamics of and sperm limitation in sex-changing fish. — Fishery Bulletin 102, 1-13.

ANGILLETTA, M. J. & DUNHAM, A. E. (2003). The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. — The American Naturalist 162, 332-342.

ANTHES, N., SCHULENBURG, H. & MICHIELS, N. K. (2008). Evolutionary links between reproductive morphology, ecology and mating behavior in opisthobranch gastropods. — Evolution 62-4, 900-916.

ARNQVIST, G., EDVARDSSON, M., FRIBERG, U. & NILSSON, T. (2000). Sexual conflict promotes speciation in insects. — PNAS 97, 10460-10464.

ATKINSON, D. & SIBLY, R. M. (1997). Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. — Trends in Ecology & Evolution 12, 235-239.

BAENA, M. L. & EBERHARD, W. G. (2007). Appearances deceive: female "resistance" behavior in a sepsid fly is not a test of male ability to hold on. — Ethology, Ecology and Evolution 19, 27-50.

BAER, B., ARMITAGE, S. A. O. & BOOMSMA, J. J. (2006). Sperm storage induces an immunity cost in ants. — Nature 441, 872-875.

BASS, C. C., PIVAK, E. D. & ANGER, K. (2007). Seasonal and interpopulational variability in fecundity, egg size, an elemental composition (CHN) of eggs and larvae in a grapsoid crab, *Chasmagnathus granulatus*. — Helgol Mar Res 61, 2225-237.

BATEMAN, A. J. (1948). Intra-sexual selection in drosophila. — Heredity 2, 349-368.

BEESE, K., ARMBRUSTER, G. F. J., BEIER, K. & BAUR, B. (2008). Evolution of female sperm-storgae organs in the carrefour of stylommatophoran gastropods. — Journal of Zoological Systematics and Evolutionary Research 47, 49-60.

BERGMANN, C. (1847). Ueber die Verhaltnisse der warmeokonomie der thiere zu ihrer grosse. — Gottinger Studien 3, 595-708.

BERRIGAN, D. (1991). The allometry of egg size and number in insects. — Oikos 60, 313-321.

BIRKHEAD, T. R. (1998). Cryptic female choice: Criteria for establishing female sperm choice. — Evolution 52, 1212-1218.

BLANCKENHORN, W. U. & DEMONT, M. (2004). Bergmann and converse Bergmann latitudinal clines in arthropods: Two ends of a continuum? — Integrative and Comparative Biology 44, 413-424.

BLOMBERG, S. P., GARLAND, T. J. & IVES, A. R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. — Evolution 57, 717-745.

BOHNING-GAESE, K. & OBERRATH, R. (1999). Phylogenetic effects on morphological, life-history, behavioral and ecological traits of birds. — Evolutionary Ecology Research 1.

BRANTE, A., CIFUENTES, S., PORTNER, H.-O., ARNTZ, W. & FERNÁNDEZ, M. (2004). Latitudinal comparisons of reproductive traits in five brachyuran species along the chilean coast. — Revista Chilena de Historia Natural 77, 15-27.

BROCKERHOFF, A. M. & McLAY, C. L. (2005). Comparative analysis of the mating strategies in grapsid crabs with special references to the intertidal crabs *Cyclograpsus lavauxi* and *Helice crassa* (Decapoda: Grapsidae) from New Zealand. — Journal of Crustacean Biology 25, 507-520.

BROSING, A., RICHTER, S. & SCHOLTZ, G. (2006). Phylogenetic analysis of the Brachyura (Crustacea, Decapoda) based on characters of the foregut with establishment of a new taxon. — Journal of Zoological Systematics and Evolutionary Research 45, 20-32.

BUBLITZ, R., SAINTE-MARIE, B., NEWCOMB-HODGETTS, C., FLETCHER, N., SMITH, M. & HARDEGE, J. (2008). Interspecific activity of the sex pheromone of the European shore crab (*Carcinus maenas*). — Behavior 145, 1465-1478.

BUCKLANDNICKS, J. & DARLING, P. (1993). Sperm are stored in the ovary of *Lacuna (Epheria) variegata* (Carpenter, 1864) (Gastropoda, Littorinidae). — Journal of Experimental Zoology 267, 624-627.

CALDWELL, R. (1991). Variation in reproductive behavior in stomatopod Crustacea. — In: Crustacean Sexual Biology (R. T. Bauer & J. W. Martin, eds). Columbia University Press, New York, p. 67-90.

CARVER, A. M., WOLCOTT, T. G., WOLCOTT, D. L. & HINES, A. H. (2005). Unnatural selection: Effects of a male focused size selective fishery on reproductive potential of a blue crab population. — Journal of Experimental Marine Biology and Ecology 319, 29-41.

CASTILHO, A. L., GAVIO, M. A., COSTA, R. C., BOSCHI, E. E., BAUER, R. T. & FRANSOZO, A. (2007). Latitudinal variation in population structure and reproductive pattern of the endemic south american shrimp *Artemesia loginaris*. — Journal of Crustacean Biology 27, 548-552.

CHARNOV, E. L. (1993). Life history invariants: some explorations of symmetry in evolutionary ecology. — Oxford University Press, Oxford.

CHARNOV, E. L. & ERNEST, S. K. M. (2006). The offspring-size/clutch-size trade-off in mammals. — American Naturalist 167, 578-582.

CHEUNG, T. S. (1968). Trans-molt retention of sperm in the female stone crab *Menippe mercenaria* (SAY). — Crustaceana 15, 117-120.

CHRISTY, J. H. (1987a). Competitive mating, mate choice and mating associations of brachyuran crabs. — Bulletin of Marine Science 41, 177-191.

—. (1987b). Female choice and breeding behavior of the fiddler crab *Uca beebei*. — Journal of Crustacean Biology 7, 624-635.

—. (2003). Reproductive timing and larval dispersal of intertidal crabs: the predator avoidance hypothesis. — Revista Chilena De Historia Natural 76, 177-185.

CHRISTY, J. H., BACKWELL, P. R. Y., GOSHIMA, S. & KREUTER, T. (2002). Sexual selection for structure building by courting male fiddler crabs: an experimental study of behavioral mechanisms. — Behavioral Ecology 13, 366-374.

CLARKE, R. K. & GORLEY, R. N. (2006). Primer v6: User Manual/Tutorial. — Primer-E Ltd, Plymouth.

CLARKE, R. K., SOMERFIELD, P. J. & CHAPMAN, G. M. (2006). On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages. — Journal of Experimental Marine Biology and Ecology 330, 55-80.

CLUTTON-BROCK, T. & HARVEY, P. (1977). Primate ecology and social organization. — Journal of Zoology 183, 1-39.

CODY, M. L. (1966). A general theory of clutch size. — Evolution 20, 174-&.

CONOVER, D. O. & SCHULTZ, E. T. (1995). Phenotypic similarity and the evolutionary significance of countergradient variation. — Trends in Ecology & Evolution 10, 248-252.

COOPER, C. B., HOCHACHKA, W. M., BUTCHER, G. & DHONDT, A. A. (2005). Seasonal and latitudinal trends in clutch size: Thermal constraints during laying and incubation. — Ecology 86, 2018-2031.

CORDOBA-AGUILAR, A., UHIA, E. & RIVERA, A. C. (2003). Sperm competition in Odonata (Insecta): the evolution of female sperm storage and rivals' sperm displacement. — Journal of Zoology 261, 381-398.

CRANE, J. (1975). Fiddler crabs of the world. — Princeton University Press, New Jersey.

CRONIN, L. E. (1947). Anatomy and histology of the male reproductive system of *Callinectes sapidus* Rathbun. — Journal of Morphology 81, 209-239.

DARNELL, Z. M., RITTSCHOFF, D., DARNELL, K. M. & MCDOWELL, R. E. (2009). Lifetime reproductive potential of female blue crabs *Callinectes sapidus* in North Carolina, USA. — Marine Ecology Progress Series 394, 153-163.

DARWIN, C. (1874). The descent of man and selection in relation to sex. — The Werner Company, Akron.

DEFEO, O. & CARDOSO, R. S. (2002). Macroecology of population dynamics and life history traits of the mole crab *Emerita brasiliensis* in Atlantic sandy beaches of South America. — Marine Ecology Progress Series 239, 167-179.

DELBARCO-TRILLO, J. & FERKIN, M. H. (2004). Male mammals respond to a risk of sperm competition conveyed by odours of conspecific males. — Nature 431, 446-449.

DERIVERA, C. E., BACKWELL, P. R. Y., CHRISTY, J. H. & VEHRENCAMP, S. L. (2003). Density affects female and male mate searching in the fiddler crab, Uca beebei. — Behavioral Ecology and Sociobiology 53, 72-83.

DEWSBURY, D. A. (1982). Ejaculate cost and male choice. — American Naturalist 119, 601-610.

DICKINSON, G. H., RITTSCHOF, D. & LATANICH, C. (2006). Spawning biology of the blue crab, Callinectes sapidus, in North Carolina. — Bulletin of Marine Science 79, 273-285.

DIESEL, R. (1990). Sperm competition and reproductive success in the decapod *Inachus phalangium* (Majidae) - a male ghost spider crab that seals off rivals sperm. — Journal of Zoology 220, 213-223.

—. (1991). Sperm competition and the evolution of mating behavior in Brachyura, with special reference to spider crabs (Decapoda, Majidae). — In: Crustacean Sexual Biology (R. T. Bauer & J. W. Martin, eds). Columbia University Press, New York, p. 145-163.

DIESEL, R., SCHUBART, C. D. & SCHUH, M. (2000). A reconstruction of the invasion of land by Jamaican crabs (Grapsidae : Sesarminae). — Journal of Zoology 250, 141-160.

DUGAN, J. E., WENNER, A. M. & HUBBARD, D. M. (1991). Geographic variation in the reproductive biology of the sand crab Emerita analoga (Stimpson) on the California coast. — Journal of Experimental Marine Biology and Ecology 150, 63-81.

DYBAS, L. K. & DYBAS, H. S. (1981). Coadaptation and taxonomic differentiation of sperm and spermathecae in featherwing beetles. — Evolution 35, 168-174.

EBERHARD, W. G. (1997). Sexual selection by cryptic female choice in insects and arachnids. — In: Mating systems in insects and arachnids (J. C. Choe & B. Crespi, eds). Cambridge University Press, Cambridge, p. 31-57.

EBERHARD, W. G., HUBER, B. A., RODRIGUEZ, R. L., BRICENO, R. D., SALAS, I. & RODRIQUEZ, V. (1998). One size fits all? Relationships between the size and degree of variation in genitalia and other body parts in twenty species of insects and spiders. — Evolution 52, 415-431.

EMLEN, D. J. & NIJHOUT, H. F. (2000). The development and evolution of exaggerated morphologies in insects. — Annual Review of Entomology 45, 661-708.

EMLEN, S. T. & ORING, L. W. (1977). Ecology, sexual selection, and evolution of mating systems. — Science 197, 215-223.

EVANS, J. P. & MAGURRAN, A. E. (1999). Geographic variation in sperm production by Trinidadian guppies. — Proceedings of the Royal Society of London Series B-Biological Sciences 266, 2083-2087.

FELSENSTEIN, J. (1985). Phylogenies and the comparative method. — The American Naturalist 125, 1-15.

FUNKE, S. & HUBER, B. A. (2005). Allometry of genitalia and fighting structures in *Linyphia triangularis* (Araneae, Linyphiidae). — Journal of Arachnology 33, 870-872.

GAGE, A. R. & BARNARD, C. J. (1996). Male crickets increase sperm number in relation to competition and female size. — Behavioral Ecology and Sociobiology 38, 349-353.

GALVANI, A. & JOHNSTONE, R. (1998). Sperm allocation in an uncertain world. — Behavioral Ecology and Sociobiology 44, 161-168.

GARDNER, C. & WILLIAMS, H. (2002). Maturation in the male giant crab, *Pseudocarcinus gigas*, and the potential for sperm limitation in the Tasmanian fishery. — Marine and Freshwater Research 53, 661-667.

GLEESON, R. A. (1991). Intrinsic factors mediating pheromone communication in the blue crab, *Callinectes sapidus*. — In: Crustacean Sexual Biology (R. T. Bauer & J. W. Martin, eds). Columbia University Press, New York, p. 17-32.

GOSSELIN, T., SAINTE-MARIE, B. & BERNATCHEZ, L. (2003). Patterns of sexual cohabitation and female ejaculate storage in the American lobster (*Homarus americanus*). — Behavioral Ecology and Sociobiology 55, 151-160.

GOULD, S. J. (1971). Geometric similarity in allometric growth - contribution to problem of scaling in evolution of size. — American Naturalist 105, 113-&.

GRAE, D. & KOZLOFF, E. N. (1999). Post-copulatory storage of sperm in the seminal bursa of the acoel flatworm *Otocelis luteola*. — Invertebrate Reproduction & Development 35, 151-154.

GRAFEN, A. (1989). The phylogenetic regression. — Philosophical transactions of the royal society of London series B-Biological Sciences 326, 119-157.

GRAHAM, M. H. (2003). Confronting multicollinearity in ecological multiple regression. — Statistical Reports 84, 2809-2815.

HARMER, A. M. T., RADHAKRISHNAN, P. & TAYLOR, P. W. (2006). Remating inhibition in female Queensland fruit flies: Effects and correlates of sperm storage. — Journal of Insect Physiology 52, 179-186.

HARTNOLL, R. G. (1969). Mating in Brachyura. — Crustaceana 16, 161-181.

HEIBO, E., MAGNHAGEN, C. & VOLLESTAD, L. A. (2005). Latitudinal variation in life-history traits in Eurasian perch. — Ecology 86, 3377-3386.

HENMI, Y. (1993). Geographic variation in life-history traits of the intertidal ocypodid crab *Macrophthalmus banzi*. — Oecologia 96, 324-330.

HENRY, L. T. (1998). Status and management of the blue crab fishery in North Carolina. — Journal of Shellfish Research 17, 456-468.

HINES, A. H. (1982). Allometric constraints and variables of reproductive effort in brachyuran crabs. — Marine Biology 69, 309-320.

—. (1986). Larval patterns in the life histories of brachyuran crabs (Crustacea, Decapoda, Brachyura). — Bulletin of Marine Science 39, 444-466.

—. (1988). Fecundity and reproductive output in 2 species of deep-sea crabs, *Geryon fenneri* and *Geryon quinquedens* (Decapoda, Brachyura). — Journal of Crustacean Biology 8, 557-562.

—. (1989). Geographic variation in size at maturity in brachyuran crabs. — Bulletin of Marine Science 45, 356-368.

—. (1991). Fecundity and reproductive output in 9 species of cancer crabs (Crustacea, Brachyura, Cancridae). — Canadian Journal of Fisheries and Aquatic Sciences 48, 267-275.

—. (1992). Constraint on reproductive output in brachyuran crabs - pinnotherids test the rule. — American Zoologist 32, 503-511.

HINES, A. H., JIVOFF, P. R., BUSHMANN, P. J., VAN MONTFRANS, J., REED, S. A., WOLCOTT, D. L. & WOLCOTT, T. G. (2003). Evidence for sperm limitation in the blue crab, *Callinectes sapidus*. — Bulletin of Marine Science 72, 287-310.

HINSCH, G. W. (1991). Structure and chemical content of the spermatophores and seminal fluid of reptantian decapods. — In: Crustacean Sexual Biology (R. T. Bauer & J. W. Martin, eds). Columbia University Press, New York, p. 290-306.

HUELSENBECK, J. P. & RONQUIST, F. (2001). MRBAYES: Bayesian inference of phylogeny. . — Bioinformatics 19, 1572-1574.

HULTGREN, K. M. & STACHOWICZ, J. J. (2008). Molecular phylogeny of the brachyuran crab superfamily Majoidea indicates close congruence with trees based on larval morphology. — Molecular Phylogenetics and Evolution 48, 986-996.

JENSEN, P. C., ORENSANZ, J. M. & ARMSTRONG, D. A. (1996). Structure of the female reproductive tract in the dungeness crab (*Cancer magister)* and implications for the mating system. — Biological Bulletin 190, 336-349.

JEYALECTUMIE, C. & SUBRAMONIAM, T. (1991). Biochemistry of seminal secretions of the crab *Scylla serrata* with reference to sperm metabolism and storage in the female. — Molecular Reproduction and Development 30, 44-55.

JIVOFF, P. (1997a). The relative roles of predation and sperm competition on the duration of the post-copulatory association between the sexes in the blue crab, *Callinectes sapidus*. — Behavioral Ecology and Sociobiology 40, 175-185.

—. (1997b). Sexual competition among male blue crab, *Callinectes sapidus*. — Biological Bulletin 193, 368-380.

—. (2003a). A review of male mating success in the blue crab, *Callinectes sapidus*, in reference to the potential for fisheries-induced sperm limitation. — Bulletin of Marine Science 72, 273-286.

—. (2003b). Summary session paper Blue Crab Symposium 2000 - Reproduction and embryonic development. — Bulletin of Marine Science 72, 265-272.

JIVOFF, P. & HINES, A. H. (1998). Female behaviour, sexual competition and mate guarding in the blue crab, *Callinectes sapidus*. — Animal Behaviour 55, 589-603.

JOHNSTON, E. E., RAND, M. S. & ZWEIFEL, S. G. (2006). Detection of multiple paternity and sperm storage in a captive colony of the central Asian tortoise, Testudo horsfieldii. — Canadian Journal of Zoology-Revue Canadienne De Zoologie 84, 520-526.

KENDALL, M. S., WOLCOTT, D. L., WOLCOTT, T. G. & HINES, A. H. (2001). Reproductive potential of individual male blue crabs, *Callinectes sapidus*, in a fished population: depletion and recovery of sperm number and seminal fluid. — Canadian Journal of Fisheries and Aquatic Sciences 58, 1168-1177.

—. (2002). Influence of male size and mating history on sperm content of ejaculates of the blue crab *Callinectes sapidus*. — Marine Ecology-Progress Series 230, 235-240.

KODRIC-BROWN, A., SIBLY, R. M. & BROWN, J. H. (2006). The allometry of ornaments and weapons. — Proceedings of the National Academy of Sciences of the United States of America 103, 8733-8738.

KOLM, N., GOODWIN, N. B., BALSHINE, S. & REYNOLDS, J. D. (2006a). Life history evolution in cichlids 1: revisiting the evolution of life histories in relation to parental care. — Journal of Evolutionary Biology 19, 66-75.

—. (2006b). Life history evolution in cichlids 2: directional evolution of the trade-off between egg number and egg size. — Journal of Evolutionary Biology 19, 76-84.

LACK, D. (1948). The significance of litter-size. — The journal of animal ecology 17, 45-50.

LARDIES, M. A. & BOZINOVIC, F. (2006). Geographic covariation between metabolic rate and life-history traits. — Evolutionary Ecology Research 8, 455-470.

LARDIES, M. A. & CASTILLA, J. C. (2001). Latitudinal variation in the reproductive biology of the commensal crab *Pinnaxodes chilensis* (Decapoda : Pinnotheridae) along the Chilean coast. — Marine Biology 139, 1125-1133.

LESTANG, S. D., HALL, N. G. & POTTER, I. C. (2003). Reproductive biology of the blue swimmer crab (*Portunus pelagicus*, Decapoda: Portunidae) in five bodies of water on the west coast of Australia. — Fisheries Bulletin 101, 745-757.

LESTER, S. E., GAINES, S. D. & KINLAN, B. P. (2007). Reproduction on the edge: large-scale patterns of individual performance in a marine invertebrate. — Ecology 88, 2229-2239.

LEVITAN, D. R. & PETERSON, C. (1995). Sperm limitation in the sea. — Trends in Ecology & Evolution 10, 228-231.

LIPCIUS, R. N. & STOCKHAUSEN, W. T. (2002). Concurrent decline of the spawning stock, recruitment, larval abundance, and size of the blue crab Callinectes sapidus in Chesapeake Bay. — Marine Ecology-Progress Series 226, 45-61.

LIPS, K. R. (2001). Reproductive trade-offs and bet-hedging in *Hyla calypsa*, a Neotropical treefrog. — Oecologia 128, 509-518.

LITZGUS, J. D. & MOUSSEAU, T. A. (2006). Geographic variation in reproduction in a freshwater turtle (Clemmys guttata). — Herpetologica 62, 132-140.

LONSDALE, D. J. & LEVINTON, J. S. (1985). Latitudinal differentiation in copepod growth - an adaptation to temperature. — Ecology 66, 1397-1407.

LOPEZ-GRECO, L. S., FRANSOZO, F. V., NEGREIROS-FRANSOZO, M. L. & DOS SANTOS, D. C. (2009). Comparative morphology of the seminal receptacles of Ocypode quadrata (Fabricius, 1787) (Brachyura, Ocypodoidea). — Zootaxa.

LORCH, P. D. & EADIE, J. M. (1999). Power of the concentrated changes test for correlated evolution. — Systematic biology 48, 170-191.

LUNG, O., TRAM, U., FINNERTY, C. M., EIPPER-MAINS, M. A., KALB, J. M. & WOLFNER, M. F. (2002). The *Drosophila melanogaster* seminal fluid protein Acp62F is a protease inhibitor that is toxic upon ectopic expression. — Genetics 160, 211-224.

MACARTHUR, R. H. & WILSON, E. O. (1967). The theory of island biogeography. — Princeton University Press, Princeton, NJ.

MACDIARMID, A. B. & BUTLER, M. J. (1999). Sperm economy and limitation in spiny lobsters. — Behavioral Ecology and Sociobiology 46, 14-24.

MAGRATH, M. J. L., SANTEMA, P., BOUMAN, K. M., BRINKHUIZEN, D. M., GRIFFITH, S. C. & LANGMORE, N. E. (2009). Seasonal decline in reproductive performance varies

with colony size in the fairy martin, *Petrochelidon ariel*. — Behavioral Ecology and Sociobiology 63, 661-672.

MALECKI, I. A., CLOETE, S. W. P., GERTENBACH, W. D. & MARTINS, G. B. (2004). Sperm storage and duration of fertility in female ostriches (Struthio camelus). — South African Journal of Animal Science 34, 158-165.

MANSFIELD, E. R. & HELMS, B. P. (1982). Detecting Multicollinearity. — The American Statistician 36, 158-160.

MARROIG, G. (2007). When size makes a difference: allometry, life-history and morphological evolution of capuchins (*Cebus*) and squirrels (*Saimiri*) monkeys (Cebinae, Platyrrhini). — BMC Evolutionary Biology 7.

MARTIN, J. W. & DAVIS, G. E. (2001). Updated classification of the recent Crustacea. — Natural History Museum of Los Angeles County, Los Angeles.

MARTINS, E. P. & HANSEN, T. F. (1997). Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. — The American Naturalist 149, 646-667.

MCINTYRE, T. M. & HUTCHINGS, J. A. (2003). Small scale temporal and spatial variation in Atlantic cod (*Gadus morhua*) life history. — Canadian Journal of Fisheries and Aquatic Sciences 60, 1111-1121.

MCKONE, M. J. & LIVELY, C. M. (1993). Statistical analysis of experiments conducted at multiple sites. — Oikos 67, 184-186.

MᴄNᴀʙ, B. K. (1971). On the ecological significance of Bergmann's Rule. — Ecology 52, 845-854.

Mᴇɴᴅᴏɴᴄᴀ, M. T. & Cʀᴇᴡs, D. (2001). Control of attractivity and receptivity in female red-sided garter snakes. — Hormones and behavior 40, 43-50.

Mɪʟᴇs, D. B. & Dᴜɴʜᴀᴍ, A. E. (2009). Comparative analyses of phylogenetic effects in the life-history patterns of iguanid reptiles. — The American Society of Naturalists 139, 848-869.

Mɪɴᴅᴇʀ, A. M., Hᴏsᴋᴇɴ, D. J. & Wᴀʀᴅ, P. I. (2005). Co-evolution of male and female reproductive characters across the Scathophagidae (Diptera). — Journal of Evolutionary Biology, 60-69.

Mᴏʟʟᴇʀ, A. P. (1991). Sperm competition, sperm depletion, paternal care, and relative testis size in birds. — American Naturalist 137, 882-906.

Mᴏʀɢᴀɴ, S. G., Gᴏʏ, J. W. & Cᴏsᴛʟᴏᴡ, J. D. (1983). Multiple ovipositions from single matings in the mud crab *Rhithropanopeus harrisii*. — Journal of Crustacean Biology 3, 542-547.

Mᴏʀʀɪsᴏɴ, C. & Hᴇʀᴏ, J. M. (2003). Geographic variation in life-history characteristics of amphibians: a review. — Journal of Animal Ecology 72, 270-279.

Mᴏᴜssᴇᴀᴜ, T., A. (1997). Ectotherms follow the converse to Bergmann's Rule. — Evolution 51, 630-632.

NAUD, M. J., SHAW, P. W., HANLON, R. T. & HAVENHAND, J. N. (2005). Evidence for biased use of sperm sources in wild female giant cuttlefish (*Sepia apama*). — Proceedings of the Royal Society B-Biological Sciences 272, 1047-1051.

OKUTANI-AKAMATSU, Y., TOMONARI, W. & AZUMA, M. (2007). Mating attraction by *Stenotus rubrovittatus* (Heteroptera: Miridae) females and its relationship to ovarian development. — Journal of Economic Entomology 100, 1276-1281.

OLSSON, M., MADSEN, T. & SHINE, R. (1997). Is sperm really so cheap? Costs of reproduction in male adders, *Vipera berus*. — Proceedings of the Royal Society of London Series B-Biological Sciences 264, 455-459.

ORENSANZ, J. M. & GALLUCCI, V. F. (1988). Comparative study of postlarval life-history schedules in four sympatric species of *Cancer* (Decapoda: Brachyura: Cancridae). — Journal of Crustacean Biology 8, 187-220.

ORENSANZ, J. M., PARMA, A. M., ARMSTRONG, D. A., ARMSTRONG, J. & WARDRUP, P. (1995). The breeding ecology of *Cancer gracilis* (Crustacea, Decapoda, Cancridae) and the mating systems of cancrid crabs. — Journal of Zoology 235, 411-437.

PAGE, R. D. M. (1996). TREEVIEW: An application to display phylogenetic trees on personal computers. — Computer applications in the biosciences 12, 357-358.

PARADIS, E., J., C. & K., S. (2004). SPE: analyses of phylogenetics and evolution in R language. — Bioinformatics 20, 289-290.

PARKER, G. A. (1970). Sperm competition and its evolutionary consequences in the insects. — Biological Reviews 45, 525-567.

—. (2006). Sexual conflict over mating and fertilization: an overview. — Philosophical Transactions of the Royal Society B-Biological Sciences 361, 235-259.

PARTRIDGE, L. & COYNE, J. A. (1997). Bergmann's rule in ectotherm: is it adaptive. — Evolution 51, 632-635.

PAUL, A. J. (1984). Mating frequency and viability of stored sperm in the tanner crab *Chionoecetes bairdi* (Decapoda, Majidae). — Journal of Crustacean Biology 4, 375-381.

PENHA-LOPES, G., RHYNE, A. L., FIGUELREDO, J., LIN, J. & NARCISO, L. (2006). Can larvae produced from stored sperm in the ornamental crab *Mithraculus forceps* (A. Milne Edwards, 1875) (Decapoda : Brachyura : Majidae) be used in aquaculture? — Aquaculture 257, 282-286.

PERAMPALADAS, K., STOLTZ, J. A. & ANDRADE, M. C. B. (2008). Mated redback spider females re-advertise receptivity months after mating. — Ethology 114, 589-598.

PIANKA, E. R. (1970). R-selection and K-selection. — American Naturalist 104, 592-&.

POLOVINA, J. J. (1989). Density dependence in spiny lobster, *Panulirus marginatus*, in the Northwestern Hawaiian Islands. — Canadian Journal of Fisheries and Aquatic Sciences 46, 660-665.

POSADA, D. (2008). jModelTest: Phylogenetic Model Averaging. — Molecular Biology and Evolution 25, 1253-1256.

QUIN, D. G., SMITH, A. P. & NORTON, T. W. (1996). Eco-geographic variation in size and sexual dimorphism in sugar gliders and squirrel gliders (Marsupialia: Petauridae). — Australian Journal of Zoology 44, 19-45.

QUINN, G. P. & KEOGH, M. J. (2002). Experimental design and data analysis for biologists. — Cambridge University Press, Cambridge.

RAETHKE, N., MACDIARMID, A. B. & MONTGOMERY, J. C. (2004). The role of olfaction during mating in the couther temperate spiny lobster *Jasus edwardsii*. — Hormones and behavior 46, 311-318.

RAMOS, M., CODDINGTON, J. A., CHRISTENSON, T. E. & IRSCHICK, D. J. (2005). Have male and female genitalia coevolved? A phylogenetic analysis of genitalic morphology and sexual size dimorphism in web-building spiders (Araneae: Araneoidea). — Evolution 59, 1989-1999.

REAKA, M. L. (1979). The evolutionary ecology of life history patterns in stomatopod Crustacea. — In: Reproductive ecology of marine invertebrates (W. E. Stancyk, ed). University of South Carolina Press, Columbia, p. 235-260.

—. (1980). Geographic range, life-history patterns, and body size in a guild of coral-dwelling mantis shrimps. — Evolution 34, 1019-1030.

—. (1986). Biogeographic patterns of body size in stomatopod Crustacea: Ecological and evolutionary consequences. — In: Crustacean Biogeography (R. H. Gore & K. L. Heck, eds). Balkema Press, Rotterdam, p. 209-235.

RICE, A. L. (1983). Zoeal evidence for brachyuran phylogeny. — In: Crustacean Phylogeny (F. R. Schram, ed). A.A. Balkema, Rotterdam, p. 313-329.

ROBLES, R., SCHUBART, C. D., CONDE, J. E., CARMONA-SUAREZ, C., ALVAREZ, F., VILLALOBOS, J. & FELDER, D. L. (2007). Molecular phylogeny of the american *Callinectes* Stimpson, 1860 (Brachyura: Portunidae), based on two partial mitochondrial genes. — Marine Biology 150, 1265-1274.

RONDEAU, A. & SAINTE-MARIE, B. (2001). Variable mate guarding time and sperm allocation by male snow crabs (*Chionoecetes opilio*) in response to sexual competition, and their impact on the mating success of females. — Biological Bulletin 201, 204-217.

RONQUIST, F. & HUELSENBECK, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. — Bioinformatics 19.

ROSENBERG, M. S. (2001). The systematics and taxonomy of fiddler crabs: A phylogeny of the genus UCA. — Journal of Crustacean Biology 21, 839-869.

RUTTENBERG, B. I., HAUPT, A. J., CHIRIBOGA, A. I. & WARNER, R. R. (2005). Patterns, causes and consequences of regional variation in the ecology and life history of a reef fish. — Oecologia 145, 394-403.

SAINTE-MARIE, B. (2007). Sperm demand and allocation in decapod crustaceans. — In: Evolutionary ecology of social and sexual systems: Crustaceans as model systems (J. E. Duffy & M. Thiel, eds). Oxford University Press, New York, p. 191-210.

SAINTE-MARIE, G. & SAINTE-MARIE, B. (1998). Morphology of the spermatheca, oviduct, intermediate chamber, and vagina of the adult snow crab (*Chionoectes opilio*). — Canadian Journal of Zoology 76, 1589-1604.

—. (1999). Reproductive products in the adult snow crab (*Chionoecetes opilio*). II. Multiple types of sperm cells and of spermatophores in the spermathecae of mated females. — Canadian Journal of Zoology-Revue Canadienne De Zoologie 77, 451-462.

SALMON, M. (1983). Courtship, mating systems, and sexual selection in decapods. — In: Studies in adaptation (S. Rebach, ed). John Wiley & Sons, Toronto, p. 143-169.

SATO, T., ASHIDATE, M., JINBO, T. & GOSHIMA, S. (2006). Variation of sperm allocation with male size and recovery rate of sperm numbers in spiny king crab *Paralithodes brevipes*. — Marine Ecology Progress Series 312, 189-199.

SCHUBART, C. D., CANNICCI, S., VANNINI, M. & FRATINI, S. (2006). Molecular phylogeny of grapsoid crabs (Decapoda, Brachyura) and allies based on two mitochondrial genes and a proposal for refraining from current superfamily classification. — Journal of Zoological Systematics and Evolutionary Research 44, 193-199.

SCHUBART, C. D., NEIGEL, J. & FELDER, D. L. (2000a). Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. — Crustacean Issues 12, 817-830.

SCHUBART, C. D., NEIGEL, J. E. & FELDER, D. L. (2000b). Molecular phylogeny of mud crabs (Brachyura: Panopeidae) form the northwestern Atlantic and the role of morphological stasis and convergence. — Marine Biology 137, 11-18.

—. (2000c). Molecular phylogeny of mud crabs (Brachyura: Panopeidae) from the northwestern Atlantic and the role of morphological stasis and convergence. — Marine Biology 137, 11-18.

SEVER, D. M., MORIARTY, E. C., RANIA, L. C. & HAMLETT, W. C. (2001). Sperm storage in the oviduct of the internal fertilizing frog *Ascaphus truei*. — Journal of Morphology 248, 1-21.

SHAPIRO, D. Y. & GIRALDEAU, L. A. (1996). Mating tactics in external fertilizers when sperm is limited. — Behavioral Ecology 7, 19-23.

SHAROV, A. F., VOLSTAD, J. H., DAVIS, G. R., DAVIS, B. K., LIPCIUS, R. N. & MONTANE, M. M. (2003). Abundance and exploitation rate of the blue crab (*Callinectes sapidus*) in the Chesapeake Bay. — Bulletin of Marine Science 72, 543-565.

SILVA, P. V., LUPPI, T. A., SPIVAK, E. D. & ANGER, K. (2009). Reproductive traits of an estuarine crab, *Neohelice* (=*Chasmagnathus*) *granulata* (Brachyura: Grapsoidea: Varunidae), in two contrasting habitats. — Scientia marina 73, 117-127.

SIMONS, A. M. (2007). Selection for increased allocation to offspring number under environmental unpredictability. — Journal Compilation 20, 813-817.

SMITH, C. C. & FRETWELL, S. D. (1974). Optimal balance between size and number of offspring. — American Naturalist 108, 499-506.

SNOW, L. S. E., ABDEL-MESIH, A. & ANDRADE, M. C. B. (2006). Broken copulatory organs are low-cost adaptations to sperm competition in redback spiders. — Ethology 112, 379-389.

SOMERTON, D. A. & MEYERS, W. S. (1983). Fecundity differences between primiparous and multiparous female Alaskan Tanner crab (*Chionoectes bairdi*). — Journal of Crustacean Biology 3, 183-186.

STEARNS, S. C. (1983). The influence of size and phylogeny on patterns of covariation among life-history traits in the mammals. — OIKOS 41, 173-187.

—. (1992). The evolution of life histories. — Oxford University Press, New York.

STEEL, M. (2005). Should phylogenetic models be trying to 'fit an elephant'? — TRENDS in Genetics 21, 307-309.

STEELE, P. & BERT, T. M. (1998). The Florida blue crab fishery: history, status, and management. — Journal of Shellfish Research 17, 441-449.

STEHLIK, L. L., SCARLETT, P. G. & DOBARRO, J. (1998). Status of the blue crab fisheries of New Jersey. — Journal of Shellfish Research 17, 475-485.

STERN, D. L. & EMLEN, D. J. (1999). The developmental basis for allometry in insects. — Development 126, 1091-1101.

STEVCIC, Z. (1971). Main Features of Brachyuran Evolution. — Systematic Zoology 20, 331-&.

STILLWELL, R. C., MORSE, G. E. & FOX, C. W. (2007). Geographic variation in body size and sexual size dimorphism of a seed-feeding beetle. — The American Naturalist 170, 358-369.

STRATHMANN, R. R. (1977). Egg size, larval development, and juvenile size in benthic marine invertebrates. — The American Naturalists 111, 373-376.

SUBRAMONIAM, T. (1991). Chemical composition of spermatophores in decapod crustaceans. — In: Crustacean Sexual Biology (R. T. Bauer & J. W. Martin, eds). Columbia University Press, New York, p. 308-319.

SZYKMAN, M., VAN HORN, R. C., ENGH, A. L., BOYDSTON, E. E. & HOLEKAMP, M. E. (2007). Courtship and mating in free-living spotted hyenas. — Behavior 144, 815-846.

TAGGART, D. A. & TEMPLESMITH, P. D. (1991). Transport and storage of spermatozoa in the female reproductive tract of the brown marsupial mouse, *Antechinus stuartii* (Dasyuridae). — Journal of Reproduction and Fertility 93, 97-110.

TEUSCHL, Y. & BLACKENHORN, W. U. (2006). The reluctant fly: what makes *Sepsis cynipsea* females willing to copulate? — Animal Behavior 73, 85-97.

TRAYNOR, R. E. & MAYHEW, P. J. (2005). A comparative study of body size and clutch size across the parasitoid Hymenoptera. — Oikos 109, 305-316.

UNDERWOOD, A. J. (1997). Experiments in ecology. — Cambridge University Press, Cambridge.

UPHOFF, J. H. J. (1998). Stability of the blue crab stock in Maryland' portion of the Chesapeake Bay. — Journal of Shellfish Research 17, 519-528.

URBANI, N., SAINTE-MARIE, B., SEVIGNY, J. M., ZADWORNY, D. & KUHNLEIN, U. (1998). Sperm competition and paternity assurance during the first breeding period of female snow crab (*Chionoecetes opilio*) (Brachyura : Majidae). — Canadian Journal of Fisheries and Aquatic Sciences 55, 1104-1113.

VAN VOORHIES, W. A. (1996). Bergmann size clines: a simple explanation for their occurrence in ectotherms. — Evolution 50, 1259-1264.

WADA, T., TAKEGAKI, T., MORI, T. & NATSUKARI, Y. (2005). Sperm displacement behavior of the cuttlefish *Sepia esculenta* (Cephalopoda: Sepiidae). — Journal of Ethology 23, 85-92.

WADDY, S. L. & AIKEN, D. E. (1991). Mating and insemination in the American lobster, *Homarus americanus*. — In: Crustacean Sexual Biology (R. T. Bauer & J. W. Martin, eds). Columbia University Press, New York, p. 126-144.

WEDELL, N. & COOK, P. A. (1999). Strategic sperm allocation in the small white butterfly *Pieris rapae* (Lepidoptera : Pieridae). — Functional Ecology 13, 85-93.

WEDELL, N., GAGE, M. J. G. & PARKER, G. A. (2002). Sperm competition, male prudence and sperm-limited females. — Trends in Ecology & Evolution 17, 313-320.

WEDELL, N., KVARNEMO, C., LESSELLS, C. K. M. & TREGENZA, T. (2006). Sexual conflict and life histories. — Animal Behaviour 71, 999-1011.

WILLIAMS, G. C. (1966). Natural selection costs of reproduction and a refinement of lacks principle. — American Naturalist 100, 687-690.

WOLCOTT, D. L., HOPKINS, C. W. B. & WOLCOTT, T. G. (2005). Early events in seminal fluid and sperm storage in the female blue crab *Callinectes sapidus* Rathbun: Effects of male mating history, male size, and season. — Journal of Experimental Marine Biology and Ecology 319, 43-55.

YAMAGUCHI, T. (1998). Longevity of sperm of the fiddler crab *Uca lactea* (de Haan, 1835) (Decapoda, Brachyura, Ocypodidae). — Crustaceana 71, 712-713.

YAMAUCHI, A. (2000). Theory of resource allocation strategy in territorial male tropical reef fishes. — Journal of Ethology 18, 115-121.

ZERA, A. J. & DENNO, R. F. (1997). Physiology and ecology of dispersal polymorphism in insects. — Annual Review of Entomology 42, 207-230.

ZWICKL, D. J. (2006). Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under maximum likelihood criterion. — PhD thesis, The University of Texas at Austin.