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Sexual Dimorphism and Behavioral Responses to Conspecific Chemical Cues in Pagurus samuelis

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Sexual Dimorphism and Behavioral Responses to Conspecific
Chemical Cues in *Pagurus samuelis*

by

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A Thesis submitted in partial satisfaction of
the requirements for the degree of
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Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree of Master of Science.

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ABSTRACT OF THE THESIS

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Anatomical differences were observed between sexes of *P. samuelis*. We measured the major cheliped and carapace lengths of hermit crabs caught off the coast of Southern California and found that male chelipeds were significantly larger relative to their carapace length than female chelipeds. Average male cheliped:carapace ratios were 1.86 while average female cheliped:carapace ratios were 1.32. We predicted that males and females would respond differently to cue waters created from their own sex. Females were exposed to female cue treatment odors and males were exposed to male cue treatment odors. We recorded hermit crabs exposed to cue treatment waters and analyzed four behaviors: withdrawn, head-extended, walking, and meral spread. Females were more likely than males to remain withdrawn in their shells when in non-agonistic cue treatment waters. Males were more likely than females to display meral spread when sensing conspecific cues. Both sexes displayed no difference in the amount of time spent stationary with head-extended across all cue treatment waters. Both sexes tended to walk more in the presence of agonistic cue waters.

CHAPTER ONE

INTRODUCTION

Research done on crustaceans has varied from anatomical studies (Evans et al., 1976; Horner et al., 1997), mating behavior (Adams and Moore, 2003; Contreras-Garduno et al., 2007), and agonistic behavior (Briffa and Elwood, 2002; Elwood et al., 2006; Hsu et al., 2006), to recognition of conspecifics (Karavanich and Atema, 1998a; Schneider et al., 2001; Gherardi and Atema, 2005; Gherardi et al., 2005), taphonomy (Shives and Dunbar, 2010), and sex-specific traits (Frix et al., 1991; Bach et al., 2006). Studies on communication and sexual differences can tie many of these topics together as some of these themes are related through communication and sexual differences.

In this thesis, I present results of a study on the hermit crab, *Pagurus samuelis,* analyzing both anatomical and behavioral differences between males and female and the impacts chemical cues have on the behavior of these hermit crabs.

I begin this chapter by stating my objectives and hypotheses. I follow with a review of the behavioral ecology of hermit crabs as a taxon, proceeding to review the literature on sexual differences within crustaceans, and then review communication and behavior of crustaceans in the context of chemical communication.

In Chapter 2 I present results of my research on hermit crab anatomical and behavioral differences between males and females. I present research on hermit crab reactions to two chemical cues from conspecifics of the same gender.

In Chapter 3 I discuss implications of my research on the understanding of the

ecology of hermit crabs and crustaceans, and conclude with some suggestions on areas of further research.

Goals and Hypotheses

My research was performed with the following goals in mind: 1) to explore differences in anatomy between male and female *Pagurus samuelis*, 2) to quantify behavioral differences between male and female *Pagurus samuelis*, and 3) to explore aspects of interaction in chemical communication and behavior in *Pagurus samuelis*.

Hypothesis 1: Male *Pagurus samuelis* have a larger cheliped:carapace ratio than females. To test this hypothesis, male and female chelipeds and carapaces were measured and the measurements were analyzed to determine evidence for sexual dimorphism.

Hypothesis 2: Male *P. samuelis* respond with higher levels of agonistic behaviors than female *P. samuelis* when exposed to chemical cues from their own sex. This hypothesis was tested by recording crab behavior during exposure to chemical cues from their own sex. Data from each sex were analyzed to determine differences in behavior between the sexes.

Hypothesis 3: *P. samuelis* increases the amount of locomotion and displays higher aggression level behaviors when sensing chemical cues from other *P. samuelis* hermit crabs in agonistic environments than when other *P. samuelis* hermit crabs are in non-agonistic and control environments. For this hypothesis, crab behaviors were recorded and analyzed to determine if there were differences in behaviors of crabs exposed to treatment cue waters and control water.

Behavioral Ecology of Hermit Crabs

Hermit crabs make up the Super-family Paguroidea (Phylum: Arthropoda, Order: Decapoda, Infraorder: Anomura). The Infraorder Anomura is made up of four superfamilies: Galatheoidea, Hippoidea, Lomisoidea, and Paguroidea. Hermit crabs are different from other crustaceans in that their abdomens are uncalcified, and twisted to the right in healthy individuals (Lancaster, 1988). Their uncalcified abdomens make them susceptible to higher levels of predation (Reese, 1968) and osmotic stress (Reese, 1968; Shumway, 1978) unless they can find suitable shelter. Thus, they mostly seek shelter and protection in empty gastropod shells. Although some hermit crab species have found shelter in sessile tubes (Caine, 1980), almost all 800 species of hermit crabs have the ability to be sheltered while mobile, making them highly successful marine invertebrates found in almost all marine environments, including tropical terrestrial shores (Hazlett, 1981).

Hermit crabs rely on their shell for many important reasons. In many cases, crabs must find larger shells in order to grow larger (Fotheringham, 1976; Angel, 2000). Desiccation and osmotic stress are inevitable without a suitable shell (Brodie, 2005; Hamasaki et al., 2011). As a crab grows larger, predation rates also increase with adequate shells becoming a limiting factor (Vance, 1972). Inadequate shells may not be able to properly protect the soft abdomen of hermit crabs. Proper development will not occur unless a shell is available (Vance, 1972; Brodie, 1999).

Hermit crabs are generally considered opportunistic detritivores. Some species are filter-feeders and use their antenna to sift their food from the surrounding water (Gerlach et al., 1976; Caine, 1980). However, most can be found eating detritus from the seafloor,

in intertidal pools, and on coastal zones.

Hermit crabs often contest with each other over shells. An attacker will signal his motivation to evict a crab by shell-rapping (Briffa and Elwood, 2000, 2001, 2002; Elwood et al., 2006). Shell-rapping consists of an attacker hermit crab holding onto the shell of another crab. The attacker brings its shell down forcefully onto the shell of the defending crab. The intensity of the rap and the number of raps per bout demonstrate the motivation level of the attacker to the defender. When an attacking crab has higher motivation to evict a crab than a defending crab has to defend its shell, the invading crab has a higher likelihood of causing an eviction.

In mating, males may exhibit pre-copulatory guarding (Goshima et al., 1998). Males will hold the shell of a female for up to 5 days before copulating. During copulation, the male grasps the female's shell with the shell apertures facing each other (Hazlett, 1996). The male then proceeds by rotating the shell of the female side-to-side in a figure eight style or by cheliped tapping. Cheliped tapping is performed by the male tapping the chelipeds against the rim of the aperture of the female's shell. The male guards the female between bouts of rotation or cheliped tapping. After several hours, the female and male mate by partially emerging from their respective shells. The male deposits the spermatophore onto the ventral surface of the female's cephalothorax, then guards the female for an additional 15 minutes after copulation. The female extrudes the eggs within one hour of copulation.

Sex-Specific Differences

Differences between sexes have been studied in many animal taxa including, but

not limited to, humans (Folkman and Lazarus, 1980; Stewart et al., 1992; Calle et al., 1999), birds (Quillfeldt et al., 2011), fishes (Roelke and Sogard, 1993; Bartolino et al., 2011), insects (Nilsen et al., 2004), and crustaceans (Frix et al., 1991; Bach et al., 2006).

In crustaceans, sex-specific differences can be evident physiologically (Pereira et al., 2011; Sroda and Cossu-Leguille, 2011a), through resource acquisition (Bach et al., 2006; Briffa and Dallaway, 2007), anatomically (Wang et al., 2011), and behaviorally (Skog, 2009). On a microscopic level, Garza-Torres et al. (2011) found a slight difference in the nuclear diameter of gametes, with the nuclear diameter of female gametes tending to be larger than male gametes, although not significantly. Females may have different requirements than males and thus, female decisions, anatomy, and behavior may be readily distinguishable from those of males.

Physiological and Hormonal Differences Between Sexes

Physiological responses can differ between sexes. Sroda and Cossu-Leguillle (2011a) found that females of the freshwater amphipod, *Gammarus roeseli,* and the killer shrimp, *Dikerogammarus villous,* were more resilient than males when exposed to sublethal copper concentrations. Females tended to have more energy reserves and had higher levels of glutathione peroxidase, an antioxidant enzyme, and lower levels of malondialdehyde, an indicator for toxic effect of potential contaminants and pollutants of aquatic ecosystems. Even when tested in nontoxic environments, female *G. roeseli* showed higher levels of lipids, proteins, and glutathione peroxidase activities than males, giving evidence that females may have a better defense system against toxins (Sroda and Cossu-Leguille, 2011b). *Carcinus maenus* females from Portuguese coasts tended to have

higher levels of metals, such as Cr, Cu, and Ni in their hepatopancreas than males (Pereira et al., 2011). Female *C. maenus* also exhibited higher levels of glutathione S-transferase at some Portuguese coast localities. Because of the differences between males and females and how they handle these toxins, Pereira et al. (2011) advised that male and female *C. maenus* be analyzed separately in chemical bioaccumulation analyses.

Some aspects of physiology show no differences between sexes. Chen and Chia (1997) measured oxyhemocyanin, protein, osmolality, and electrolyte levels in the mud crab, *Syclla serrata*. They found no difference in levels between males and females even though there were size differences between males and females. Similar testing was done on the giant river prawn, *Macrobrachium rosenbergii* (Cheng et al., 2001). No significant differences were found between sexes, except that very large males had lower levels of oxyhemocyanin, Ca^{2+} and Mg^{2+}, and a lower ratio of oxyhemocyanin to protein (Cheng et al., 2001). The striped shore crab, *Pachygrapsus crassipes*, displayed no differences in fatty acid constitution between sexes (Sjoeben et al, 2010). The striped shore crab's fatty acid amount and constitution was not necessarily dependent on just temperature, but may have also changed due to energy resources, reproduction, day length, and molting. Biggs and McDermott (1973) found that the hermit crab, *Pagurus longicarpus*, showed no significant differences between sexes in the ability to tolerate fluctuating temperature and salinity levels.

Anatomical Differences

Anatomical differences are also apparent between sexes of some crustaceans. The crayfish, *Procambarus clarkii,* shows sexual dimorphism (Wang et al., 2011). Males tend

to be heavier, yet have shorter tails than their female counterparts. *Carcinus maenus* exhibits a difference between males and females, with males being larger and heavier than females (Pereira et al., 2011). Chen and Chia (1997) noted a size difference between sexes in the mud crab, *Scylla serrata*. Male carapace width was, on average, larger than female carapace width.

Thiel et al. (2010) found that the dancing shrimp, *Rhynchocinetes bruicei,* displayed very little sexual dimorphism, although three male morphotypes were present. The largest male morphotype was termed robustus, and the two smaller male morphotypes are typus and intermedius. Female sizes were insignificantly smaller than the typus males. *Macrobrachium rosenbergii* also show evidence of male morphotypes. Small males and females were similar in size and weight (Cheng et al., 2001).

Perhaps one of the best examples of sexual dimorphism in the crustacean world is evident in fiddler crabs (Genus: *Uca*). Male fiddler crabs have much larger chelae than females, which may give them an advantage in predator avoidance (Bildstein et al., 1989). Weissburg and Derby (1995) showed that both female claws in *Uca pugilator* are much more sensitive to chemical stimulation than the male feeding claw. The male major claw is not used for feeding, but for mating rituals and for agonistic contests among male conspecifics. Weissburg et al. (2001) found that limbs from female *Uca pugilator* that were actually transplanted onto males in place of their major claw, still retained features and behavioral traits normally identified as female. Female feeding claws on male host bodies showed much higher sensitivity to chemical stimulants than native male feeding claws. They found that the nerves of the transplanted limb did not innervate areas of the central nervous system that are responsible for chemical sensory input, yet the limb was

nevertheless stimulated chemically. Thus, neuronal organization itself is different between males and females in this species.

In *Uca mjoebergi*, the male major claw actually reflects more light per unit area than the female claw (Cummings et al., 2008). This is advantageous for males to more easily attract females in their claw-waving mating rituals. Interestingly, Cummings et al. (2008) found that the dorsal carapace of *U. mjoebergi* showed very little dimorphism between the sexes. Both male and female carapaces blend better with the surroundings from above, while the male major claw is visible from below for mating. This would be advantageous, as avian predators are more likely to be looking down from above.

Some hermit crab males have a larger major cheliped than females, as in *Pagurus bernhardus* (Briffa and Dallaway, 2007) and *P. criniticornis* (Mantelatto et al., 2007). This trait may also have been developed more dramatically in males since larger male hermit crabs win more often than smaller male conspecifics in competitions for female hermit crabs, as in *Coenobita compressus* (Contreras-Garduno et al., 2007), and for food (Ramsay et al., 1997). Larger sizes and larger chelipeds in male hermit crabs may be a reproductive advantage when males are competing for females.

Behavioral Differences Between Sexes

Sex-specific differences are often displayed in behavior. Crustacean behavior, in turn, is often affected by anatomy (Frix et al., 1991), energy preservation (Billock and Dunbar, 2008), resources acquisition (Briffa and Williams, 2006; Billock and Dunbar, 2011), predator avoidance (Frix et al., 1991; Hazlett, 1999; Schneider and Moore, 2000), reproduction (Hayden et al., 2007), and communication (Moore and Bergman, 2005).

Resource Acquisition and Defense

Sex-specific behavior in decapods can impact resource acquisition. When feeding, the male sentinel crab, *Macrophthalmus convexus*, showed much lower scoop rates per minute than females (Schuwerack et al., 2006). Male *M. definitus* also had lower scoop rates than females, but only significantly different from their conspecific females during the first quarter of the lunar cycle. Schuwerack et al. (2006) noted that male *M. convexus* pinched their food more before bringing the food to their mandibles. They noted that more food uptake per scoop may make up for their lower scoop rate overall.

Molis et al. (2011) found female *C. maenus* did not consume periwinkles, while 45 - 50% of the males ate periwinkles that were smaller than 18 mm. Weissburg and Derby (1995) found that feeding behavior in the fiddler crabs, *Uca pugilator* and *U. pugnax,* was dependent on sex. Females are much more sensitive to chemical food stimulants than males and were able to feed at lower food levels than males.

Another important resource is shelter. Peeke et al. (1998) found *Homarus americanus* males to be more successful at defending shelters than females. Females were weak in defending their shelters from other female intruders. Males may defend their shelters more efficiently because of the need for this resource in mating. Females seek out males in shelters (Atema, 1986) and thus have less need to defend their own shelters.

Male *Pagurus longicarpus* were more likely to be found in shells covered with the epibiont, *Hydractinia symbiolongicarpus*, than females (Bach et al., 2006). There were proportionally more ovigerous females than non-ovigerous females in shells with *Hydractinia*. Bach et al. (2006) attributed this to the possibility that hermit crab egg

predation can occur if a female were to try to switch into or out of shells covered with *Hydractinia*. Therefore, females are more likely to choose shells without this epibiont. Females are also less likely to switch out of a *Hydractinia*-covered shell if they are ovigerous.

Responses to stimuli were different between sexes in *P. bernhardus*. Appel and Elwood (2009) drilled holes in the shells of *P. bernhardus*, into which electric probes were placed. They shocked the abdomens of male and female hermit crabs and found that shocked females were more likely to evacuate their shells than shocked male conspecifics.

Female *P. bernhardus* are more likely than males to switch to new shells and to quickly withdraw or thrust their abdomens into their new shells (Appel and Elwood, 2009). Interestingly, female *P. bernhardus* appears to fight harder than males to evict conspecifics from their shells (Briffa and Dallaway, 2007). Males were more likely to initiate fights. If the defender was female, the attacker was more persistent even though there was no difference between males and females in the ability to defend their shell. Overall, male *P. bernhardus* appeared to have the advantage over females in gaining the vital resource of a snail shell, but only because males initiated more fights.

Predator Avoidance

One important behavior for crustaceans is predator avoidance, which can differ between sexes. In the fiddler crabs, *Uca pugilator* and *U. pugnax,* sex affected the way in which crabs avoided predators, with females retreating further in burrows than males when an imitation avian predator was introduced into their environment (Frix et al.,

1991). The hermit crab, *Clibanarius vittatus*, also exhibited a difference between sexes when sensing one of its predators, the stone crab, *Minnipe mercenaria* (Hazlett and Rittschof, 2000). Males were less disturbed in the presence of predators and would continue to exhibit pre-copulatory mating behaviors. The presence of predators significantly reduced a female's attractiveness to males. The authors suggested that females may change their chemical cues or stop sending out pheromones so that males do not find them attractive for mating.

Aggression

Aggression levels can be different between sexes in crustaceans. In the crayfish, *Orconectes rusticus*, reproductive state influenced aggression and fight outcomes (Martin and Moore, 2010). Reproductive males were the most dominant, while non-reproductive females were the most subordinate. However, reproductive females would usually win in contests against non-reproductive males. The European lobster, *Homarus gammarus* (Skog, 2009) showed evidence that females are more aggressive overall than males. Female *H. gammarus* escalated into higher levels of aggression, such as claw locking, significantly more often than males.

Recognition

Skog (2009) found that recognition patterns differed between males and females of *H. gammarus.* Females that lost a recent fight with another female distinguished unfamiliar conspecifics as a winner when the unfamiliar conspecific had recently won agonistic contests. The loser from the previous fight behaved subordinately much more

quickly. Even when meeting unfamiliar conspecifics, female losers tended to lose to females that had won previously. Males exhibited individual recognition and fought at equal intensity when interacting agonistically with any unfamiliar conspecific. Male losers from a previous fight won 33% of the time with a winner from a previous fight, showing dominance reversals are not uncommon within males.

Chemical Cues, Mating and Reproduction

Behavior is often impacted by mating and reproduction. Many of the behavioral differences between sexes may be attributable to chemical cues given off or sent by one sex to the opposite sex. Successful chemical communication is extremely important in finding (Atema and Cowan, 1986) and competing for mates (Prenter et al., 2006). Urine signals and molt odors appear to be necessary in identifying the gender of crustaceans during reproductive periods.

Chemical Communication Through Urine and Cues

Two studies showed the crayfishes, *Orconectes rusticus* and *Pacifastacus leniusculus*, used urine to signal reproductive maturity and gain access to females (Simon and Moore, 2007; Berry and Breithaupt, 2008). Simon and Moore (2007) found that *O. rusticus* appeared to have a degree of control over urine output. When reproductive male and female *O. rusticus* were paired, they excreted more urine than when only one of them was in a reproductive state, or if both of them were not in reproductive states. Berry and Breithaupt (2008) found that urine from a female crayfish, *Pacifastacus leniusculus,* was able to significantly stimulate male conspecifics to initiate breeding attempts with a

female dummy, caused the heart to skip a few heart beats, and then increased the overall heart rate of the male. Male urine and control water did not significantly alter the male crayfish in the same fashion.

The lobster, *H. americanus,* also releases urine to chemically signal reproductive readiness (Atema and Cowan, 1986; Bushmann and Atema, 1996). Bushman and Atema (1996) found that urine is important in finding mates in *H. americanus* because female lobster urine causes the male lobster to reduce aggression and engage in mating behavior. Male urine also establishes the status of the male with the female, since females prefer dominant males (Bushmann and Atema, 2000). Female *H. americanus* molt about 0.5 hr before mating (Atema, 1986). A combination of urine and molt odors is important for males to recognize these females as reproductive (Atema and Cowan, 1986). *Homarus americanus* males reacted very strongly to female urine (Atema and Cowan, 1986). Females did not react to male urine as definitively as males did to female urine. Atema and Cowan (1986) found evidence that urine and body odors from males, and urine and body odors from females, elicited different responses from conspecifics of the opposite sex. This showed that chemical cues in urine were different based on sex.

Carcinus maenus males only responded with sexual behaviors when they were exposed to urine from a pre-moult female crab (Bamber and Naylor, 1997). Urine samples from premoult males and intermoult males and females did not cause any sexual behavioral responses, but urine from pre-moult female crabs elicited sexual responses from receptive males. *Callinectes sapidus* showed evidence that reception of chemical cues were highly important for mating (Bushmann, 1999). When male antennules were ablated or nephropores were blocked, there were less incidences of successful pair

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formation.

In *Procambarus,* males were willing to mate as long as a chemical cue from a female was present (Aquiloni and Gherardi, 2008). Males remained aggressive when given only visual cues of a female conspecific. By exposing a male to the female cue, the male behaved in a less aggressive manner.

In the grapsid crab, *Cyclograpsus lavauxi*, females usually molt after the breeding season (Brockerhoff and McLay, 2005), which may indicate that premolt odors may also be a sign of reproductive readiness. Several decapod species, such as the lobster, *Homarus americanus,* and the hermit crab, *Pagurus longicarpus,* communicate reproductive status through pheromones or other body odors (Atema and Cowan, 1986; Gherardi and Tricarico, 2007).

Reproductive Behavioral Differences Between Sexes

Males and females have very specific roles they must fulfill in order to reproduce successfully. The male shore crab, *Carcinus maenus*, displayed decreased feeding activities during the peak mating and reproductive months of summer, lowering the likelihood of cannibalism of its soft-bodied female mate, while females were active feeders year round (Hayden et al., 2007). Hayden et al. (2007) found this is primarily due to exposure of crustecdysone by the female during its post-molt state. Bamber and Naylor (1997) found that when male *C. maenus* were restricted from their sexual pose of rising up on pereopods 2-4 with pereopods 5 extended behind, that receptive females still continued with their part of the ritual and placed themselves upside down with the dorsal side of the carapace touching the floor of the test tank and flexing their abdomens. This

study showed that females are much more active in the mating process than was previously thought.

The female crayfish, *Orconectes quinebaugensis*, appeared to lower aggression during fall reproductive months, perhaps to invest energy in other areas (Warren et al., 2009), while males remained more agonistic overall. Warren et al. (2009) suggested that males have a reproductive advantage if they have higher dominance in the hierarchy. Their continued aggression can aid them in the reproductive hierarchy, while reproductive females can focus more on egg formation than agonistic interactions.

Pre-molt *H. americanus* females seek out dominant males in shelters to mate and reproduce with (Atema et al., 1979) which is different from *Procambarus clarkia*, in which males appear to seek out females (Aquiloni and Gherardi, 2008). Indeed, Cowan (1991) found that ablating male *H. americanus* antennules did not interfere with courtship and mating, but ablation of female antennules had very drastic results. Female *H. americanus* that had the antennules excised exhibited aberrant behavior by molting outside of a shelter, molting at odd times (normal times for molting were in the morning), and mating, but not cohabiting. Many of these females were injured after molting and one experienced death. This demonstrated that in *H. americanus*, female olfaction during mating is more important than male olfaction. In the case of the land hermit crab, *Coenobita compressus*, Contreras-Garduno, et al. (2007) found that males will compete with each other for females. However, females will ultimately choose which male to mate with.

Aquiloni and Gherardi (2008) found *Procambarus clarkii* males and females responded differently to signals for mating. Both reacted aggressively by raising their

chelipeds to the opposite sex if only visual stimuli were used, but males showed non-aggressive gestures with lowered chelipeds, as long as female chemical cues were present, even if no visual cue was present. Females remained aggressive until both visual and chemical cues were presented together. The authors suggested that males may be the mate searchers in this species since they utilize odors more in the search for mates.

Booksmythe et al. (2008) found that the female fiddler crab, *Uca mjoebergi,* travels less in search for mates, while male counterparts were much more willing to travel longer distances to find a mate. Female *U. mjoebergi* prefer more attractive males over less attractive mates only when the attractive male's distance was within 20 cm of the female. Males were considered more attractive if they initiated the mating ritual of claw-waving before other males. In order for males to offset the disadvantage of being too far away from a female, males were willing to leave their burrows unguarded, travel farther distances, and come within 5 cm of a tethered female to convince the female to come with them.

The male hermit crab, *Pagurus filholi*, exhibits pre- and post-copulatory guarding, and will choose females to mate with if they are within 5 days of spawning (Goshima et al., 1998). When females are not ready to spawn, they are likely to consider conspecifics as competitors. During reproductive months, males become resources for the female and the female's behavior changes as the female is dragged by the male in pre-copulatory guarding behavior.

Although so much of reproduction is dependent on chemical cues, crustaceans are highly dependent on chemical stimuli in many other areas of their lives.

Chemical Communication

Communication in crustaceans is very important. Many factors influence the way in which crustaceans communicate. One important factor is competition for resources. Motivation levels of an animal to acquire resources may differ in individuals (Billock and Dunbar, 2008), and thus may lead to different behaviors and higher levels of competition for the resources (Pechenik et al., 2001). Larger individuals of hermit crabs often have a higher degree of limiting resources in finding large shells (Shih and Mok, 2000), thus increasing their aggressiveness and motivation level for those resources.

Marine invertebrates may opt not to physically compete for resources, since doing so will deplete energy sources and may cause injury. Visual or tactile communication can then be utilized. Adamo and Hanlon (1996) found that the male cuttlefish, *Sepia officinalis*, gives visual cues through face darkening to male conspecifics to signal levels of aggression. Darker faces mean higher aggression. The hermit crab, *Pagurus bernhardus,* displays its cheliped to display its size in an agonistic interaction (Elwood et al., 2006). Hermit crabs also exhibit a tactile form of communication in shell rapping, which takes place between an attacker and defender in competition for a gastropod shell. The power of the shell rapping by the attacker informs the defender of the motivational level of the attacker (Briffa and Elwood, 2000, 2002).

Olfaction or chemical communication may be just as important as visual communication. Relaying chemical messages may increase efficiency, and reduce energy consumption and potential for physical harm when interacting with conspecifics and predators. In aquatic animals, the importance of chemical communication is heightened by turbid waters when visibility is reduced (Dodson et al., 1994; Correia et al., 2007).

Predators and Predation Events

Crustaceans may be warned of the presence of predators by signals sent out from conspecifics that have encountered predators and escaped (Schneider and Moore, 2000), by the odor of the predator itself (Chiussi et al., 2001; Briffa et al., 2008), or by sensing dead conspecifics in the water around them (Hazlett et al., 2006). The crayfish, *Procambarus clarkii,* increased its output of urine when under stress by a predator and also released a chemical in its urine when it was exposed to predators (Schneider and Moore, 2000). Although the crayfish were not harmed by the predator, they were in visual contact with the predator. Schneider and Moore (2001) then exposed conspecifics to the water that surrounded these stressed conspecifics. When other conspecifics sensed this chemical from stressed crayfish, the conspecifics walked faster and farther than they did in non-stressed crayfish cue waters, and moved away from the source of the signal. These investigators found that stressed crayfish were aiding in the survival of conspecifics by sending out specific chemicals about predators. Thus, it appears that a different chemical is released in crayfish urine when they sense predators than when they engage in other activities.

Juvenile blue crabs, *Callinectes sapidus*, will not respond to visual cues of predators unless chemical cues of estuarine origins are introduced (Diaz et al., 2003). The juvenile mangrove crab, *Aratus pisonii*, can sense predator odor and, on average, orients itself 21% more often towards visual cues for shelter when predator odor is introduced (Chiussi, 2003). The crayfish, *Orconectes virilis,* decreases the time spent grooming, moving its chelipeds or antennae, or exhibiting feeding movements when predator odor is introduced (Hazlett, 1999). When food odor is introduced, the crayfish spends more time

moving and exhibiting feeding movement and less time in its burrow than when predator odor is introduced.

Crustaceans also react to different chemical cues released from crushed conspecifics (Chiussi et al., 2001; Diaz et al., 2003; Hazlett et al., 2006; Bulinski, 2007). They can sense conspecific haemolymph in the surrounding water. Researchers have used crushed conspecifics to simulate a predation event in many crustacean studies. Crustaceans that encounter an environment with crushed conspecifics usually respond by hiding, as in the case of the crayfish, *O. virilis* (Hazlett et al., 2006), or exhibiting an escape response, as in the blue crab, *C. sapidus* (Diaz et al., 2003). However, Bulinski (2007) found that the hermit crab, *Pagurus granosimanus,* actually switched shells more often when exposed to crushed conspecifics. Bulinski (2007) suggests that this may be because a dead conspecific may signal that a shell has become available for habitation.

The hermit crab, C*libanarius vittatus*, exhibits a flight response when a predator odor is introduced to the water (Rittschof and Hazlett, 1997). The hermit crab, *Clibanarius antillensis* (Chiussi et al., 2001), responded significantly more often towards or away from visual targets when appropriate chemical cues of gastropod and calcium odors, predator odors, and seagrass odors were given than when no odors were given.

Hierarchies and Agonistic Interactions

Crustaceans show evidence of individual recognition. Individuals of the crayfish, *Orconectes rusticus,* recognize conspecifics they have encountered previously, and engage in agonistic interactions for a shorter time when they meet a familiar conspecific (Schneider et al., 2001). These crayfish also sense the status of conspecifics and thus can

either fight one that seems to be subordinate, or not fight an individual that seems to be more dominant. Urine plays an important part in these behaviors. Those crayfish with blocked nephropores spent much more time and energy engaging in agonistic interactions (Schneider et al., 2001).

The big-clawed snapping shrimp, *Alpheus heterochaelis*, may use urine as a means of chemical messaging in establishing dominance. It is more aggressive towards conspecifics that have previously lost to another conspecific, and are less aggressive towards a conspecific that has won in a recent fight (Obermeier and Schmitz, 2003).

The lobster, *Homarus americanus,* increases its urine output when it encounters other conspecifics and engages in agonistic interactions. On average, aggressive individuals released seven times more urine than nonaggressive, defending individuals (Breithaupt et al., 1999). Lobsters can even recognize conspecifics they have met before by their urine, and create social hierarchies (Karavanich and Atema, 1998c; Karavanich and Atema, 1998b). When a known, or familiar, dominant individual releases urine, the subordinate lobster immediately retreats, thereby avoiding a potentially costly agonistic interaction (Karavanich and Atema, 1998b). Another study on *H. americanus* by Breithaupt and Atema (2000) found lobsters that won agonistic interactions with a conspecific usually released urine early on in the fight. They suggested that urine release coupled with aggressive behavior may let the receiver know the status of the lobster and thus discourage the subordinate from fighting a long and injurious fight. By demonstrating that these crustaceans can control their urine output, Breithaupt and Atema (2002) have provided a good foundation for examining hypotheses regarding chemical use by specific crustaceans during messaging in different situations.

The hermit crab, *Pagurus bernhardus*, has been shown to release an unidentified chemical when engaging in agonistic interactions with other hermit crabs (Briffa and Williams, 2006). Their study showed how a focal male hermit crab reacted to odors left by other male hermit crabs that had been involved in aggression through shell swapping. These focal hermit crabs spent more time in their shell, less time in locomotion, and less time foraging for food when they sensed aggressive conspecific behaviors.

Resource Acquisition

Urine is also important for the Caribbean spiny lobster, *Panuliris argus,* in finding shelter (Horner et al., 2008). This study on *Panuliris argus* showed how the aesthetic region of the lateral flagellum of the antenna was the sensitive area for urine pheromones when in search of suitable shelter. Urine in this species is an aggregation pheromone (Horner et al., 2006). Aesthetic-ablated lobsters sought shelter as much as aesthetic-intact lobsters, but aesthetic-intact lobsters preferred shelters emanating conspecific urine, while aesthetic-ablated lobsters showed no significant preference for shelters with conspecific urine or odor-free seawater (Horner et al., 2008).

The hermit crab, *Pagurus longicarpus* Say 1817, has a more complex system for individual recognition than was supposed invertebrates were capable of (Gherardi et al., 2005). When a focal crab sensed an empty shell in the presence of a familiar crab that had a high-quality shell, the focal crab was more likely to investigate the shell quicker and for a longer period of time than in the presence of a familiar crab that had a low-quality shell. This showed that these crabs were utilizing their memory, so that if a familiar crab had a high-quality shell and vacated it for a better one, other crabs more readily took over the

shell that was empty.

Hormones and Behaviors

Many studies have been performed on crustacean hemolymph, biogenic amines, and hormones. The purposes of these studies have varied from understanding how hormones affected dominant and subordinate behavior (Antonsen and Paul, 1997; Sneddon et al., 2000; Briffa and Elwood, 2007), and crustacean circulatory systems (Tsukamoto and Kuwasawa, 2003; Yamagishi et al., 2004), to crustacean physiology (Sy and Airriess, 2002; Tierney et al., 2003). Hormonal levels have been shown to play a part in invertebrate behavior, and may play an important role in chemical communication.

Hormones in crustacean hemolymph have been connected with different behaviors. Antonsen and Paul (1997) found they could manipulate certain behaviors by injecting biogenic amines into the squat lobster, *Munida quadrispina*. These lobsters are usually nonaggressive and do not set up any type of observed dominance hierarchy. Injecting serotonin into these usually docile creatures caused them to become much more aggressive. One animal exhibited aggression towards a potential predator instead of exhibiting an escape response. Lobsters injected with octopamine became more likely to exhibit escape responses. These researchers claimed that aminergic neurons were heavily responsible for some types of behavior in the squat lobster.

Huber and Delago (1998) showed that manipulating serotonin levels in crayfish can cause a subordinate crayfish, *Astacus astacus,* to fight longer than normal. Subordinate animals would escalate fighting instead of retreating when infused with serotonin. Although higher serotonin levels caused a subordinate crayfish to fight longer,

it did not ensure it would be the winner. Panksepp et al. (2003) infused crayfish, *Astacus astacus,* with serotonin and noted aggression. They found that the amines themselves were not responsible for certain behaviors in the crayfish. After size-matching dominant and subordinate crayfish, they found no difference in levels of dopamine and serotonin in hemolymph when compared to controls. Their conclusion was that amines only fine tune and modulate behavior and are not necessarily the causal element of behavior in crayfish. Panksepp et al. (2003) state that there are more elements to consider when observing animal behavior than just biogenic amines. Circumstances, motivational state, and neurochemical mechanisms all play a crucial role in determining animal behavior.

Such close relationships between these hormones and the nervous system must impact the behavior of the animal in very important ways. Laxmyr (1984) measured levels of serotonin, dopamine, noradrenaline, octopamine, and dihydroxyphelalanine in the nervous systems of five different decapod crustaceans using high pressure liquid chromatography. The animals were *Carcinus maenas, Eupagurus bernhardus, Homarus vulgaris, Pandalus borealis,* and *Leander squilla.* Octopamine had the highest levels of all hormones for most of the species. All substances were present in all species, except *Leander squilla.* Neither octopamine nor serotonin levels were detected in this species because of insufficient sample size. The study by Laxmyr (1984) merely established the presence of these biogenic amines and amino acids in the central nervous system (CNS) of these crustacean species, but did not determine the direct effect of the amines on behavior. Laxmyr (1984) states that the presence of noradrenaline and dopamine in the CNS of these animals elevates the chance that these are neurotransmitters in the CNS of crustaceans.

Marine invertebrates have much more complex systems than previously recognized. It was previously thought that physical strength alone was the factor determining the outcome of fights over resources or in establishing dominance. However, Moore and Bergman (2005) showed how chemical communication, previous history, and the neurochemical state of opponents often determine outcomes of crayfish fights, not simply physical strength.

Stressful conditions can also cause changes in levels of specific compounds in the hemolymph (Lacoste et al., 2001; Chang, 2005). Chang (2005) investigated Crustacean Hyperglycemic Hormone (CHH) and heat stress proteins, and found that CHH levels in crabs can vary under differing oxygen levels, salinities, and temperatures. He found that individuals of the Norway lobster, *Nephrops norvegicus*, with parasites had higher levels of CHH. With heat-shock treatment and salinity changes, these lobster exhibited higher heat shock protein levels or had elevated mRNA expression than normal. A similar study was done on the oyster, *Crassostrea gigas* (Lacoste et al., 2001). Researchers exposed the oyster to different types of stressors: shaking, temperature change, and salinity change. They found increased levels of measured noradrenaline (NA) and dopamine (DA) in the hemolymph after the oysters were exposed to these stressors.

Research on ovigerous *H. americanus* showed that there was no difference in levels of hormone in the hemolymph when females were stripped of their eggs or left with their eggs (Figler et al., 2004). Nonstripped females were usually more aggressive than stripped females. Researchers found no significant difference between serotonin levels of nonstripped and stripped females, and thus, concluded that their maternal aggressiveness did not directly stem from serotonin. A study has also been done showing

how varying serotonin levels in juvenile *H. americanus* affect their behavior in shelter competition (Peeke et al., 2000). Lobsters with high (> 3.0 mg/kg) dosages of serotonin, had lower ability to compete for a shelter than saline-injected lobsters. Although serotonin appears to increase aggression in many animals, it appears to decrease ability to compete for resources in this species.

Sneddon et al., (2000) showed that activity alone does not raise or lower hormone levels in the shore crab, *Carcinus maenas*. These researchers caused crabs to walk on treadmills and found that walking alone did not alter the hormone levels. They found that agonistic interactions were the cause of increased octopamine levels in losers and decreased levels in winners. In contrast, serotonin levels increased in both winners and losers and may have been related to the fact that both were fighting and engaging in aggressive behavior. Still, winners of fights had higher levels of serotonin than losers. The intensity of the interactions was positively correlated with levels of dopamine. Sneddon et al. (2000) found octopamine, serotonin, and dopamine to be related to agonistic behavior, while tyramine and norepinephrine had no part in the behavior.

Serotonin and dopamine levels have been studied in hermit crab hemolymph (Briffa and Elwood, 2007). Levels of these monoamines differ according to the role the hermit crab played, either as attacker or defender. Dopamine levels were usually higher in attackers or initiators of fights. Serotonin levels were high in those individuals that successfully evicted conspecifics from their shells.

Although there are conflicting studies regarding the role of hormones in aggressive behavior, hormones do appear to have a strong relationship with dominance. If dominant animals tend to release more urine in agonistic interactions than subordinate

ones and dominant animals have higher levels of certain types of hormones, there may be evidence of hormone-like molecules in the urine of these animals. Very few studies have looked at the contents of urine, although research thus far has shown that some type(s) of urine-borne signals are responsible for chemical communication (Atema and Cowan, 1986; Breithaupt and Atema, 1993; Schneider and Moore, 2000; Breithaupt and Eger, 2002). To date, the only study that identified a urine-borne signal was work on the shore crab, *Carcinus maenus*, by Hardege et. al. (2002), who found that the female sex pheromone does not appear to be a type of steroid. Sulphate metabolites have also been noted in the urine of lobsters (Huber et al., 1997).

Purpose of Study

Sex and chemical communication can have large impacts on behavior in crustaceans. Understanding communication and sex-specific differences in crustaceans may be one of the steps to understanding toxic effects on aquatic ecosystems. Male and female crustaceans may have different tolerances to toxins in the water. Sroda and Cossu-Leguille (2011a) found that female *Gammarus roeseli* had higher tolerance to toxic copper. Researchers now have a stronger basis for studying both sexes when analyzing effects of toxins on species. In research, understanding sexual dimorphism can allow for sex-specific collection of animals. This would allow less sacrifice of animals when performing sex-specific research. There are also commercial benefits. Wang et al. (2011) found that female *P. clarkii* yield more abdominal meat than males. They suggested that taking out large aggressive male *P. clarkii* from populations in aquafarms may promote the growth of larger females and may promote the growth of smaller, less aggressive

conspecific males.

We have used the hermit crab, *Pagurus samuelis,* which is an abundant animal along Southern California coasts. *Pagurus samuelis* has been used in various studies of aggression (Courches.E and Barlow, 1971), shell selection (Hahn, 1998), and behavior (Billock and Dunbar, 2008; Shives and Dunbar, 2010; Billock and Dunbar, 2011). Hermit crabs are unique in that one of their major resources- the shell- can be a sign of a mate, a competitor, its home, or its food. I explored sexual dimorphism in *P. samuelis*. Cephalothorax and chelae measurements were taken in both male and female hermit crabs to determine if there is evidence of sexual dimorphism in this species. Individuals were analyzed to determine if there were differences in behavior between sexes when hermit crabs were exposed to chemical cues from conspecifics of the same sex. Visual cues were absent in our research on *P. samuelis* behavior to ensure the absence of any confounding factors on a study designed to test reactions to chemical cues.

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CHAPTER TWO

SEXUAL DIMORPHISM AND BEHAVIORAL RESPONSES TO CONSPECIFIC

CHEMICAL CUES IN THE HERMIT CRAB *PAGURUS SAMUELIS*

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Abstract

We compared anatomical differences between sexes of *P. samuelis,* measuring the major cheliped and carapace lengths of hermit crabs, caught off the coasts of Southern California. Male chelipeds were significantly larger relative to their carapace lengths than female chelipeds. Average male cheliped:carapace ratios were 1.86 while average female cheliped:carapace ratios were 1.32. We suggest that intrasexual selection is the reason for dimorphism in this species. Behavioral gender differences were studied for responses to cue waters created from their own sex. We recorded hermit crabs exposed to cue treatment waters and analyzed four behaviors: withdrawn, head-extended, walking, and meral spread. In non-agonistic cue treatment waters, females were more likely than males to remain withdrawn in their shells. Males were more likely than females to display meral spread when sensing conspecific cues. Both sexes displayed no difference in the amount of time spent stationary with head-extended across all cue treatment waters. Both sexes tended to walk more in the presence of agonistic cue waters.

Keywords: Chemical communication, crustaceans, sexual differences

Introduction

Chemical communication in crustaceans can be just as important as visual or tactile communication. The importance of chemical communication may be heightened by darkness or turbid waters when visibility is reduced (Dodson et al., 1994; Correia et al., 2007). Relying on chemical messages may increase efficiency and reduce energy consumption and potential for physical harm when interacting with conspecifics or avoiding predators. Some species will interpret their visual surroundings based on chemical input (Diaz et al., 2003), part of what Billock and Dunbar (2011) termed "Contextual Decision Hierarchies." Chemical communication can also be essential in social contexts, such as establishing social hierarchies (Moore and Bergman, 2005).

Competition for resources can influence methods of communication. Motivation levels of an animal to acquire resources may differ among individuals (Billock and Dunbar, 2008), and thus lead to a variety of behaviors and higher levels of competition or aggression for resources (Pechenik et al., 2001). Female hermit crabs, for example, may seek different types of shells than males (Wait and Schoeman, 2012; Yoshino et al., 2011) depending on ovigery and volume of the shell, leading to sexual differences in the use of resources. Large hermit crabs often encounter difficulties in finding suitably large shells (Shih and Mok, 2000), leading to increased aggressiveness for this essential resource and, potentially, different communication methods.

One way hermit crabs communicate their motivation levels to conspecifics is by powerful shell rapping. When hermit crabs contest over a gastropod shell that is already occupied, the attacker holds the shell of the opponent and brings its own shell into contact with the other shell, initiating a series of shell raps. The power of the rap itself, and the

intervals between shell rapping bouts communicate to the defender the strength and motivation level of the attacker (Briffa and Elwood, 2000, 2002). In intersexual agonistic contests, females of *P. bernhardus* initially fought harder through shorter intervals between shell rappings than males did (Briffa and Dallaway, 2007), but there was no difference in actual eviction success between the sexes.

Several decapod species rely heavily on chemical cues (Atema, 1986; Gherardi and Tricarico, 2007). These cues may be used to lower the likelihood of cannibalism of soft-bodied mates (Hayden et al., 2007), and to communicate sex (Atema and Cowan, 1986) and reproductive status (Simon and Moore, 2007). Females of the crayfish *Orconectes quinebaugensis*, for example, appear to lower aggression during fall reproductive months, perhaps to invest energy in other areas, while males remain more agonistic overall (Warren et al., 2009). Warren et al. (2009) suggested that the continued aggression by males can aid them in acquiring higher social status in the dominance hierarchy, whereas reproductive females focus more on egg formation and reproduction than on agonistic interactions, thereby giving dominant males a reproductive advantage. Males of the hermit crab *Pagurus filholi* exhibit pre- and post-copulatory guarding, and can perceive and preferentially choose females to mate with that are within 5 days of spawning (Goshima et al., 1998).

Although crustaceans may not be able to control their odors all the time, especially in the case of injured individuals, studies have demonstrated that they exhibit a degree of control over the release of their chemical messages (Breithaupt et al., 1999; Breithaupt and Atema, 2000; Schneider and Moore, 2000; Breithaupt and Eger, 2002). The hermit crab *P. bernhardus*, for example, releases an unidentified chemical when

engaging in agonistic interactions with other hermit crabs (Briffa and Williams, 2006). Focal male hermit crabs in the study reacted to odors left by other male hermit crabs that had engaged in aggressive shell swapping. These focal hermit crabs spent more time in their shell, less time in locomotion, and less time foraging for food when they sensed the presence of aggressive conspecifics.

In some crustacean species, anatomical size or size of body parts appear to determine agonistic contest outcome. Mowles et. al (2011) found that winners in shell fights have larger abdominal muscle mass:body mass ratios than losers. The hermit crab *Pagurus bernhardus* displays its cheliped to show its size in agonistic interactions (Elwood et al., 2006; Arnott and Elwood, 2010). Larger hermit crabs are more likely to have larger chelipeds, which would increase the potential of winning agonistic contests. Larger male hermit crabs win more often than smaller conspecifics in competition for female hermit crabs (Contreras-Garduno et al., 2007; Yoshino et al., 2011) and for food (Ramsay et al., 1997). Males contesting over females in the species *Diogenes nitidimanus* appear to have a much greater advantage when they have larger major cheliped:body mass ratios (Yoshino et al, 2011).

Some hermit crab species exhibit sexual dimorphism in cheliped size. Males possess larger chelipeds than females, as documented in the hermit crabs *Pagurus bernhardus* (Briffa and Dallaway, 2007), *P. criniticornis* (Mantelatto et al., 2007), *Isocheles sawayai* (Fantucci et al., 2009), and *Diogenes nitidimanus* (Koga et al., 2010). In the case of *P. bernhardus*, possessing a larger chela does not appear to give one crab an advantage over another when contesting over gastropod shells with conspecifics (Briffa and Dallaway, 2007). However, males of *D. nitidimanus* with larger major chelipeds gain

a significant advantage when contesting for females (Yoshino et al., 2011), thus giving evidence that this trait may be a consequence of intrasexual selection.

The hermit crab *Pagurus samuelis* occurs abundantly along the West coast of North America. This species was analyzed in prior studies of aggression (Courches and Barlow, 1971), shell selection (Hahn, 1998), and behavior (Billock and Dunbar, 2008; Shives and Dunbar, 2010), but sexual differences in behavior have not been examined. Hermit crabs are unique in that one of their major resources, the shell, may be a visual cue for a mate, a competitor, its home, or its food (Billock and Dunbar, 2008). This suggests that chemical cues may be important in determining what type of resource a shell really is.

The purpose of this study was to explore the potential for and presence of sex-specific responses to chemical cues in *P. samuelis*. We began by testing the hypothesis that sexual dimorphism exists for cheliped size in *P. samuelis*. We then evaluated the hypothesis that *P. samuelis* exhibits different behavioral responses to same-sex agonistic, versus same-sex non-agonistic, chemical cues. Our experimental design also allowed us to test for sex-specific and size-related differences in the behavioral responses.

Methods

Capture and Housing

We hand-captured 150 *Pagurus samuelis* specimens on the coast of southern California (Moss Street Beach: 33°31'38,61"N, 117°46'18.01"W; Little Corona Del Mar: 33°35'33.51"N, 117°52'04.21"W), and housed them in 40-L aquaria. Each aquarium contained approximately 12 L of constantly aerated seawater, 1 – 2 cm of sand and

pebbles for substrate, and one large rock with a portion above the 7-cm-deep water. We obtained seawater from the Kirkhoff Marine Laboratory at Newport Beach. Crabs were maintained at a constant temperature of 21.5 ± 2.4 °C with ambient light, and fed 2–3 times a week with commercial frozen shrimp.

After 1–2 weeks of acclimation in the laboratory, hermit crabs were individually placed in small plastic containers (80 – 120 cm^2 floor area) with 350 ml seawater and constant aeration. Subjects remained isolated 10–30 days prior to testing, during which they were fed and the water was changed 2–3 times per week. We withheld food 2 days prior to testing, and changed the water 1 day prior.

Sexual Dimorphism Measurements

Hermit crabs were removed from their shells by gently cracking the shells open with a table vise. We weighed and sexed all 150 hermit crabs, and measured the calcified anterior portion of the cephalothorax (shield) and major cheliped to the nearest 0.5 mm using calipers. For consistency, one of us (MK) obtained all measurements. We determined sex using a dissecting microscope to detect the gonopore found in females at the coxa of the third periopods (McLaughlin, 1974).

After initial collection of hermit crabs, and noting apparent sexual dimorphism in cheliped size, we attempted to predict the sex of individuals in the field while collecting the last 47 specimens. Thus, we recorded our impression of sex based on relative cheliped size, and compared our sex assignments to actual sex determined subsequently using a microscope.

Cue Water Preparation

Hermit crabs were subjected to three water treatments with different chemical cues present: 1) same-sex agonistic cues, 2) same-sex non-agonistic cues, and 3) a control devoid of hermit crab cues. Accordingly, three types of cue water were prepared for each sex. Agonistic-male cue water was prepared by placing four hermit crabs in 400 ml of UV-filtered seawater with aeration for 4 hr prior to focal crab exposure to cue water. Agonistic-female cue water was prepared in the same way using four female hermit crabs. To elicit agonistic behavior during cue water preparation, each hermit crab was previously provided only damaged shells with holes or borings ($\geq$4 mm width) in the shell structure. Eviction of conspecifics was noted within the agonistic cue treatment water in the first 5 min. Non-agonistic cue water was prepared by placing a single hermit crab (of appropriate sex) in a plastic container with 100 ml of UV-filtered seawater with aeration for 4 hr. The crab was also previously provided with a damaged shell during isolation. Control water was comprised of 100 ml of UV-filtered seawater devoid of hermit crab chemicals. All seawater used in experimentation was filtered through a UV-filter and a 200-μm mesh filter.

Briffa and Williams (2006) used water from 40 crabs left in a tank with 4.2 L of seawater for 16 hr to create their agonistic cue waters. We opted not to leave the crabs for so long, as cannibalism was often noted for crabs left together for that duration. Cannibalism would allow hemolymph to mix into the water, thus acting as a confounding factor in chemical communication. We sought to eliminate any chemical cues that were not derived from the cue animals as odors or urine.

Experimental Setup of Behavioral Observations

We randomly selected 20 crabs - 10 of each sex - and tested them in a room with no external light. The arena was a small, white, plastic Tupperware container (10-cm diameter) with 100 ml of treatment seawater. The arena was surrounded by a thick black cloth so crabs would not be disturbed by investigator movements in the room. Trials were videotaped using a Logitech QuickCam Orbit AF (Logitech, Newark, California) placed 25 cm above the water level. Crabs were individually exposed to, and recorded in, one of the three different cue waters every 3 days. Each crab was tested in each of the three cue waters that were prepared for its own sex. The order of exposure to different cue waters was randomized.

For each trial, an isolated crab was taken from its container and placed into the circular arena filled with 100 ml of cue treatment water. Videotaping and experimental observations commenced immediately. After 2 min, the arena was gently shaken by hand until the crab withdrew completely into its shell. This mechanical stimulus was performed to observe behavioral changes when the crab sensed conspecifics chemically and then experienced a type of environmental mechanical stimulus or shock. Observations continued for another 2 min. Data were pooled across the 4 min, and differences in reactions between sexes were analyzed.

We analyzed behavior from the videos using JWatcher software (UCLA and The Animal Behaviour Lab, Macquarie University, Sydney). During video review, we recorded the following behaviors and postures for individual hermit crabs: withdrawn (body inside its shell so that the anterior portion was not visible); head-extended (anterior portion out of shell but stationary, eyestalks and pereopods of crab visible to the

observer); walking (crab locomoting in the arena); and meral spread (major and minor cheliped fully extended anteriorly). We then computed the proportion of time during the 4-min trial that each crab devoted to each behavior.

Data Analyses

All data were analyzed using SPSS 13.0 (Statistical Package for the Social Sciences, Inc., Chicago, IL, 2002), with $\alpha = 0.05$. We employed both statistical significance (null hypothesis testing) and practical significance (effect size or magnitude) to assess the relevance of our data. Measures of effect size offer advantages to statistical significance in that they are independent of sample size, they better identify meaningful differences, and they are more readily compared among different data sets and different studies (Cohen, 1988; Nakagawa, 2004; Nakagawa and Cuthill, 2007).

To evaluate sexual dimorphism in cheliped size, we used analysis of covariance (ANCOVA) and discriminant function analysis (DFA; Mertler and Vannatta, 2002). Mass, carapace length, and cheliped length were $\log_{10}$-transformed to better meet parametric assumptions. Although carapace length could not be normalized and was homoscedastic only after rank-transformation, the results of tests were identical using both transformations, so we report only those involving log-transformation. For the ANCOVA, we treated cheliped length as the dependent variable, sex as the independent variable, and carapace length as the cofactor. Effect sizes were computed as partial eta-squared (η^2), with values of ~0.01 loosely regarded as small, ~0.06 medium, and ≥ 0.14 large (Cohen, 1988). The DFA models, including mass, carapace length, and cheliped length as predictors of sex, were constructed using SPSS defaults with leave-one-out classification

to cross-validate results. To compare our prediction of sex of individual hermit crabs in the field with actual sex determined microscopically in the lab, we used Chi-square analysis (Zar, 1996) and computed Phi (φ) for effect size, with values of ~0.1, 0.3, and 0.5 loosely deemed small, medium, and large, respectively (Cohen, 1988).

To compare behavioral responses between sexes and among the three chemical cue conditions, we conducted four separate 2×3 ANOVAs, one for each behavior, with the behavior (proportion of time exhibited during trial) as the dependent variable, sex as a between-subjects factor, and treatment as a within-subjects factor. We could not use an omnibus model that included the four behaviors within a single independent variable because the percentages summed to 100%, which confounded the interaction terms. Furthermore, statistical power constrained by sample size was too weak to include body size as a covariate, or to include all four behaviors as dependent variables within a multivariate analysis of variance model (MANOVA; Mertler and Vannatta, 2002). For each ANOVA model, several cells failed to meet the assumption of normality, and because transformations (arcsin, log, rank) failed to improve this situation, we simply analyzed the original data. Fortunately, ANOVA is robust to departures from normality (Mertler and Vannatta, 2002). Because two of the four models failed the repeated-measures assumption of sphericity, we applied Greenhouse-Geisser adjustments to the degrees-of-freedom for the effects that included repeated measures (Mertler and Vannatta, 2002). Effect sizes were computed as partial eta-squared (η^2) values. Post-hoc multiple comparisons were achieved using least significant differences (LSD; Mertler and Vannatta, 2002).

Because body size may have influenced behavioral responses in the chemical cues

experiment, male and female body size was compared using a *t*-test (Zar, 1996;

parametric assumptions were met) and Cohen's *d* for the effect size, with values of ~0.2,

~0.5, and ≥0.8 loosely considered small, medium, and large effect sizes, respectively

(Cohen, 1988). We conducted bivariate Pearson's correlations (*r*; Zar, 1996) for each sex

separately to examine the relationship between body size and frequency of each behavior

(averaged for each individual across the three chemical cues), with *r* values of ~0.10,

~0.30, and ≥0.50 loosely regarded as small, medium, and large effect sizes, respectively

(Cohen, 1988). However, because one hermit crab of each sex exhibited a relatively high

level of meral spread, a non-parametric Spearman's correlation (r_s; Zar, 1996) more

appropriately described the association of this behavior with body size.

Following Nakagawa (2004), we chose not to adjust α for multiple tests. Mean

values presented here are reported with standard deviation unless otherwise indicated.

Results

Sexual Dimorphism in *Pagurus samuelis*

From the ANCOVA model, a significant interaction existed between sex and

carapace length for cheliped length ($F_{1,146}$ = 21.15, *P* < 0.001, partial η^2 = 0.13),

indicating that relative cheliped length differences between males (*N* = 85) and females

(*N* = 65) increased with growth in body size (Fig. 1). Although the interaction indicated

violation of the ANCOVA assumption of equal regression slopes (Zar, 1996), existence of

the interaction confirmed sexual dimorphism in cheliped size, with males having

relatively larger chelipeds than females (Fig. 1). The effect size for carapace length ($F_{1,146}$

= 231.45, *P* < 0.001, partial η^2 = 0.61) far exceeded that of both sex ($F_{1,146}$ = 11.03, *P* <

0.001, partial $\eta^2 = 0.07$) and the interaction of sex and carapace length (noted above).

The DFA model also confirmed that anatomical differences between sexes were significant (Wilks' $\Lambda = 0.32$, $\chi^2 = 166.2$, df $= 3$, $P < 0.001$). Cheliped length proved better than mass and carapace length at discriminating between males and females
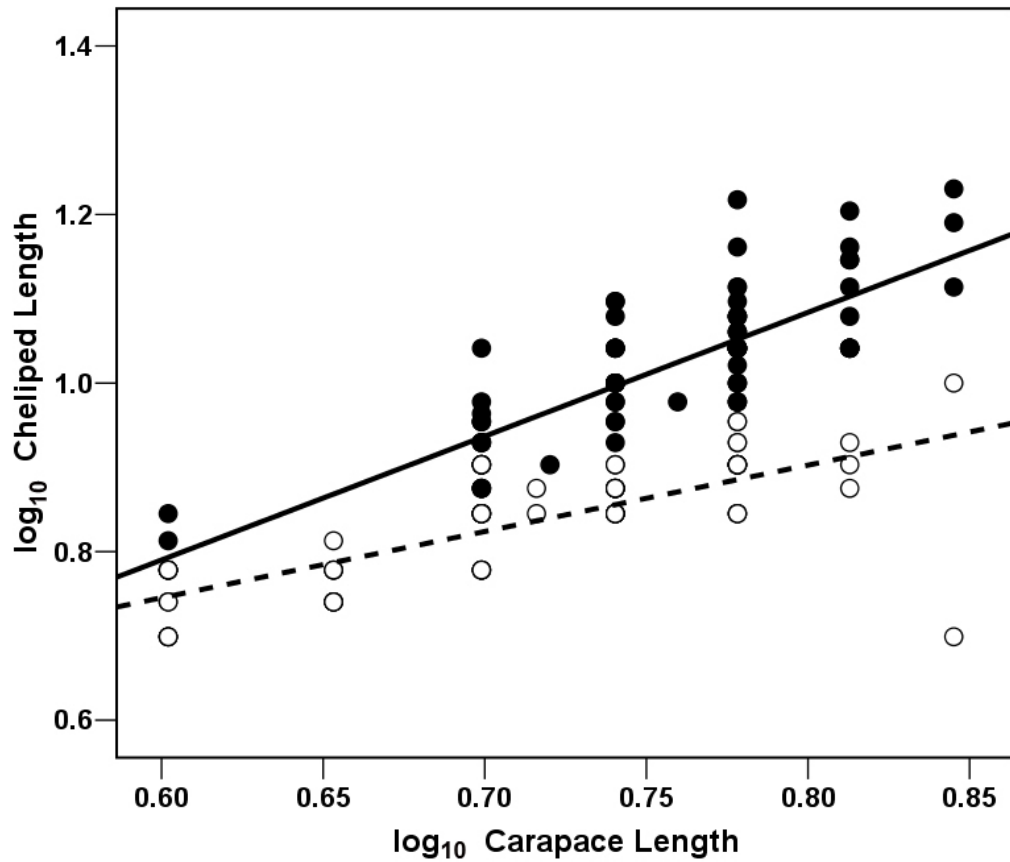
Fig 1. Significant correlation between cheliped length and carapace length and significant interaction between cheliped length and sex (Wilks' $\Lambda = 0.32$, $\chi^2 = 166.2$, df = 3, $P < 0.001$). ●- males, ○- females, solid line- fit line for males, dotted line- fit line for females.

(standardized coefficients were 1.722, -0.68, and -0.52, respectively). Males ($N = 85$) and females ($N = 64$; mass was missing for one individual) were correctly assigned 94.1% and 96.9% of the time, respectively (95.3% for all crabs), for both original and cross-validated classification.

Our attempt to identify sex in the field based on relative cheliped size proved much more successful than expected by chance ($\chi^2 = 29.07$, df $= 1$, $P < 0.001$, $\varphi = 0.79$). We correctly guessed the sex of 90.5% of 21 males and 88.5% of 26 females, which compared favorably to predictions based on morphological measurements (the DFA results).

<div align="center">Behavioral Responses to Chemical Cues</div>

When the 20 hermit crabs (10 of each sex) were tested for behavioral responses in the presence of agonistic, non-agonistic, and control chemical cues, walking was the most frequently observed behavior, followed by head-extended, withdrawn (remaining in shell), and meral spread, respectively (Fig. 2). For withdrawn behavior (remaining in shell), neither main effect (sex, chemical cue condition) nor the interaction were significant (Table 1). However, the relatively large effect size for the interaction (partial $\eta^2 = 0.12$) suggests that females were more likely than males to remain withdrawn in the presence of non-agonistic chemical cues relative to agonistic and control cues (Fig. 2). This effect is almost certainly due to the size difference between males and females. For head-extended behavior, the lack of significant effects and relatively small effect sizes (Table 1) suggest that this behavior did not differ between the sexes or among the three chemical cues (Fig. 2). For walking, there was no interaction and no effect of sex;

<div align="center">53</div>

Table 1. Behavior Table

Behavior	Sex			Condition			Interaction		
	$F_{(1,18)}$	P	η^2	$F_{(2,36)}$	P	η^2	$F_{(2,36)}$	P	η^2
Withdrawn	2.10	0.164	0.11	2.35[a]	0.138	0.12	2.64[a]	0.116	0.13
Head-extended	0.90	0.355	0.05	1.61	0.215	0.08	1.36	0.269	0.07
Walking	0.05	0.826	0.00	3.40	0.044	0.16	1.24	0.301	0.06
Meral spread	3.73	0.069	0.17	2.41[a]	0.130	0.12	1.36[a]	0.264	0.07

[a]Greenhouse-Geisser adjustment applied to degrees-of-freedom: $F_{(1.15,20.75)}$
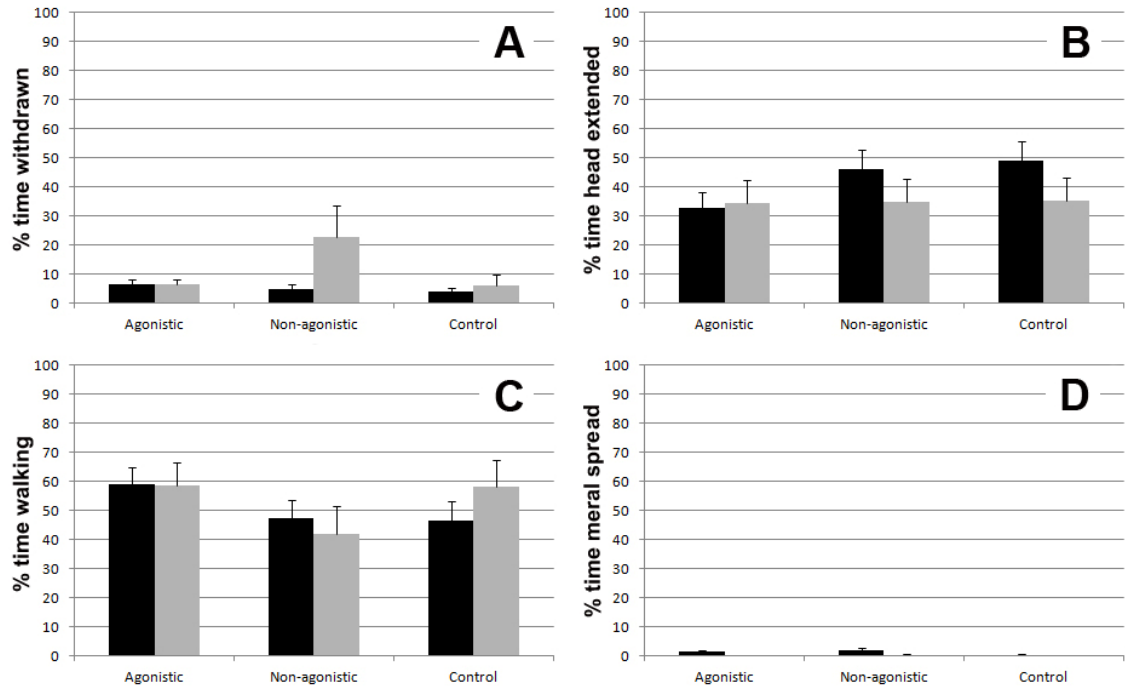
<Figure 2 about here.> Fig. 2. Percent of time spent in observed behaviors. Black bars-males, gray bars- females, error bars- SE. Panels A, B, C and D present the % of time the observed crabs were described as withdrawn, with head extended, walking and with meral spread, respectively.

however, the chemical cue condition was significant ($P = 0.044$, partial $\eta^2 = 0.16$; Table 1), with post-hoc comparisons (LSD) indicating that ambulation by both sexes occurred more frequently in the presence of agonistic cues than in the presence of non-agonistic cues (Fig. 2). For meral spread behavior, no effects were significant, but the large effect size for sex (partial $\eta^2 = 0.17$; Table 1) suggests that males exhibited meral spread with greater frequency than females because of size differences between the sexes. Males in this experiment averaged 34.4% more in mass than females (mean $\pm$ SD: 0.77 ± 0.19 g and 0.57 ± 0.18 g, respectively; $t = 2.37$, df $= 18$, $P = 0.029$, Cohen's $d = 1.06$). Accordingly, sex differences in behavior could have resulted because of body size disparity between the sexes. Among the 10 females, body size was moderately and negatively associated with withdrawn ($r = -0.34$, $P = 0.34$) and head-extended ($r = -0.34$, $P = 0.34$), but positively and more strongly associated with walking ($r = 0.55$, $P = 0.10$) and meral spread ($r_s = 0.51$, $P = 0.13$). Among the 10 males, body size was strongly and negatively associated with withdrawn ($r = -0.69$, $P = 0.026$), independent of head-extended ($r = 0.03$, $P = 0.93$) and walking ($r = 0.16$, $P = 0.66$), and somewhat positively associated with meral spread ($r_s = 0.24$, $P = 0.50$). Thus, the two consistent relationships between body size and behavior in both males and females were that larger individuals of both sexes spent less time withdrawn and more time in meral spread than smaller individuals. When data were pooled for the two sexes ($N = 20$), the associations of body size with withdrawn ($r = -0.46$, $P = 0.040$) and meral spread ($r_s = 0.45$, $P = 0.046$) became significant.

Discussion

This study yielded two noteworthy findings. First, we showed that sexual dimorphism in cheliped size exists in *P. samuelis*, which we suggest may have resulted from intrasexual selection. Second, we found that the behavioral responses of *P. samuelis* varied depending on the presence of same-sex agonistic chemical cues in the water. Although we interpret the results while emphasizing practical significance (effect size) because of sample size constraints, our conclusions are nevertheless supported by statistical significance.

Sexual Dimorphism

We found significant differences in cheliped:carapace ratios between the sexes of *P. samuelis,* providing evidence of sexual size dimorphism, which also exists among other Paguroideans (Bach et al., 2006). In our study, the major chelipeds of males were relatively larger than those of females. The two main reasons for sexual dimorphism are sexual selection (Yoshino et al., 2011) or ecological advantage (Slatkin, 1984). Examples in Shine's (1989) appendix show that sexual dimorphism in the order Decapoda can often be attributed to sexual selection versus ecological causes. However, crustaceans in other subphylums show evidence of sexual dimorphism due to ecological functions (Gilbert & Williamson, 1983; Kornicker, 1985; Shine, 1989). Shine (1989) also suggests that modifications between sexes can occur if one sex was predominantly capturing prey or acquiring resources while the other sex was escaping from danger. Filling separate ecological niches could allow for sexual dimorphism to occur.

In the case of *P. samuelis*, we suggest that sexual dimorphism is apparent due to

reproductive advantage and, more specifically, to intrasexual selection, since in the case of the close relative *P. bernhardus*, having a larger chela did not give any advantage in contests over gastropod shells (Briffa and Dallaway, 2007). No observations, thus far, have been made of any sexual differences in the use or acquisition of resources. Also, larger *P. bernhardus* males win more often in contests over resources, such as females and food, than smaller males (Contreras-Garduno et al., 2007; Ramsay et al., 1997). Sanvicente-Anorve and Hermoso-Salazar (2011) found that in the land hermit crab, *Coenobita clypeatus*, males tended to be larger than females. They attribute this to the fact that females invest much more energy into reproduction.

Large male chelipeds may be advantageous for reproductive behavior, as male hermit crabs tend to compete aggressively for females (Hazlett, 1996). In the hermit crab *Diogenes nitidimanus*, larger males and males with larger chelipeds tend to outcompete smaller males in contests over females (Asakura, 1987). Female size had no impact on being chosen by males for reproduction. Males of *D. nitidimanus* are, on average, larger than females (Asakura, 1995). Larger male chelipeds may also be helpful in the many species of crustaceans that engage in precopulatory guarding (Goshima et al., 1998; Hazlett, 1996). However, having a larger, stronger cheliped may come with a higher metabolic cost, and therefore limit sexual dimorphism in this trait (Doake et al., 2010).

For *Pagurus samuelis*, however, females may actually be more aggressive than males (personal observation). Tanks in which males and females were separated by sex showed that females had more evidence of aggression, with many more females losing appendages than males in male tanks. However, we did not quantify these observations and the loss of appendages among females may be due to other factors, such as stress of

capture or autotomy.

Whereas males likely expend more energy and resources toward developing larger major chelipeds, female *P. samuelis* may expend more energy towards egg production rather than size development. In the case of the hermit crab, *Calcinus tubularis*, egg clutch size was not impacted by female size (Gherardi, 2004). In the wild, 98% of all males were found inhabiting shells, while 84% of females were found in tubes. Contests between genders revealed that males that were previously housed in shells won more contests than females regardless of the females' previous dwelling. When females that had shells contested with males that lived in tubes, females usually won. In this case it appears that, first, having a shell and, second, being male gives a crab an advantage over resources when competing with conspecifics. These data provide evidence that *Calcinus tubularis* males expend more energy in gaining better resources, such as gastropod shells, while females tend to focus on egg production or other activities. Because of the difference in motivation levels for various resources, females may not benefit as much as males from having a larger cheliped.

During the reproductive autumn season, males of the crayfish *Orconectes quinebaugensis* were much more agonistic overall than reproductive females (Warren et al., 2009). Thus, larger males may have greater genetic fitness than smaller males, while female size does not appear to impact reproductive advantage.

Stein (1976) found that the larger chelae in the male crayfish, *Orconectes propinquus*, was primarily for social hierarchy among the males. During the breeding season, males took on a breeding form with the large chelae, and males with the largest chelae were most successful in breeding attempts. Interestingly, when predation risk was

highest for the crayfish in the summer months, males were in the non-breeding form when the chelae are much smaller in proportion to their body size. Thus, in this species, a larger chela was not necessarily an advantage in predator defense or they would show this form during times of high predation risk. Females would also exhibit larger chelae if it was advantageous in food acquisition. Because this is not the case, Stein (1976) concluded that sexual dimorphism in *O. propinquus* was primarily for reproductive purposes.

<p style="text-align:center">Behavioral Differences between Sexes</p>

In this study, we subjected male and female hermit crabs to three different chemical cues (same-sex agonistic odors, same-sex non-agonistic odors, and a control with absence of conspecific odors) and recorded four behavioral responses. We anticipated different responses to the chemical cues, and possibly sex-specific or size-related differences as well. Behavioral differences between the sexes were fairly subtle.

Females were more likely than males to remain withdrawn in their shells in non-agonistic cue treatment waters than in agonistic and control cue treatment waters. These females may have displayed this defensive posture more in this treatment water than agonistic and control waters as a way of limiting the opportunity of encountering a lone conspecific in need of a better shell.

In agonistic cue treatment waters, both sexes increased the amount of time spent walking compared to non-agonistic cue waters. This difference in behaviors between the two treatment waters suggests that when a crab senses many conspecifics engaging in agonistic contests, the crab will exhibit a flight response while a female crab specifically

may opt to remain withdrawn if she senses only a single conspecific. Also, time allotted to walking in agonistic cue treatment water may be higher than normal because the crabs used in creating treatment waters all had inadequate shells. The focal crab may have been attempting to escape from the environment in which it sensed other crabs contending with each other for an adequate shell, or to investigate for a better shell of its own (Bulinski, 2007).

The meral spread behavior is often seen in decapods in agonistic or aggressive situations (Breithaupt and Atema, 2000). In our study, males were more likely to exhibit this behavior than females in agonistic environments. Also, our research shows that larger males generally have larger major chelipeds (Fig. 1). If males are more likely to engage their chelipeds in agonistic behavior, and if larger males win in contests over females (Contreras-Garduno et al., 2007), sexual selection may be a component in the reason why sexual dimorphism is apparent in this species.

The amount of time spent in the head-extended posture showed no difference among the three treatments or the sexes. This behavior appears to be neither aggressive nor defensive and may be the posture for processing their environment. Both males and females appear to need the same amount of time for processing stimuli regardless of their environment.

We found evidence that *P. samuelis* behavior is impacted by chemical cues. In the highly social context that hermit crabs inhabit, chemical cues are important in agonistic contests over resources, reproduction, and predator avoidance. Further studies may more clearly demonstrate if there are behavioral differences between sexes when they sense chemical cues from a conspecific of the opposite sex.

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CHAPTER THREE

DISCUSSION

Sexual dimorphism

I found significant differences in cheliped:shield ratios between sexes of *P. samuelis*. Male major chelipeds were significantly larger relative to body size than females. Although having a stronger, larger cheliped may require more metabolic energy, the larger cheliped may aid male hermit crabs in competing for females (Hazlett, 1996) and in pre-copulatory guarding (Goshima et al., 1998; Hazlett, 1996). Pre-copulatory guarding in hermit crabs involves the male grasping the shell of the female until copulation occurs.

Studies on the role of gender in agonistic contests are conflicting. According to Yoshino and Goshima (2002), male *Pagurus filholi* were more dominant than females when they were contesting in shell fights. They found that larger males could evict females from their shells faster than smaller males could evict females. In this species, larger animals are more likely male than female (Yoshino et al., 2001). In another study, *Pagurus bernhardus* males were more likely to initiate attacks (Briffa and Dallaway, 2007). However, once a crab began attacking, gender had no role in the success of the attacker. Cheliped size also had no relationship to success or failure of an attacker. Briffa and Dallaway (2007) noted that male *P. bernhardus* appeared more successful in fights because they initiated more often than females, but males did not actually have any advantage over females when contesting for shells. The difference in results between

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Yoshino and Goshima (2002) and Briffa and Dallaway (2007) may be attributed to a difference in species or a difference in design. Briffa and Dallaway (2007) stated that males appeared to have higher success in evicting defending crabs, but they realized this was due to the fact that males were more likely to initiate the attacks. Yoshino and Goshima (2002) may not have factored in the higher likelihood of a male to initiate attacks than females, thus giving them different results from Briffa and Dallaway's study (2007).

Sexual size dimorphism may be evident in crustaceans for several reasons. Larger males overall tend to be more successful in gaining and defending resources, such as females, in the hermit crabs, *Clibanarius digueti* (Harvey, 1990), *Clibanarius vittatus* (Hazlett, 1996), and *Coenobita compressus* (Contreras-Garduno et al., 2007). This allows for higher genetic success for larger size to be passed down to their progeny. In *Clibanarius* species, large intruding males could interrupt a courting pair and mate with the female when the original mating male was smaller than the intruder (Harvey, 1990; Hazlett, 1996).

In the land hermit crab, *Coenobita compressus*, larger males win more often than smaller males when competing for females (Contreras-Garduno et al., 2007). This type of competition, found in various species, may give crustacean males an advantage if they are larger in size and have larger chelipeds than other males. Females focus much more of their energy on egg production and protection in many crustacean species (Page and Cooper, 2004; Warren et al., 2009). Thus, males, and not females, have the larger claw in many decapod species.

Larger-chelaed crustaceans often have a greater advantage than smaller-chelaed

crustaceans when competing for resources or avoiding predators. Stein (1976) found that in the crayfish, *Orconectes propinquus*, males have chelae that are up to twice as large as females, overall. He found the greatest evidence for larger chelae in males to be in support of the idea of reproductive success. Prey and food capture is important, but if the primary advantage of larger chelae was simply food acquisition, females might also be expected to have large chelae. Larger chelae have been hypothesized to be an advantage during times of predator defense. Stein (1976) found that the smallmouth bass, *Micropterus dolomieui,* a natural predator, pick out crayfish with smaller chelae. However, male crayfish of this species molt between two forms, a breeding form and non-breeding form. The non-breeding form is most common for approximately eight weeks during the summer months. In this form, the male chelae are much smaller, proportional to the body, than in the breeding form. Fish predation is highest during summer. If larger chelae were advantageous as predator defense, then male crayfish might be expected to show the larger-chelae during non-breeding times, instead of the observed smaller-chelae. Again, if the primary function of large chelae was predator defense, females might also be expected to exhibit this enlarged body structure. Males with larger chelae were significantly more successful in reproduction than males with relatively smaller chelae during competition for females (Stein, 1976).

The advantages of exhibiting a sexually dimorphic trait must outweigh the disadvantages. Male fiddler crabs have a large major claw and a smaller minor feeding claw. Having such a large appendage can be metabolically expensive. However, Bildstein et al. (1989) found that female fiddler crabs were more prone to avian predation than males. Darnell and Munguia (2011) observed differences in body temperature increases

when exposed to radiant heat in intact males, males with the major claw removed, males with the minor claw autotomized, and females. Males with the major claw autotomized showed similar patterns of body temperature increases to females when exposed to radiant heat. Males with only the minor claw removed showed patterns similar to intact males and did not increase in body temperature as much as females or males with no major claw. Thus, thermoregulation can offset the energetic costs of having such a large appendage that is necessary for breeding in males.

Larger chelae did not appear to give males an advantage over females in *O. propinquus* (Stein, 1976) and larger chelipeds did not appear to gives males an advantage over females in *P. bernhardus* (Briffa and Dallaway, 2007). Like *O. propinquus* and *P. behernardus*, having larger chelipeds and body mass may aid male *Pagurus samuelis* hermit crabs to have higher social status and thus, have improved mating success, but not necessarily better access to resources than their smaller-chelaed female conspecifics, although social status was not tested in the current study.

Abrams (1988) put forth three reasons why sexual dimorphism may be evident in hermit crab species: 1) differences in allocation of energy for growth; 2) competition between males for females favor the larger individual, thus, males increase in size more quickly than females; and 3) competition for shells causes character displacement between sexes. Ultimately, sexual dimorphism cannot necessarily be more advantageous for one sex over the other because the other gender would likely, over time, also display those sex-defining characteristics (Stein, 1976) due to environmental pressures. Sexual dimorphism must be apparent for reproductive reasons, for various roles that sex plays in mating, or to fill sex-specific niches.

Chemical communication and Behavior

My research showed significant behavioral differences when the hermit crab, *Pagurus samuelis*, was exposed to different treatment waters. All crabs used in making cue treatment waters were housed in inadequate shells. Thus, agonistic cue treatment water contained hermit crabs that were contesting over gastropod shells. All crabs used in the creation of treatment cue waters were the same gender as the focal crab. Single cue treatment water contained a single hermit crab of the same gender as the focal crab, while control water was pure seawater. Crab behaviors were very predictable and significant overall. Animals spent the most time walking and the least time exhibiting meral spread. After walking, the second most common behavior was stationary with the anterior portion of the body visible, and the third most common behavior was withdrawn in the shell.

Briffa and Williams (2006) found that *P. bernhardus* stayed withdrawn in its shell more when it sensed agonistic interactions or shell fights among conspecifics. Although there were no significant interactions between sex and chemical cue conditions, females in my study tended to stay in their shells more when in single cue treatment waters than when in agonistic or control cue conditions. Crabs in my study increased the amount of time spent walking when in agonistic cue treatment water, suggesting that crabs may have been exhibiting a stronger escape or locomotion response when in agonistic cue treatment water than when in the single cue treatment water or control water. When in single cue water, they may have tried to protect themselves from any potential agonistic encounter with a single hermit crab in an inadequate shell. Alternatively, they may have been waiting for some type of stimulus.

The agonistic meral spread posture was very seldom observed, even when crabs were exposed to agonistic cue treatment waters. Meral spreads and other agonistic or aggressive postures were observed often in *H. americanus* in the case of predator avoidance (Schneider and Moore, 2000), and agonistic interactions between conspecifics (Breithaupt and Atema, 2000). In contrast to the current study, however, both previous studies used visual cues. Breithaupt and Eger (2002) also found that *Astacus leptodactylus* showed aggression towards conspecifics. This posture may be highly dependent on visual stimuli. *Pagurus samuelis* is a highly social crab and can be found feeding in large groups in the intertidal zone. Agonistic postures may only be necessary when a crab is directly competing for resources.

Visual stimulation, in combination with chemical cues, has been shown to alter behavior in crustaceans. The hermit crab, *Clibanarius antillensis,* responded much more strongly to visual cues when appropriate chemical cues were present (Chiussi et al., 2001). Hermit crabs oriented themselves towards targets representing gastropod shells when calcium and gastropod odors were introduced. They also oriented themselves more to striped targets representing seagrass when seagrass odor was introduced. In the crayfish, *Procambarus clarkii*, males were willing to mate when only the female chemical signal was given (Aquiloni and Gherardi, 2008). However, females required both male visual presence as well as chemical stimulation for mating. The blue crab, *Callinectes sapidus,* reacted by moving away from visual targets if there was a chemical cue, regardless of what that cue was - predator odors, crushed conspecific odors, or waters from estuaries (Diaz et al., 2003).

While I found significant differences in behavior between treatment waters and

control waters overall, I found no significant behavioral differences between sexes of *P. samuelis* when exposed to the treatment cue waters from conspecifics in agonistic interactions or single hermit crab water. However, I found large effect sizes for the interaction between sex and chemical cue condition for females in the non-agonistic cue condition for the withdrawn behavior. Meral spread behavior data analyses showed that although there were no significant effects, there was also a large effect size showing that males were more likely to exhibit this behavior than females.

My study focused on reactions to cue waters created by the same sex as the treatment crab. Female *P. samuelis* responded to female cue waters in the same way that male *P. samuelis* responded to male cue waters. One of the weaknesses of my study is that I did not test responses of male hermit crabs to female cue treatment waters, or female hermit crabs to male cue treatment waters. However, there is good reason to believe that such experiments would be beneficial in our understanding of chemical communication and behavioral differences between sexes of *Pagurus samuelis,* since cues from the opposite sex may also cause behavioral changes. There appeared to be higher number of missing or autotomized appendages in tanks containing only females than in tanks of only males. This could be due to higher levels of aggression between females than is apparent between males, or to increased autotomy overall in females. However, this was not quantified in the current study.

There are many ways in which behavior may be altered by chemical cues from the other sex, with reproductive behaviors providing the best examples. In the hermit crab, *Pagurus filholi,* males could sense when a female was within 5 days of spawning (Goshima et al., 1998). Only when a female was within that period of time were they

constrained by males in pre-copulatory guarding. In the hermit crab, *Clibanarius vittatus*, male crabs reacted significantly to female odors by increasing the amount of time spent in locomotion and even grasping other smaller males when water from tanks that housed only female hermit crabs was introduced into the male tank (Hazlett, 1996). Male *P. clarkii* were aggressive towards female conspecifics when given only visual cues, but became willing to mate when presented with female conspecific cues (Aquiloni and Gherardi, 2008). In the crayfish, *Homarus americanus,* males responded very strongly to female urine by raising their bodies higher off the ground (Atema, 1986). Female shore crabs from the species *Carcinus maenas* released hydroxyecdysone during the summer reproductive months to deter males from cannibalistic behavior while the females were in their soft moulting stage (Hayden et al., 2007). During that time, females continued to feed intensely, while males significantly reduced their feeding activities.

Even in agonistic environments, individuals of a species may alter behavior based on the sex of conspecifics encountered. Briffa and Dallaway (2007) found that *Pagurus bernhardus* attackers, regardless of sex, can sense when a female is defending its shell. The attacker is more persistent than when a male is defending its shell. However, in the end, males and females had the same ability to defend. A similar phenomenon was observed intersexually and intrasexually in *Pagurus filholi* when males defended their shells in agonistic shell fights for significantly longer times than females (Yoshino and Goshima, 2002). Males fought longer overall. Thus, behaviors change not only in reproductive settings, but in agonistic settings between sexes, as well.

Martin and Moore (2010) found that *O. rusticus* exhibits varying levels of aggression based on reproductive state in fights against conspecifics of the opposite sex.

Reproductive males won significantly more often when fighting against reproductive and non-reproductive females. Reproductive females won significantly more fights when the male was non-reproductive than when the male was reproductive. In intersexual agonistic fights, when both crayfish were reproductive, fights lasted longer and potentially ended in matings. Simon and Moore (2007) found that crayfish in this species excreted more urine during intersexual agonistic contests when both animals were reproductive than when only one was reproductive or neither were reproductive, giving more evidence for the role of chemical communication in mating rituals.

In general, pheromones, cues, odors, signals from the natural habitat, conspecifics, predators, and other species have all been shown to alter behavior in crustaceans. The hermit crab, *Clibanarius vittatus*, increases overall locomotion when a predator odor is introduced to its environment (Rittschof and Hazlett, 1997). Evidence shows that crayfish establish a hierarchical system, partly based on chemical communication (Moore and Bergman, 2005). The crayfish, *Orconectes rusticus* (Schneider et al., 2001), and the lobster, *Homarus americanus* (Karavanich and Atema, 1998a; Karavanich and Atema, 1998b), can recognize conspecifics it has met before by their urine, thus decreasing time spent in agonistic contests between familiar conspecifics. The lobster, *H. americanus*, uses urine to send signals during agonistic contacts and always in connection with aggressive behavior (Breithaupt et al., 1999). They found that aggressive lobsters and eventual winners release more urine at the onset of agonistic contests. The mangrove crab, *Aratus pisonii*, finds shelter when predator odor is introduced (Chiussi, 2003). The crayfish, *Orconectes virilis*, also alters its behavior when predator odor is introduced (Hazlett, 1999).

Chemical cues can alter behavior in measurable ways. Crustaceans are highly dependent on chemical cues for their survival and for information regarding both their environments and their interactions with other organisms. The decisions they make, the contests they win or lose, and the shelters they choose, may all be dependent on the correct interpretation of chemical cues.

Directions of Future Research

The role of sex-specific differences in anatomy and behavior in *Pagurus samuelis* has received investigative attention. Sexual dimorphism is mainly apparent for each sex to fill a sex-specific role or niche, or for reproductive advantage within one sex (Stein, 1976). Thus research determining the effects, the roles, and the necessity of sexual dimorphism should be pursued to understand the ecological and behavioral roles that sexual dimorphism plays in *P. samuelis.*

Future studies in resource utilization differences between sexes, intrasexual versus intersexual agonistic contests, and responses to agonistic cue treatment waters of the opposite sex would be beneficial. Studies in resource utilization differences would help determine potential issues in population decline if one sex was declining due to certain limited resources. Comparing differences in intersexual contests versus intrasexual contests would give a much better understanding of motivation levels between sexes and behavioral differences between sexes. Differences in reaction to chemical cues from males or females in agonistic contests would give evidence for significant differences in the chemical cues between sexes. Chemical cues from the opposite gender in agonistic

situations may allow less energy to be spent in fighting when conspecifics understand the

motivation levels for those resources based on the conspecific's sex.

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